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# SECONDARY METABOLITE PRODUCTION BY *FUSARIUM* SPECIES ISOLATED FROM MAIZE KERNELS

by

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# SECONDARY METABOLITE PRODUCTION BY *FUSARIUM* SPECIES ISOLATED FROM MAIZE KERNELS

**K. Kpodo, U. Thrane, and M. Jakobsen**

## SUMMARY

The potential of the *Fusarium* isolates from maize kernels to produce mycotoxins other than fumonisins in addition to other secondary metabolites on Yeast extract sucrose agar and Potato sucrose agar was exhibited through the secondary metabolite profile analyses. Based on the UV spectra and retention indices, most of the *Fusarium verticillioides* isolates tested produced compounds related to pigments such as fusarubin and nectriafurone. The mycotoxin fusarin C was produced by two *F. verticillioides* isolates whilst one isolate produced fusaproliferin. Over 75% of the *F. semitectum* isolates tested produced either one or both of the antifungal α-pyrones, fusapyrone and deoxyfusapyrone in addition to the mycotoxin/antibiotic equisetin which was also produced by four of five isolates of *F. equiseti*.

# 1. INTRODUCTION

The genus *Fusarium* was first described in 1809 by Link as fungi with fusiform, non-septate asexual spores borne on a stroma and was included in 1821 by Fries in the order Tuberculariae (Booth, 1971). With the development of pure cultures, the presence of a stroma was abandoned as an essential character of this genus in favour of the presence of fusoid macroconidia with a foot cell bearing some kind of a heel. Currently, this is accepted as the most reliable character of the genus *Fusarium* (Nirenberg, 1981; Nelson et al., 1983; Burgess et al., 1994).

Currently, the classification of *Fusarium* has been dealt with by several authors with the development of illustrated guides. The system of Snyder and Hansen (1945) formed the basis of an illustrated guide to the identification of the *Fusaria* (Toussoun and Nelson, 1968). Another illustrated guide to the identification of the genus, based predominantly though not wholly on the classification system of Wollenweber and Reinking (1935), was published by Nelson et al., (1983). Other classifications available are Booth (1971), Nirenberg and Gerlach (1982) and Burgess et al. (1994) who have also developed laboratory manuals for *Fusarium* research.

The morphology of macroconidia is regarded as the primary characteristic for defining most species of *Fusarium* (Wollenweber, 1913; Toussoun and Nelson, 1975; Nelson et al., 1983). It is not however a reliable criterion for separating species in section *Liseola* because there is considerable overlap in the morphology of the macroconidia between species. Therefore these species are usually distinguished by the morphology and mode of formation of microconidia. It is however known that identification based exclusively on morphology is difficult due to variations in conidial appearance within the same species or resemblance between different species. So other characteristics have to be considered for taxonomic purposes. Profiles of secondary metabolites have proven a good supplement to morphology and have been effective in differentiating between species in *Penicillium* and *Fusarium* (Anderson, 1991; Frisvad and Filtenborg, 1989; Thrane, 1989).

In the recent past, research concerning the production of secondary metabolites (including mycotoxins) has been very intensive. Despite this, the secondary metabolites produced are still unclear. This is due to the misidentification or insufficient characterization of the producer-strains which is based primarily on morphological criteria which are difficult to recognize. Other reasons for misidentification are that *Fusarium* strains can easily deteriorate in culture and the fact that different taxonomic systems may be used between which there may be no, or very little, cross-reference possible.

A large number of known fungal secondary metabolites are not classified as mycotoxins. Some may have toxic effects on insects (insecticides), plants (herbicides), or on microorganisms (antibiotics)

or they may have pharmacological effects on vertebrates or act synergistically with known mycotoxins on vertebrates. Though secondary metabolites are also known to be good indicators of fungal contamination of foods and feeds and of mycotoxins produced in smaller amounts, not much work has been conducted on the identification of metabolites produced by *Fusarium* species.

The purpose of this study therefore is to test the ability of *Fusarium* isolates from Ghanaian maize kernels to produce secondary metabolites on Potato sucrose agar (PSA) and Yeast extract sucrose agar (YES) and to identify the metabolites produced by comparison to UV spectra from literature.

## 2. MATERIALS AND METHODS

### 2.1 Maize samples

Fifteen maize kernel samples (each sample weighing approximately 500 g) were obtained from two retail markets and two processing sites in Accra, Ghana. The retail markets, Mallam Atta, and Kaneshie markets are designated MA and KA respectively. The two processing sites at Osu Amantra (AM) and Osu Ajumako (AJ) are major processing sites each with a capacity of several tonnes a week. All the samples were destined for human consumption and were in apparently good condition. Maize kernel samples were stored at 4°C prior to mycological examination and secondary metabolite profile analysis.

### 2.2 Isolation and identification of *Fusarium* spp.

For each of the fifteen maize kernel samples, 200 kernels were selected at random and surface disinfected by soaking in 1% NaOCl for 1 minute after which they were plated (5 kernels per plate) onto Dichloran 18% Glycerol agar (DG 18; Hocking and Pitt, 1980) and Czapek-Dox Iprodione Dichloran agar (CZID; Abildgren et al., 1987) under aseptic conditions. A total of 100 kernels per sample were plated on each medium.

The plates were incubated at 25°C for 5 days under alternating cycles of 12 h of near ultraviolet (NUV) light and darkness. Representative *Fusarium* colonies on the CZID plates were plated onto Spezieller Nährstoffarmer Agar (SNA) defined by Nirenberg (1976). *Fusarium* colonies on DG 18 showing peculiar characteristics were also isolated unto SNA plates. Each SNA plate was supplied with a piece of sterile filter paper to promote sporulation. Plating on SNA plates was from a suspension of conidia picked up from a colony in a drop of 0.2% molten agar containing 0.05% detergent (spore suspension). The SNA plates were incubated under alternating light for 7 days after which *Fusarium* species were then single-point inoculated onto fresh SNA plates, potato sucrose agar (PSA) (Booth, 1971) and three-point inoculated on Yeast extract sucrose agar (YES) (Frisvad, 1981).

Inoculated SNA plates incubated at 25°C in alternating light for 7 days were used for morphological characterisation and identification according to the descriptions by Burgess et al. (1994) and Samson et al. (1995). Morphological characters such as the nature of mycelia and type of growth, if micro-conidia are in chains, on heads (bunches), or solitary and the presence and shape of macro-conidia. Slide preparations were made with lactofuchsin to observe the shape of micro-conidia and the presence of monophialides or polyphialides.

PSA plates were incubated for 7 days in alternating light at 25°C for colour development after which substrate pigmentation was noted and recorded. Plates were incubated for a further 7 days in

alternating light at 25ºC after which selected plates were used for secondary metabolite profile determinations. Inoculated YES plates were incubated in the dark at 25ºC for 14 days after which they were used for secondary metabolite profile determinations.

### 2.3 Selection of *Fusarium* isolates

A total of 52 isolates were selected from the PSA and YES plates and subjected to secondary metabolite profile determinations. Isolates were selected to cover all samples and species as well as to cover the whole range of pigmentation observed on the PSA and YES plates.

### 2.4 Extraction procedure

The extraction procedure used was based on that of Smedsgaard (1997). Agar plugs were cut out from the 14-day old cultures using a 4 mm cork borer. For the PSA plates, 3 plugs were taken from around the point of inoculation in the centre. For the YES plates which were previously 3 point inoculated, 3 plugs were taken. Two plugs were taken from around the middle and the third one taken towards the edge of the plate. The plugs were transferred with a needle into screw-cap vials and stored at -20°C till extracted. The extraction solvent used consisted of 1% formic acid in chloroform-methanol-ethyl acetate (2:1:3 v/v) solution. Five hundred microlitres of the extraction solvent was added to each vial containing the agar plugs after which vials were sonicated for 60 minutes. The extract was then transferred using Pasteur pipettes into fresh vials and evaporated overnight in a fume chamber. The residue was dissolved in 500 µl of methanol (HPLC grade) through sonication for 20 minutes, then filtered through a 0.45 µm filter (Minisart, RC 15 Sartorius AG-37070, Gottingen, Germany) into a vial ready for HPLC analysis. Analysis was carried out the same day otherwise extracts were stored at -20°C till analysed.

### 2.5 High performance liquid chromatography (HPLC) analysis

The extracts were analysed by HPLC on a Hewlett Packard HP 1100 system equipped with two pumps, a built-in Diode-Array-Detector (DAD), an auto sampler injection system (3.0 µl) and an external computer control device HP 9000 Model 310. The column used for separation was an HP Hypersil BDS C-18 (125mm x 2 mm x 3 µm) maintained at 40°C. The gradient solvent system was composed of 0.05% trifluoroacetic acid in deionised water (solvent A) and 0.05% trifluoroacetic acid in acetonitrile (solvent B). An initial concentration of 10% B was raised to 50% in 30 min and finally to 90% in 10 min, held at 90% for 3 min, lowered to 10% in 6 min and held at 10% for 1 min at a flow rate of 0.3 ml/minute.

A series of alkylphenones was used as an external standard prior to HPLC analyses to calculate retention time indices (RI) of all peaks (Frisvad and Thrane, 1987). For each detected metabolite, the UV-spectrum in the range 200 to 900 nm was recorded and printed. The *Fusarium* isolates were compared based on the excretion of metabolites with the same RI and identical spectra. The individual secondary metabolites produced by the isolates were identified by comparing the profiles and retention indices to those of reference standards and to published literature.

The alkylphenone retention time index (RI) of metabolites was calculated from the equation

$$RI = \frac{(T_{sm} - T_{p1})\,\Delta z \,.\, 100}{T_{p2} - T_{pl}} + z \,.\, 100$$

where;

$T_{sm}$ = retention time of the secondary metabolite

$T_{p1}$ = retention time of the alkylphenone which elutes before the secondary metabolite

$T_{p2}$ = retention time of the alkylphenone which elutes after the secondary metabolite

$z$ = the number of carbon atoms in the alkylphenone that elutes before the secondary metabolite.

$\Delta z$ = the difference between the number of carbon atoms in the alkylphenones that elute before and after the secondary metabolite.

# 3. RESULTS

## 3.1 Characterisation and identification of *Fusarium* isolates

Six different *Fusarium* species were identified. These were *F. verticillioides*, *F. semitectum*, *F. equiseti*, *F. oxysporum*, *F. graminearum* and *F. chlamydosporum*. The dominant *Fusarium* species isolated from the maize kernels was *F. verticillioides*.

Isolates of *F. verticillioides* always showed clavate shaped microconidia present in very long chains on monophialides on Spezieller nährstoffarmer agar (SNA) plates. Mycelium was floccose with a powdery appearance on PSA and colony pigmentation ranged from colourless (unpigmented) through beige to various shades of purple. *Fusarium semitectum* isolates showed floccose mycelium on SNA. Macroconidia were fusoid, cigar-shaped or showed a distinct asymmetrical curvature (hunched) and were present on polyphialides. Growth on PSA was abundant with pale yellow, beige, or peach pigmentation. *Fusarium equiseti* showed sparse feathery growth on SNA. Falcate and 'hunched' macroconidia were observed in bunches on monophialides. Growth was abundant on PSA and pigmentation was light brown, peach or lemon coloured.

Only two isolates were identified as *F. oxysporum*. They showed abundant growth on SNA. Both microconidia and macroconidia were observed on monophialides. Microconidia were borne on false heads and were oval in shape whilst macroconidia were falcate in shape. One isolate each of *F. chlamydosporum* and *F. graminearum* was identified. *F. graminearum* showed very rapid growth on PSA (25°C) with a bright wine coloured pigmentation on the reverse and floccose yellow growth on the obverse. Macroconidia were slender and sickle shaped.

## 3.2 Secondary metabolite profiles of selected isolates

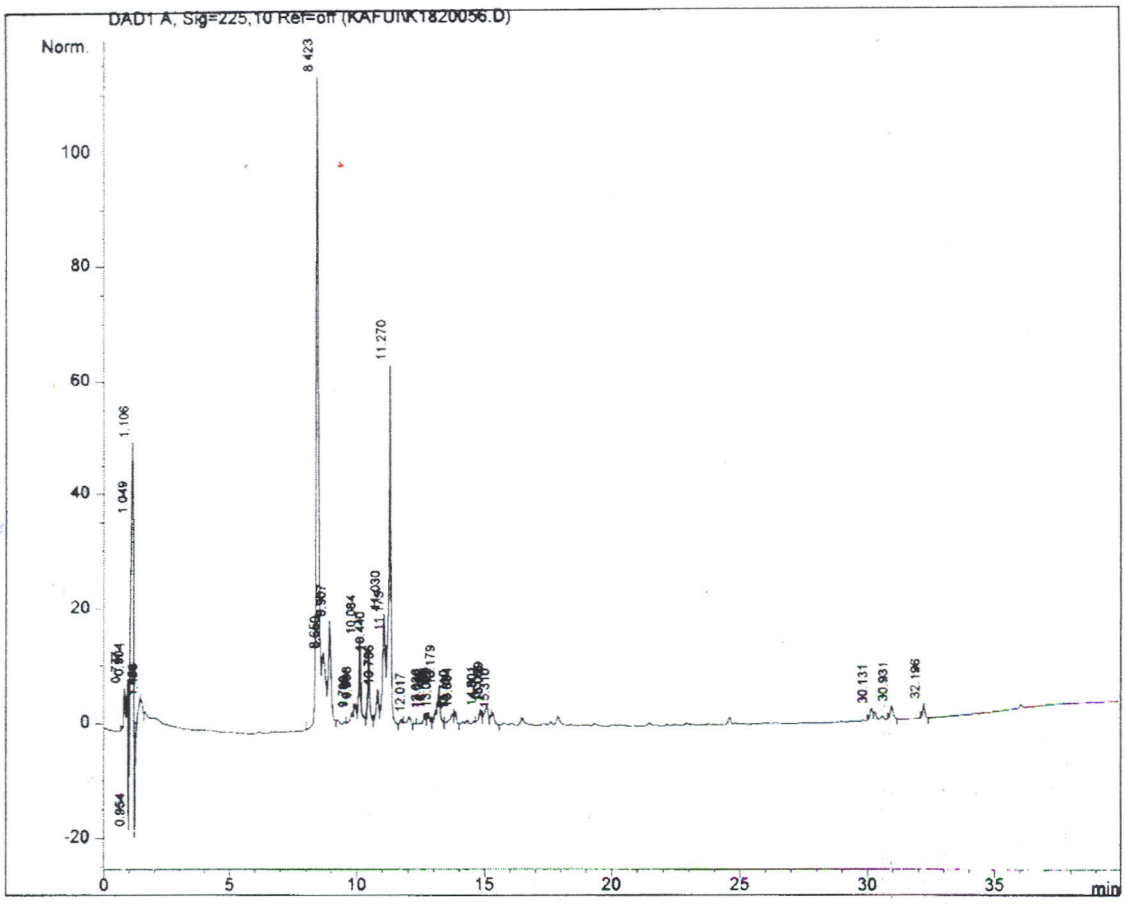
A total of 52 *Fusarium* isolates were subjected to secondary metabolite profile analysis. They consisted of 27 isolates of *F. verticillioides*, 16 of *F. semitectum*, 5 of *F. equiseti*, 2 of *F. oxysporum* and one each of *F. chlamydosporum* and *F. graminearum*. A complete list of the isolates, their colony pigmentation on Potato Sucrose agar plates incubated at 25°C for 7 days, and the "known" metabolites produced are presented in Appendix 1.

### 3.2.1 *Fusarium verticillioides*

The secondary metabolite profiles obtained for the various *Fusarium* species showed that each species appeared to have a characteristic HPLC chromatogram. For *F. verticillioides* however, two types of profiles could be identified. The majority (group 1) of isolates showed profiles similar to that shown in Figure 1. A few isolates (group 2) namely KA 6+3, KA 4+10, MA 1+18, and MA 1+29

showed a different type of profile shown in Figure 2.

**Figure 1 Typical secondary metabolite profile of *Fusarium verticillioides* isolates in group 1**




Most isolates in group 1 consistently produced six metabolites (Appendix 1). The UV spectra of the six metabolites (A, B, C, D, E, F) consistently produced by the isolates in group 1 on Potato sucrose agar are shown in Figure 3. These isolates had retention times of 8.20, 8.69, 9.87, 10.24, 10.84 and 11.07 minutes respectively. Their retention indices were 784, 796, 825, 834, 848 and 854 respectively. Comparison with standards showed them to be all related to various pigments. Metabolites A, C, D, E and F appeared to be naphthoquinone compounds whilst metabolite B showed a UV spectrum similar to nectriafurone and could be said to belong to this chromophore family.

All the four isolates in group 2 also produced several identical metabolites. The UV spectra of four of these metabolites are shown in Figure 4. These metabolites could not be identified by comparison to standards however they have been reported to be often seen in profiles of *Fusarium*

*proliferatum* isolates (Thrane, personal communication).

One isolate MA 2-1 produced a compound with a retention time of 16.20 minutes, a retention index of 999 and a maximum absorption around a wavelength of 368 nm. By comparison with UV spectra available from literature (Singh et al., 1991) the UV spectrum corresponded to that of the mycotoxin fusarin C (Figure 5a). This isolate showed a low growth rate (7 mm) on Potato sucrose agar incubated at 37°C for 7 days.

**Figure 2 Typical secondary metabolite profile of *Fusarium verticillioides* isolates in group 2**

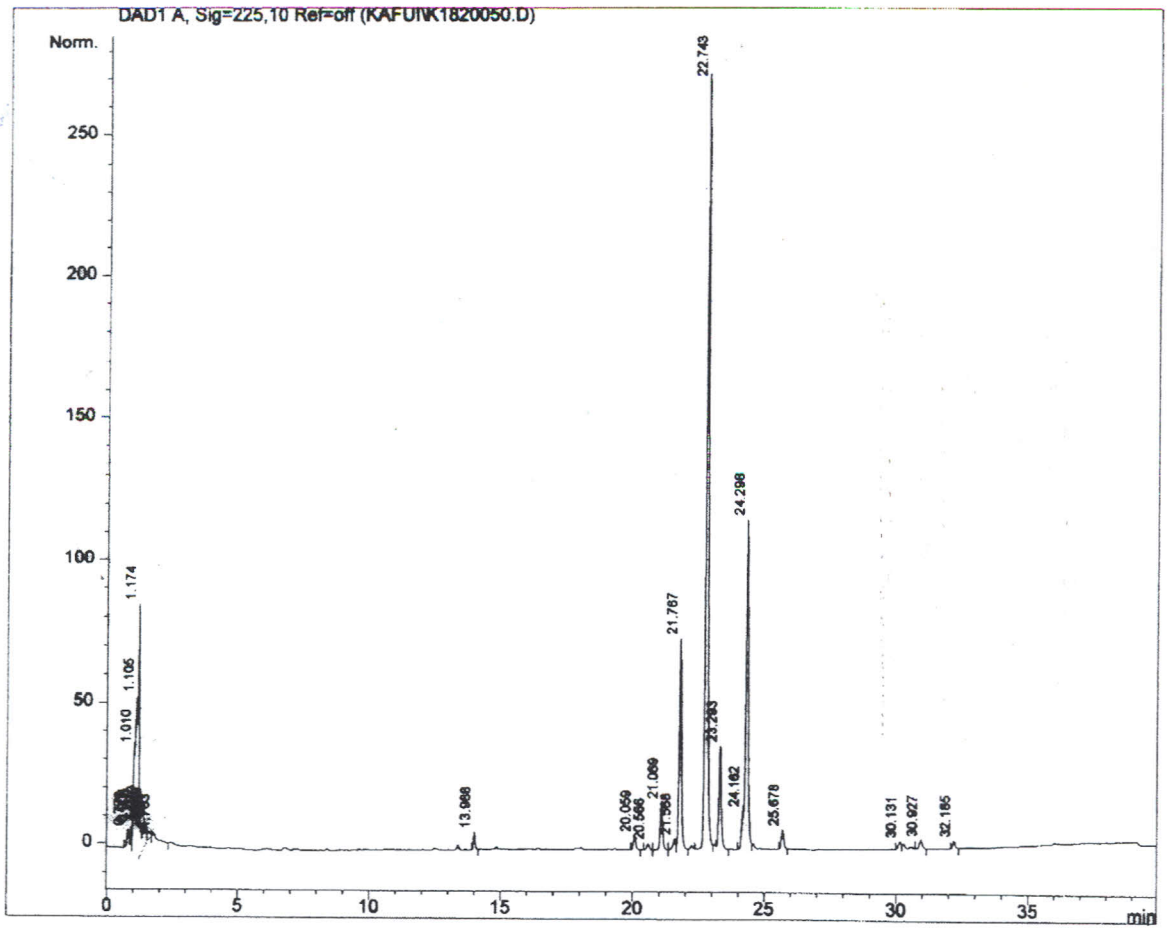


Figure 3 UV spectra of metabolites consistently produced by group 1 isolates of *F. verticillioides*

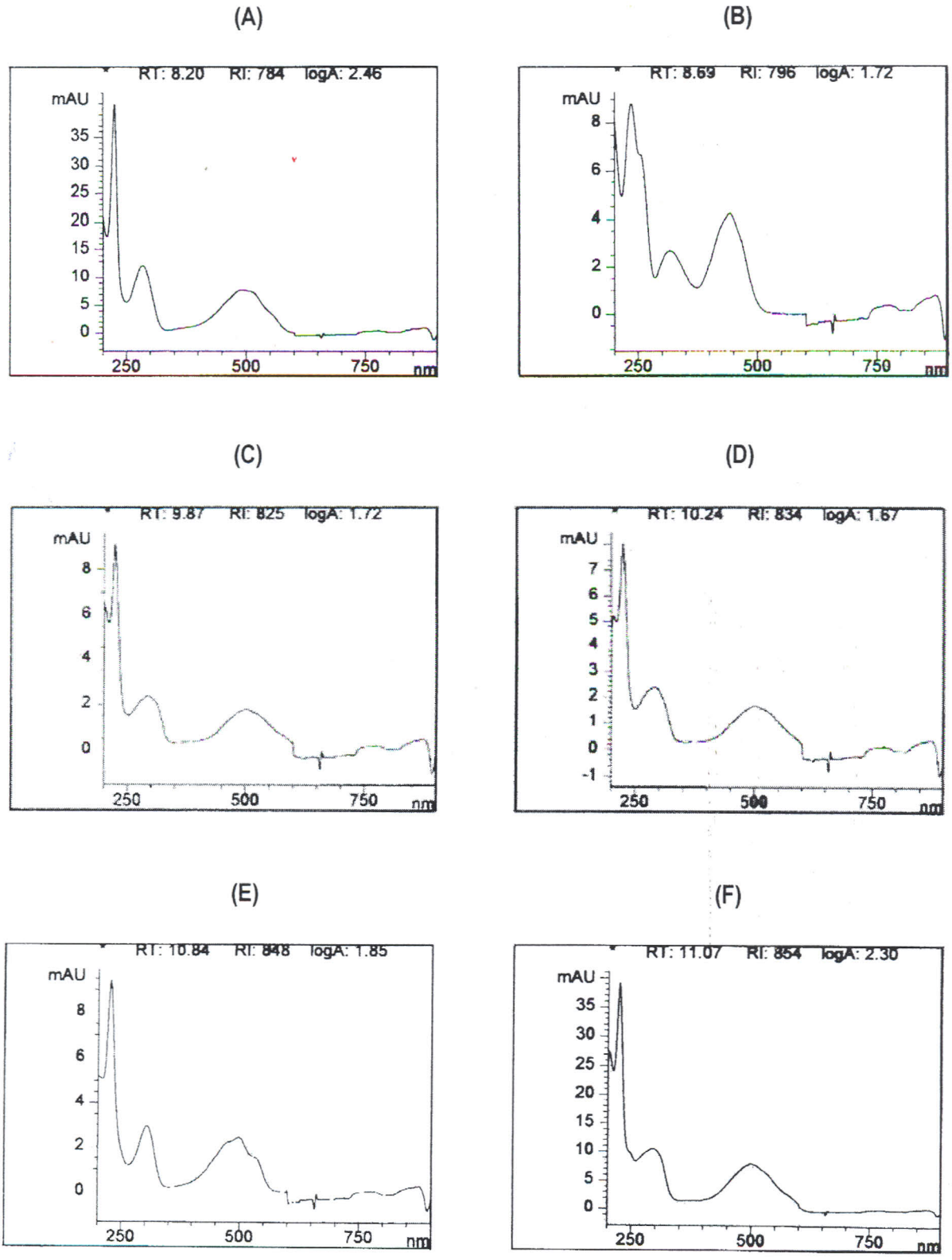
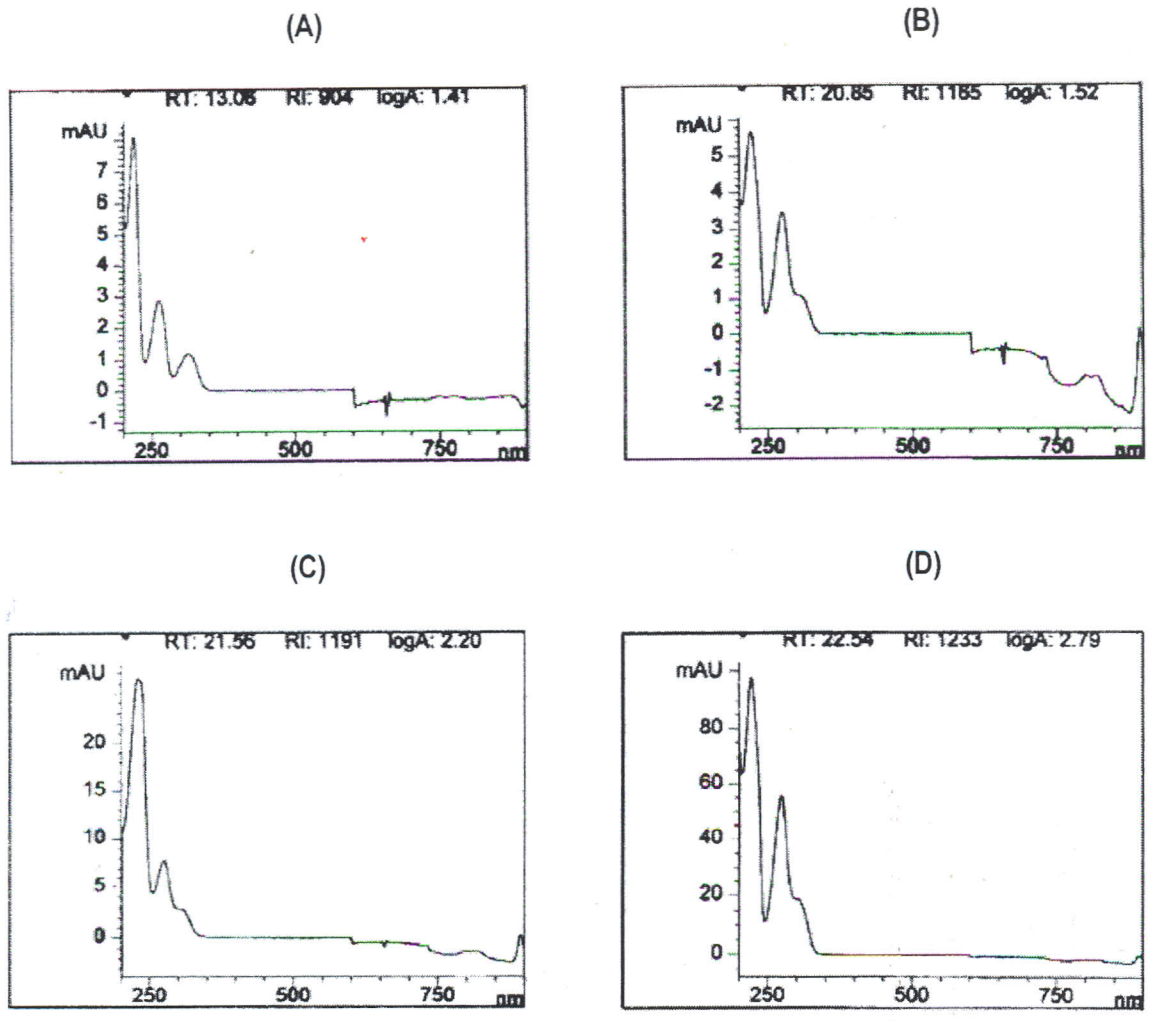


Figure 4 **UV spectra of metabolites consistently produced by group 2 isolates of *F. verticillioides***




### 3.2.2 *Fusarium semitectum*

Several metabolites were produced by each of the sixteen *F. semitectum* isolates analysed. Thirteen of the isolates produced a metabolite with a UV spectrum similar to equisetin (Figure 5b). This metabolite was identified by a retention time of 26.1 minutes and a retention index of 1384. The UV spectrum showed maximum absorption around wavelengths of 235 nm and 294 nm and corresponded to that obtained from literature for equisetin (Singh et al., 1991). Six of the isolates (AJ 1-4, AM 1-9, MA 3+4, KA 1+2, MA 7+7, MA 4+12) in addition produced a metabolite with a UV spectrum similar to zearalenone. This metabolite showed a retention time of around 16.99 minutes, a retention index of around 1023 (Appendix 1) and a UV spectrum with maximum absorption at around 237, 275 and 315 nm. By comparison to standards, other zearalenone-related metabolites namely α-zearalenol, β-zearalenol, and zearalanone, were produced by some of these isolates (Appendix 1).

Three of the *F. semitectum* isolates (KA 5+1, KA 5+2, MA 1+27) did not produce any of the metabolites similar to equisetin and zearalenone.

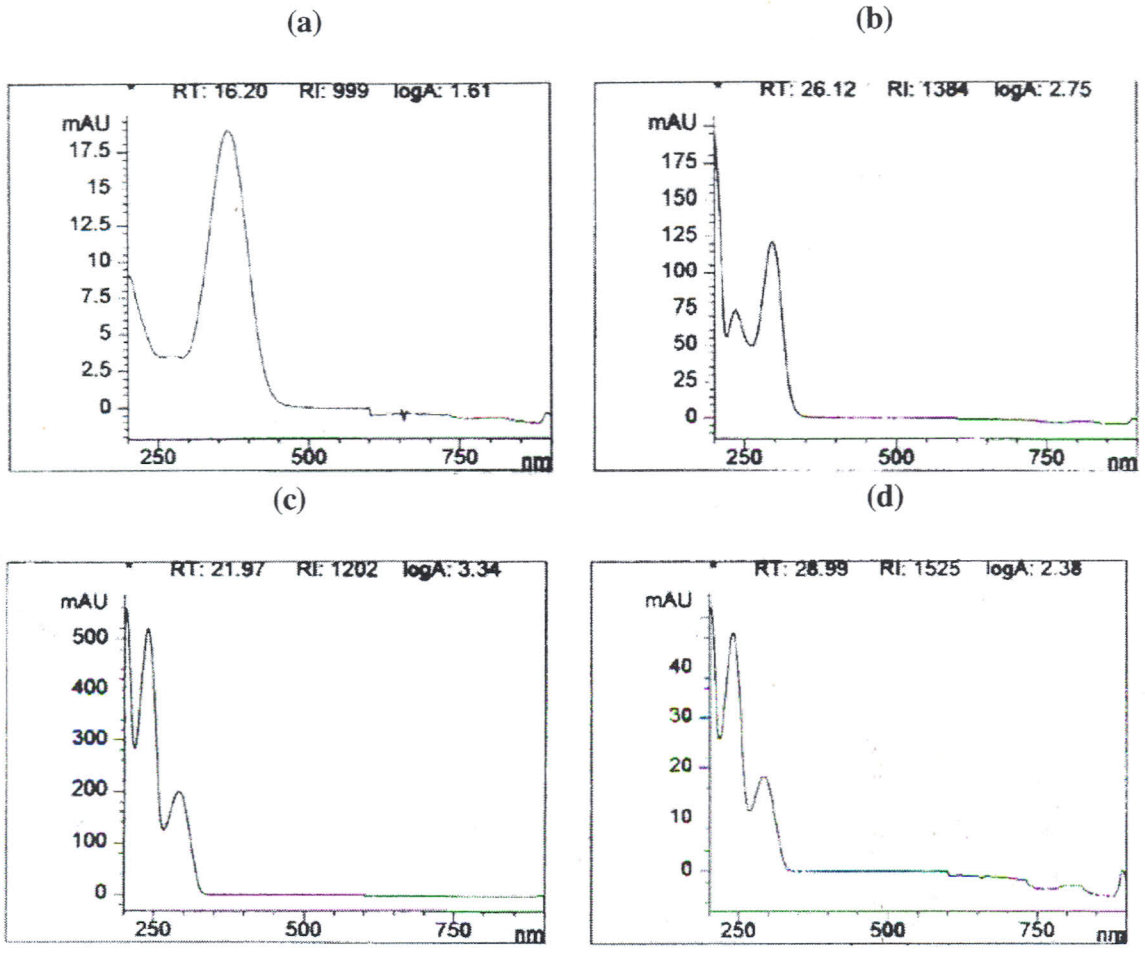



**Figure 5** UV spectra of (a) fusarin C, (b) equisetin, (c) fusapyrone, and (d) deoxyfusappyrone

The production of the metabolites fusapyrone and deoxyfusapyrone appeared to be a general characteristic of the isolates in this species. Nine of the sixteen *Fusarium semitectum* isolates tested produced both fusapyrone and deoxyfusapyrone together whilst three isolates (MA 5+28, AM 1-1, and KA 1-5) produced only deoxyfusapyrone. Four isolates (AJ 1-4, AM 1-9, MA 2+5, and KA 1+2) did not produce any of these two metabolites (Appendix 1). The UV spectra of fusapyrone and deoxyfusapyrone are shown in Figures 5c and 5d respectively. Ergosterol was produced by four of the isolates. Generally, production of metabolites by isolates of *F. semitectum* seemed to be better on Yeast extract sucrose agar than on Potato sucrose agar as indicated by the HPLC chromatograms (not shown).

### 3.2.3 *Fusarium equiseti*

Five isolates of *F. equiseti* were analysed for secondary metabolites. One isolate (AJ- 1-2) produced a metabolite with similarities to the 'equisetin-like' metabolite produced by the isolates of *F. semitectum*. Three other isolates produced metabolites with UV spectra very similar to equisetin but with slight variations in retention times and retention indices. Isolate AM 1-4 produced a metabolite with retention time 26.1 and retention index of 1382. Isolates KA 3+13 and MA 5+14 produced metabolites with similar UV spectra and retention indices of 1385 and 1386 respectively. Isolate MA 1+16 did not produce any of these metabolites but was found to produce a compound similar to deoxyfusapyrone (Thrane, personal communication). Two other isolates (KA 3+13 and MA 5+14) were also found to produce deoxyfusapyrone. No fusapyrone was produced by any of the five *F. equiseti* isolates tested. Several other metabolites of unknown identity were however produced.

### 3.2.4 *Fusarium oxysporum, F. chlamydosporum* and *F. graminearum*

The isolates of *F. oxysporum, F. chlamydosporum, F. graminearum* analysed all produced different HPLC profiles as shown in Figures 6, 7, and 8. Both isolates of *F. oxysporum* tested produced fusapyrone and deoxyfusapyrone as well as a fusarubin-like compound with a retention time of 8.21 minutes and a retention index of 784. A nectriafurone-like compound with a retention time of 8.70 minutes and a retention index of 796 was also produced by both isolates. These same metabolites (A and B respectively) were produced by several *F. verticillioides* isolates. Isolate MA 2+10 in addition produced a compound which after comparison to standards was identified as moniliformin. *Fusarium graminearum* produced fusarin C (Appendix 1). Both *F. graminearum* and *F. chlamydosporum* produced a compound identified as aurofusarin at a retention time of 17.40 with a retention index of 1037. It was not possible to identify most of the metabolites produced by the isolates of these three Fusarium species by comparison with literature and due to the limited number of isolates analysed it was not possible to define any metabolites consistently produced by these species.

**Figure 6 Secondary metabolite profile of *F. oxysporum***

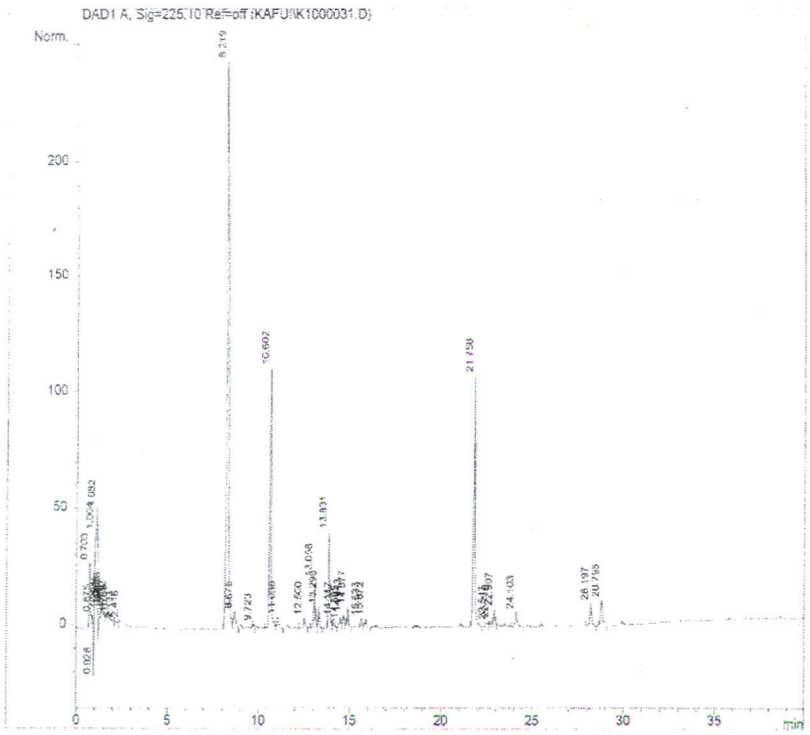



**Figure 7 Secondary metabolite profile of *F. chlamydosporum***

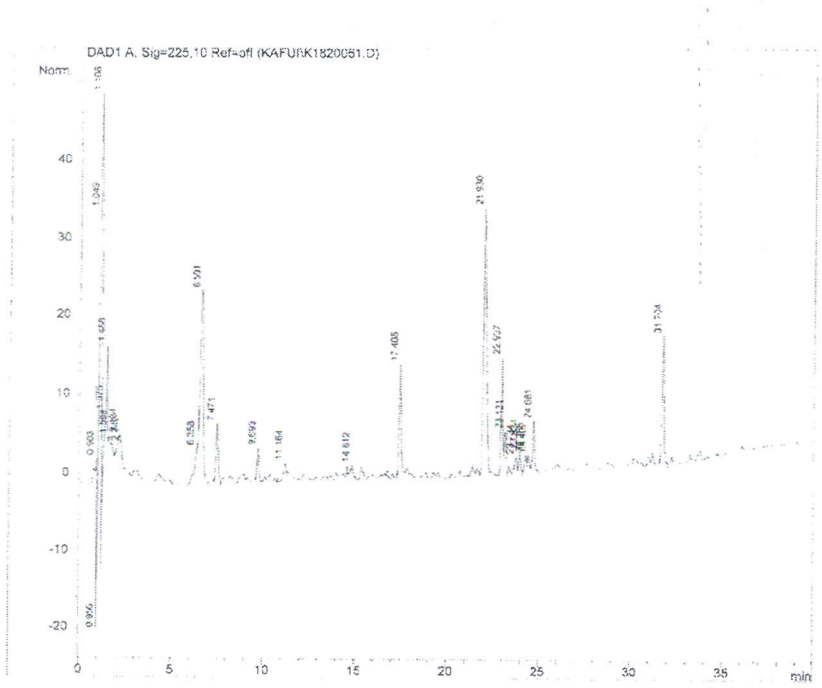
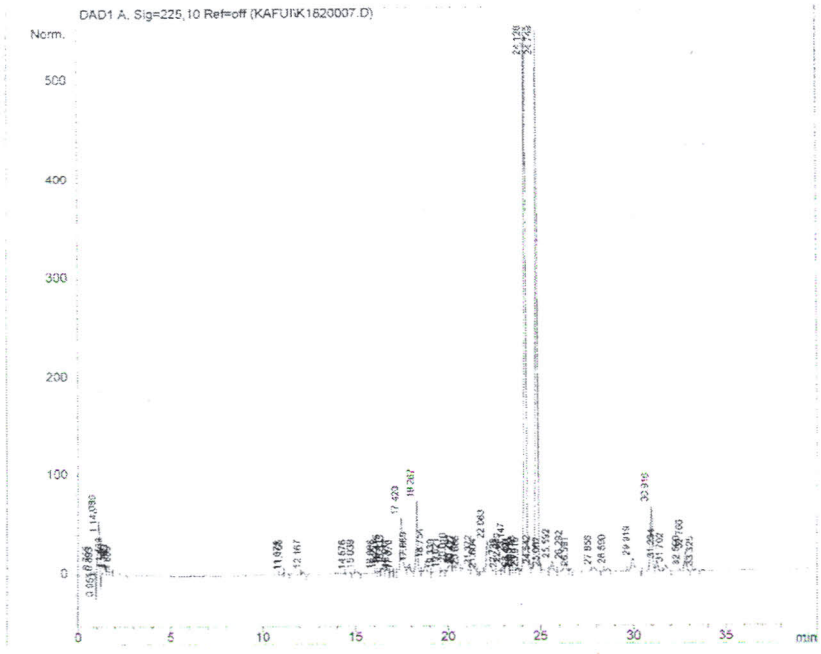


**Figure 8 Secondary metabolite profile of *F. graminearum***




## 4 DISCUSSSION

The genus *Fusarium* is known to contain several species which are important pathogens of maize and other cereals, causing root, stem and ear rot, with severe reduction in crop yield (Bottalico et al., 1989). Apart from being pathogenic, certain isolates are able to produce secondary metabolites such as mycotoxins, phytotoxins, antibiotics and pigments which can accumulate in the infected plants or in stored grains. Secondary metabolites are important for survival and communication and are of high ecological importance and may be defined as products of cellular metabolism that are more restricted in their distribution, being found in less than every species in a single fungal family (Campbell, 1984).

The use of morphological characteristics of *Fusarium* colonies is restricted to identification at species level (Nelson et al. 1983). Although groups of highly infectious isolates can be distinguished within the species by using the pathogenicity test towards host plants or vegetative compatibility grouping (Bridge et al., 1993), when characterisation of individual isolates within the species is required such as in studies on mycotoxin production, other identification techniques are needed. The use of the secondary metabolite profile assay becomes very useful because it is able to demonstrate

variations in profiles of secondary metabolites excreted by fungal cultures in solid culture media (Thrane, 1990; Frisvad and Filtenborg 1983).

***Fusarium verticillioides***

In these studies, based on the secondary metabolite profiles it was possible to vaguely group the *F. verticillioides* isolates into two groups although morphologically no distinct groups were seen. It was observed that most of the *F. verticillioides* isolates (group 1) produced compounds related to pigments such as fusarubin and nectriafurone. According to Steyn et al. (1979), the main dark-red pigment obtained from the cultivation of *F. verticillioides* reported as *Fusarium moniliforme* on solid media was 8-O-methylbostrycoidin and established the structure as 6,8-dimethoxy-5-hydroxy-3-methyl-2-azaanthraquinone. Other dark-red pigments produced by *Fusarium moniliforme* were identified as 8-O-methyljavanicin, 8-O-methylsolaniol, and 8-O-methylfusarubin (Steyn et al., 1979). Similar studies by Visconti et al. (1983) on the production of red pigments on maize kernels by isolates of *Fusarium moniliforme* showed the production of these same four pigments in addition to 3-8-O-O-dimethylfusarubin. In the current study, the *Fusarium verticillioides* isolates showed a wide variation in colour on PSA ranging from colourless through pink to dark purple and it is likely the colour variations may be due to the presence or absence of these five dark red pigments. The production of the fusarubin-like compounds named A and C by most of the isolates may account for the red colouration expressed on the PSA plates. From the results obtained and presented (Appendix 1), it was however not possible to relate the exact colouration on the PSA plates to the secondary metabolites produced. Isolates belonging to "group 1" showed the production of compounds associated with the naphthoquinones and other pigments. Isolates in the second group produced metabolites usually observed in profiles of *F. proliferatum* isolates (Thrane, personal communication). Based on the observed morphological and growth rate characteristics of the isolates it was not possible to make a distinction between the two groups.

Apart from the pigments *F. verticilliodes* isolates have been found to produce other metabolites such as fumonisins (Gelderblom et al., 1988); fusaric acid, fusarin C, fusariocins, gibberellins, moniliformin (Marasas et al., 1984); cyclonerodiol (Cross et al., 1971); bikaverins (Balan et al., 1970) and N-jasmonoyl-isoleucines (Cross and Webster, 1970). In this study, two isolates (MA 2-1, AM 1-2) produced fusarin C which is a mutagenic mycotoxin (Gelderblom et al., 1984) and has also has been found to be immunosuppressive by Bacon et al. (1990). In addition isolate MA 2-1 produced two other compounds with UV spectra similar to fusarin C. *F. verticillioides* is also known to produce fusarins (Marasas et al., 1984; Miller et al., 1995) and the two compounds with UV spectra similar to fusarin C may be fusarins.

One isolate (AM 1-2) in addition to fusarin C produced fusaproliferin, a recently described mycotoxin identified from maize cultures of *F. proliferatum* isolated from maize (Randazzo et al., 1993). Fusaproliferin has been found to be toxic to brine shrimps (*Artemia salina* L.), insect cells and the International Agency for Research into Cancer (IARC)/LCL 171 human nonneoplastic B-lymphocyte cell line (Logrieco et al., 1996) as well as to cause teratogenic effects on chicken embryos (Ritieni et al., 1997a). Fusaproliferin has been detected in maize kernel cultures of several isolates of *F. proliferatum* and *F. subglutinans* (Morreti et al., 1996; Munkvold et al., 1998) and has been detected in nine naturally contaminated samples of visibly mouldy (mostly *F. proliferatum*) maize ears in Italy at levels as high as 500 mg/kg (Ritieni et al., 1997b). Reports on the production of this mycotoxin by *F. verticillioides* are lacking and so far, only one report is found of the production of trace amounts (< 5mg/kg) of fusaproliferin by one strain (MRC 4321) of *F. verticillioides* (Shephard et al., 1999). The identity of the above-mentioned isolate (AM 1-2) as *F. verticillioides* will have to be confirmed. Apart from presenting with a yellowish-wine colour on PSA, it showed all the morphological and growth rate characteristics of *F. verticillioides*.

Most of the other metabolites listed as produced by *F. verticillioides* were not detected in the present work. This may be due to the fact that under standardised conditions different strains of a particular *Fusarium* species may not produce a certain metabolite in equal quantity (Ishii et al., 1985) or some metabolites may be produced in low levels and are therefore not detected. Another reason could be that as indicated by Bu'Lock (1980), only a limited number of strains are able to produce a particular metabolite. This variation may be due to natural differences in the genes or mutations resulting from subculturing (Burnett, 1984; El-Bahrawy et al., 1985; Duncan and Bu'Lock, 1985) or UV-radiation and mutagenic chemicals (Erokhina, 1969; Parisot et al., 1981; Avalos et al., 1985).

***Fusarium semitectum***

Isolates of *F. semitectum* have been associated with the production of moniliformin (Rabie et al., 1982), zearalenone (Marasas et al., 1984), and more recently with the production of two antifungal α-pyrones named fusapyrone and deoxyfusapyrone (Evidente et al., 1994). It is important to note that most of the *F. semitectum* isolates tested produced these metabolites except moniliformin. The production of beauvericin an insect toxin by isolates of F. semitectum has also been reported by Gupta et al. (1991).

Twelve of the sixteen isolates of *F. semitectum* subjected to secondary metabolite analysis produced either fusapyrone or deoxyfusapyrone or both. Fusapyrone and deoxyfusapyrone are metabolites originally isolated from rice culture of *F. semitectum* and have exhibited toxic activity

towards a broad spectrum of filamentous fungi including some important plant pathogenic and mycotoxigenic fungi such as *Alternaria alternata*, *Aspergillus flavus*, *Penicillium* spp., *Cladosporium* spp. (Evidente et al., 1994; Perrone et al., 1995). They are also reported to show inhibitory activity on several human pathogenic fungi including *Candida* spp. and *Aspergillus fumigatus* but appear to be selective in their toxic activity and are scarcely active towards yeasts (Evidente et al., 1999). The potential for their use as pesticides of natural origin therefore exists. There are no reports on the toxicity of these metabolites to humans. The fact that most of the *F. semitectum* isolates were able to produce these two compounds warrants further studies on the possible natural occurrence of these two metabolites in Ghanaian maize.

In this study zearalenone and in some cases its derivatives, zearalanone, α-zearalenol and β-zearalenol were produced by six of the sixteen isolates studied. Zearalenone is a mycotoxin known to have estrogenic properties in various animal species (Mirocha and Christensen 1974; Peraica et al., 1999). Its presence in maize has been reported by some researchers (Thiel et al., 1982; Scudamore et al., 1998) and has been named together with zearalenols as natural contaminants of food and feed substrates (Bilgrami and Choudhary, 1998). The ability of *F. semitectum* which was isolated in relatively high amounts from the maize kernels from Ghana to produce this mycotoxin may be considered a potential threat to consumers.

Preliminary studies conducted on the occurrence of this toxin and its derivative α-zearalenol in fermented maize dough (Kpodo et al., 1996) showed the absence of the toxin in 20 samples. The raw material (maize kernels) was not analysed in this study and it is not known if the toxins were present and were "destroyed" during processing into maize dough. Furthermore, a limited number of samples were screened in the study by Kpodo et al. (1996). It is also known that a period of low temperature (12-14°C) is required for significant production of the toxin zearalenone (Newberne, 1987). These temperatures are not normally attained in Ghana and one can say it is unlikely that this toxin will be produced in the climatic conditions pertaining in Ghana.

The production of equisetin by thirteen of the sixteen isolates of *F. semitectum* in this study is interesting in the light of current findings. Equisetin and a homologue of equisetin recovered from *Fusarium heterosporum* and *Phoma* spp. respectively have been characterised as a human immunodeficiency virus (HIV) integrase inhibitor by several researchers (Singh et al., 1998; Hazuda et al., 1999). The production of equisetin by isolates of *F. equiseti* has been reported by Marasas et al. (1984). Only one report of the production of equisetin by *F. semitectum* (= *F. pallidoroseum*) is available (Wheeler et al., 1999). These researchers found that equisetin, an antibiotic suppressed germination and inhibited growth of various monocotyledonous and dicotyledonous seeds.

***Fusarium equiseti***

In this study, four of the five isolates tested produced equisetin or related compounds. Other metabolites reported to be produced by this *Fusarium* species include zearalenones and trichothecene type A (Marasas et al., 1984). None of these was detected in this work. No fusapyrone was produced by the five isolates of *F. equiseti* tested however three of the isolates produced metabolites with UV spectra similar to that of deoxyfusapyrone.

***Fusarium oxysporum***

Production of the two antifungal metabolites fusapyrone and deoxyfusapyrone was again demonstrated by the two isolates (MA 2+10, MA 2+11) of *F. oxysporum* tested. Isolate MA 2+10 also produced moniliformin in addition to fusapyrone and deoxyfusapyrone. Moniliformin has been shown to be toxic to chickens (Allen et al., 1981). Other metabolites reported to be produced by *F. oxysporum* are bikaverins (Kreitman and Nord, 1949), enniatins and lycomarasmin (Gäumann, 1951); fusaric acid and moniliformin (Marasas et al., 1984); oxysporone (Adesogan and Alo, 1979);

In this study other metabolites produced by the two isolates of *F. oxysporum* were the fusarubin-like and nectriafurone-like compounds earlier designated as compounds A and B respectively (Appendix 1). These two compounds were also produced by several of the "group 1" *F. verticillioides* isolates. It is known (Thrane, Personal communication) that most isolates from *Fusarium* species produce red pigments and members belonging to the sections *Liseola*, *Elegans*, and *Martiella* produce naphtoquinones whilst all other red-pigment producing fusaria have their red colour attributed to the aurofusarins.

***Fusarium graminearum* and *F. chlamydosporum***

Only one isolate each of *F. graminearum* and *F. chlamydosporum* was isolated and tested in this work. Although one is tempted to suggest that these two *Fusarium* species are not common contaminants of maize in Ghana, maximum caution has to be exercised bearing in mind the limited numbers of samples and the localised area from which the maize samples were taken.

Several metabolites have been associated with *F. graminearum*. These include trichothecenes type B, zearalenones, 4-acetamido-2-butenoic acid and butenolide (Marasas et al., 1984); Fusarin C (Gelderblom et al., 1984); culmorin (Greenhalgh et al., 1984) and aurofusarins (Ashley et al., 1937). In this study only fusarin C and aurofusarin (Appendix 1) were the identified metabolites produced by the single isolate analysed. The metabolite aurofusarin is reported to be responsible for the characteristic dark red wine colour produced by isolates of *F. graminearum* and *F. chlamydosporum* on PSA (Thrane, Personal communication). The aurofusarins include aurofusarin, fuscofusarin and

rubrofusarin (Takeda et al., 1968).

Although several metabolites were produced by the isolate of *F. chlamydosporum*, only aurofusarin was identified and this can be said to confirm the identity of this isolate by the wine pigmentation exhibited on PSA coupled to the observed morphological characteristics. *F. chlamydosporum* has been reported to produce moniliformin (Marasas et al., 1984); chlamydosporol (Savard et al., 1990; Solfrizzo et al., 1994) and other chlamydosporol related compounds such as chlamydospordiol and isochlamydosporol (Solfrizzo et al., 1994).

## 5. CONCLUSIONS AND RECOMMENDATIONS

Through the secondary metabolite profile analyses conducted, there appears to be the possibility of several secondary metabolites including mycotoxins such as equisetin, fusarin C, fusaproliferin, moniliformin and zearalenone being present on maize kernels. Studies on the ability of the various *Fusarium* isolates to produce these mycotoxins on maize substrates as well as their natural occurrence in maize and maize products need to be conducted. Other important compounds such as fusapyrone and deoxyfusapyrone together with the antibiotic/mycotoxin equisetin with high medicinal potential were produced by several of the *F. semitectum*, *F. equiseti* and *F. oxysporum* isolates.

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## APPENDIX 1: *Fusarium* isolates subjected to secondary metabolite profile analysis and the "known" metabolites produced

| Isolate Number | *Fusarium* Species | Colony Pigmentation on PSA[a] | RT | RI | "Known" metabolites |
|---|---|---|---|---|---|
| [P]MA 1+7 | *F. verticillioides* | Purple | 8.06 | 781 | Naphthoquinone compound (A) |
| | | | 8.57 | 794 | Nectriafurone-like (B) |
| | | | 9.65 | 820 | Naphthoquinone (C) |
| | | | 9.82 | 824 | Naphthoquinone compound (D) |
| | | | 10.80 | 848 | Naphthoquinone compound (E) |
| | | | 11.06 | 854 | Naphthoquinone compound (F) |
| [P]MA 1+18 | *F. verticillioides* | Purple | 13.05 | 903 | Cpd. seen in *F. proliferatum* (G) |
| | | | 20.85 | 1164 | Cpd. seen in *F. proliferatum* (I) |
| | | | 21.55 | 1191 | Cpd. seen in *F. proliferatum* (J) |
| | | | 22.53 | 1233 | Cpd. seen in *F. proliferatum* (K) |
| [P]MA 1+29 | *F. verticillioides* | Pale purple | 13.06 | 904 | Cpd. seen in *F. proliferatum* (G) |
| | | | 20.85 | 1165 | Cpd. seen in *F. proliferatum* (I) |
| | | | 21.56 | 1191 | Cpd. seen in *F. proliferatum* (J) |
| | | | 22.54 | 1233 | Cpd. seen in *F. proliferatum* (K) |
| [Y]MA 2-1 | *F. verticillioides* | Dark purple | 14.48 | 947 | Fusarin-C-like compound |
| | | | 15.86 | 989 | Fusarin-C-like compound |
| | | | 16.20 | 999 | Fusarin C |
| [P]MA 3+3 | *F. verticillioides* | Colourless | 8.21 | 784 | Naphthoquinone compound (A) |
| | | | 8.69 | 796 | Nectriafurone-like (B) |
| | | | 10.84 | 849 | Naphthoquinone compound (E) |
| | | | 11.07 | 854 | Naphthoquinone compound (F) |
| [P]MA 4+3 | *F. verticillioides* | Orange-purple | 8.20 | 784 | Naphthoquinone compound (A) |
| | | | 8.69 | 796 | Nectriafurone-like (B) |
| | | | 9.87 | 825 | Naphthoquinone compound (C) |
| | | | 10.24 | 834 | Naphthoquinone compound (D) |
| | | | 10.84 | 849 | Naphthoquinone compound (E) |
| | | | 11.07 | 854 | Naphthoquinone compound (F) |
| [P]MA 4+6 | *F. verticillioides* | Pinkish-cream | 8.21 | 784 | Naphthoquinone compound (A) |
| | | | 8.69 | 796 | Nectriafurone-like (B) |
| | | | 10.85 | 849 | Naphthoquinone compound (E) |
| | | | 11.07 | 854 | Naphthoquinone compound (F) |

**Appendix 1 (Continued)**

| Isolate Number | *Fusarium* Species | Colony Pigmentation on PSA[a] | RT | RI | "Known" metabolites |
|---|---|---|---|---|---|
| [P]MA 2+8 | *F. verticillioides* | Pale purple | 8.21<br>8.70<br>11.08 | 784<br>796<br>854 | Naphthoquinone compound (A)<br>Nectriafurone-like (B)<br>Napthoquinone compound (F) |
| [Y]MA 2+12 | *F. verticillioides* | Pinkish purple | - | - | A few metabolites were produced which could be identified |
| [Y]MA 2+24 | *F. verticillioides* | Colourless | 8.22 | 784 | Naphthoquinone compound (A) |
| [P]MA 2+26 | *F. verticillioides* | Pale purple | 8.22<br>8.70<br>9.88<br>10.25<br>11.08 | 784<br>796<br>825<br>834<br>854 | Naphthoquinone compound (A)<br>Nectriafurone-like (B)<br>Naphthoquinone compound (C)<br>Napthoquinone compound (D)<br>Napthoquinone compound (F) |
| [P]MA 6+21 | *F. verticillioides* | Purple | 8.42 | 610 | Naphthoquinone compound |
| [Y]MA 7+1 | *F. verticillioides* | Pale purple | 8.43 | 611 | Naphthoquinone compound |
| [P]MA 7-3 | *F. verticillioides* | Colourless | 8.42<br>8.91 | 610<br>641 | Naphthoquinone compound<br>Nectriafurone-like compound |
| [P]KA 1+1 | *F. verticillioides* | Pale purple | 8.42<br>8.91 | 610<br>641 | Naphthoquinone compound<br>Nectriafurone-like compound |
| [P]KA 3+2 | *F. verticillioides* | Beige | - | - | A few metabolites were produced which could not be identified |
| [P]KA 4+1 | *F. verticillioides* | Pale purple | 8.41<br>8.90<br>10.08<br>10.44 | 610<br>641<br>716<br>737 | Naphthoquinone compound<br>Nectriafurone-like compound<br>Naphthoquinone compound<br>Naphthoquinone compound |

**Appendix 1 (Continued)**

| Isolate Number | *Fusarium* Species | Colony Pigmentation on PSA[a] | RT | RI | "Known" metabolites |
|---|---|---|---|---|---|
| [p]KA 5+4 | *F. verticillioides* | Purple | 8.42 | 610 | Naphthoquinone compound |
| | | | 8.91 | 641 | Nectriafurone-like compound |
| [y]AM 1+1 | *F. verticillioides* | Orange | 8.43 | 611 | Naphthoquinone compound |
| | | | 8.91 | 642 | Nectriafurone-like compound |
| [y]AJ 1+3 | *F. verticillioides* | Pale purple | 8.43 | 611 | Naphthoquinone compound |
| | | | 8.91 | 642 | Nectriafurone-like compound |
| [p]MA 4+7 | *F. verticillioides* | Orange-purple | 8.21 | 784 | Naphthoquinone compound (A) |
| | | | 8.70 | 796 | Nectriafurone-like (B) |
| | | | 9.88 | 825 | Naphthoquinone compound (C) |
| | | | 10.25 | 834 | Naphthoquinone compound (D) |
| | | | 10.85 | 849 | Naphthoquinone compound (E) |
| | | | 11.07 | 854 | Naphthoquinone compound (F) |
| [p]MA 4+14 | *F. verticillioides* | Orange-purple | 8.20 | 784 | Naphthoquinone compound (A) |
| | | | 8.69 | 796 | Nectriafurone-like (B) |
| | | | 9.87 | 825 | Naphthoquinone compound (C) |
| | | | 10.24 | 834 | Naphthoquinone compound (D) |
| | | | 10.84 | 849 | Naphthoquinone compound (E) |
| | | | 11.07 | 854 | Naphthoquinone compound (F) |
| [y]MA 4+14 | | | 29.94 | 1579 | Ergosterol (Q) |
| [p]MA 5-3 | *F. verticillioides* | Purple | 8.20 | 784 | Naphthoquinone compound (A) |
| | | | 8.69 | 796 | Nectriafurone-like (B) |
| | | | 9.87 | 825 | Naphthoquinone compound (C) |
| | | | 10.24 | 834 | Naphthoquinone compound (D) |
| | | | 10.84 | 848 | Naphthoquinone compound (E) |
| | | | 11.07 | 854 | Naphthoquinone compound (F) |
| [p]MA 5-13 | *F. verticillioides* | Purple | 8.21 | 784 | Naphthoquinone compound (A) |
| | | | 8.69 | 796 | Nectriafurone-like (B) |
| | | | 9.87 | 825 | Naphthoquinone compound (C) |
| | | | 10.24 | 834 | Naphthoquinone compound (D) |
| | | | 10.84 | 849 | Naphthoquinone compound (E) |
| | | | 11.07 | 854 | Naphthoquinone compound (F) |

**Appendix 1 (Continued)**

| Isolate Number | *Fusarium* Species | Colony Pigmentation on PSA[a] | RT | RI | "Known" metabolites |
|---|---|---|---|---|---|
| [p]KA 4+10 | *F. verticillioides* | Colourless | 14.00 | 931 | Cpd. seen in *F. proliferatum* (G) |
| | | | 20.08 | 1130 | Cpd. seen in *F. proliferatum* (H) |
| | | | 21.09 | 1168 | Cpd. seen in *F. proliferatum* (I) |
| | | | 21.78 | 1195 | Cpd. seen in *F. proliferatum* (J) |
| | | | 22.76 | 1237 | Cpd. seen in *F. proliferatum* (K) |
| [p]KA 6+3 | *F. verticillioides* | Pinkish orange | 13.99 | 931 | Cpd. seen in *F. proliferatum* (G) |
| | | | 20.06 | 1129 | Cpd. seen in *F. proliferatum* (H) |
| | | | 21.07 | 1168 | Cpd. seen in *F. proliferatum* (I) |
| | | | 21.77 | 1194 | Cpd. seen in *F. proliferatum* (J) |
| | | | 22.74 | 1236 | Cpd. seen in *F. proliferatum* (K) |
| [y]KA 6+3 | | | 30.12 | 1582 | Ergosterol (Q) |
| [y]AM 1-2 | *F. verticillioides* | Yellow-wine | 16.40 | 1003 | Fusarin C |
| | | | 20.96 | 1163 | Fusaproliferin |
| | | | 30.13 | 1582 | Ergosterol |
| [y]MA 1+27 | *F. semitectum* | Pale yellow | 21.76 | 1199 | Fusapyrone |
| | | | 28.80 | 1522 | Deoxyfusapyrone |
| [p]MA 1+27 | | | 21.76 | 1199 | Fusapyrone |
| [y]MA 2+5 | *F. semitectum* | Lemon | 25.91 | 1381 | Equisetin |
| | | | 26.48 | 1406 | Equisetin-like compound |
| [p]MA 3+4 | *F. semitectum* | Pale yellow | 16.60 | 1013 | Zearalanone |
| | | | 16.76 | 1018 | Zearalenone |
| | | | 21.76 | 1199 | Fusapyrone |
| [y]MA 3+4 | | | 16.77 | 1019 | Zearalenone |
| | | | 21.76 | 1199 | Fusapyrone |
| | | | 25.91 | 1381 | Equisetin |
| | | | 28.81 | 1523 | Deoxyfusapyrone |
| [y]MA 4+10 | *F. semitectum* | Pale purple | 21.77 | 1199 | Fusapyrone |
| | | | 25.91 | 1381 | Equisetin |
| | | | 28.79 | 1522 | Deoxyfusapyrone |

**Appendix 1 (Continued)**

| Isolate Number | *Fusarium* Species | Colony Pigmentation on PSA[a] | RT | RI | "Known" metabolites |
|---|---|---|---|---|---|
| [p]MA 4+12 | *F. semitectum* | Pale yellow | 14.61 | 951 | α-zearalenol |
| | | | 16.60 | 1013 | Zearalanone |
| | | | 16.76 | 1018 | Zearalenone |
| | | | 21.76 | 1199 | Fusapyrone |
| [y]MA 4+12 | | | 16.76 | 1018 | Zearalenone |
| | | | 21.76 | 1199 | Fusapyrone |
| | | | 25.91 | 1381 | Equisetin |
| | | | 28.80 | 1522 | Deoxyfusapyrone |
| [y]MA 5+28 | *F. semitectum* | Beige | 25.91 | 1381 | Equisetin |
| | | | 28.80 | 1522 | Deoxyfusapyrone |
| [p]MA 7+7 | *F. semitectum* | Lemon-peach | 13.18 | 907 | β-zearalenol |
| | | | 16.81 | 1017 | Zearalanone |
| | | | 16.97 | 1022 | Zearalenone |
| | | | 21.97 | 1202 | Fusapyrone |
| | | | 28.99 | 1525 | Deoxyfusapyrone |
| [y]MA 7+7 | | | 16.99 | 1023 | Zearalenone |
| | | | 21.98 | 1202 | Fusapyrone |
| | | | 26.12 | 1384 | Equisetin |
| | | | 29.00 | 1526 | Deoxyfusapyrone |
| | | | 30.14 | 1583 | Ergosterol |
| [p]KA 1+2 | *F. semitectum* | Peach | 16.83 | 1018 | Zearalanone |
| | | | 16.99 | 1023 | Zearalenone |
| [y]KA 1+2 | | | 19.99 | 1023 | Zearalenone |
| | | | 26.13 | 1384 | Equisetin |
| [y]KA 1-5 | *F. semitectum* | Pale yellow | 26.13 | 1384 | Equisetin |
| | | | 29.01 | 1526 | Deoxyfusapyrone |
| [p]KA 4+3 | *F. semitectum* | Brown | 21.99 | 1203 | Fusapyrone |
| | | | 26.14 | 1385 | Equisetin |
| [y]KA 4+3 | | | 21.99 | 1203 | Fusapyrone |
| | | | 26.12 | 1384 | Equisetin |
| | | | 29.01 | 1526 | Deoxyfusapyrone |
| | | | 30.14 | 1583 | Ergosterol |

**Appendix 1 (Continued)**

| Isolate Number | *Fusarium* Species | Colony Pigmentation on PSA[a] | RT | RI | "Known" metabolites |
|---|---|---|---|---|---|
| [y]KA 5+1 | *F. semitectum* | Lemon-peach | 21.97 | 1202 | Fusapyrone |
| | | | 29.00 | 1525 | Deoxyfusapyrone |
| [y]KA 5+2 | *F. semitectum* | Peach | 21.98 | 1202 | Fusapyrone |
| | | | 29.00 | 1526 | Deoxyfusapyrone |
| [y]KA 6-3 | *F. semitectum* | Lemon-peach | 21.27 | 1175 | Fusapyrone-like compound |
| | | | 21.97 | 1203 | Fusapyrone |
| | | | 26.12 | 1384 | Equisetin |
| | | | 28.99 | 1525 | Deoxyfusapyrone |
| [y]AM 1-1 | *F. semitectum* | Lemon | 26.11 | 1383 | Equisetin |
| | | | 28.97 | 1524 | Deoxyfusapyrone |
| | | | 30.11 | 1581 | Ergosterol |
| [p]AM 1-9 | *F. semitectum* | Peach | 14.84 | 956 | α-zearalenol |
| | | | 16.83 | 1018 | Zearalanone |
| | | | 16.99 | 1023 | Zearalenone |
| [y]AM 1-9 | | | 16.99 | 1023 | Zearalenone |
| | | | 26.10 | 1383 | Equisetin |
| [y]AJ 1-4 | *F. semitectum* | Peach | 16.99 | 1023 | Zearalenone |
| | | | 26.09 | 1383 | Equisetin |
| | | | 30.10 | 1580 | Ergosterol |
| [p]MA 1+16 | *F. equiseti* | Yellow | 28.79 | 1522 | Deoxyfusapyrone |
| [y]MA 1+16 | | | 28.80 | 1522 | Deoxyfusapyrone |
| [y]MA 5+14 | *F. equiseti* | Pale yellow | 26.11 | 1386 | Equisetin |
| | | | 29.03 | 1527 | Deoxyfusapyrone |
| [p]KA 3+13 | *F. equiseti* | Bright lemon | 26.14 | 1385 | Equisetin |
| | | | 29.01 | 1526 | Deoxyfusapyrone |
| [y]KA 3+13 | | | 26.14 | 1385 | Equisetin |
| | | | 29.02 | 1527 | Deoxyfusapyrone |

**Appendix 1 (Continued)**

| Isolate Number | *Fusarium* Species | Colony Pigmentation on PSA[a] | RT | RI | "Known" metabolites |
|---|---|---|---|---|---|
| [y]AM 1-4 | *F. equiseti* | Peach | 26.09 | 1382 | Equisetin |
| | | | 30.11 | 1581 | Ergosterol |
| [y]AJ 1-2 | *F. equiseti* | Lemon-peach | 26.12 | 1384 | Equisetin |
| | | | 30.14 | 1583 | Ergosterol |
| [p]MA 2+10 | *F. oxysporum* | Pink-purple | 8.21 | 784 | Naphthoquinone compound (A) |
| | | | 8.70 | 796 | Nectriafurone-like (B) |
| | | | 21.76 | 1199 | Fusapyrone |
| | | | 28.79 | 1522 | Deoxyfusapyrone |
| [y]MA 2+10 | | | 0.70 | 601 | Moniliformin |
| | | | 8.22 | 784 | Naphthoquinone compound (A) |
| | | | 21.76 | 1199 | Fusapyrone |
| | | | 28.80 | 1522 | Deoxyfusapyrone |
| [p]MA 2+11 | *F. oxysporum* | Pink-purple | 8.70 | 796 | Nectriafurone-like (B) |
| | | | 21.76 | 1199 | Fusapyrone |
| | | | 25.48 | 1362 | Often occurs with fusapyrone |
| | | | 28.79 | 1522 | Deoxyfusapyrone |
| [y]MA 2+11 | | | 8.22 | 784 | Naphthoquinone compound (A) |
| | | | 21.76 | 1199 | Fusapyrone |
| [p]MA 7-6 | *F. chlamydosporum* | Wine | 17.39 | 1036 | Aurofusarin |
| [y]MA 7-6 | | | 17.40 | 1037 | Aurofusarin |
| [p]MA 5-5 | *F. graminearum* | Bright red/wine | 17.43 | 1038 | Aurofusarin |
| [y]MA 5-5 | | | 16.41 | 1004 | Fusarin C |
| | | | 17.42 | 1037 | Aurofusarin |

PSA[a] : Potato sucrose agar after 7 days incubation at 25°C
RT = Retention Time
RI= Retention Index
_ [p] metabolites identified on agar plugs taken from PSA plates
_ [y] metabolites identified on agar plugs taken from YES plates

# Food Research Institute

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