Available Online at ESci Journals
ESci Journal of Plant Pathology
ISSN: 2305-106X (Online), 2306-1650 (Print)
http://www.escijournals.net/EJPP

TOXIGENICITY OF FUSARIUM SPECIES IN *GIBBERELLA FUJIKUROI* SPECIES COMPLEX (GFSC) ASSOCIATED WITH STALK AND EAR ROT DISEASE OF CORN

[a]Titi Darnetty*, [b]Baharuddin Salleh
[a] *Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University, Limau Manis, Padang, Indonesia.*
[b] *School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.*

A B S T R A C T

Fusarium stalk and ear rot disease did not only cause significant losses of yield but also produced mycotoxins that are harmful to animals and human. This study was conducted to elucidate three major mycotoxins i.e. fumonisin B1 ($FUMB_1$), moniliformin (MON), and beauvericin (BEA) produced by the *Fusarium* spp. isolated from corn showing typical stalk and ear rot symptoms in Indonesia, Malaysia and Thailand. Twenty selected strains of *Fusarium* species in *Gibberella fujikuroi* species complex i.e. *F.verticillioides*, *F. proliferatum*, *F. subglutinans,* and *F. konzum* were analyzed for production of the three mycotoxins by using an Ultra Performance Liquid Chromatography (UPLC). All strains of *F. verticillioides* and *F. proliferatum* produced $FUMB_1$ at high levelsand MON at low levels. Many strains of *F. verticillioides* (67%) and *F. proliferatum* (50%) did not produce BEA while the others produced BEA at low levels. Two strains of *F. subglutinans* did not produce $FUMB_1$ but produced MON at low levels. One strain of *F. subglutinans* produced BEA and the other one did not produce the toxin. Two strains of *F. konzum* produced both MON and BEA at low levels but only one strain produced $FUMB_1$ at a low level. These mycotoxins have not been reported from *Fusarium* spp. in *Gibberella fujikuroi* species complex isolated form stalk and ear rot diseases of corn in these areas. Therefore, concerted efforts must be made to educate all stake holders about the presence and health hazards of these mycotoxins.

Keywords: Moniliformin (MON), fumonisin ($FUMB_1$), beauvericin (BEA).

INTRODUCTION

Many species of *Fusarium* in *Gibberella fujikuroi* species complex (Gfsc) such as *F. verticillioides, F. proliferatum,* and *F. subglutinans* have been known as the causal agents of stalk and ear rot diseases on corn in all major corn producers of the world (Referance). In addition, these *Fusarium* species are capable of producing a variety of mycotoxins, such as fumonisins (FUM), moniliformin (MON), and beauvericin (BEA) (Bottalico *et al*., 1989; Logrieco *et al*., 1995; Moretti *et al*., 1997; Chulze *et al*., 1998; Shephard *et al*., 1999). The occurrence of mycotoxins in this cereal grain is of great concern worldwide, because their presence in feeds and food is often associated with chronic or acute mycotoxicoses in livestock and, to a lesser extent, also in human (Charmley *et al*., 1995).

FUM are natural contaminants of cereal grains worldwide and are mostly found in corn and products derived from corn (Bullerman, 1996; Visconti, 1996; Weidenbörner *et al*., 2001), and is produced by a variety of *Fusarium* species mainly those in Gibberella fujikuroi species complex such as *F. verticillioides, F. proliferatum,* and some other related species e.g. *F. fujikuroi*, *F. konzum*, and *F. nygamai* (formally *F. moniliforme*; teleomorph *Gibberella fujikuroi* species complex) (Gelderblom *et al*., 1988; Thiel *et al.,* 1991; Nelson *et al.,* 1992). *F. verticillioides* is a pathogen of agriculturally important cereals, especially corn, and it is considered as one of the most important toxigenic fungi worldwide; as the main source of FUM contamination of food and feed products (Nelson *et al*., 1993; Moss, 1996). The most abundant FUM produced in nature is FUM B_1 ($FUMB_1$) that caused several chronic and acute diseases in human and animals (Gelderblom *et al*., 1988; Desjardins, 2006). According to Desjardins (2006), high levels of $FUMB_1$ have been found consistently in corn infected by strains of *F. verticillioides* and *F. proliferatum,* but $FUMB_1$

* Corresponding Author:
Email: Darnetty_06@yahoo.com

production was discontinuous among the other species of the Gfsc. As stated by Nelson *et al.* (1991), strains of *F. verticillioides* generally produce moderate to high levels of fumonisins (400 - 4000 mg/kg). Results of a study conducted by Acuna *et al.* (2005) showed that almost all strains of *F. verticillioides* (97.1%) and *F. proliferatum* (97.1%) produced $FUMB_1$ (5.6 - 25846.4 mg/kg) and (6.9 - 3885.0 mg/kg) respectively, while *F. subglutinans* did not produce FUM.

Like FUM, MON also was found widely in food and feeds infected by strains of *Fusarium* species in *Gibberella fujikuroi* species complex such as *F. proliferatum*, *F. subglutinans*, *F. avenaceum*, and *F. tricinctum* (Gutleb *et al*., 2002; Logrieco *et al*., 2002). Results of a survey conducted by Desjardins (2006) showed that in the Gfsc, MON production was widespread but not universal. Most notably, many strains of *F. proliferatum, F. subglutinans*, and *F. thapsinum* produced high levels of MON, but *F. verticillioides* very rarely produced MON. Individual species do vary widely in the frequency of MON-producing strains and in the levels of MON produced. Also stated by Leslie *et al.* (1995) that *F. verticillioides* produce little or no MON.

Beauvericin (BEA) is a mycotoxin produced by several *Fusarium* species, mainly by *F. proliferatum*, *F. semitectum,* and *F. subglutinans* (Moretti *et al*., 1997; Logrieco *et al*., 1998; Shephard *et al*., 1999). Although most of FUM-producing *Fusarium* species also produced BEA, however, the frequency and levels of BEA differ between individual strains (Desjardins, 2006). *F. verticillioides* and *F. proliferatum* are important corn pathogens causing stalk and ear rot all over the world including Southeast Asia, can produce a variety of mycotoxins including BEA both in pre-harvested and in stored products (Bottalico *et al*., 1989; Logrieco *et al*., 1995; Moretti *et al*., 1997; Chulze *et al*., 1998; Shephard *et al*., 1999). According to Leslie *et al.* (2004), *F. konzum* produce variable but generally low to moderate amounts of BEA (4 - 320 µg/g). Besides the threats, mycotoxin profiles also are important chemotaxonomic criteria in the identification and characterization of *Fusarium* species. Nelson *et al.* (1993), and Thrane and Hansen (1995) have revealed species-specific metabolite profiles of *Fusarium* species. Also, Fotso *et al.* (2002) found that individual species of *Fusarium* produced different classes and levels of secondary metabolites. For example, gibberellic acid (GA) could be used to differentiate *F. fujikuroi* with several other closely related species in *Gibberella fujikuroi* species complex (Nur Ain Izzati, 2007). So far, most studies of *Fusarium* mycotoxin profiles were using strains from the temperate region which might be different than those produced by tropical strains.

This study was conducted to elucidate toxigenecity of three most important mycotoxins i.e. $FUMB_1$, MON, and BEA produced by *Fusarium* species in *Gibberella fujikuroi* species complex isolated from corn showing typical stalk and ear rot symptoms in Indonesia, Malaysia, and Thailand.

MATERIALS AND METHODS

Strains and Chemicals: A total of 20 strains representing four *Fusarium* species i.e. *F. verticillioides* (12 strains), *F. proliferatum* (4 strains), *F. subglutinans* (2 strains), and *F. konzum* (2 strains) isolated from stalk and ear rot–infected corn plants were used to study the mycotoxin toxigenicity (Table 1). $FUMB_1$, MON, and BEA standards, sodium borate ($Na_2B_4O_7$), 2-mercaptoethanol, 2-ME ($HOCH_2CH_2SH$), tetrabutylammonium hydrogen sulphate (TBAHS). ($C_{16}H_{35}N{\cdot}H_2SO_4$), and ortho-phthaldialdehyde, OPA were purchased from Sigma-Aldrich, USA. Sodium dihydrogen phosphate monohydrate (NaH_2PO_4), *potassium dihydrogen phosphate* (KH_2PO_4), potassium chloride (KCl) from Univar, Australia. Orthophosphoric acid (H_3PO_4), acetonitrile, ACN (CH_3CN) HPLC grade, methanol, MeOH (CH_3OH) HPLC grade, n-heptane (C_7H_{16}) HPLC grade and dichloromethane (CH_2Cl_2) were purchased from Fisher Scientific, UK and C18-SPE column from Supelco, USA.

Fusarium Culture Preparation: About 15 g of washed and dried corn grits were placed into 100 ml Erlenmeyer flask, plugged with cotton wool and covered with aluminum foil, and autoclaved for 45 min at 15 psi, 121°C. The sterile corn grits in Erlenmeyer flasks were inoculated with 1 ml conidial suspension (1 x 10^7 conidia/ml). and then added with 5 ml of sterile distilled water to keep the moisture of corn grits. The Erlenmeyer flask was daily hand-shaken to homogenize the conidial suspension and incubated for 30 days in the dark.

Mycotoxin Extraction, Clean up, and Chemical analysis: All cultured media were extracted, cleaned up, and assayed for the following mycotoxins by using an Ultra Performance Liquid Chromatography (UPLC) as follows.

Fumonisin B_1 ($FUMB_1$): The method employed for fumonisin extraction was adapted from Nelson *et al.* (1991).

Table 1. Strains of *Fusarium* species in Section Liseola isolated from corn showing typical stalk and ear rot symptoms in Indonesia, Malaysia and Thailand used for mycotoxin toxigenicity analysis.

Strains code	Location	Region	Country	Organ Ear, Stalk	Species name
R106O	Bukit Tamiang	Perlis	Malaysia	Ear	*F. verticilioides*
P445O	MARDI Bertam	Penang	Malaysia	Ear	*F. verticilioides*
K2404O	Gurun	Kedah	Malaysia	Stalk	*F. verticilioides*
K2405O	Gurun	Kedah	Malaysia	Stalk	*F. verticilioides*
OLN0188O	Bogor	West Java	Indonesia	Ear	*F. verticilioides*
OLN0200O	TulungAgung	East Java	Indonesia	Ear	*F. verticilioides*
OLN0227O	Medan	North Sumatra	Indonesia	Ear	*F. verticilioides*
OLN0260O	Medan	North Sumatra	Indonesia	Stalk	*F. verticilioides*
OLN0266O	Agam	West Sumatra	Indonesia	Ear	*F. verticilioides*
OLN0344O	Padang-Pariaman	West Sumatra	Indonesia	Stalk	*F. verticilioides*
HLN0155O	Tak Fa	Nankhon Sawan	Thailand	Stalk	*F. verticilioides*
HLN0180O	Songkhla	Hat Yai	Thailand	Stalk	*F. verticilioides*
Q5565O	Sri Aman	Sarawak	Malaysia	Ear	*F. proliferatum*
OLN0187O	Bogor	West Java	Indonesia	Ear	*F. proliferatum*
OLN0381O	Banda Aceh	Aceh	Indonesia	Ear	*F. proliferatum*
HLN0160O	Tak	Tak	Thailand	Stalk	*F. proliferatum*
S4895O	Ranau	Sabah	Malaysia	Stalk	*F. subglutinans*
OLN0186O	Bogor	West Sumatra	Indonesia	Ear	*F. subglutinans*
OLN0343O	Padang-Pariaman	West Sumatra	Indonesia	Stalk	*F. konzum*
OLN0492O	Agam	West Sumatra	Indonesia	Stalk	*F. konzum*

About 10 g of inoculated corn grits were suspended with 50 ml of acetonitrile: water (50:50, v/v), and homogenized in a Waring blender for 5 minutes. The mixture was filtered through a Whatman no. 4 filter paper and separated by using a separatory funnel; the bottom layer was taken and evaporated to near dryness at 65°C by using a rotary evaporator (Buchi 461, Switzerland). The solvent residue was dissolved in 1 ml of methanol.

The filtrate was cleaned up on a C-18 solid phase extraction (SPE) column. The filtrate (1 ml) was added to 2.5 mL of 1% aqueous KCl and was passed through the C18-SPE column, which had been preconditioned with methanol (5 ml), followed by 5 ml of 1% aqueous KCl. The column was then washed with 3 ml of 1% aqueous KCL, followed by 2 ml of acetonitrile: 1% aqueous KCl (10:90, v/v). The fumonisins were eluted off the C_{18}-SPE column with 2 ml of acetonitrile: water (50:50, v/v) and was ready for the pre-column derivatization.

O-Phthaldialdehyde (OPA) derivatising agent was prepared by adding methanol (1ml) to OPA (40 mg) and diluted with 5 mL 0.1 mol L^{-1} sodium borate, then 50 µl of 2-ME was added to the mixture. This reagent is stable in the dark for up to 8 days in capped, aluminum foil-covered vials (Abramovic *et al*. 2005). The OPA reagent (200 µl) was added to a sample or standard solution (50 µl) and was mixed well. Five µL of this solution was injected into the UPLC unit, within four minutes after the derivatization. The mobile phase was methanol: mixture (0.1% sodium dehydrogen phosphate with pH 3.35 adjusted with o-phosphoric acid) (78:22). Detection of fumonisin was by fluorescence (FLR) detector with wavelength set at 305 nm for excitation and 432 nm for emission. Calculation of $FUMB_1$ concentrations of tested samples was based on peak areas of samples compared with those of standards.

Moniliformin (MON): MON extraction was carried out by following the techniques described by Burmeister *et al*. (1979). About 10 g of inoculated corn grits were soaked with 100 ml of 1% tetrabutylammonium hydrogen sulphate (TBAHS) in ddH_2O and shaken for 30 minutes. Then the shaken corn grits were filtered through a Whatman no.4 filter paper and defatted with 10 ml of n-hexane in a separating funnel and the bottom layer was taken. Dichloromethane (same volume as

sample) was added to the sample solution and mixed gently. The mixture was separated by using a separatory funnel and the sample solution at bottom layer was taken and evaporated in a Buchi (Switzerland) rotary vacuum evaporator at 55°C. Then, the soluble residue was dissolved in 1 ml dichloromethane, filtered through 0.2 um Millipore filter, and kept in a bottle at 5°C until used. The filtrate was cleaned up by the C_{18}-SPE column as described for $FUMB_1$. Mobile phase was acetonitrile and mixture (50 ml of 40% tetrabutylammonium hydrogen sulphate, 100 ml of 1.1 M potassium dehydrogen phosphate, 850 ml ddH_2O, pH 6.5 adjusted by 5N KOH) (8:92). MON was detected by a photodiode array (PDA) detector at wavelength 229 nm.

Beauvericin (BEA): Procedure for beauvericin detection was adapted from Logrieco *et al.* (1998). About 15 g of corn grits were soaked overnight with 75 ml of acetonitrile: methanol: water (16:3:1; v/v), and milled in a Waring blender for five minutes. The milled corn grits were filtered through a Whatman no. 4 filter paper, and then defatted twice with 25 ml of n-hexane in a separating funnel. The bottom layer was evaporated to near dryness at 80°C by using a vacuum rotary evaporator (Buchi 461, Switzerland). The solvent residue was dissolved in 50 ml of methanol: water (1:1 v/v) and extracted twice with 25 ml of dichloromethane. Then, the solution was evaporated and redissolved in 1 ml of methanol, filtered through 0.2 um Millipore filter, and kept in 5 ml Bijoux bottle at 5°C condition until used. The filtrate was cleaned up by the C_{18}-SPE column as described for $FUMB_1$. Mobile phase used for BEA detection was methanol: water (85:15; v/v) with flow rate a 0.5 ml/min. BEA was detected by a photodiode array (PDA) detector at wavelength 205 nm.

RESULTS AND DISCUSSION

The results of this study showed that each strain of *Fusarium* species has a different ability to produce the three mycotoxins, $FUMB_1$, MON, and BEA. Representative production of $FUMB_1$, MON, and BEA by each strain were indicated in Figure 1. Concentrations of three mycotoxins produced by *Fusarium* species were shown in Table 2. $FUMB_1$ were produced in high concentrations up to 32756.3 µg/g mainly by strains of *F. verticillioides* and *F. proliferatum,* although there were some strains that produced $FUMB_1$ with rather low concentrations. In general, the productions of $FUMB_1$ by *F. verticillioides* were higher than those by *F. proliferatum*. These two fungi were known as the major FUM-producing species (Gelderblom *et al*., 1988; Thiel *et al*., 1991; Nelson *et al*., 1992). Also, the two species of *Fusarium* always produce FUM in high levels, while others in Gfsc do not always produce FUM as stated by Desjardins (2006). On the contrary, two strains of *F. subglutinans* tested did not produce the $FUMB_1$. As mentioned by Nelson *et al.* (1992) and Leslie *et al.* (2004) that *F. subglutinans* produced little or no $FUMB_1$. The results of this study were similar with those by Acuna *et al.* (2005) in which almost all strains of *F. verticillioides* and *F. proliferatum* produced $FUMB_1$ at the levels ranging from 5.6 to 25846.4 mg/kg and from 6.9 to 3885.0 mg/kg respectively, while *F. subglutinans* did not produce $FUMB_1$. In the presence study, one strain of *F. konzum* produce $FUMB_1$ at low level, and the other strain did not produce the toxin. This is in accordance with that proposed by Leslie *et al.* (2004) that *G. konza* (teleomorph of *F. konzum*) strains produced little or no fumonisins (up to 120 µg/g by one strain).

Moniliformin (MON) derived from *F. moniliforme*, the fungal species that was identified as the original toxin producer in the United State in 1993 (Cole *et al*., 1973; Marasas *et al.,* 1984). However, after re-investigation of MON producing strains indicated that some strains were misidentified as *F. proliferatum* and the others were *F. nygamai* (Marasas *et al*, 1988). Subsequent studies have confirmed that MON rarely produced by *F. moniliforme* and the amount of MON is not more than trace levels (now renamed as *F. verticillioides*) (Nelson *et al.* 1993; Leslie *et al.* 1996). Rabie *et al.* (1982) stated that the species now recognized as *F. verticillioides* does not generally produce significant levels of MON even though some strains may produce the mycotoxin when grown on corn. However, in this study all strains of *F. verticillioides* produced MON at low levels. The results of this study are not unexpected because *F. verticillioides* has been found to produce MON even though in a very small amount (Leslie *et al*., 1995) and interestingly, results of a research conducted by Moretti *et al* (2004) indicated that all strains from banana fruits identified morphologically as *F. verticillioides* and biologically as MP-A produced MON while none of the strains produced FUM and the *F. verticillioides* strains from corn produced $FUMB_1$ (20 – 5645 µg/g) but did not produce MON. Based on these results, it can be concluded that there are strains of *F. verticillioides* that can produce MON at relatively high levels. Therefore, the results of the study by Moretti *et al.* (2004) cancorroboratethe results of our

findings. MON formation by strains of *F. proliferatum* and *F. subglutinans* in the present study was also in accordance with the results of several researchers such as Marasas *et al.* (1984), Abbas *et al.* (1989), Chelkowski *et al.* (1990), Leslie *et al.* (1996), and Desjardins *et al.* (1997). Two strains of *F. konzum* produced MON at low levels and there has been no previous report of MON production by *F. konzum*.

BEA production is widely distributed in *Fusarium* species including both FUM-producing and trichothecene-producing species (Bottalico *et al*, 1995; Shephard *et al*., 1999; Desjardins *et al*., 2000; Fotso *et al*., 2002; Logrieco *et al*., 2002, and Leslie *et al*., 2004). In the present study, eight strains of *F. verticillioides* did not produce BEA while the other four strains produced BEA at the low levels (0.6-2.8 µg/g). These results are in agreement with those reported by Desjardins (2006), that *F. verticillioides* produced little or no BEA. Two of the four tested strains of *F. proliferatum* and one of two tested strains of *F. subglutinans* produced BEA at low levels.

The productions of BEA by the strains of the two species are in accordance with the results of studies that have been published by many researchers such as Krska *et al.* (1996), Moretti *et al.* (1997), Logrieco *et al.* (1998), and Shephard *et al.* (1999). In this study the amounts of BEA produced by *F. proliferatum* and *F. subglutinans* were very low when compared with the results of the research that have been reported by all researchers mentioned above. According to Desjardins (2006) individual species do vary widely in frequency of BEA-producing strains and in the level of BEA produced.

Table 2. Toxigenicity of strains of *Fusarium* spp. in *Gibberella fujikuroi* species complex isolated from corn showing typical stalk and ear rot symptoms in Indonesia, Malaysia and Thailand.

Fusarium species	Strains	Concentration (µg/g)		
		FUM B_1	MON	BEA
F.verticillioides	R106O	15996.7	1.4	ND
	P445O	25367.1	1.9	0.8
	K2404O	20624.7	1.9	ND
	K2405O	4550.4	1.7	ND
	OLN0188O	12606.3	1.8	ND
	OLN0200O	29471.4	2.6	2.8
	OLN0227O	16329.6	2.5	2.7
	OLN0260O	32756.3	0.7	ND
	OLN0266O	9926.8	3.2	ND
	OLN0344O	715.6	4.9	ND
	HLN0155O	843.5	1.4	ND
	HLN0180O	27692.9	1.9	0.6
F. proliferatum	Q5565O	156.2	1.7	ND
	OLN0187O	13217.6	2.3	3.2
	OLN0381O	8933.1	1.2	ND
	HLN0161O	21423.1	3.0	0.1
F. subglutinans	S4895O	ND	1.6	3.9
	OLN0186O	ND	1.1	ND
F. konzum	OLN0343O	30.1	2.1	1.2
	OLN0492O	ND	0.9	1.0

$FUMB_1$= Fumonisin B_1; MON= Moniliformin; BEA= Beauvericin; ND= Not Detected.

In conclusion, all strains of *F. verticillioides* and *F. proliferatum* produced $FUMB_1$ at high levels ranging from 715.6 to 32756.3 µg/g and 156.2 to 21423.1 µg/g, respectively while produced MON at low levels from 0.7 to 4.9 µg/g and 1.2 to 3.0 µg/g, respectively. In general, production of $FUMB_1$ by strains of *F. proliferatum* was relatively low compared to *F. verticillioides.* Many strains of *F. verticillioides* (67%) and *F. proliferatum* (50%) did not produce BEA while the others produced BEA at low levels, up to 2.8 and 3.2 µg/g, respectively. Two strains of *F. subglutinans* tested did not produce $FUMB_1$ but produced MON at low levels, 1.1 and 1.6 µg/g.

One strain of *F. subglutinans* produced BEA (3.9 µg/g) and the other one did not produce the toxin. Two strains of *F. konzum* produced both MON and BEA at low levels but only one strain produced $FUMB_1$ at a low level.

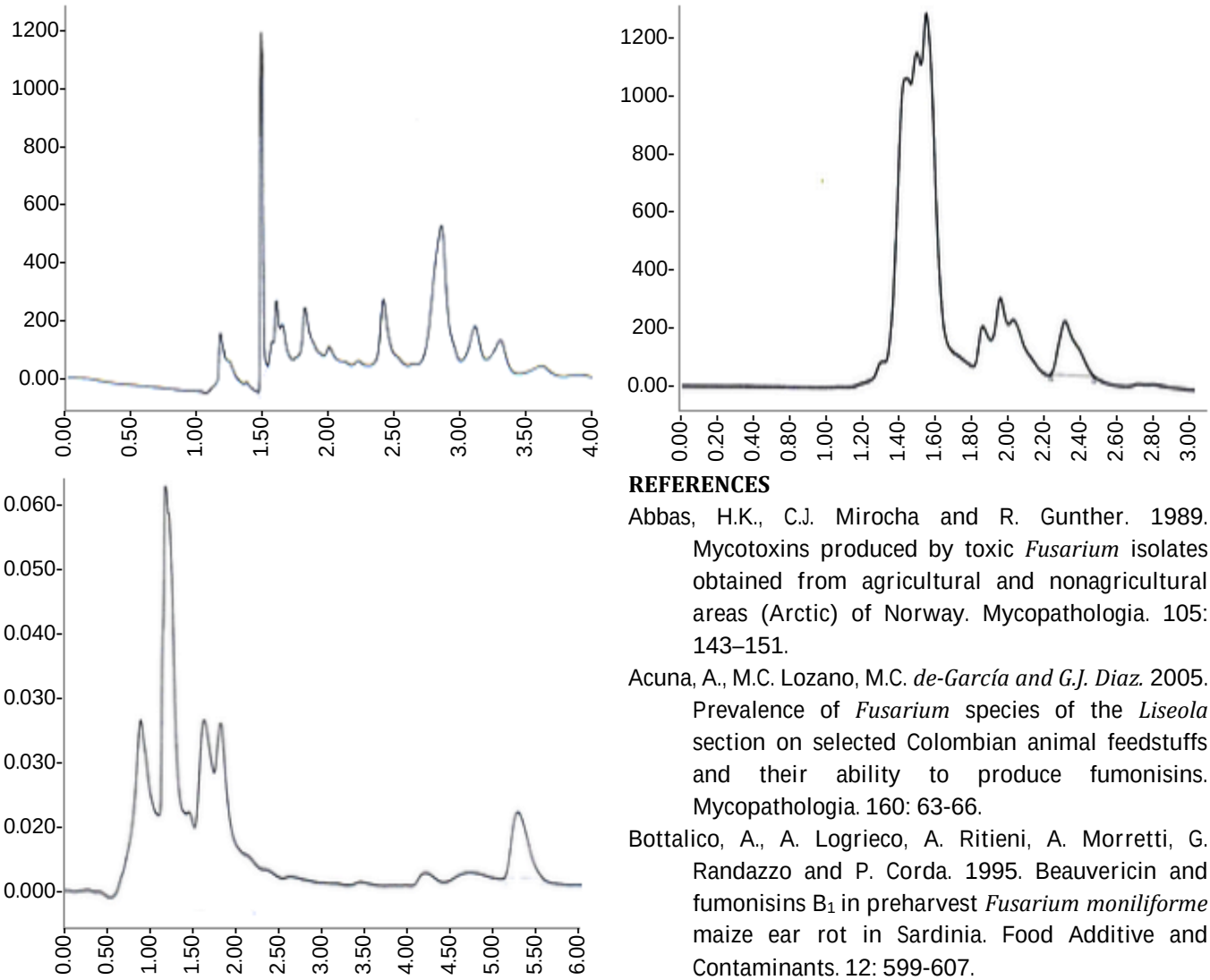

Figure 1. UPLC chromatogram of $FUMB_1$, 843.3 µg/g (A), MON, 4.9 µg/g (B), and BEA, 2.8 µg/g) (C) obtained from corn grits inoculated with *F. verticillioides,* (HLN0155), (OLN0344O) and (OLN0200O) respectively.

These profiles of mycotoxins have not been reported from *Fusarium* spp. in *Gibberella fujikuroi* species complex isolated form stalk and ear rot diseases in Indonesia, Malaysia, and Thailand. Therefore, concerted efforts must be made to educate all stake holders, especially corn farmers and those involve in poultry and animal farming, about the presence and health hazards of these mycotoxins.

ACKNOWLWDGEMENTS

We are thankful to the RU Research Grant 1001/PBIOLOGI/811182 provided by Universiti Sains Malaysia (USM).

REFERENCES

Abbas, H.K., C.J. Mirocha and R. Gunther. 1989. Mycotoxins produced by toxic *Fusarium* isolates obtained from agricultural and nonagricultural areas (Arctic) of Norway. Mycopathologia. 105: 143–151.

Acuna, A., M.C. Lozano, M.C. *de-García and G.J. Diaz.* 2005. Prevalence of *Fusarium* species of the *Liseola* section on selected Colombian animal feedstuffs and their ability to produce fumonisins. Mycopathologia. 160: 63-66.

Bottalico, A., A. Logrieco, A. Ritieni, A. Morretti, G. Randazzo and P. Corda. 1995. Beauvericin and fumonisins B_1 in preharvest *Fusarium moniliforme* maize ear rot in Sardinia. Food Additive and Contaminants. 12: 599-607.

Bottalico, A., A. Logrieco and A. Visconti. 1989.*Fusarium* species and their mycotoxins in infected cereals in field and in stored grains. In: Chelkowski, J. (ed) Fusarium-Mycotoxins, Taxonomy and Pathogenicity. Topics in Secondary Metabolites. Elsevier, Amsterdam, Netherlands. pp 85–119.

Bullerman, L. B. 1996. Occurrence of *Fusarium* and fumonisins on food grains and in foods. *In*: Fumonisins in Food. L.S. Jackson, J.W. Devries, and L.B. Bullerman, eds. Plenum Press: New York. pp 27-38.

Burmeister, H.R., A. Ciegler and R.F. Vesonder. 1979. Moniliformin, a metabolite of *Fusarium moniliforme*: Purification and Toxicity. Applied and Environmental Microbiol. 37: 11 – 13.

Charmley, L.L., H.L. Trenholm, D.A. Prelusky and A.

Rosenberg. 1995. Economic losses and decontamination. Natural Toxins. 3: 199–203.

Chelkowski, J., M. Zawadzki, P. Zajkowski, A. Logrieco and A. Bottalico. 1990. Moniliformin production by *Fusarium* species. Mycotoxins Res. 6: 41-45.

Chulze, S.N., M.L. Ramirez, M. Pascale and A. Visconti. 1998. Fumonisin production by, and mating population of, *Fusarium Gibberella fujikuroi* species complex isolates from maize in Argentina. Mycological Res. 102: 141–144.

Cole, R.J., J.W. Kirksey, H.G. Cutler, B.L. Doubnik and J.C. Peckham. 1973. Toxin from *Fusarium moniliforme*: effects on plants and animals. Science. 179: 1324-1326.

Desjardins, A.E. 2006. *Fusarium* mycotoxins: chemistry, genetics, and biology. APS St. Paul.

Desjardins, A.E. and T.M. Hohn. 1997. Mycotoxins in plant pathogenesis. Molecular Plant-Microbe Interactions. 10: 147–152.

Desjardins, A.E., H.K. Manandhar, R.D. Plattner, G.G. Manandhar, S.M. Poling and C.M. Maragos. 2000. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. Applied of Environmental Microbiol. 66: 1020-1025.

Fotso, J., J.F. Leslie and J.S. Smith. 2002. Production of beauvericin, moniliformin, fusaproliferin, and fumonisin B_1, B_2, and B_3 by ex-type strains of fifteen *Fusarium* species. Applied and Environmental Microbiology 68: 5195 – 5197.

Gelderblom, W.C.A.J., W.F.O. Jaskiewicz, P.G. Marasas, R.M. Thiel, R.M. Horak, R. Vleggar and N.P.J. Kriek. 1988. Fumonisin mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. Applied of Environmental Microbiol. 54: 1806-1811.

Gutleb, A.C., E. Morrison and A.J. Murk. 2002. Cytotoxicity assays for mycotoxins produced by *Fusarium* strains: a review. Environmental Toxicology and Pharmacol. 11: 309 – 320.

Krska, R., M. Lemmens, R. Schuchmacher, M. Grasserbauer, M. Pronczuk, H. Wisniewska and J. Chelkowski. 1996. Accumulation of the mycotoxin beauvericin in kernels of corn hybrids inoculated with *Fusarium subglutinans.* J. of Agricultural and Food Chemistry. 44: 3665–3667.

Leslie, J.F. 1995.*Gibberella fujikuroi*: Available populations and variable traits. Canadian J. of Botany. 73: 282-291.

Leslie, J.F. and K.K. Klein. 1996. Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics. 144: 557–567.

Leslie, J.F., K.A. Zeller, A. Logrieco, G. Mule, A. Moretti and A. Ritieni. 2004. Species diversity of and toxin production by *Gibberella fujikuroi* species complex strains isolated from native prairie grasses in Kansas. Applied and Environmental Microbiol. 70: 2254-2262.

Logrieco, A., A. Moretti, G. Castella, M. Kostecki, P. Golinski, A. Ritieni and J. Chelkowski. 1998. Beauvericin production by *Fusarium* species. Applied and Environmental Microbiol. 64: 3084-3088.

Logrieco, A., A. Moretti, A. Ritieni, A. Bottalico and P. Corda. 1995. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Dis. 79: 727-731.

Logrieco, A., G. Mule, A. Moretti and A. Bottalico. 2002. Toxigenic *Fusarium* species and mycotoxins associated with maize ear rot in Europe. European J. of Plant Pathol. 108: 597–609.

Marasas, W.F.O., T.S. Kellerman, W.C.A. Gelderblom, J.A.W. Coetzer, P.G. Thiel and J.J. Van Der Lugt. 1988. Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. Onderstepoort J. Veterinary Res. 55: 197-203.

Marasas, W.F.O., P.E. Nelson and T.A. Toussoun. 1984. *Toxigenic* Fusarium *species.* The Pennsylvania State University Press.

Moretti, A., A. Logrieco, A. Bottalico, A. Ritieni, V. Fogliano and G. Randazzo. 1997. Diversity in beauvericin and fusaproliferin production by different populations of *Gibberella fujikuroi* (*Fusarium fujikuroi* species complex). Sydowia. 48:44–56.

Moretti, A., G. Mule, A. Susca, M.T. Gonzalez-Jaen and A. Logrieco. 2004. Toxin profile, fertility and AFLP analysis of *Fusarium verticillioides* from banana fruits. European J. of Plant Pathol. 110: 601-609.

Moss, M.O. 1996. Mycotoxins. Mycological Res. 100: 513-523.

Nelson, P.E. 1992. Taxonomy and biology of *Fusarium moniliforme*. Mycopathologia. 117: 29–36.

Nelson, P.E., A.E. Desjardins and R.D. Plattner. 1993. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. Annual Review of Phytopathol. 31:233–252.

Nelson, P.E., R.D. Plattner, D.D. Shackelford and A.E. Desjardins. 1991. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Applied and Environmental Microbiol. 57: 2410 – 2412.

Nur-Ain-Izzati, M.Z., A.A. Razak and B. Salleh. 2009. Bakanae disease of rice in Malaysia and Indonesia: etiology of the causal agent based on morphological, physiological and pathogenicity characteristics. J. of Plant Protection Res. 48: 475-485.

Rabie, A.J., W.F.O. Marasas, I.P.G. Thiel, A. Lubben and L.R. Vleggaar. 1982. Moniliformin production and toxicity of different *Fusarium* species from Southern Africa. Applied and Environmental Microbiol. 43: 517-521.

Shephard, G.S., V. Sewram, T.W. Nieuwoudt, W.F.O. Marasas and A. Ritieni. 1999. Production of the mycotoxins fusaproliferin and beauvericin by South African isolates in the *Fusarium Gibberella fujikuroi* species complex. J. of Agricultural and Food Chemistry. 47: 5111–5115.

Thiel, P.G., W.F.O. Marasas, E.W. Sydenham, G.S. Shephard, W.C.A. Gelderblom and J.J. Nieuwenhuis. 1991. Survey of fumonisin production by *Fusarium* species. Appl. Environ. Microbiol. 57:1089–1093.

Thrane, U. and U. Hansen. 1995. Chemical and physiological characterization of taxa in the *Fusarium sambucinum* complex. Mycopathologia. 129: 183 –190.

Visconti, A. 1996. Fumonisins in maize genotypes grown in various geographic areas. In: Jackson L.S., De Vries, J.W., and Bullermann, L.B. (eds) Advances Experimental Medicine and Biology – Fumonisins in Food. Plenum Press, New York, USA pp 193–204.

Weidenbörner, M. 2001. Foods and fumonisins. Eur. Food Res. Technol. 212: 262-273.