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BENZENE DERIVATIVES PRODUCED BY *FUSARIUM GRAMINEARUM*

SHORT COMMUNICATION

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Using NMR spectroscopy benzene derivatives were detected in mycelia of *Fusarium graminearum*, a pathogen of wheat and maize. In previous studies *F. graminearum* was found to cause cancer to humans and benzene derivatives were detected in breath of cancer sufferers. Surprisingly, no study found benzene derivatives to be the cancerous agents in *F. graminearum*. In this study we detected benzene derivatives in *F. graminearum* and propose to study their role as cancer agents.

Keywords: Fusarium graminearum – benzene derivatives – nuclear magnetic resonance

Fusarium graminearum Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch] is a pathogen of wheat and maize which causes head blight and cob rot in these respective plant hosts. Upon infection of the wheat kernel and the maize cob, *F. graminearum* produces mycotoxins which apart from reducing yield and downgrading quality [3, 4, 5], are harmful to humans and animals. Fumonisins, one of the groups of mycotoxins produced by *F. graminearum,* have been associated with oesophageal cancer in humans [9] and leukoencephalomacia in equine species and rabbits [2, 6, 7] among other serious illnesses. Because of these health hazards, research in the chemistry of *F. graminearum* has been focused on profiling mycotoxins and their harm to humans and animals. Most of these research efforts have been based on the isolation and characterisation of single compounds or a group of compounds. An overview of the entire metabolome of *F. graminearum* has not received sufficient attention from researchers up until now. In the present era of metabolomics it is possible to capture a snapshot of the entire metabolome of *F. graminearum* to discover compounds other than the most studied group of compounds, the mycotoxins. In our attempt to profile the metabolome of *F. graminearum* we focused on benzene derivatives some of which are presumed to have the same health hazards as mycotoxins. Benzene derivatives were identified in breath from lung cancer patients [10], clearly linking them to this disease. A culture of *F. graminearum* strain G44A isolated from wheat was grown

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at 25 °C on Potato Dextrose Agar (PDA). After five days of incubation mycelia were scrapped with a scalpel and ground with liquid nitrogen using a mortar and pestle. Four fractions of the ground mycelia weighing 0.10 g were made for the extraction of secondary metabolites, with each fraction representing a technical replication. Methanol-d_4, 99.8 atom % D and deuterium oxide, 99.9 atom % D provided by Sigma-Aldrich® Corporation were used for the secondary metabolite extraction. Each fraction of the ground mycelia was suspended in a 1.5 ml solution of methanol-d_4/deuterium oxide (50:50 v/v). The mixture was vortexed vigorously, sonicated for 20 minutes and centrifuged briefly at room temperature. The supernatant which contained secondary metabolites was dispensed into nuclear magnetic resonance (NMR) tubes for analysis. Nuclear magnetic resonance spectroscopic analysis was done using a 600 MHz Varian NMR instrument to obtain 1D proton spectra of the four samples. Phase correction and baseline correction of the spectra were done using ACD/NMR Processor [1] and represented compounds were identified using NMRShiftDB [8]. For each spectrum from each of the replications the output of

Table 1
List of compounds detected in *Fusarium graminearum* isolate G44A.
The compounds were detected in all four technical replicates

	NAME OF THE COMPOUND
1	Trimethyl-[(E)-2-phenyl-2-triethylgermloxyethenyl]silane
2	Dipropan-2-ylbenzene-1,2-dicarboxylate
3	5-methyl-5-nitro-2-phenyl-1,3,2-dioxaphosphinane
4	Dichloro-ethyl-diphenyl-$I^{5}-arsane
5	Dichloro-diethyl-phenyl-$I^{5}-arsane
6	3-chloro-5-methyl-5-phenyl-4H-1,2-oxazole
7	5-(2-methylpropanol)-1,3-dihydrobenzimidazol-2-one
8	[ditert-butylphosphoryl(phenyl)methyl]benzene
9	2,4,6-triphenyl-4,5-dihydro-1H-pyrimidin-6-ol
10	[1-(chloromethylsulfanyl)-4,4-dimethylpent-2-ynyl]benzene
11	7-nitro-2-(4-nitrophenoxy)-3-propan-2-ylquinazolin-4-one
12	2-chloroethylbenzene
13	3-phenylpropylbenzene
14	(3E)-3-[methyl(phenyl)hydrazinylidene]-1-phenylpropan-1-one
15	N-methyl-N,3-diphenyl-2H-azirine-2-carboxymide
16	(3E)-1-(4-chlorophenyl)-3-[methyl(phenyl)hydrazinylidene]propan-1-one
17	[2-isothiocyanato-3-(4-methoxyphenyl)propyl]thiocynate
18	(2R,3R)-2-dimethoxyphosphoryl-3-phenyloxirane
19	(3E)-1-(4-methoxyphenyl)-3-[methylphenyl)hydrazinilidine]propan-1-one
20	(3E)-3-[methyl-(4-nitrophenyl)hydrazinylidene]-1-phenylpropan-1-one

NMRShiftDB was 300 of the compounds represented by spectrum with the identities between the queries and the database entries represented in percentages. From the compounds represented by the spectra, known benzene derivatives were selected and presented in Table 1 above. Only benzene derivatives with names which could be clearly seen among the output of 300 compounds were selected.

We assume that most of the benzene derivatives produced by *F. graminearum,* at 25 °C on PDA can also be produced during grain infestation under natural conditions in the field. To our knowledge, none of the compounds listed in Table 1 have yet been individually reported to cause cancer. As a result, we intend to continue to chemically profile *F. graminearum* in an attempt to identify the full range of potentially hazardous compounds produced by this plant pathogen in its plant host. Medically guided fractionation method using high performance liquid chromatography is one of the methods to be used in a follow-up study, thus allowing us the privilege of targeting and profiling potentially active/hazardous metabolites.

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