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Some of phytotoxic and antimicrobial compounds extracted from culture filtrates of *Fusarium proliferatum* FP85

Fateme Rasekhi[1*], Mohammad A. Tajick[1], Heshmatollah Rahimian[1], Salman Sharifimehr[2]

[1]*Sari Agricultural Sciences and Natural Resources University, Department of Plant Protection, Sari,Iran*

[2]*Genetic and Agricultural Biotechnology Institue of Tabarestan,Sari, Iran*

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Abstract

Fusarium is one of the most important plant pathogens around the world which causes great damages to agriacultural crops. The fungus produces a wide variety of volatiles and bioactive metabolites like mycotoxins, fatty acids and hydrocarbons on artificial medium with antifungal, antibacterial, antiviral and phytotoxic properties. After 21 days inoculating the species *Fusarium proliferatum* FP85 on PDB, the culture filtrates were subjected to metabolites extraction by EtOAc and phase separating and evaporation. The sediment were dissolved in one ml MtOH, for qualitative detection by GC-MS. The GC-MS results revealed different metabolites such as Pyridine, 3-butyl-; Pyrazine, 3-ethyl-2 ,5-dimethyl-; Palmitic acid; Stearic acid; Oleic acid; Adipic acid; Piperitenone oxide and Phthalic acid, butyl isobutyl ester. Some of detected compounds are phytotoxins and toxic compounds against fungi and bacteria and posses many biological activities.

***Corresponding Author:** Fateme Rasekhi ✉ rasekhifateme@yahoo.com

Introduction

Fungi produce a wide rang of bioactive compounds that have remarkable importance for human. Some of these compounds have beneficial applications in the medicin, industry, and agricultural applications (Siddiquee *et al*., 2012). Chemical metabolites produced by these fungi possess anti-fungal, anti-bacterial and anti-viral activity and contains potent mycotoxins that cause biological control of plant diseases and sometimes are effective in the growth of vegetation. The *Fusarium* species are important pathogens of small grain cereals, especially in the temperate regions of the world. Infection with *Fusarium* commonly results in reduction of the quality and yield of the crop. Additionally, many of these fungi are capable of producing phytotoxic secondary metabolites that cause wilting, necrosis, growth inhibition and inhibition of seed germination in some plants. The important group of these compoundes are mycotoxins which may evoke adverse effects on human and animal health if they gain access to the food and feed chain. Some have herbicide properties (Kokkonen *et al*., 2010; Meca *et al*., 2009). In order to determine the secondary metabolites, various techniques such as GC-MS, HPLC and TLC are used. in this study, the GC-MS method has been used for the qualitative detection of metabolites of *Fusarium proliferatum* FP85.

Materials and methods

Culture conditions

The fungus isolate was inoculated on PDA (Potato dextrose agar: 200 g potato, 15 g dextrose and 15 g agar on 1 L dionised water). The agar plates were incubated at 25 °C for 7 days with daylight alternating with darkness in cycles of 12 h. Then, for long-term storage, spore suspensions were prepared and cultured on a liquid medium PDB (Potato dextrose broth: 200 g potato, 20 g dextrose on 1 L dionised water). At last 10 inoculated flasks were incubated at 28 °C For 21 days under darkness.

Metabolites extraction

Medium was maintained for 24 h at 4 ° C before extraction and After 21 days after fungal growth on PDB,the broth were filtered through Whatman no. 1 paper in a Buchner funnel. The metabolites extracted using 50 mL of ethyl acetate in 20 ml liquid medium for 30 min by a decanter was performed in three steps:

- First, 20 mL of ethyl acetate was poured into the funnel containing liquid medium and shaken several times and a few minutes after the formation of the phase, supernatant (containing ethyl acetate with metabolites) of the pellet (containing liquid medium) were isolated and kept on erlenmeyer flask A.

- Next 20 mL of ethyl acetate was added to the lower phase was separated before and like the previous step supernatant was transferred to flask A .

- As a final step, 10 mL of ethyl acetate was added to the broth of funnel, after enough shaking, supernatant again was added to flask A like before.

The samples were centrifugated in 7000 rpm for 5 min and 1-2 g Anhydrous sodium sulfate (Na_2SO_4) was added. Then evaporated to dryness at 45 ° C on a rotary evaporator and one ml methanol was added to it. At last filtered through 0.22 µm Poly-Tri-Fluoro-E thylene (PTFE).

GC-MS

Separation of volatile compounds was achived using a GC-MS system from Agillent company model 7890. An Hp-5MS fused silica capillary column (Hewlett-Packed, 30 m, 0.25 mm i.d., 0.25 µm film Thickness, cross-linked to 5% phenyl methyl siloxane stationary phase) was used . The entire system was controlled by MS ChemStation software (Hewlett- Packed, version A.01. 01). Electron impact mass spectra were recorded at 70 eV. Ultra-high purity helium (99.999%) was used as the carrier gas at flow rate of 1mL/min. The injection volume was 1µL and all the injections were performed in a splitless mode. Injector temperatures was 250 °C. Temperature program was used: 60 °c (2 min)–30 °c /min–170 °c (5 min)–7 °c /min–250 °c (10 min) (Langseth *et al*., 1998).

All used chemicals and solvents were HPLC-grade and obtained from Merck Company.

Results

list of metabolites obtained by GC-MS are shown in Table 1. Mycotoxins: P-Benzoquinone, derivatives of Pyridine and Pyrazine, fatty acids: Stearic acid, Palmitic acid and Oleic acid essential oils: Carvone, Piperitenone oxide and Farnesol and volatile compounds: Heptacosane, Hexadecane, Heneicosane, Tetratriacontane, Octadecane,Eicosane, Heptadecane and Pentatriacontane Including extracellular metabolites were produced on PDB medium. chromatogram of GC-MS analysis is shown in Figure 1.

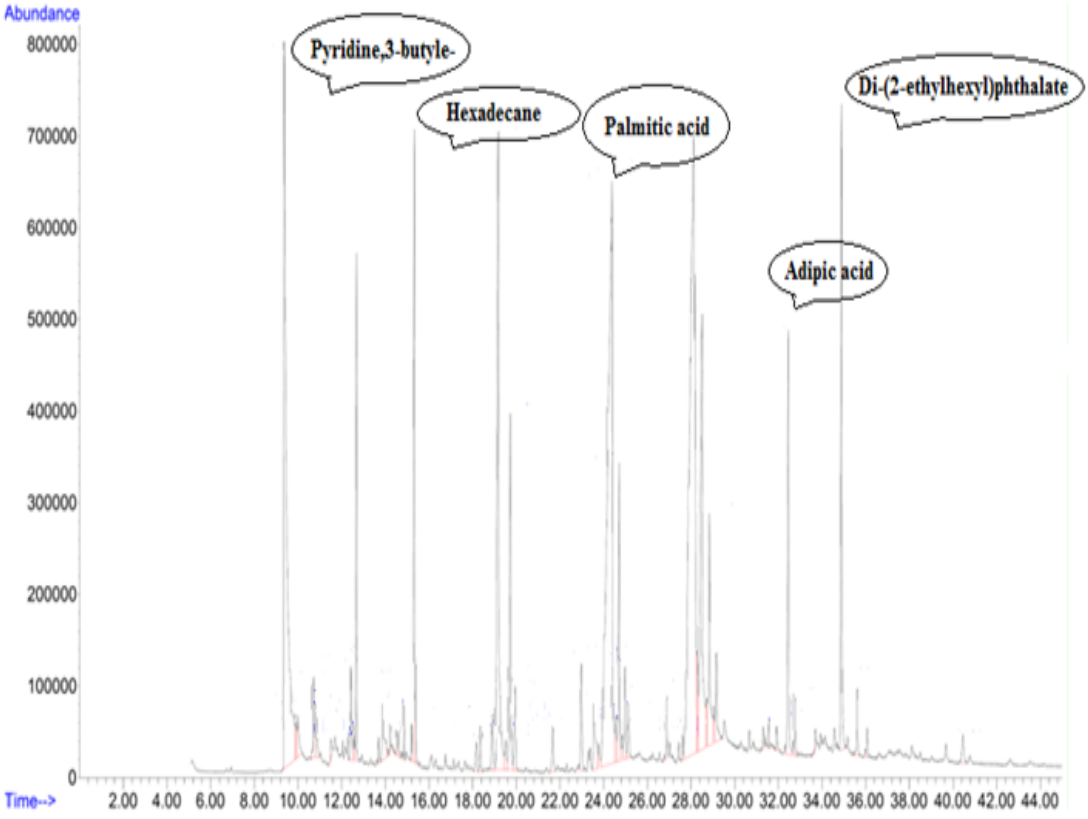

Fig 1. GC-MS chromatogram of Fusarium proliferatum FP85 after 21 days incubation in a PDB medium at 28ºC .

Table 1. GC-MS analysis of the compounds produced by *Fusarium proliferatum* FP85 after 21 days incubation in a PDB medium at 28 ºC.

Compounds	Retentin time(min)	PeakArea(%)

Pyridine, 3-butyl-	9.382		11.89
Cis-dihydrocarvone	10.003		0.51
Carvone	10.714		0.63
Cyclohexane	10.850		0.38
Pyrazine, 3-ethyl-2,5-dimethyl-	11.517		0.17
Piperitenone oxide	12.422		0.64
Tetradecane	12.681		2.39
1-Tetradecanol	13.697		0.14
p-Benzoquinone	13.871		1.01
Phenol, 2,4-bis(1,1-dimethylethyl)-	14.220		0.17
Pentadecane, 7-methyl-	14.531		0.17
Tridecane, 5-methyl-	14.609		1.16
Heptacosane	14.874		0.28
1-Nonadecene	15.204	0.16	
Hexadecane	15.327		3.72
Cyclopentane, 1-hexyl-3-methyl-	15.398		0.58
Heneicosane	18.179		0.30
10-Methylnonadecane	18.354		0.36
Tetratriacontane	18.891		1.06
Benzyl Benzoate	19.169		7.05
1-Nonadecene	19.512		0.31
Octadecane	19.725		3.15
5-Octadecene	19.945		0.78
Phthalic acid, butyl isobutyl ester	21.672		0.44
7,9-Ditert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	22.979		0.90
Palmitic acid			
Thiosulfuric acid	24.383		16.83
Eicosane	24.551		0.41
Xylazine	24.713		2.18
Oleic acid	25.133		0.48
Stearic acid	26.905		22.15
Heptadecane	28.503		6.29
S-Butylcyclohexane	28.846		2.26
Adipic acid	29.163		0.86
Di-(2-ethylhexyl)phthalate	32.456		3.30
Pentatriacontane	34.894		4.46
2,4-Pentadien-1-ol	35.612		0.48
Farnesol	36.072		0.21
	40.457		0.36

Discussion

Toxins, fatty acids and hydrocarbons were obtained. 3-Butylpyridine is a member of Pyridine mycotoxin that are known to possess a range of biological activities such as analgesic, antifungal, antimalarial, antiinflammatory, antibacterial, anti-HIV, antitumoral and antiviral properties. Some studies report their phytotoxic activities against *Sorghum bicolor, Cucumis sativus* and *Ipomoea grandifolia* (Jacinto Demuner *et al*., 2009). Results of Goshaev, (1993) showed this compound is the causative agents of Fusarial wilt of the cotton, tomatoes, and other agricultural crops. He also reported that the capacity for causing the symptoms of wilt is 22 times greater for 3-Butylpyridine than for the fusaric acid. Pyrazine derivatives in addition to phytotoxic, herbicides and fungicides activities, in the cases of pharmaceutical, posses anti-viral, anti-cancer and anti-mycobacterial properties. They also found naturally in many vegetables, insects, terrestrial vertebrates, and marine organisms and they are produced by microorganisms during their primary or secondary metabolisms (Dolezal and Kralova., 2011).

Fatty acids were produced with high concentrations in a medium of this speciess. A fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated like Hexadecanoic (Palmitic) acid, Stearic (Octadecanoic acid) acid or unsaturated like Oleic (9-Octadecenoic) acid. The antifungal and bactericidal properties of some fatty acids are well known and some studies reported well antitumor activity of fatty acids. The free carboxyl group is necessary for activity, because ester formation generally decreased bactericidal activity of the fatty acids. Reduction of the carboxyl group to the aldehyde or alcohol or the change to an amine or amide group increased bacteriostatic effects (Kabara *et al*., 1972). Fatty acids have phytotoxic activity too (Yu and Matsui, 1994).

The antibacterial and antifungal activities of essential oils are directly related to their ability to penetrate the cell walls of bacteria or fungi and therefore due to their solubility in the phospholipid bilayer of cell membrane (Verstegen, 1994). Carvone is one of the essential oils that its antibacterial activities against *Clavibacter*, *Curtobacterium*, *Rhodococcus*, *Erwinia*, *Xanthomonas*, *Ralstonia* and *Agrobacterium* genera were reported (Iacobellis *et al*., 2006). This compound is also active against cancer cell lines (Aydin *et al*., 2013). Piperitenone oxid and 7,9-Ditert-butyl-1-oxaspiro (4,5) deca-6 ,9-diene-2 ,8-dione are other essential oils that have a high antibacterial activity against several bacterial species, especially gram-positive bacteria (Marinkovic *et al*., 2002; Nadaf *et al*., 2013). Activity and toxicity of farnesol strongly depends on enviromantal and growth conditions. Twenty to fifty micromolar farnesol inhibits or kills other fungi, opaque *Candida albicans* cells, several mammalian cell lines, and some bacteria (Langford *et al*., 2010). Verstegen, (1994) assessed phytotoxicity and antifungal activity of some essential oils. Heptacosane, Hexadecane, Heneicosane, Tetratriacontane and Octadecane are hydrocarbons were produced by this strain. These compounds also inhibited the growth of some plant pathogens (Ozdemir *et al*., 2004; Siddiquee *et al*., 2012). Adipic acid is a di-carboxylic acid that decreases the growth of tobacco plant (Hirabayashi *et al*., 2001). Phthalic acid, butyl isobutyl ester is another metabolite which is known as a toxic compound to cells and has antifungal, herbicidal and phytotoxic activity (Dib *et al*., 2010; Zhang *et al*., 2011). Xylazine is a sedative used in anesthesia. There are few reports about to its toxicity.

Conclusion

The aim of present study was to investigate and identifying the secondary metabolites of *Fusarium proliferatum* FP85. Pathogenesis of some toxins of this strain for human and laboratory and domestic animals was domenstrated (Meca *et al*., 2009). According to the pathogenicity of this isolate on plants and the presence of high amounts of phytotoxic metabolites derived from these analysis, It is suggested that by further investigation in this field, we can establish a link between these two features

and fungal identification. It also recommended that by applying more accurated tools and techniques and by ragarding physical and environmental conditions of producing secondary metabolites, charecterisation of metabolites with more details and evaluating of the rate of toxins production were obtained.

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