Mycologia Iranica 3(1): 47 – 55, 2016 Original Article DOI: 10.22043/mi.2017.41532.1072

Identification of volatile organic compounds of *Trichoderma* spp. using static headspace gas chromatography-mass spectrometry

M. Shahiri Tabarestani
Department of Plant Protection, Faculty of Plant Production, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran
&
Faculty of Agricultural Sciences, Payame Noor University, Tehran, Iran

K. Rahnama ✉
Department of Plant Protection, Faculty of Plant Production, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran

M. Jahanshahi
Nanotechnology Research Institute, School of Chemical Engineering, Babol Noshirvani University of Technology, Mazandaran, Iran

S. Nasrollahnejad
Department of Plant Protection, Faculty of Plant Production, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran

M. H. Fatemi
Faculty of Chemistry, Mazandaran University, Babolsar, Iran

Abstract: Fungi release wide spectrum of volatile organic compounds (VOCs) that belong to several chemical groups with different biochemical origins such as monoterpenes, sesquiterpenes, alcohols, aldehydes, aromatic compounds, esters, furans, ketones, sulfur and nitrogen compounds. *Trichoderma* species are the most studied fungal biocontrol agents and are successfully used as biofungicides and biofertilizers in greenhouse and field. Volatile metabolites play a key role in mycoparasitism of *Trichoderma* spp., as well as in their interactions with plants and other organisms in their environments. Based on the antibiotic activity of these fungi against the fungal pathogens, further consideration of their VOCs profiles, has been offered. In this study, VOCs of native *Trichoderma* species from Iran (*T. harzianum, T. virens* (6011), *T. atroviridae* (1-3)) have been identified by static headspace gas chromatography-mass spectrometry. The most of detected compounds were related to monoterpenes and sesquiterpenes. These are including; dl-limonene; beta-himachalene; beta-cubebene; cadinene; caryophyllene; alpha-gurjunene; farnesol; thujopsene; beta-bisabolene and alpha-farnesene. Based on antifungal effects of these compounds, biological control of these *Trichoderma* species can be related to them. These VOCs could be potential sources for purposes of chemotaxonomy and natural fungicides to protect crops from the fungal pathogens without environmental problems.

Key words: GC–MS, static headspace, *Trichoderma* species, volatile organic compounds.

INTRODUCTION

Fungi produce various volatile organic compounds (VOCs) that due to their small sizes and high vapor pressure are readily able to diffuse through the atmosphere and soils at normal temperature and pressure. VOCs generally have low to medium water solubility and often have a distinctive odor (Hung et al. 2015). Up to now, approximately 500 VOCs have been detected in fungal metabolites. From more than 100,000 species of described fungi, only about 100 species have been studied for VOC production (Korpi et al. 2009; Hung et al. 2015). VOCs play important signaling roles in fungal natural environments. Many ecological interactions are mediated by VOCs, between fungi, plants and bacteria (Morath et al. 2012). They appear as intermediate and final products of different metabolic pathways and principally devoted to mono- and sesquiterpenes, alcohols, ketones, lactones, esters, fatty acids, sulfur-containing compounds, simple pyranes and benzene derivatives (Korpi et al. 2009). These metabolites are involved in different biological processes such as biocontrol or communication between microorganisms and their living environment (Bitas et al. 2013). They can mediate defense against predators, parasites and diseases, and may be produced for competition between species (Stoppcher et al. 2010).

Fungal strains of the genus *Trichoderma* are well-known producers of volatile compounds. The VOCs profile of a known species or strain will vary depending on the substrate, duration of incubation,

Submitted 11 April 2016, accepted for publication 18 June 2016
✉ Corresponding author e-mail: kamranrahnama1995@gmail.com

type of nutrients, temperature, and other environmental parameters (Siddiquee et al. 2012; Tait et al. 2013). VOCs of the filamentous biocontrol fungi like *Trichoderma* spp., act antibiotically against range of plant pathogenic moulds and can confer plant growth promoting effects as well as systemic resistance to plants, thus rendering plants less susceptible to the fungal pathogens (Vinale et al. 2008). The ability of *Trichoderma* spp. to produce a significant number of volatile (e.g. pyrones, sesquiterpenes) and nonvolatile secondary metabolites (e.g. peptaibols) has been reviewed recently (Reino et al. 2008).

The *Trichoderma* strains have been used as biocontrol agents with different mechanisms, such as, mycoparasitism, antibiosis, competition for nutrients, cell wall-lytic enzyme activity, and induction of systemic resistance to pathogens *in planta* (vinal et al. 2006; Norouzi et al. 2014; Habibi et al. 2015). Determination of volatile fungal metabolites usually is determined by gas chromatography (GC) methods and has been detected for different fungal genera such as *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Trichoderma*. After culture of the fungi in liquid (Pinches and Apps 2007) or on solid growth medium (Nemcovic et al. 2008), volatiles can be extracted in different ways, such as with organic solvents (Reithner et al. 2005), solid phase extraction using C18 or silica gel columns (Keszler et al. 2000), online gas enrichment on adsorption tubes or various headspace (HS) techniques: e.g. static headspace, dynamic headspace (purge and trap) and solid phase microextraction (Stoppcher et al. 2010). In static headspace analysis, the volatiles in the sample are allowed to equilibrate with the air in an airtight container. After equilibration, a known volume of air is collected from the sample, frequently in a gas-tight syringe, and injected directly into the gas chromatograph (GC). After GC separation on nonpolar stationary phases, the constituents of complex mixtures of VOCs can be identified by mass spectrometry (MS) (Siddiquee 2014). Mass spectrometric detection can detect individual volatiles from complex mixtures. Structure characterization and confirmation of identity is usually achieved by comparison of mass spectra with library spectra (Jeleń, 2003; Stoppcher et al. 2010).

The objective of this research was to detect volatile organic compounds from the headspace of *Trichoderma* cultures by using static headspace gas chromatography-mass spectrometry. This is a powerful approach for the direct profiling of VOCs, because fungi are cultured directly in headspace vials and HS-GC-MS measurement is realized in a fully automated method (Guler et al. 2015).

MATERIALS AND METHODS

Fungal isolates and growth conditions

In this study, three native biocontrol *Trichoderma* species were used. *Trichoderma harzianum* (NCBI GeneBank accession No. JX173852.1), *T. virens* (6011) accession No. KP671477.1, and *T. atroviridae* (1-3) were obtained from the Mycology Laboratory, Department of Plant Protection, Gorgan University of Agricultural Sciences and Natural Resources. Morphological identification of the last isolate has been confirmed by Dr. Zafari (Bu-Ali Sina University). It was compatible with type specimen from Mashhad collection, too (Zafari et al. 2002). They were isolated from the soil of canola and cucurbits farms in Gorgan and were successful in biological control of different phytopathogens (Abdolahian et al. 2012; Norouzi et al. 2014; Habibi et al. 2015). All the fungal strains were maintained on potato dextrose agar (PDA) (Merck, Germany) slants at room temperature and subcultured bimonthly. From actively growing margins of PDA cultures, a 5 mm diameter plug of each *Trichoderma* species, was placed on the centre of slants consisting of 5 mL of sterile PDA in 20 mL headspace vials. The control vials were consisted of only sterile PDA culture (without *Trichoderma* plug). Three replicates were considered for each treatment. The vials were sealed with screw-caps containing gas-tight silicone/teflon septa and incubated at 22 °C for 5 days. A single GC-MS measurement was carried out, for all fungal cultures and control vials.

HS-GC/MS conditions

After 10 min of equilibration at 90 °C, extraction of volatiles from the headspace of the fungal cultures was carried out by the aid of a COMBI PAL autosampler (CTC ANALYTICS, Switzerland). For the detection of fungal VOCs, a GC Agilent 7890A equipped with an Agilent 5975C mass selective detector was used. GC–MS analyses were performed with ionization energy of 70ev. Identification of volatile metabolites was conducted using a nonpolar capillary colum (DB-5): 60 m, 0.25 mm, 0.25 µm. Oven program: 40 °C (hold 2 min), 10 °C/min to 200 °C, 25 °C/min to 260 °C (hold 25 min).

Injector temperature was hold at 250 °C (splitless mode) and detector temperature was set at 280 °C. The carrier gas was helium (He) at the flow-rate of 1 ml/min. The scan range was 45-550 m/z. Fungal metabolites were identified by comparison of the obtained mass spectrum with mass spectral libraries (NIST08.L).

RESULTS

According to NIST08.L mass spectra library of the GC–MS analysis, 30 volatile compounds were identified in the headspace of cultures.

The retention time and abundance of these compounds are shown in (Tables1-3). The detected VOCs in the culture samples, included cycloalkene, alcohol, ketone, ester, organic acid, monoterpene, sesquiterpene, sulphur and nitrogen compounds. Most of detected compounds by this method were related to monoterpenes and sesquiterpenes. Chemical structures of some identified VOCs are illustrated in Fig. 1 (https://pubchem.ncbi.nlm.nih.gov). In all three species, limonene is the common compound.

DISCUSSION

Volatile organic compounds have been shown to be involved in interactions between filamentous fungi and their living environment. Thus, analytical methods for the identification of volatile compounds are the key to considering their formation and functions in the biological interactions.

Some identified VOCs were previously reported in various standard laboratories as shown in references list in Tables 1-3. Isoamyl alcohol, limonene and 2, 2-dimethoxy-1,2-diphenyl-ethanone have been identified in all three species in this study (Tables 1-3). Limonene had the most frequency in these three species. This compound is biosynthesised from acetyl-CoA via the intermediate mevalonate. It has been shown antitumor activities in animal models and in cell culture experiments (Wagner et al. 2003). Khethr et al. (2008) investigated the antibacterial and antifungal activities of limonene against five pathogenic bacterial and fungal strains, and reported that this compound has antibacterial effect, without any antifungal activity.

They also declared that this compound was the major component in the *Trichoderma* extract. Tajick et al. (2014) has been detected limonene in secondary metabolites of *Penicillium purpurogenum*.

The following VOCs, just detected in *T. atroviridae* (1–3): ethanol, beta–bisabolene, epizonarene, farnesol, beta–guaiene, alpha–gurjunene, beta–himachalene, beta–sesquiphellandrene, widdrene, zingiberene, diethylac–etylene, benzoic acid–4nitroso–ethyl ester and propanoic acid. In this isolate, ethanol and isoamyl alcohol had major amounts after limonene (Table 3). Based on antifungal effects of these compounds, its biocontrol activity can be related to them.

Four compounds have been recognized only in *T. harzianum*: cembrene, beta–elemene, alpha–muurolene and 6–methyl–5–nonen–4–one (Table 1). Unique metabolites were identified in *T. virens* (6011) include cadinene, calamenene, caryophyllene, beta-eudesmol, alpha–farnesene, 1,2,3,4,5-pentamethyl-1,3-cyclopentadiene and 2–amino–5,7–dimethyl thiazolo[4,5–b]pyridine (Table 2).

Sivasithamparam and Ghisalberti (1998) declared that different species of one family and different isolates of one species, can often produce significantly different compounds. It means that secondary metabolites express the individuality of species in chemical terms. They also stated that, widely separate species could produce the same class of the secondary metabolite and sometimes even the same secondary metabolites.

Zeringue et al. (1993) identified also alpha-gurjunene, caryophyllene, cadinene, alpha-muurolene in aflatoxigenic strains of *Aspergillus flavus.* Ethanol, beta-bisabolene, alpha-farnesene, beta-himachalene, dl-limonene, beta-sesquiphellandrene, caryophyllene and zingiberene have been detected in *T. atroviride* and *T. viride* (Stoppacher et al. 2010; Polizzi et al. 2011; Polizzi et al. 2012; Hung et al. 2013).

The bisabolenes are a large group of sesquiterpenes that various biological activities (nematicidal and antimicrobial activities) have been reported for them (Wu et al. 2011).

Table 1. Volatile metabolites of the biocontrol fungus *Trichoderma harzianum* identified by HS-GC-MS.

Compounds	RT (min)	Abundance (%)	Producing species	References
Isoamyl alcohol	7.177	9.49		
dl-Limonene	12.27	21.5	*Trichoderma atroviridae*	Nemcovic et al. 2008
			T. viridae	Hung et al. 2013
			T. atroviridae	Sidiquee 2014
			Penicillium sp.	Tajick et al. 2014
6-methyl-5-Nonen-4-one	14.227	6.09		
Beta-Elemene	18.200	0.51	*Periconia Britannica*	Polizzi et al. 2012
			Penicillium decumbens	Polizzi et al. 2012
			Aspergilus ustus	Polizzi et al. 2012
Alpha-Muurolene	18.687	0.62	*A. ustus*	Polizzi et al. 2012
Beta-Chamigrene	21.101	0.67	*P. decumbens*	Polizzi et al. 2012
			T. longibrachiatum 594	Citron et al. 2011
			T. harzianum 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
2,2-dimethoxy-1,2-diphenyl- Ethanone	22.818	1.26		
Cembrene	24.031	1.20		

RT: Retention Time

Table 2. Volatile metabolites of the biocontrol fungus *T. virens* (6011) identified by HS-GC-MS.

Compounds	RT(min)	Abundance (%)	Producing species	References
Isoamyl alcohol	10.107	1.84		
dl-Limonene	12.627	15.81	*Trichoderma atroviridae*	Nemcovicet al. 2008
			T. viridae	Hung et al. 2013
			T. atroviridae	Sidiquee 2014
			Penicillium purpurogenum	Tajick et al. 2014
Cadinene	18.089	1.01	*Aspergillus ustus*	Polizzi et al. 2012
	19.120	3.22	*T. longibrachiatum* 594	Citron et al. 2011
Calamenene			*T. harzianum* 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
Alpha-Farnesene	19.308	2.35	*T. atroviridae*	Nemcovicet al. 2008
			T. atroviridae	Stoppacher et al. 2010
			T. atroviridae	Polizzi et al. 2011
			Aspergillus fumigatus	Bazemore et al. 2012
			T. viridae	Hung et al. 2013
			T. atroviridae	Sidiquee 2014
			A. fumigatus	Heddergott et al. 2014
Beta-Cubebene	19.361	1.47	*A. ustus*	Polizzi et al. 2012
Caryophyllene	19.589	1.02	*Phoma* sp.	Strobel et al. 2011
			Fusarium oxysporum	Minerdi et al. 2011
			Periconia britannica	Polizzi et al. 2012
			F. oxysporum	Bitas et al. 2013
1,2,3,4,5-pentamethyl-1,3-Cyclopentadiene	19.859	3.18		
Beta-Chamigrene	19.859	3.18	*Penicillium decumbens*	Polizzi et al. 2012
			T. longibrachiatum 594	Citron et al. 2011
			T. harzianum 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
2-Amino-5,7-dimethylthiazolo[4,5-b]pyridine	20.544	16.76		
Beta-Eudesmol	21.166	0.89		
2,2-dimethoxy-1,2-diphenyl-Ethanone	22.818	1.26		
5-Methoxy-2,8,8-trimethyl-4H,8H-benzo [1,2-b:3,4-b']dipyran-4-one	24.037	1.15		

RT: Retention Time

Sesquiterpenes share the same metabolic precursor mevalonate as the monoterpenes and are converted to the final structures by the action of sesquiterpene synthases. They presented a structurally complex compound class that showed antimicrobial and antiviral activities (Fraga 2012; Stoppacher et al. 2010).

Kundu et al. (2013) demonstrated significant antifungal activity of cadinene derivatives that makes them as a source of antifungal agent for the development of a natural fungicide.

Matasyoh et al. (2013) presented antifungal activity of cadinene and beta–bisabolene against mycotoxigenic *Aspergillus*, *Fusarium* and *Penicillium* species. Dahham et al. (2015) demonstrated antimicr-obial activities of caryophyllene against pathogenic bacterial and fungal strains. Caryophyllene could enhance plant growth and increase stress resistance (Morath et al. 2012; Bitas et al. 2013). Caryophyllene oxide, an oxygenated terpenoid, well known as preservative in food, drugs and cosmetics, has been shown *in vitro* antifungal effect against dermatophytes (Yang et al. 1999). Azevedo et al. (2013) reported that 7-hydroxycalamenene-rich oils presented high antimicrobial activity. Siddiqui et al. (2013) reported *M. scandens* extract had a remarkable antifungal effect against *Rhizoctonia solani*, *Pythium graminicola* and *Fusarium oxysporum*. They clarified that the key role for their antifungal activities was related to the presence of phenolic compounds, oxygenated monoterpenes and sesquiterpene hydrocarbons such as beta–caryophyllene, d-cadinene, alpha–cubebene, caryophyllene oxide, beta-himachalene and beta–farnesene. These compounds have already been detected in this study.

Berberović & Milota (2011) showed high inhibitory effects of thujopsene against wood decay fungi. Farnesol is a natural pesticide for mites and is a pheromone for several other insects. It is used by the commensal, opportunistically pathogenic fungus *Candida albicans* as a quorum sensing molecule that inhibits filamentation (Hornby et al. 2001).

Citron et al. (2011) demonstrated, some sesquiter–pens such as calamenene, beta–sesquiphellandrene, zingiberene, epizonaren, beta–bisabolene, beta–chami–grene and beta–sesquiphellandrene had minor percentage in *T. longibrachiatum*, *T. harzianum* and *T. viride* medium cultures.

Table 3. Volatile metabolites of the biocontrol fungus *T. atroviridae* (1-3) identified by HS-GC-MS.

Compounds	RT(min)	Abundance (%)	Producing species	References
Ethanol	4.422	16.87	*Trichoderma viridae*	Hung et al. 2013
Isoamyl alcohol	7.188	7.48		
dl-Limonene	12.627	21.14	*T. atroviridae*	Nemcovicet al. 2008
			T. viridae	Hung et al. 2013
			T. atroviridae	Sidiquee 2014
			Penicillium purpurogenum	Tajick et al. 2014
Widdrene	19.073	0.92	*P. decumbens*	Polizzi et al. 2012
Alpha-Gurjunene	19.261	1.12		
Zingiberene	19.319	1.87	*T. atroviridae*	Stoppacher et al. 2010
			P. polonicum	Polizzi et al. 2012
			T. atroviridae	Polizzi et al. 2012
			T. atroviridae	Sidiquee 2014
			T. longibrachiatum 594	Citron et al. 2011
			T. harzianum 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
Beta-Sesquiphellandrene	19.653	3.70	*T. atroviridae*	Nemcovicet al. 2008
			T. atroviridae	Stoppacher et al. 2010
			P. polonicum	Polizzi et al. 2012
			T. atroviridae	Polizzi et al. 2012
			T. longibrachiatum 594	Citron et al. 2011
			T. harzianum 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
Beta-Bisabolene	20.497	2.53	*T. atroviridae*	Stoppacher et al. 2010
			P. polonicum	Polizzi et al. 2012
			T. atroviridae	Polizzi et al. 2012
			T. atroviridae	Sidiquee 2014
			T. longibrachiatum 594	Citron et al. 2011
			T. harzianum 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
Benzoic acid, 4-nitroso- ethyl ester	20.544	1.76		
Beta -Guaiene	20.579	1.14		
Farnesol	20.644	2.24	*Candida albicans*	Hornby et al. 2001
Epizonaren	20.732	4.37	*T. longibrachiatum* 594	Citron et al. 2011
			T. harzianum 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
Diethylacetylene	20.831	1.78		
Beta-Himachalene	21.136	0.34	*T. viridae*	Hung et al. 2013
			P. decumbens	Polizzi et al. 2012
			T. longibrachiatum 594	Citron et al. 2011
			T. harzianum 714	Citron et al. 2011
			T. viride 54	Citron et al. 2011
2,2-dimethoxy-1,2-diphenyl- Ethanone	22.818	1.80		
5-Methoxy-2,8,8-trimethyl-4H,8Hbenzo[1,2-b:3,4-b']dipyran-4-one	24.037	2.71		
Propanoic acid	14.731	0.33		

RT: Retention Time

Several researchers have reported that monoterpenes and sesquiterpenes and their oxygenated derivatives have potential to inhibit microbial pathogens (Cakir et al. 2004; Siddiqui et al. 2013). In this research, monoterpenes and sesquiterpenes were also included the most of detected compounds.

Trichoderma VOCs with antifungal effects can become a suitable alternative for synthetic fungicides in agro-industries as natural fungicides against phyto-pathogens. In recent years, natural fungicides are acquiring increasing interest because of their relatively safe status, wide acceptance by consumers and utilization for multi-purpose functional uses. Therefore, it would also be suggested to study the effects of *Trichoderma* VOCs against other important fungi for development of the new antifungal agents to control serious fungal diseases in plants as well as purposes of chemotaxonomy.

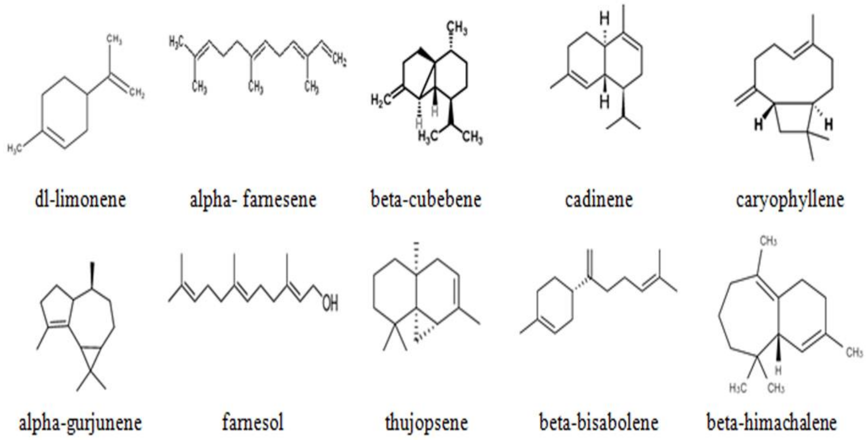

Fig. 1. Chemical structures of some identified VOCs in *Trichoderma* species (pubchem.ncbi.nlm.nih.gov).

ACKNOWLEDGEMENTS

The technical assistance of Dr. Zafari (Department of Plant Protection, Bu-Ali Sina University, Hamedan, Iran) for identification of *Trichoderma* species is gratefully acknowledged. We are kindly appreciated from Professor Joan W. Bennett (Department of plant pathology, University of New Jercy, USA) for excellent comments and critical review.

REFERENCES

Abdolahi M, Rahnama K, Mamarabadi M, Ommati F, Zaker M. 2012. The in vitro efficacy of Trichoderma isolates against Pythium aphanidermatm, the causal agent of sugar beet root rot. Journal of Research Agricultural Sciences 8: 79–87.

Azevedo MMB, Chaves FCM, Almeida CA, Bizzo HR, Duarte RS, Takaki GMC, Alviano CS, Alviano DS. 2013. Antioxidant and antimicrobial activities of 7–Hydroxy–calamenene–rich essential oils from Croton cajucara Benth. Molecules 18: 1128–1137.

Bazemore RA, Jason F, Leland C, Podila GK. 2012. Biomedically important pathogenic fungi detection with volatile biomarkers. Journal of Breath Research 6: 016002.

Berberović A, Milota MR. 2011. Impact of wood variability on the drying rate at different moisture content levels. Forest Products Journal 61: 435–442.

Bitas V, Kim HS, Bennett JW, Kang S. 2013. Sniffing on microbes: diverse roles of microbial volatile organic compounds in plant health. Molecular Plant-Microbe Intractions 26: 835–843.

Cakir A, Kordali S, Zengin H, Izumi S, Hirata T. 2004. Composition and antifungal activity of essential oils isolated from Hypericum hyssopifolium and Hypericum heterophyllum. Flavour and Fragrance Journal 1: 62–68.

Citron CA, Riclea R, Brock NL, Dickschat JS. 2011. Biosynthesis of acorane sesquiterpenes by Trichoderma. RSC Advances 1: 290–297.

Dahham SS, Tabana YM, Iqbal MA, Ahamed MB, Ezzat MO, Majid AS, Majid AM. 2015. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene beta- caryophyllene from the essential oil of Aquilaria crassna. Molecules 20: 11808–29.

Fraga BM, 2012. Natural sesquiterpenoids. Natural Product Reports 29: 1334–1366.

Guler Z, Karaca F, Yetisir H. 2015. Identification of volatile organic compounds (VOCs) in different colour carrot (Daucus carota L.) cultivars using static headspace/gas chromatography/mass spectrometry. Food Science & Technology 1: 1117275. http://dx.doi.org/10.1080/23311932.2015.1117275.

Habibi R, Rahnama K, Taghinasab M. 2015. Evaluating the effectiveness of native Trichoderma species in production of extracellular enzymes during interaction with plant pathogenic fungus Fusarium oxysporum. Journal of Applied Research Plant Protection 4: 73–85.

Heddergott C, Calvo AM, Latgé JP. 2014. The volatome of Aspergillus fumigatus. Eukaryotic Cell 13: 1014–1025.

Hornby JM, Jensen EC, Lisec AD, Tasto JJ, janke B, Shoemaker R, Dussault P, Nickerson KW. 2001. Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol. Applied and Environmental Microbiology 67: 2982–2992.

Hung R, Lee S, Bennett JW. 2013. Arabidopsis thaliana as a model system for testing the effect of Trichoderma volatile organic compounds. Fungal ecology 6: 19–26.

Hung R, Lee S, Bennett JW. 2015. Fungal volatile organic compounds and their role in ecosystems. Applied Microbiology Biotechnology 99: 3395–3405.

Jelen HH. 2003. Use of solid phase microextraction

(SPME) for profiling fungal volatile metabolites; Letters in Applied Microbiology 36: 263–267.

Keszler A, Forgács E, Kótai L, Vizcaíno JA, Monte E, García-Acha I. 2000. Separation and identification of volatile components in the fermentation broth of Trichoderma atroviride by solid phase extraction and gas chromatography-mass spectrometry. Journal of Chromatographic Science 38: 421–424.

Korpi A, Jarnberg J, Pasanen AL. 2009. Microbial volatile organic compounds. Critical Reviews in Toxicology 39: 139–193.

Khethr FBH, Ammar S, Saidana D, Daami M, Chriaa J, Liouane K, Mahjoub MA, Helal AN, Mighri Z. 2008. Chemical composition, antibacterial and antifungal activities of Trichoderma sp. growing in Tunisia. Annals of microbiology 58: 303–308.

Kundu A, Saha S, Walia S, Shakil NA, Kumar J, Annapurna K. 2013. Cadinene sesquiterpenes from Eupatorium adenophorum and their antifungal activity. Journal of Environmental Science and Health 48: 516–22.

Matasyoh JC, Wagara IN, Nakavuma JL, Chepkorir R. 2013. Chemical composition and antifungal activity of Piper capense oil against mycotoxigenic Aspergillus, Fusarium and Penicillium species. International Journal of Biological and Chemical Sciences 7: 1441–1451.

Minerdi D, Bossi S, Maffei ME, Gullino ML, Garibaldi A. 2011. Fusarium oxysporum and its bacterial consortium promote lettuce growth and expansin A5 gene expression through microbial volatile organic compound (MVOC) emission. FEMS Microbiology Ecology 76: 342–351.

Morath SU, Hung R, Bennett JW. 2012. Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. Fungal Biology Reviews 26: 73–83.

Nemcovic M, Jakubıkova L, Vıden I, Vladimır F. 2008. Induction of conidiation by endogenous volatile compounds in Trichoderma spp. FEMS Microbiology Letters 284: 231–236.

Norouzi S, Rahnama K, Rabbani Nasab H, Taqi Nasab M. 2014. Evaluation of efficacy of Trichoderma and Bacillus isolates in biological control of melon Fusarium wilt. Biocontrol in Plant Protection 2: 43–55.

Pinches SE, Apps P. 2007. Production in food of 1, 3-pentadiene and styrene by Trichoderma species. International Journal of Food Microbiology 116: 182–185.

Polizzi V, Adams A, Picco AM, Adriaens E, Lenoir J, Peteghem, CV, Saeger SD, Kimpe ND. 2011. Influence of environmental conditions on production of volatiles by Trichoderma atroviride in relation with the sick building syndrome. Building and Environment 46: 945–954.

Polizzi V, Adams A, Saeger SD, Peteghem CV, Moretti A, Kimpe ND. 2012. Influence of various growth parameters on fungal growth and volatile metabolite production by indoor molds. Science of the Total Environment 414: 277–286.

Reino JL, Guerriero RF, Gala RH, Collado IG. 2008. Secondary metabolites from species of the biocontrol agent Trichoderma. Phytochemistry Reviews 7: 89–123.

Reithner B, Brunner K, Schuhmacher R, Peissl P, Seidl V, Krska R, Zeilinger S. 2005. The G protein α subunit Tga1 of Trichoderma atroviride is involved in chitinase formation and differential production of antifungal metabolites. Fungal Genetics and Biology 42: 749–760.

Siddiquee S, Cheong BE, Taslima K, Hossain K, Hasan MM. 2012. Separation and identification of volatile compounds from liquid cultures of Trichoderma harzianum by GC-MS using three different capillary columns. Journal of Chromatographic Science 50: 358–367.

Siddiquee S. 2014. Recent advancements on the role and analysis of volatile compounds (VOCs) from Trichoderma. In: Biotechnology and biology of Trichoderma. (VK Gupta, M Schmoll, AH Estrella, RS Upadhyay, I Druzhinina, MG Tuohy, eds):139–175. Elsevier's Science & Technology Rights Department in Oxford, UK.

Siddiqui SL, Islam RA, Islam RE, Jamal AHM, Tanzima P, Atiqur R. 2013. Chemical composition and antifungal properties of the essential oil and various extracts of Mikania scandens (L.) Willd. Arabian Journal of Chemistry. http://dx.doi.org/10.1016/j.arabjc.2013.07.050.

Sivasithamparam K, Ghisalberti EL. 1998. Secondary metabolism in Trichoderma and Gliocladium. In: Trichoderma and Gliocladium. (CP Kubicek, GE Harman, eds): Vol. 1, 139–188. Taylor & Francis Ltd., London,UK.

Stoppacher N, Kluger B, Zeilinger S, Krska R, Schuhmacher R. 2010. Identification and profiling of volatile metabolites of the biocontrol fungus Trichoderma atroviride by HS-SPME-GC-MS; Journal of Microbiological Methods 81:187–193.

Strobel G, Singh SK, Riyaz– Ul– Hassan S, Mitchell AM, Geary B, Sears J. 2011. An endophytic/ pathogenic Phoma sp. from creosote bush producing biologically active volatile compounds having fuel potential. FEMS Microbiology Letters 320: 87–94.

Tait E, Perry JD, Stanforth SP, Dean JR. 2013. Identification of volatile organic compounds produced by bacteria using HS–SPME–GC–MS. Journal of Chromatographic Science 363–373. DOI: 10.1093/chromsci/bmt042.

Tajick Ghanbari MA, Mohammadkhani HS, Babaeizad V. 2014. Identification of some secondary metabolites produced by four Penicillium species. Mycologia Iranica 1: 107–113.

Vinale F, Marra R, Scala F, Ghisalberti EL, Lorito M, Sivasithamparam K. 2006. Major secondary metabolites produced by two commercial

Trichoderma strains active against different phytopathogens. Letters in Applied Microbiology 43: 143–148.

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, Lorito M. 2008. A novel role for Trichoderma secondary metabolites in the interactions with plants. Physiological and Molecular Plant Pathology 72: 80–86.

Wagner KH, Elmadfa I. 2003. Biological relevance of terpenoids: overview focusing on mono-, di- and tetraterpenes. Annals of Nutrition and Metabolism 47: 95–106.

Wu SH, Zhaoa LX, Chena YW, Huangb R, Miaoa CP, Wanga J. 2011. Sesquiterpenoids from the Endophytic Fungus Trichoderma sp. PR–35 of Paeonia delavayi. Chemistry & Biodiversity 8: 1717–1723.

Yang D, Michel L, Chaumont JP, Millet-Clerc J. 1999. Use of caryophyllene oxide as an antifungal agent in an in vitro experimental model of onychomycosis. Mycopathologia 148:79–82.

Zafari D, Ershad D, Zare R, Alizadeh A. 2002. A contribution to the identification of Trichoderma in Iran. Iranian Journal of Plant Pathology 38: 21–45.

Zeringue HJ, Bhatnagar D, Cleveland TE. 1993. C15H24 Volatile compounds unique to aflatoxigenic strains of Aspergillus flavus. Applied and Environmental Microbiology 7: 2264–2270.

شناسایی ترکیبات آلی فرار *Trichoderma* spp. به روش کروماتوگرافی گازی - طیف سنجی جرمی با تکنیک Static Headspace

مائده شهیری طبرستانی [۱و۲]، کامران رهنما [۱] ✉ ، محسن جهانشاهی [۳]، سعید نصراله نژاد [۱] و محمد حسین فاطمی [۴]

۱-گروه گیاهپزشکی، دانشگاه کشاورزی و منابع طبیعی گرگان، گرگان، ایران

۲- گروه کشاورزی، دانشگاه پیام نور، تهران، ایران

۳- آزمایشگاه تحقیقاتی نانو بیوتکنولوژی، پژوهشکده فناوری نانو، دانشگاه صنعتی نوشیروانی بابل، بابل، ایران

۴- دانشکده شیمی، دانشگاه مازندران، بابلسر، ایران

چکیده: قارچ‌ها طیف وسیعی از ترکیبات آلی فرار آزاد می‌کنند که به چندین گروه شیمیایی با منشأهای بیوشیمیایی مختلف نظیر منوترپن‌ها، سسکوئی‌ترپن‌ها، الکل‌ها، آلدئیدها، ترکیبات آروماتیک، استرها، فوران‌ها، کتون‌ها و ترکیبات حاوی گوگرد و نیتروژن تعلق دارند. گونه‌های تریکودرما از عوامل بیوکنترل قارچی هستند که بیشتر مورد مطالعه قرار گرفتند و به‌طور موفقیت‌آمیزی به‌عنوان قارچ‌کش و تقویت‌کننده‌های بیولوژیکی در گلخانه و مزرعه استفاده می‌شوند. ترکیبات فرار در مایکوپارازیتیسم گونه‌های تریکودرما و تعاملشان با گیاهان و دیگر موجودات زنده محیط اطرافشان، نقش کلیدی دارند. باتوجه به فعالیت آنتی‌بیوتیکی این قارچ‌ها دربرابر بیمارگرهای قارچی، بررسی بیشتر ترکیبات آلی فرار این گونه‌ها پیشنهاد می گردد. در این تحقیق، ترکیبات آلی فرار گونه‌هایی از تریکودرمای بومی خاک مزارع (*T. harzianum*, *T. virens* (6011), *Trichoderma atroviridae* (1–3)) به روش کروماتوگرافی گازی- طیف‌سنجی جرمی با تکنیک Static Headspace شناسایی شدند. اکثر ترکیبات شناسایی شده، مربوط به گروه منوترپن‌ها و سسکوئی‌ترپن‌ها هستند که شامل dl–limonene، beta–himachalene، beta–cubebene، cadinene، caryophyllene، alpha–gurjunene، farnesol، thujopsene، beta–bisabolene و alpha–farnesene می‌باشند. باتوجه به اثرات ضدقارچی ترکیبات فوق، کنترل بیولوژیکی گونه‌های مورد مطالعه می‌تواند مربوط به حضور این ترکیبات باشد. ترکیبات اخیر می‌توانند، جهت اهداف کموتاکسونومی و قارچ‌کش‌های طبیعی به کار رفته و بدون بروز مشکلات زیست‌محیطی، محصولات گیاهی را دربرابر بیمارگرهای قارچی محافظت نمایند.

کلمات کلیدی: GC–MS، static headspace، *Trichoderma*، ترکیبات آلی فرار

مکاتبه کننده: کامران رهنما Email: kamranrahnama1995@gmail.com

تاریخ دریافت: ۱۳۹۵/۰۱/۲۳ تاریخ پذیرش: ۱۳۹۵/۰۳/۲۹