Appl Biol Chem (2018) 61(2):243–250
https://doi.org/10.1007/s13765-018-0352-x

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

Inhibitory effects of three monoterpenes from ginger essential oil on growth and aflatoxin production of *Aspergillus flavus* and their gene regulation in aflatoxin biosynthesis

Young-Sun Moon[1] · Hoi-Seon Lee[2] · Sung-Eun Lee[1]

Received: 22 December 2017 / Accepted: 18 February 2018 / Published online: 1 March 2018
© The Korean Society for Applied Biological Chemistry 2018

Abstract Ginger (*Zingiber officinale*) essential oil (ZOE) possesses strong antibacterial and antifungal activities. In this study, the antifungal activity of ZOE against *Aspergillus flavus* was investigated, and a chemical analysis was carried out to identify compounds that control fungal growth. A total of 37 compounds were identified by gas chromatographic analysis with a mass detector, and the antifungal and antiaflatoxigenic properties of three constituents, γ-terpinene, isoborneol, and citral, against *A. flavus* were tested. All compounds exhibited strong antifungal activity at 1000 μg/mL, and the antifungal activity of γ-terpinene and citral remained until treatment with tenfold diluted solution. The decrease in aflatoxin production by the three compounds was observed until treatment with 10 μg/mL. To evaluate their antiaflatoxigenic activity, RT-qPCR was used to compare the expression of 11 genes involved in aflatoxin biosynthesis by *A. flavus*. Among the three compounds, γ-terpinene and citral markedly reduced the expression of most of the tested genes but a different pattern of downregulation of the expression was observed. γ-Terpinene did not downregulate *aflR*, *aflS*, and *yap*, whereas citral did not alter the expression of *aflC* and *aflG*. Therefore, γ-terpinene and citral may have the potential to control *A. flavus* growth and aflatoxin production in agricultural products, including at the storage stage.

Keywords Aflatoxins · Antiaflatoxigenic activity · Antifungal · *Aspergillus flavus* · Citral · γ-Terpinene

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13765-018-0352-x) contains supplementary material, which is available to authorized users.

Young-Sun Moon, Hoi-Seon Lee and Sung-Eun Lee authors have contributed equally to this work.

✉ Sung-Eun Lee
selpest@knu.ac.kr

[1] School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Republic of Korea

[2] Department of Bioenvironmental Chemistry, Chonbuk National University, Jeonju 54896, Republic of Korea

Introduction

The phytochemicals in ginger (*Zingiber officinale*) can control viruses, including *Feline calicivirus*, and bacteria [1–3]. The major constituents of fresh ginger are phenol derivatives such as gingerols, which are converted into shogaols during heat treatment [4]. These natural compounds exhibit strong pharmacological activities, such as lowering of lipid and glucose content in the blood [5], antiplatelet activity by decreasing production of platelet thromboxane-B2 [6], and anti-inflammatory effects [7].

In a previous study, *Zingiber officinale* essential oil (ZOE) was extracted by steam distillation and its constituents were identified by gas chromatography with a mass detector (GC–MS) [8]. ZOE showed antibacterial activity against eight pathogenic bacteria (125–500 μg/mL) and antifungal activities against two fungal strains (250 μg/mL) [9]. They identified sabinene, (E)-1-(3′,4′-dimethoxyphenyl)buta-1,3-diene, terpinen-4-ol, γ-terpinene, and β-phellandrene as the major constituents of ZOE among the 49 compounds determined [9]. In addition, ZOE exhibited antibacterial activity against bacteria isolated from fish and shellfish [10]. ZOE also inhibited reproduction of the cattle tick *Rhipicephuls microplus* [11].

Aspergillus flavus produces cancer-stimulating compounds, such as aflatoxins. Birds are particularly susceptible to these natural compounds [12, 13]. Selenium and zinc can neutralize aflatoxin toxicity in poultry through dietary supplementation [13]. Interestingly, many essential oils have been used as reducing agents against aflatoxin contamination by *A. flavus* or *A. parasiticus* in agricultural products [14, 15]. The direct inhibition of aflatoxin biosynthesis in *A. flavus* or indirect inhibition pathways that reduce the respiration ability in aflatoxin-producing fungi are responsible for the antiaflatoxigenic properties [16].

Recently, we identified natural compounds containing a methylenedioxy moiety that exhibit antifungal and anti-aflatoxigenic activities against *A. flavus* by direct inhibition of aflatoxin production through the suppression of certain major genes involved in the biosynthesis [17, 18]. 4-Hydroxy-7-methyl-3-phenylcoumarin also possessed similar inhibitory properties against *A. flavus* by strongly suppressing *aflK*-expressing versicolorin B synthase [19].

In the present study, the antifungal activity of ZOE against *A. flavus* was investigated, and a chemical analysis was performed using GC–MS. The antifungal and anti-aflatoxigenic activities of the three compounds, which are easily available in the market, against *A. flavus* were evaluated. Finally, the modes of antiaflatoxin action of the three compounds were evaluated and confirmed by RT-qPCR by comparing the gene expression after treatment.

Materials and methods

Chemicals and microorganisms

ZOE was purchased from Lhasa Karnak Herb Company (Berkeley, CA, USA). Tested γ-terpinene, isoborneol, and citral were also purchased from Sigma-Aldrich Co. (St. Louise, MO, USA). *Aspergillus flavus* ATCC 22546 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA).

Preparation of spore solution

For the preparation of the isolate, *A. flavus* was grown on malt extract agar (MEA: Difco Laboratories, Sparks, MD, USA) and this isolate generated aflatoxin B1 and B2, but not G1 and G2 [19]. It was grown on MEA medium at 30 °C for 5 days until fungal spores formed. After spore formation, they were collected from slants by shaking with 0.05% (v/v) of Tween 80 and stored at − 70 °C in 20% glycerol solution (v/v).

Chemical analysis of ZEO using GC–MS

Chemical analysis for ZOE was undertaken using a HP-6890 (Agilent Technologies, Wilmington, DE, USA) gas chromatograph equipped with a DB-5 fused silica capillary column (60 m × 0.25 mm, 0.25 µm film thickness) interfaced with a 59,731 V (Agilent) mass detector. The GC–MS was connected to a computer equipped with Wiley 7 spectra library. The condition of GC–MS was column temperature ranged from 60 to 250 °C with an elevation of 5 °C. Injector temperature was set at 250 °C, and injection volume was 1.0 µl with a split ratio of 1:50. The determined mass range was 50–600 amu. Compounds were identified by comparing their mass spectra with those in Wiley 7 mass spectral database for GC–MS (Table S1).

Aflatoxin analysis using HPLC-fluorescence detector

Fungal spore suspension adjusted to 10^6 density was inoculated into the liquid culture media comprising potato dextrose broth (25 mL) (Difco Laboratories). All the test compounds were spiked into the corresponding liquid media serially, and the culture was incubated at 25 °C for 5 days under shaking conditions. All experiments were performed in triplicate for each concentration of the tested compounds.

Following cultivation in liquid medium for 5 days, the fungal growth was measured using a filter paper to weigh the mycelial and sclerotial residues with overnight drying in an oven. Separately, the mycelia from each treatment were subjected to the extraction procedure using an ultrasonic cleaner, and aflatoxin B (AFB) and G were analyzed using an HPLC-fluorescence detector [19]. The average of the three replicates was calculated for each experiment. One-way ANOVA was used for comparisons between two groups, and statistical significance was set at $p < 0.05$ [20].

Real-time qPCR after isolation of total RNA

To understand the inhibition of fungal growth and aflatoxin production, real-time qPCR was employed. Fungal mycelia in liquid media were carefully collected and total RNA was extracted using the QIAzol Lysis reagent, supplied by QIAGEN Inc. (Dusseldorf, Germany), after grinding to a fine powder under addition of liquid nitrogen. Total RNA extracted from the treated fungi were quantified with µDrop™ Plate (Thermo Fisher Scientific Inc., Waltham, MA, USA), and the extracted RNAs were quantified using 1% of agarose gel with ethidium bromide. Complementary DNA (cDNA) for extracted RNAs (2 µg) was synthesized using Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA).

RT-qPCR was performed using Rotor-Gene SYBR Green PCR Kit (QIAGEN Inc.) with an appropriate amount of cDNA (100 ng). Primers for genes, such as *yap, aflR, aflS, aflK, aflD, aflQ,* and *18S rRNA*, were synthesized by Genotech (Daejeon, Korea) (Table S2) and used to understand the relationship between aflatoxin biosynthesis and the active compound [18]. Forty cycles of thermal cycling were performed for amplification (denaturation at 95 °C for 30 s, annealing at 60 °C for 20 s, and elongation at 72 °C for 30 s, followed by an additional step at 95 °C for 5 min). RT-qPCR was performed in triplicate for each treatment. Significant differences in gene expression were calculated using delta/delta Ct methods [21]. Data were standardized with *18S rRNA*, and the gene expression between the treatment group and controls was compared.

Results

The components of ZEO were identified by GC–MS analysis (Table S1). After the analysis, γ-terpinene, isoborneol, and citral were chosen to investigate their inhibitory effects on the growth of *A. flavus* and aflatoxin production with the assessment of their molecular modes of inhibitory action.

The three major components of ZEO showed a strong inhibitory effect against *A. flavus* growth at 1000 µg/mL. This inhibition by isoborneol decreased approximately twofold when the fungi were treated with 250 µg/mL of ZEO and disappeared at 100 µg/mL. γ-Terpinene exhibited 50% inhibitory effect on the fungal growth at 100 µg/mL, but this effect was not observed at 10 µg/mL. Citral exhibited a similar inhibitory pattern as the other two monoterpenes (Table 1).

Aflatoxin production after treatment with the three compounds was determined (Table 2), and the three compounds completely inhibited aflatoxin biosynthesis at 100 µg/mL. At 10 µg/mL, γ-terpinene and citral showed some antiaflatoxigenic activities.

To understand the inhibitory effect of γ-terpinene on aflatoxin production, the expression of 11 genes, which are involved in aflatoxin biosynthesis, was analyzed by RT-qPCR (Fig. 1). The expression of *aflC*, *aflD*, *aflE*, *aflK*, *aflO*, and *aflQ* genes was inhibited by approximately 80% compared with that of the control at 100 µg/mL. However, *aflR*, *aflS*, and y*ap* were not affected by the treatment (Fig. 1). At tenfold dilution, γ-terpinene downregulated only five genes by approximately 50% and upregulated *aflG* gene approximately threefold (Fig. 1).

Citral downregulated the expression of most of the tested genes, except *aflC* and *aflG*, at 100 µg/mL (Fig. 2). However, the inhibition was higher at 10 µg/mL than at 100 µg/mL. In addition, *aflR* was the only gene to be more strongly inhibited by citral at 100 µg/mL.

Discussion

In a previous study, ZEO exhibited antifungal activity against *A. flavus* and antiaflatoxigenic activity [22]; the mycelial growth of *A. flavus* was completely inhibited at 150 µg/mL. The major components in ZEO are monoterpenes such as β-phellandrene (0.95%), camphene (0.61%), and β-pinene (0.61%) as well as sesquiterpenes such as zingiberene (29.54%), β-sesquiphellandrene (18.42%), and germacrene D (3.58%) (Onyenekwe and Hashimoto, 1999). However, higher amounts of monoterpenes such as camphene (3.0%), β-phellanfrene (1.4%), 1,8-cineole (1.9%), borneol (2.1%), neral (7.4%), and geranial (25.9%) and lower amounts of sesquiterpenes such as zingiberene (9.5%), β-sesquiphellandrene (5.1%), and germacrene B (0.3%) in ZEO have also been reported (Singh et al., 2008).

Table 1 Effects of monoterpenes present in ginger essential oil on *Aspergillus flavus* growth

Compound	Concentration (µg/mL)	Dry weight (mg)	Growth rate (%)
Isoborneol	1000[1]	0[a]	0
	500[2]	7.3 ± 1.4[b]	11.9 ± 2.2
	250[2]	32.3 ± 28.6[c]	45.7 ± 40.5
	100[3]	58.5 ± 10.9[d]	93.2 ± 17.4
γ-Terpinene	1000[4]	16.7 ± 18.9[a]	21.3 ± 24.1
	100[2]	32.1 ± 22.0[b]	52.2 ± 35.7
	10[2]	76.0 ± 5.1[c]	123.5 ± 8.4
Citral	1000[4]	0[a]	0
	100[2]	43.9 ± 38.0[b]	71.3 ± 61.8
	10[2]	63.7 ± 11.9[b]	103.5 ± 19.3

*Different letters indicate statistically different from the control ($p < 0.05$). These values are used for the control when compared to each experiment

Concentrations in control after tests: [1]74.0 ± 4.7; [2]61.5 ± 5.9; [3]62.8 ± 15.1; [4]78.4 ± 10.0

Table 2 Effects of monoterpenes derived from ginger essential oil on aflatoxin production

Compound	Concentration (μg/mL)	Aflatoxin production rate (%) compared to the control			
		AFB1	AFB2	AFG1	AFG2
Isoborneol	500	ND	ND	ND	ND
	250	ND	ND	ND	ND
	100	ND	ND	ND	ND
γ-Terpinene	100	ND[a]	ND[a]	ND	ND
	10	53.0 ± 3.9[b]	39.0 ± 3.3[b]	ND	ND
Citral	100	ND[a]	ND	ND	ND
	10	13.0 ± 1.7[b]	ND	ND	ND

N.D. Not detected

*Different letters indicate statistically different from the control ($p < 0.05$). These values are used for the control when compared to each experiment

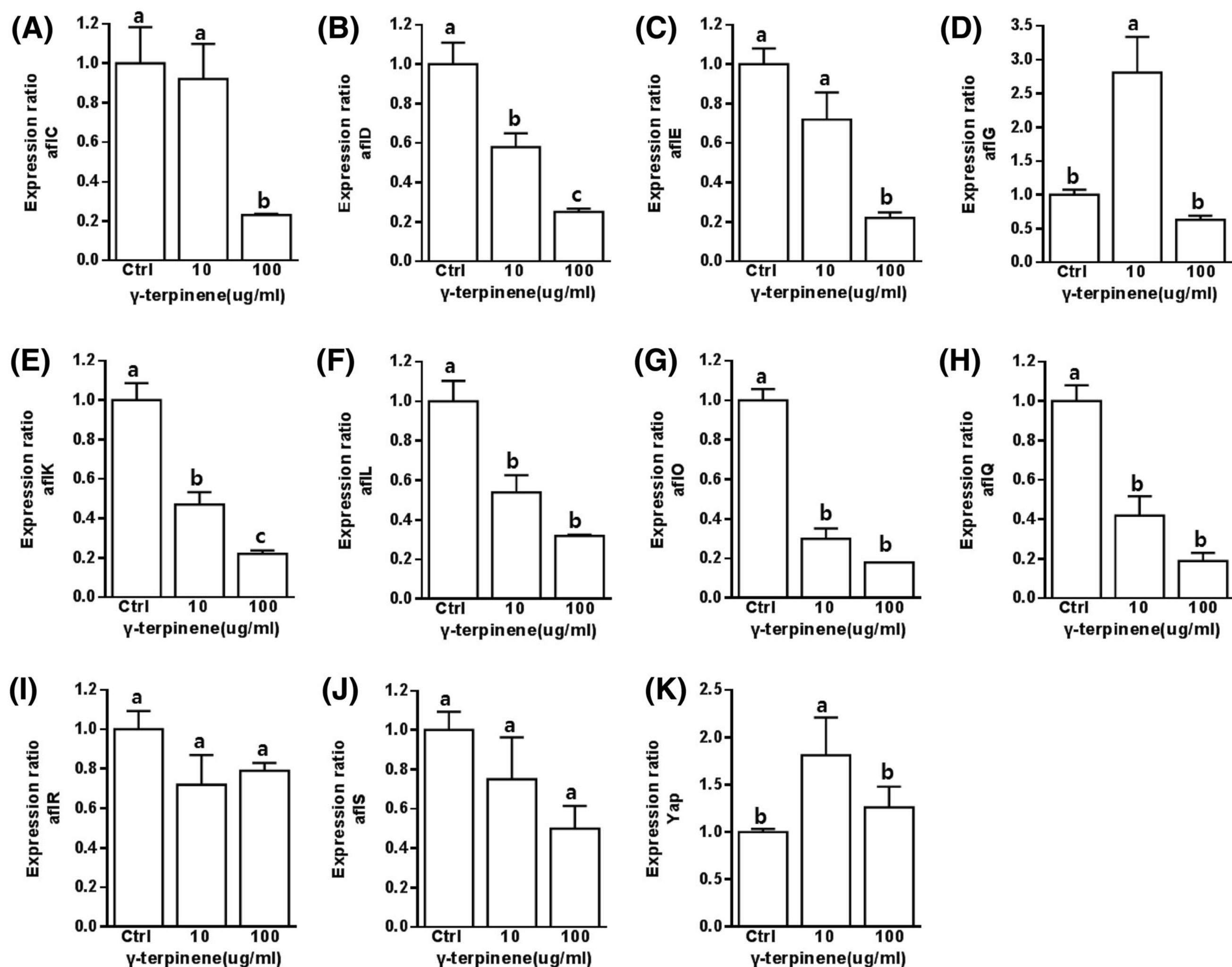

Fig. 1 Expression of genes involved in aflatoxin biosynthesis in *Aspergillus flavus* after the treatment of γ-terpinene. (**A**), *aflC*; (**B**), *aflD*; (**C**), *aflE*; (**D**), *aflG*; (**E**), *aflK*; (**F**), *aflL*; (**G**), *aflO*; (**H**), *aflQ*; (**I**), *aflR*; (**J**), *aflS*; (**K**), *yap*. *18S rRNA* was used for the standardization of gene expression. Different letters indicate significantly different from the control group ($p < 0.05$)

In our study, γ-terpinene (1.9%), campene (17.2%), shisool (19.86%), citral (3.41%), γ-curcumene (3.03%), and ar-curcumene (6.16%) were the main volatile components extracted from ZEO (Table S1). ZEO contained 0.22%

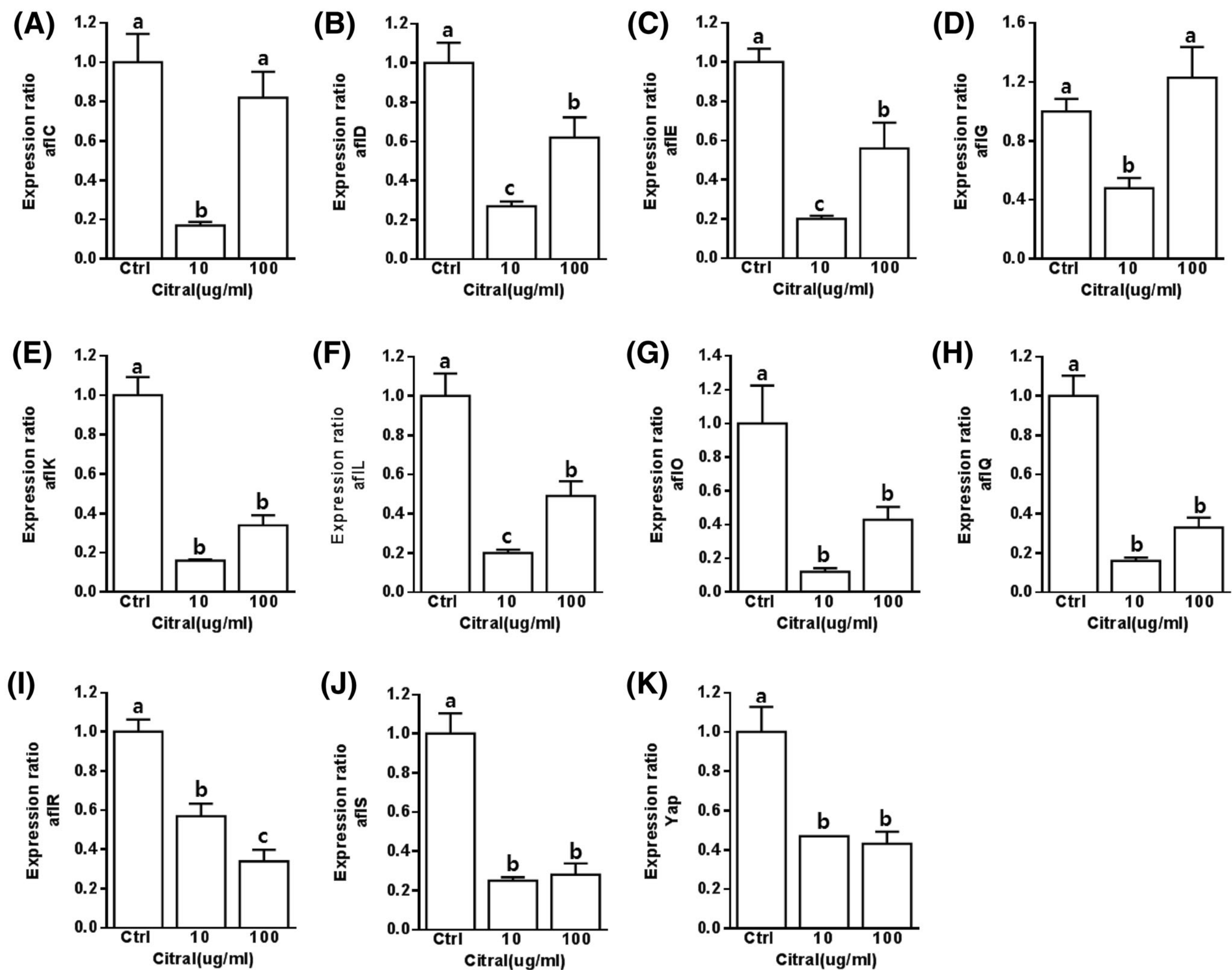

Fig. 2 Expression of genes involved in aflatoxin biosynthesis in *Aspergillus flavus* after the treatment of citral. (**A**), *aflC*; (**B**), *aflD*; (**C**), *aflE*; (**D**), *aflG*; (**E**), *aflK*; (**F**), *aflL*; (**G**), *aflO*; (**H**), *aflQ*; (**I**), *aflR*; (**J**), *aflS*; (**K**), *yap*. *18S rRNA* was used for the standardization of gene expression. Different letters indicate significantly different from the control group ($p < 0.05$)

zingiberene, and its proportion was much lower than that reported earlier [23, 24].

Compositional difference in essential oils extracted from the same plant sources may be due to the extraction methods for essential oils [25]. They used two different extraction methods, hydrodistillation and supercritical fluid extraction (SCFE), to obtain essential oils from cumin. Then, the authors analyzed these two essential oils by GC–MS [25]. Cumin essential oil obtained by hydrodistillation had a higher proportion of cuminaldehyde (52.6%) than that in the essential oil (37.3%) obtained by SCFE extraction method. In the case of cuminic alcohol, the essential oil obtained by SCFE contained higher portions than that by hydrodistillation [25]. In addition, the essential oil composition may differ with weather condition during cultivation, e.g., guava cultivated during the dry season primarily comprises nerolidol [26]. Finally, one of primary parameters for the difference in composition may be related to the plant chemo-type [27]. Therefore, essential oils, including ZEO, need to be standardized before use in the agricultural or other industries [28, 29].

The antifungal activity and antiaflatoxigenic activities of three monoterpenes, γ-terpinene, isoborneol, and citral, against *A. flavus* were evaluated in this study. γ-Terpinene is a major component of several essential oils that are known to inhibit *Aspergillus* spp. growth and aflatoxin production [22, 30, 31]. However, its antifungal and antiaflatoxigenic activities against *A. flavus* have not been evaluated yet. Isoborneol has not been reported to exhibit antifungal and antiaflatoxigenic activities against *A. flavus*. In our study, γ-terpinene and isoborneol strongly inhibited *A. flavus* growth at 1000 μg/mL and aflatoxin production at 100 μg/mL. However, their mode of action in terms of the downregulation pattern of aflatoxin biosynthesis genes was

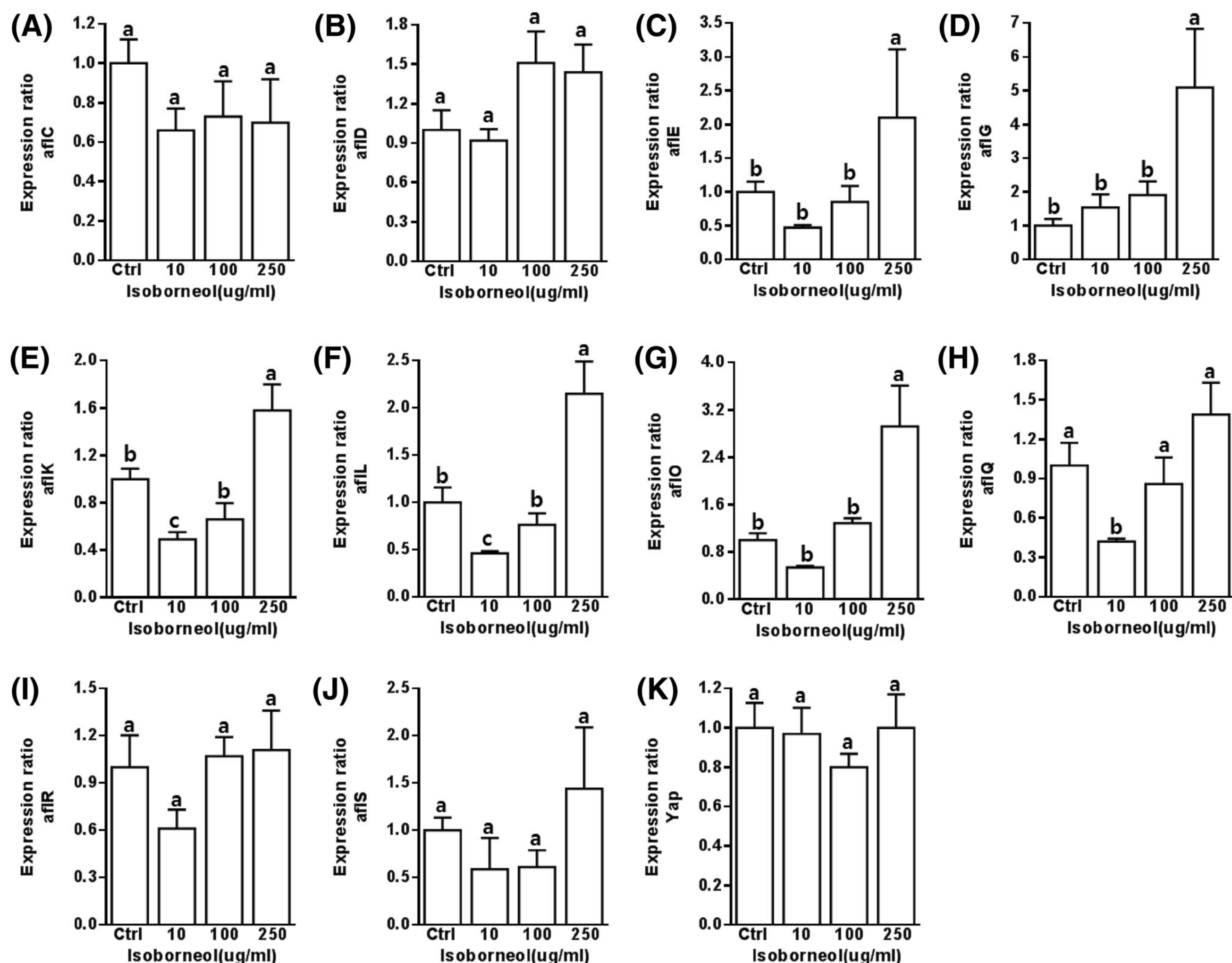

Fig. 3 Expression of genes involved in aflatoxin biosynthesis in *Aspergillus flavus* after the treatment of isoborneol. (**A**), *aflC*; (**B**), *aflD*; (**C**), *aflE*; (**D**), *aflG*; (**E**), *aflK*; (**F**), *aflL*; (**G**), *aflO*; (**H**), *aflQ*; (**I**), *aflR*; (**J**), *aflS*; (**K**), *yap*. *18S rRNA* was used for the standardization of gene expression. Different letters indicate significantly different from the control group ($p < 0.05$)

different. γ-Terpinene downregulated 7 genes at 100 μg/mL, while isoborneol did not downregulate genes at the same concentration. γ-Terpinene showed a similar pattern of gene downregulation as eugenol and cinnamaldehyde, which inhibited aflatoxin production by modifying the major genes for aflatoxin biosynthesis in *A. flavus* [32]. Another aflatoxin-producing fungi, *A. parasiticus*, has been used to study the inhibitory effect of eugenol on its growth and aflatoxin production [33]. Eugenol downregulated aflatoxin biosynthetic genes *ver-1*, *nor-1*, *pks A*, *omt A*, and *aflR* at 62.5–125 μg/mL [33]. *ver-1* is equivalent to *aflM* in this study, *nor-1* to *aflD*, *pksA* to *aflC*, and *omtA* to *aflP* [34]. In our study, the expression of *aflC* was only 20% in the presence of 100 μg/mL of γ-terpinene. *aflD* was downregulated approximately twofold and fivefold in the presence of 10 and 100 μg/mL of γ-terpinene, respectively. However, the expression of *aflR* was not affected. Therefore, the inhibition of major genes involved in the biosynthesis of aflatoxins in *A. flavus* contributes toward the inhibitory action of γ-terpinene.

In the case of isoborneol, *aflK*, *aflL*, and *aflQ* were downregulated approximately twofold at 10 μg/mL. It is likely that isoborneol possesses a different mode of inhibitory action on aflatoxin production, suggested by the penetration of H_2O_2 generated after treatment with isoborneol [35].

In a previous study, citral showed antifungal and antiaflatoxigenic activities against *A. flavus* growth and aflatoxin biosynthesis, respectively [32]. They demonstrated that at 2.80 mmol/L, citral completely inhibited *A. flavus* growth and aflatoxin production [32]. It also downregulated *aflT* completely and inhibited the expression of *aflM*, *aflP*, *aflL*, *aflR*, and *aflD* 257-, 29-, 3.5-, and 2.5-fold compared with the control group. In our study, citral treatment downregulated *aflC*, *aflD*, *aflE*, *aflG*, *aflK*, *aflL*, *aflO*, *aflQ*, *aflR*, *aflS*, and *Yap* in *A. flavus*. All the tested

genes were affected by the addition of 10 μg/mL citral (Fig. 3). We did not use *aflT* in this study. However, our results were similar to those reported [32] that *aflL*, *aflR*, and *aflD* were downregulated following treatment with citral. This indicates that citral inhibits aflatoxin production in *A. flavus* by interfering with the expression of aflatoxin biosynthesis genes. In addition to the downregulation of the expression of aflatoxin-producing genes, another inhibitory mechanism has been suggested—the secretion of H_2O_2 after treatment with citral [35]. With these monoterpenoids, other major constituents such as turmerone from turmeric essential oil may also need to determine their antifungal and antiaflatoxigenic activities to *A. flavus* to develop ecofriendly used fungicides in agricultural fields [36].

Conclusively, three natural compounds (isoborneol, γ-terpinene, and citral) in ZOE showed antifungal and anti-aflatoxigenic activities against *A. flavus*. Aflatoxin production by *A. flauvs* was strongly inhibited at 100 μg/mL of γ-terpinene and citral. These two compounds downregulated the primary genes involved in aflatoxin biosynthesis in *A. flavus* at 10 μg/mL. However, their inhibitory action on gene expression was different, and γ-terpinene suppressed *aflC*, *aflD*, *aflE*, *aflK*, *aflO*, and *aflQ* genes, which express polyketide synthase, reductase, NOR-reductase, VERB synthase, O-methyltransferase B, and oxidoreductase, respectively. Citral downregulated nine genes among the tested genes, except *aflC* and *aflG*, which express polyketide synthase and P450 monooxygenase, respectively. Isoborneol did not suppress any of the tested genes. The different inhibitory patterns of gene expression in *A. flavus* warrant further studies.

Acknowledgments This research was supported by Kyungpook National University Bokhyeon Research Fund (2015).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Hasan S, Danishuddin M, Khan AU (2015) Inhibitory effect of *Zinziber officinale* towards Streptococcus mutans virulence and caries development: in vitro and in vivo studies. BMC Microbiol 15:1
2. Aboubakr HA, Nauertz A, Luong NT, Agrawal S, El-Sohaimy SA, Youssef MM, Goyal SM (2016) In vitro antiviral activity of clove and ginger aqueous extracts against feline calicivirus, a surrogate for human norovirus. J Food Prot 79:1001–1012
3. Chitra K, Manikandan A, Antony SA (2016) Effects of poloxamer of *Zingiber officinale* extracted green synthesis and antibacterial studies of silver nanoparticle. J Nanosci Nanotechnol 16:758–764
4. Wohlmuth H, Leach DN, Smith MK, Myers SP (2005) Gingerol content of diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe). J Agric Food Chem 53:5772–5778
5. Kadnur SV, Goyal RK (2005) Beneficial effects of *Zingiber officinale* Roscoe on fructose induced hyperlipidemia and hyperinsulinemia in rats. Indian J Exp Biol 43:1161–1164
6. Thomson M, Al-Qattan KK, Al-Sawan SM, Alnaqeeb MA, Khan I, Ali M (2002) The use of ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent. Prostaglandins Leukot Essent Fatty Acids 67:475–478
7. Grzanna R, Lindmark L, Frondoza CG (2005) Ginger–a herbal medicinal product with broad anti-inflammatory actions. J Med Food 8:125–132
8. Gupta S, Pandotra P, Ram G, Anad R, Gupta AP, Husain K, Bedi YS, Mallavarapu GR (2011) Composition of a monoterpenoid-rich essential oil from the rhizome of *Zingiber officinale* from north western Himalayas. Nat Prod Commun 6:93–96
9. Verma RS, Joshi N, Padalia RC, Singh VR, Goswami P, Verma SK, Iqbal H, Chanda D, Verma RK, Darokar MP, Chauhan A, Kandwal MK (2017) Chemical composition and antibacterial, antifungal, allelopathic and acetylcholinesterase inhibitory activities of cassumunar-ginger. J Sci Food Agric 98:321–327
10. Snuossi M, Trabelsi N, Ben Taleb S, Dehmeni A, Flamini G, De Feo V (2016) *Laurus nobilis*, *Zingiber officinale* and *Anethum graveolens* essential oils: composition, antioxidant and antibacterial activities against bacteria isolated from fish and shellfish. Molecules 21:1414
11. Pazinato R, Volpato A, Baldissera MD, Santos RC, Baretta D, Vaucher RA, Giongo JL, Boligon AA, Stefani LM (2016) *In vitro* effect of seven essential oils on the reproduction of the cattle tick *Rhipicephalus microplus*. J Adv Res 7:1029–1034
12. Kumar P, Mahato DK, Kamle M, Mohanta TK, Kang SG (2017) Aflatoxins: a global concern for food safety, human health and their management. Front Microbiol 7:2170
13. Mughal MJ, Peng X, Kamboh AA, Zhou Y, Fang J (2017) Aflatoxin B1 induced systemic toxicity in poultry and rescue effects of selenium and zinc. Biol Trace Elem Res 178:292–300
14. Hu Y, Zhang J, Kong W, Zhao G, Yang M (2017) Mechanisms of antifungal and anti-aflatoxigenic properties of essential oil derived from turmeric (*Curcuma longa* L.) on *Aspergillus flavus*. Food Chem 220:1–8
15. Kedia A, Dwivedy AK, Pandey AK, Kumar RR, Regmi P, Dubey NK (2015) Efficacy of chemically characterized *Foeniculum vulgare* Mill seed essential oil in protection of raw tobacco leaves during storage against fungal and aflatoxin contamination. J Appl Microbiol 119:991–998
16. Sakuda S, Yoshinari T, Furukawa T, Jermnak U, Takagi K, Limura K, Yamamoto T, Suzuki M, Nagasawa H (2015) Search for aflatoxin and trichothecene production inhibitors and analysis of their modes of action. Biosci Biotechnol Biochem 80:43–54
17. Moon YS, Choi WS, Park ES, Bae IK, Choi SD, Paek O, Kim SH, Chun HS, Lee SE (2016) Antifungal and antiaflatoxigenic methylenedioxy-containing compounds and piperine-like synthetic compounds. Toxins 8:E240
18. Choi H, Lee BH, Moon YS, Kim K, Lee HS, Lee SE (2017) Antifungal and antiaflatoxigenic effects of a fumigant, ethanedinitrile, on *Aspergillus flavus*. Appl Biol Chem 60:631–636
19. Moon YS, Kim L, Chun HS, Lee SE (2017) 4-hydroxy-7-methyl-3-phenylcoumarin suppresses aflatoxin biosynthesis via downregulation of alfK expressing versicolorin B synthase in *Aspergillus flavus*. Molecules 22:E712
20. SAS (2001) SAS User's Guide, 4th edn. SAS Institute, Cary, NC
21. Rao X, Huang X, Zhou Z, Lin X (2013) An improvement of the $\hat{2}$(delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostat Bioinform Biomath 3:71–85

22. Nerilo SB, Rocha GHO, Tomoike C, Mossini SA, Grespan R, Mikcha JMG, Machinski M Jr (2016) Antifungal properties and inhibitory effects upon aflatoxin production by *Zingiber officinale* essential oil in *Aspergillus flavus*. Int J Food Sci Technol 51:286–292
23. Onyenekwe PC, Hashimoto S (1999) The composition of the essential oil of dried Nigerian ginger (*Zingiber officinale* Roscoe). Eur Food Res Technol 209:407–410
24. Singh G, Kapoor IPS, Singh P, de Heluani CS, de Lampasona MP, Catalan CAN (2008) Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. Food Chem Toxicol 46:3295–3302
25. Saha S, Walia S, Kundu A, Sharma K, Singh J, Tripathi B, Raina A (2016) Compositional and functional difference in cumin (*Cuminum cyminum*) essential oil extracted by hydrodistillation and SCFE. Cogent Food Agric 2:1143166
26. Shaabana HAE, El-Ghoraba AH, Shibamoto T (2012) Bioactivity of essential oils and their volatile aroma components: review. J Essent Oil Res 24:203–212
27. Tucker AO, Maciarello MJ, Landrum LR (1995) Volatile leaf oils of American Myrtaceae III. *Psidium cattleianum* Sabine, *P. friedrichsthalianum* (Berg) Niedenzu, *P. guajava* L., *Psidium guineense* Sw., and *Psidium sartorianum* (Berg) Niedenzu. J Essent Oil Res 7:187–190
28. Rantzsch U, Vacca G, Duck R, Gillissen A (2009) Anti-inflammatory effects of Myrtol standardized and other essential oils on alveolar macrophages from patients with chronic obstructive pulmonary diseases. Eur J Med Res 14(Suppl 4):205–209
29. Papamaroupa M, Gillissen A (2016) Is Myrtol® standardized a new alternative toward antibiotics? Pharmacogn Rev 10:143–146
30. Tian J, Ban X, Zeng H, Huang B, Wang Y (2011) Chemical composition and antifungal activity of essential oil from *Cicuta virosa* L. var. *latisecta* Celak. Int J Food Microbiol 145:464–470
31. Kohiyama CY, Ribeiro MMY, Mossini SAG, da Silva Bomfim N, Nerilo SB, Rocha GHO, Grespan R, Mikcha JMG, Macinski M Jr (2015) Antifungal properties and inhibitory effects upon aflatoxin production of *Thymus vulgaris* L by *Aspergillus flavus* Link. Food Chem 173:1006–1010
32. Liang D, Xing F, Selvaraj JN, Liu X, Hua H, Zhou L, Zhao Y, Wang Y, Liu Y (2015) Inhibitory effect of cinnamaldehyde, citral, and eugenol on aflatoxin biosynthetic gene expression and aflatoxin B1 biosynthesis in *Aspergillus flavus*. J Food Sci 80:M2917–M2924
33. Janhanshiri Z, Shams-Ghahfarokhi M, Allameh A, Razzaghi-Abyaneh M (2015) Inhibitory effect of eugenol on aflatoxin B1 production in *Aspergillus parasiticus* by downregulating the expression of major genes in the toxin biosynthetic pathway. World J Microbiol Biotechnol 31:1071–1078
34. Yu J, Chang PK, Ehrlich KC, Cary JW, Bhatnagar D, Cleveland TE, Payne GA, Linz JE, Woloshuk CP, Bennett JW (2004) Clustered pathway genes in aflatoxin biosynthesis. Appl Environ Microbiol 70:1253–1262
35. Li J, Li J, Lu Z, Liu Y, Li CM (2015) Transient transmembrane secretion of H_2O_2: a mechanism for the citral-caused inhibition of aflatoxin production from *Aspergillus flavus*. Chem Commun 51:17424–17427
36. Hwang KW, Son D, Jo HW, Kim CH, Seong KC, Moon JK (2016) Levels of curcuminoid and essential oil compositions in tumerics (*Curcuma longa* L) grown in Korea. Appl Biol Chem 59:209–215