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Original Article

Plant Seedling Growth Stimulation and Antifungal Activities of Volatile Organic Compounds Emitted by *Aspergillus flavus* Endophyte

Fatma Fakhry. Abdel-Motaal [1*], Noha M. Kamel [1], Soad A. El-Zayat [1], Abou El-Hamd H. Mohamed [2], Doaa B. Darwish [3]

[1] Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt
[2] Chemistry Department, Faculty of Science, Aswan University, Aswan 81528, Egypt
[3] Botany Department, Faculty of Science, Mansoura University, Mansoura 35511, Egypt

Corresponding Author: Fatma Fakhry. Abdel-Motaal, PhD, Associate Professor, Botany Department, Faculty of Science, Aswan University, Aswan 81528, Egypt. Tel: +20-1023523399, E-mail: fatma.fakhry@aswu.edu.eg

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Abstract

Introduction: Volatile Organic Compounds (VOCs) produced by Plant Growth Promoting fungi (PGPF) have recently been investigated due to their role in plant growth promotion and defense. Whereas many VOCs produced by PGPF promote seed growth. It is known that VOCs, among several other mechanisms, are responsible for the antagonistic activity produced by microorganisms. In this study, we focused on a comparative study between the VOCs emitted by the endophyte *A. flavus* AFEG-2017 and its host plant (*Eleocharis geniculata)* and the role of these VOCs in the growth promotion and biological control of some economic plants.

Materials and Methods: VOCs from AFEG-2017 and *E. geniculata* were extracted using ethyl acetate. Then, the analysis of emitted VOCs was accomplished by using gas chromatography mass (GC-MS). Seedling stimulation assay was investigated on seeds of *Trigonella foenum-graecum*, *Solanum lycopersicum*, *Portulaca oleracea*, and *Lepidium sativum*. In addition, the antifungal activity of VOCs was evaluated against some plant pathogenic fungi.

Results: The GC-MS analysis of the volatile emitted from A. flavus (AFEG-2017) resulted in 25 organic and bioactive compounds; of them 2-(2-hydroxy-3-isobutoxypropyl) pent-4-enoic acid, hydrazide was the most abundant compound. The findings of the present study advocate that linalool, linalyl acetate, geranyl acetate, oleic acid, 1-eicosanol, and 1-chloro-octadecane are suitable as biocontrol agents against phytopathogenic fungi. In addition, the volatiles of *Euphorbia geniculata* showed 28 bioactive compounds, in which Phytol was the most abundant one. The VOCs produced by AFEG-2017 enhanced the seedling growth of *T. foenum-graecum*, *S. lycopersicum*, *P. oleracea,* and *L. sativum*. Also, VOCS showed inhibition in the tested pathogenic fungi growth like *Fusarium oxysporum* which showed the highest inhibition percentage in the growth (40%).

Conclusions: This study proved that there is a harmony between VOCs produced by the medicinal plant *E. geniculata* and its endophyte *A. flavus*. These volatiles could successfully accelerate plant seeding and limit the growth of some important phytopathogens.

Keywords: *Aspergillus flavus*, Volatile Organic Compounds, Growth Promotion, Antifungal Activity

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Introduction

The medicinal plant *Euphorbia* has traditionally been used to treat skin diseases, gonorrhoea, and intestinal parasites in traditional healthcare systems. Phytochemical investigation of different parts of *Euphorbia* plants indicated that they are rich in highly bioactive isoprenoid constituents. Diterpenoids, such as jatrophanes and myrsinols, are the main constituents of this genus.[1,2]

Endophytic microorganisms that are not harmful to the host plants but help their host in many ways, are highly diverse and metabolically very sound. Clear twining between *Eleocharis geniculata* and its associated endophytes secondary metabolites was proved. There is an interesting link in the production of terpenes, tannins, steroids, flavenoid and alkaloids between this medicinal plant and the endophyte *Aspergillus flavus*.[3] The genus *Aspergillus* is one of the most prevalent members of the *A. flavus*-oryzae group of moulds. This mould has been discovered to grow on a variety of agricultural goods at a variety of temperatures and relative humidity levels. *Aspergillus flavus* exhibits a strong lipolytic activity and, under certain conditions, also amylolytic and proteolytic activities. Also, it can produce active VOCs like 3-methylbutanol and 3-octanone.[4]

VOCs are low molecular weight compounds that can vaporize and enter the gas phase at normal atmospheric pressure and temperatures. VOCs have a low to medium solubility in water and frequently have a unique odor. Growing fungi are shown to produce VOCs as a mixture of compounds of many molecular sizes in several studies using modern analytical

equipment in which numbers, types, and amounts of individual VOCs are variable. Chemically, this gas phase mixture may contain acids, aromatics, aldehydes, alcohols, heterocycles, esters, terpenes, ketones etc.[5]

In the last decade, VOCs emitted by microorganisms have been described for their ability to induce growth.[6] Because there has been so little research on endophytic fungi and their volatile organic metabolites, there is a good chance of discovering unknown numbers of novel fungal genera existing as plant-associated microbes as well as various novel VOCs with significant bioactivity. Plant pathogens are increasingly being controlled with endophytic fungal VOCs, which have been found to induce positive changes in plant growth,[7] since, the disease management strategy for plants by using chemical fungicides has resulted in adverse effects to human health and environment.[8] In addition toimproving host survival in desert habitats and thus assisting the host in competition with other plants, it also helps repel or attract insects.[5,9]

Important plants like, *Trigonella foenum-graecum, Solanum lycopersicum, Portulaca oleracea, and Lepidium sativum* which are used in medicine, nutrition, antioxidants, minerals and are sources of vitamins[10,11] need biological and safe means to stimulate their growth and protect these plant from fungal infection.

Thus, in this study, we focused on a comparative study between the VOCs emitted by the endophyte *A. flavus* AFEG-2017 with that of the host plant (*E. geniculata*). Also, we aimed to find the role of *A. flavus* AFEG-2017 VOCs on growth promotion and biological control of the economic plants, *T. foenum-graecum, S. lycopersicum, P. oleracea,* and *L. sativum.*

Materials and Methods

Fungal Strain and Plant Materials

AFEG-2017 (accession number LC480514) previously isolated as endophyte from *E. geniculata*[3] was inoculated in potato dextrose agar media and incubated at 28 ± 2 °C for five days before use. *Euphorbia geniculata* was collected from Aswan-Daraw road, Aswan, Egypt.

Extraction of VOCs from AFEG-2017 and E. geniculata

AFEG-2017 was grown in 200 ml PD Broth (PDB) media in 500 ml conical flasks under stationary condition at 28 °C. After two weeks of incubation, Ethyl Acetate (EtOAc) was added to the fungal culture and mixed with a homogenizer for 2 min. The organic layer was separated using a separating funnel. *Euphorbia geniculata* was dried, grinding well, extracted with EtOAc and both extracts (fungi and plant) were concentrated under pressure at 40 °C in 130 rpm rotary evaporator.

GC-MS Analysis of VOCs Released by AFEG-2017 and E. geniculata

Analysis of VOCs emitted by AFEG-2017 and *E. geniuclata* were accomplished by using gas chromatography-mass (GC-MS. A trace Ultra GC (Thermo Scientific Corp., USA), accompanying a thermo-mass spectrometer detector (ISQ single Quadrupole Mass spectrometer). One microliter of diluted samples (1:10 hexane, v/v) was injected. Mass spectra were obtained by Electron Ionization (EI) at 70 eV, using a spectral range of *m/z* 40-450. The identification of the chemical constituents of the VOCs was de-convoluted using AMDIS software (www.amdis.net) and identified by its retention indices (relative to *n*-alkanes C_8-C_{40}) using Wiley spectral library collection and NSIT library database. Then, the VOCs of AFEG-2017 were compared with the VOCs of *E. geniculata.*

Seedling Stimulation Assay

Seeds of *T. foenum-graecum, S. lycopersicum, P. oleracea,* and *L. sativum* were surface-sterilized by sodium hypochlorite (2 min) followed by ethanol 70% (1 min) then washed by sterilized distilled water and dried well. The sterilized seeds were placed in 12 cm Petri dishes with filter paper opposite to AFEG-2017 culture (three-days old) in a smaller plate (5.0 cm). The length and weight of the tested seeds was measured after a two weeks incubation period (28 °C).

Plant Pathogenic Fungi

Four phytopathogenic fungi, *Alternaria phragmospora* APT-3, and Eupenicillium *brefeldianum* EJT-1, isolated from the infected tomato fruits, in addition to, *Alternaria alternata* AAP-1 from infected pepper fruits, were obtained from the Mycology Lab, Faculty of Science, Aswan University. The *Fusarium oxysporum* FOP-1 from the rotting roots of palm, were obtained from the Agriculture Research Center in Aswan.

Assay of Antifungal Activity of VOCs

The VOCs of AFEG-2017 were tested for its ability to inhibit the phytopathogenic fungi by following the protocols developed by Strobel et al., (2001) with some modification.[12] Briefly, in 12 cm petri dish, two small petri dishes (6.0 cm) were plated. In the first plate, AFEG-2017 was grown in PDA media in which a 2-cm-wide strip of agar was removed in the middle of the plate. The fungus was incubated at 28 °C for three days for the optimum production of VOCs. Then, the tested organism was inoculated on the other plate. The plates were wrapped with Parafilm and incubated again for three days. The diameter growth of the tested organism was measured and the inhibition percentage was calculated.

$$\% \text{ Inhibition} = \frac{Dc - Ds}{Dc} \times 100$$

Where:

Dc = Average of mycelial growth diameter in control,

Ds = Average of mycelial growth diameter in treatment.[13]

Statistical Analysis

All experiments in triplicate were individually assessed. Data were subjected to One-way Analysis of Variance (ANOVA). Significant differences between the control and treatments at the level of $p \leq 0.05$ were obtained by Tukey tests. Tukey test was deployed to compare differences among means by using the MINITAB software. Values shown in the figures are the means ± Standard Errors (SEs) of four independent replicates. Box blot was carried out using R program (R-4.1.1, https://www.r-project.org/).

Results

GC-MS Analysis of VOCs Released by AFEG-2017 and E. geniculata

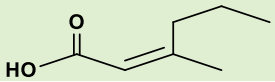
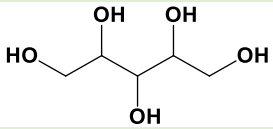
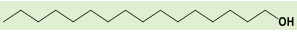
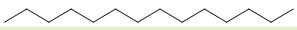
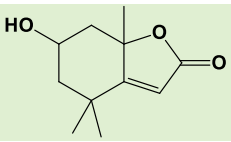
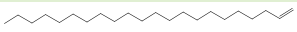
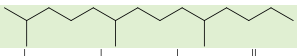
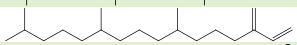
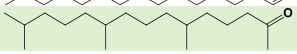
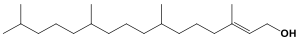
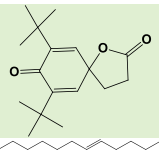
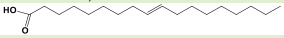
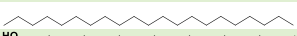
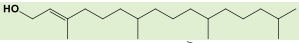
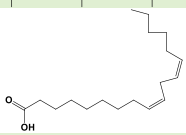
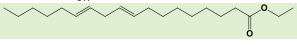
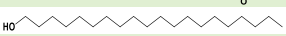
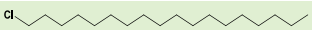
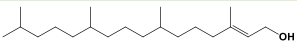
GC-MS analysis of the VOCs from AFEG-2017 and *E. geniculata* resulted in various organic and bioactive compounds for each identified with reference to Wiley and NIST mass spectral database which corresponds to each compound's identity (Tables 1 and 2).

The identified metabolites of *E. geniculata* plant included 28 compounds; the most abundant constituent was Phytol with a relative value of 50.35% followed by 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-Ol with the relative value of 6.81% (Table 1, Figure 1).

Also, 25 compounds were identified emitted by AFEG-2017 and the most abundant compound was 2-(2-hydroxy-3-isobutoxypropyl)-pent-4-enoic acid, hydrazide with the relative value (21.74%) followed by Linalool (10.58%) (Table 2 and Figure 2).

Interestingly, four compounds linked between the host plant *E. geniculata* and its endophyte, AFEG-2017 (Table 3).

Table 1. Volatiles Organic Compounds (VOCs) Emitted by the Medicinal Plant *E. geniculata*

Chemical Compounds	Structure	Formula	M.W	RT	Area %
Z-3-Methyl-2-hexenoic acid		$C_{29}H_{40}O_{10}$	548	5.18	0.68
Ribitol		$C_5H_{12}O_5$	152	6.02	1.84
1-Hexadecanol		$C_{16}H_{34}O$	242	11.73	1.28
Tetradecane		$C_{14}H_{30}$	198	11.89	0.72
1-Nonadecene		$C_{19}H_{38}$	266	15.81	3.11
Nonadecane		$C_{19}H_{40}$	268	15.95	1.75
6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzo- furan-2(4H)-one-		$C_{11}H_{16}O_3$	196	19.18	1.01
1-Docosene		$C_{22}H_{44}$	308	19.54	4.19
Tetradecane, 2,6,10-trimethyl		$C_{17}H_{36}$	240	19.66	1.97
Neophytadiene		$C_{20}H_{38}$	278	20.36	2.15
2-Pentadecanone, 6,10,14-trimethyl		$C_{18}H_{36}O$	268	20.46	0.56
3,7,11,15-Tetramethyl-2-hexadecen-1-Ol		$C_{20}H_{40}O$	296	20.78	0.46
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione		$C_{17}H_{24}O_3$	276	21.77	1.33
9-Octadecenoic acid (*Z*)-		$C_{18}H_{34}O_2$	282	22.53	3.37
10-Heneicosene		$C_{21}H_{42}$	294	22.94	3.75
Heneicosane		$C_{21}H_{44}$	296	23.03	0.71
Phytol		$C_{20}H_{40}O$	296	24.86	50.35
9,12-Octadecadienoic acid (Z,Z)-		$C_{18}H_{32}O_2$	280	25.31	2.89
Ethyl (9Z,12Z)-9,12-Octadecadieno		$C_{20}H_{36}O_2$	308	25.67	1.11
1-Eicosanol		$C_{20}H_{42}O$	298	26.05	1.66
1-Chlorooctadecane		$C_{18}H_{37}Cl$	288	26.13	0.67
3,7,11,15-Tetramethyl-2-hexadecen-1-Ol		$C_{20}H_{40}O$	296	26.44	6.81

Continue

Heptacosane		$C_{27}H_{56}$	380	27.58	0.91
1-Docosanol		C22H46O	326	28.90	0.45
Docosane		C22H46	310	28.97	1.21
Pentatriacontane		C35H72	492	30.32	1.64
Dotriacontane		$C_{32}H_{66}$	450	31.60	0.88
7-Methyl-Z-tetradecen-1-Ol acetate		$C_{17}H_{32}O_2$	268	32.85	0.70

Table 2. Volatiles Organic Compounds (VOCs) Emitted by the Endophyte AFEG-2017

Chemical Compounds	Structure	Formula	RT	M.W	Area %
3,7-Dimethylocta-1,6-dien -3-Ol (Linalool)		$C_{10}H_{18}O$	4.42	154	10.58
3,7-Dimethylocta-1,6-dien-3-yl acetate (linalyl acetate)		$C_{12}H_{20}O_2$	6.94	196	4.48
Lavandulyl acetate		$C_{12}H_{20}O_2$	8.95	196	1.04
3,7-Dimethylocta-2,6-dien-1-yl acetate (Geranyl acetate)		$C_{12}H_{20}O_2$	9.29	196	1.03
2,6-Dibutyl-2,5-cyclohexadiene-1,4-dione		$C_{14}H_{20}O_2$	10.80	220	1.16
1,4-Benzenediol, 2-(1,1-dimethylethyl)-5-(2 propenyl)-		$C_{13}H_{18}O_2$	11.63	206	2.82
Nerolidol		$C_{15}H_{26}O$	12.44	222	1.05
Oleic acid		$C_{18}H_{34}O_2$	12.86	282	0.60
Dodecanoic acid (Lauric acid)		$C_{12}H_{24}O_2$	15.36	200	8.18
1-Hexadecanol		$C_{16}H_{34}O$	16.06	242	2.74
Vinyl decanoate		$C_{12}H_{22}O_2$	16.16	198	1.91
2-(2-Hydroxy-3-isobutoxypropyl)- pent-4-enoic acid, hydrazide		$C_{12}H_{24}N_2O_3$	17.51	244	21.74
1,3,5-Triazine-2,4-diamine, 6-chloro-*N*-ethyl-		$C_5H_8ClN_5$	17.74	173	1.60
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione		$C_{17}H_{24}O_3$	18	276	6.65

Continue

9,12-Octadecadienoic acid (*Z,Z*)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{38}O_4$	18.47	354	0.98
9-Octadecenoic acid (*Z*)-	$C_{18}H_{34}O_2$	18.62	282	3.24
1-Eicosanol	$C_{20}H_{42}O$	18.98	298	6.97
1-Chloro-octadecane	$C_{18}H_{37}Cl$	19.06	288	2.51
9,12-Octadecadienoyl chloride, (*z,z*)-	$C_{18}H_{31}ClO$	19.23	298	1.41
9-Octadecenoic acid (*Z*)-	$C_{18}H_{34}O_2$	20.24	282	1.41
1-Docosene	$C_{22}H_{44}$	21.64	308	4.90
12-Methyl-*E,E*-2,13-octadecadien-1- Ol	$C_{19}H_{36}O$	22.97	280	1.32
1-Docosanol	$C_{22}H_{46}O$	24.10	326	2.20
Dotriacontane	$C_{32}H_{66}$	25.31	450	1.33
7-Methyl-8-tetradecenyl acetate	$C_{17}H_{32}O_2$	26.52	268	0.88

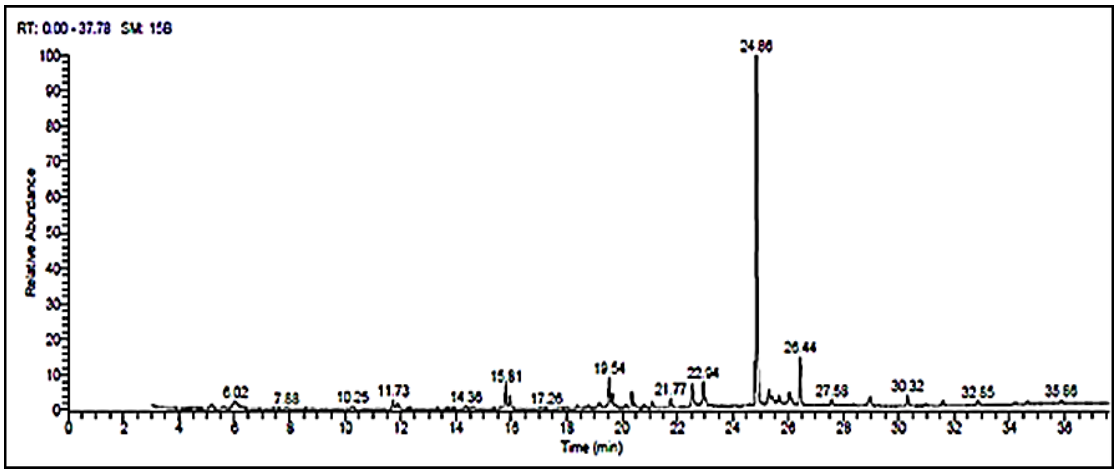


Figure 1. GC-MS Chromatogram Showing VOCs Emitted by *E. geniculate*.

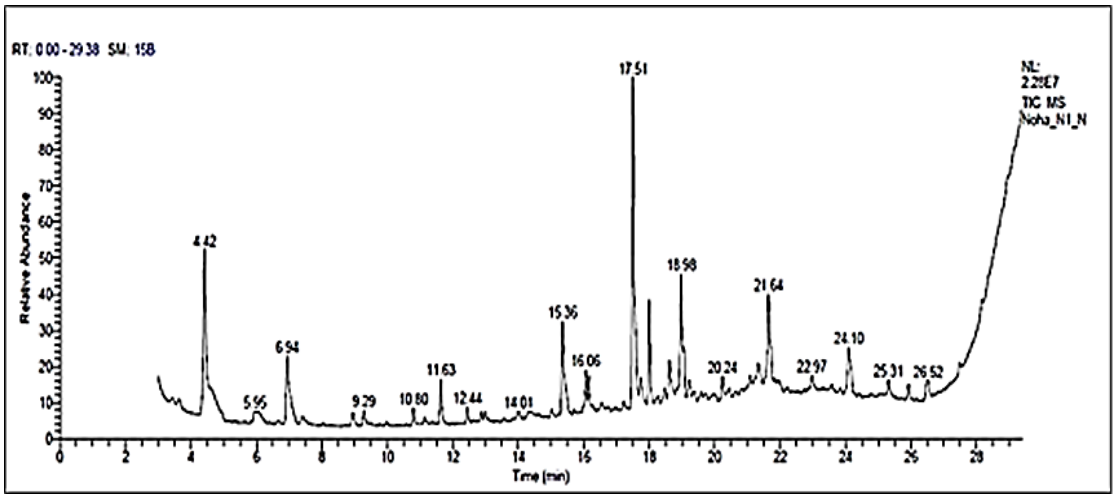


Figure 2. Chromatogram Obtained from GC-MS Analysis of VOCs Emitted by AFEG-2017 on PDB Medium after 2 Weeks Inoculation. Each peak in the chromatogram identified a detected compound.

Effect of VOCs of AFEG-2017 on Seedling

Our results clearly confirmed the seedling promoting activity of VOCs emitted by the PGPF AFEG-2017 in the medicinal and valuable plants like *T. foenum-graecum*, *S. lycopersicum*, *P. oleracea*, and *L. sativum*. When the seeds of these plants were subjected to VOCs of AFEG-2017, a recognizable increase in the length and weight of the seedling were detected (Figure 3).

Table 3. VOCs Uniformity between *E. geniculata* and AFEG-2017

No	chemical compounds	Formula	Molecular Weight
1	1-Eicosanol	C20H42O	298
2	9-Octadecenoic acid (z)-	C18H34O2	282
3	1-Chlorooctadecane	C18H37Cl	288
4	1-Hexadecanol	C16H34O	242

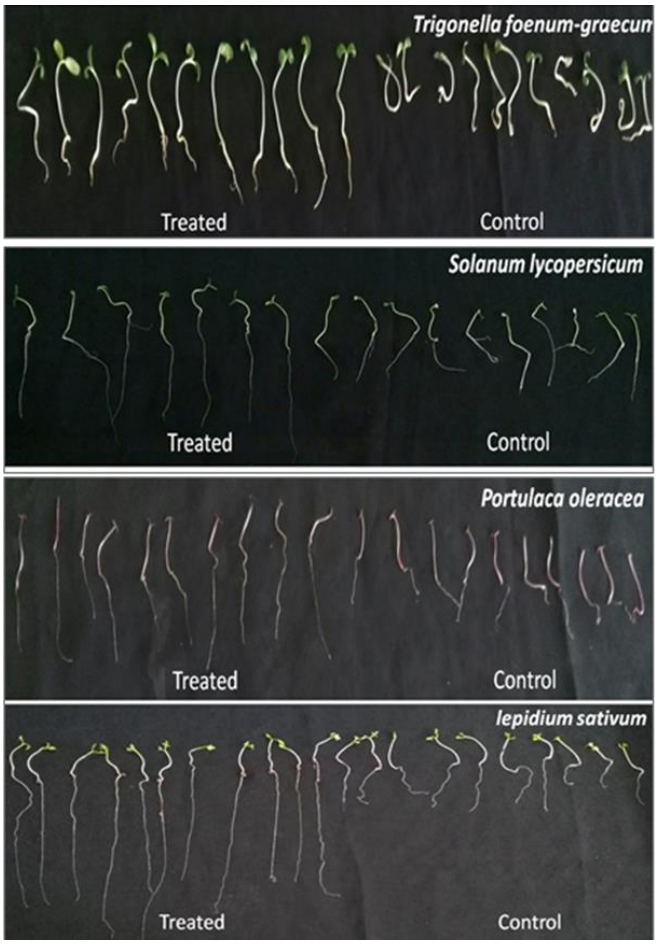


Figure 3. Seedling Promotion of *T. foenum-graecum, S. lycopersicum, P. oleracea,* and *L. sativum* when Exposed to VOCs of AFEG-2017 Grown on PDA Medium.

The length of *S. lycopersicum* seedlings subjected to VOCs of AFEG-2017 was ranged between 8.0 and 10.5 cm compared with control (5.0 to 7.6 cm). This enhancement by AFEG-2017 VOCs was recognized also with *L. sativum* seedling (7.0 to 12.8 cm) in length compared with the control (5.3 to 7.6 cm). In addition, *T. foenum-graecum* seedling length with VOCs started with 6.5 and reached 8.2 cm in comparison with the control which started with 5.0 and reached 6.6 cm. *Portulaca oleracea* seedling length ranged between 2.7 and 4.8 cm compared to the control which ranged between 1.7 and 3.5 cm (Figure 4). The fresh weight of *T. foenum-graecum*, *L. sativum*, *P. oleracea*, and *S. lycopersicum* treated with VOCs of AFEG-2017 was 0.11, 0.0158, 0.0159, and 0.0023 gm respectively, in comparison with the control which was not subjected to VOCs (0.061, 0.008, 0.0126, and 0.00093 gm respectively (Figure 4)).

VOCs of AFEG-2017 Restricted the Growth of Phytopathogenic Fungi

VOCS emitted by *AFEG-2017* successfully restricted the growth of four phytopathogenic fungi (Figure 5). The pathogen *F. oxysporum* showed the highest inhibition percentage in the growth (40%) followed by *E. brefeldianum* (26%), *A. phragmospora* (17.8%), and *A. alternata* (12%).

Discussion

Abdel-Motaal et al., (2020) reported that *A. flavus* isolated endophytically from the medicinal plant, *E. geniculata* stimulated the growth of tomato plant and can improve the resistance of this plant to an aggressive plant pathogen like *A. phragmospora* which cause early blight disease.[14] Kaminśki et al., (1972) proved that *A. flavus* produced some active VOCs; of them: 3-methylbutanol, 3-octanone, 3-octanol, 1-octen-3-Ol, 1-octanol, and *cis*-2-octen-1-Ol.[4] Both oct-1-en-3-Ol and *cis*-2-octen-1-Ol are thought to be responsible for the characteristic musty-fungal odor of certain fungi; the latter compound may be a useful chemical index of fungal growth.

Park et al., (2015) recently reported that VOCs emitted by *Pseudomonas fluorescens* have been shown to stimulate plant growth and have been developed both *in vitro* and *in vivo*.[15] Also, a clear increase was observed in the fresh weight, shoot length and root length of tomato plants following exposure to *Bacillus subtilis* VOCs.[16] Many *Trichoderma* strains commonly found in soil have been extensively studied for their beneficial effects on plant growth. They have been found to emit VOCs mixtures that probably mimic plant metabolites and significantly enhance plant growth in *Arabidopsis* and tomato as measured by biomass and plant size depending on the duration of exposure.[17]

Lee et al., (2016) proved that different strains of *Trichoderma* emitted VOCs which have a positive role in plants growth. Both interactions between mixtures of VOCs produced by active growing fungi and time exposure to these VOCs influence the plant development. The bio-stimulatory strains tended to have a larger number of complex terpenes which may explain the variation in growth induced by different *Trichoderma* strains.[17]

Previous studies have found that beneficial rhizosphere bacteria produce VOCs that enhance plant growth.[18,19]

Similarly, volatile mixtures emitted from the biocontrol fungus *Trichoderma viride* enhanced the growth of *Arabidopsis*,[20] and volatiles of *Cladosporium cladosporioides* enhanced the growth of tobacco plants.[21] Minerdi et al., (2009) recorded that in lettuce, VOCs emitted from a consortium of *Fusarium oxysporum* and bacteria also promoted growth.[22] Recently, *Cladosporium halotolerans* VOCs stimulated the growth of many vegetables.[23]

Besides growth promotion, fungal VOCs benefit plants by providing defense against pathogens of their hosts.[24] For example, an endophyte, *Muscodor albus*, produces VOCs which include esters, alcohols, acids, lipids, and ketones that inhibit and kill plant pathogenic fungi.[12,25] Strobel et al., (2011) reported that a range of plant pathogens, including

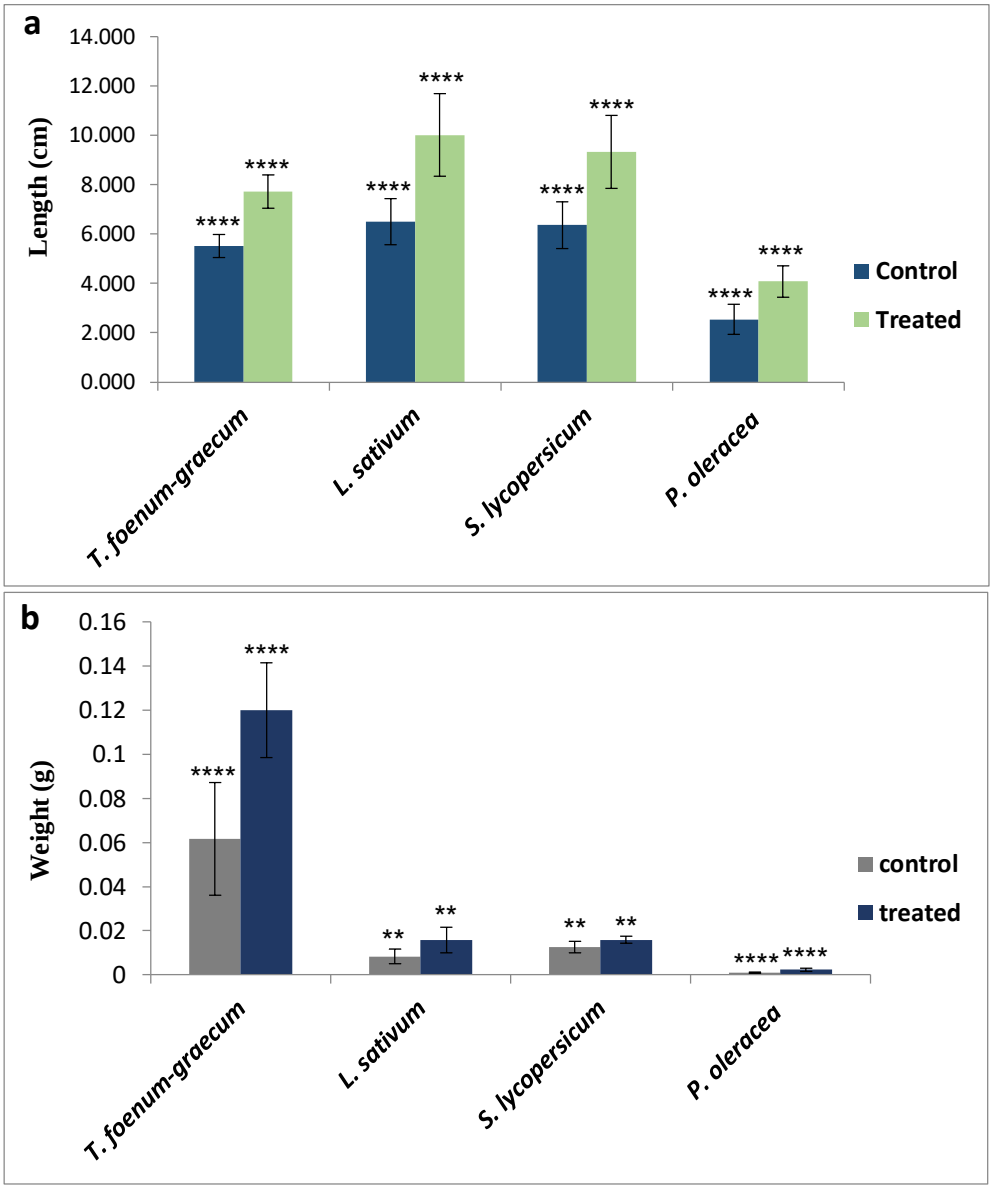


Figure 4. Effect of AFEG-2017 VOCs on **(a)** the Length (cm) and **(b)** the Weight (gm) of Different Seeds (*T. foenum-graecum, S. lycopersicum, P. oleracea, and L. sativum*). Asterisks indicate significance of inhibition (*low; **moderate; ***high, and ****very high).

Verticillium, *Ceratocystis*, *Cercospora*, and *Sclerotinia*, were inhibited or killed by the mixture of VOCs produced from *Phoma* sp. isolated from creosote bush.[26]

Our results of GC Mass analysis of AFEG-2017 showed that there are many emitted VOCs like 2-(2-hydroxy-3-isobutoxypropyl) pent-4-enoic acid, hydrazide, lavandulyl acetate, linalool, and nerol which are of considerable importance. Popiołek, (2017) reported that hydrazide compounds are present in many bioactive molecules and display a wide variety of biological activities, such as antifungal, antibacterial, anticancer, and anti-inflammatory.[27] Series of hydrazones derivatives showed high antibacterial activity against *Mycobacterium tuberculosis*.[28] In addition, Kumar et al., (2011) reported that 3-ethoxy-4-hydroxybenzylidene/4-nitrobenzylidene

hydrazides have antibacterial activity against *Candida albicans* and *Aspergillus niger*.[29] D'auria et al., (2005) and Özcan et al., (2018) reported that lavandulyl acetate is the EOs of *Lavandula stoechas* and the EOs of *Lavandula angustifolia* Mill. (Lavender oil) and its main components, linalyl acetate and linalool had antifungal activity against pathogenic fungi.[30,31] Linalool also exhibits antimicrobial activity against *C. albicans* and bacteria associated with oral diseases.[32] Lee et al., (2007) reported that Nerolidol is a sesquiterpenoid component of EOs which has inhibitory effect on *E. coli* by altering bacterial cell permeability, also has antifungal activity against *Microsporurn gypseurn*.[33] Also, Oleic acid has antifungal activity against the plant pathogenic fungi *Crinipellis perniciosa* and *Rhizoctonia solani*.[34]

The antifungal efficacy of Nerol (2, 6-octadien-1-Ol, 3, 7-dimethyl) has been proved against *A. flavus* causing food spoilage by

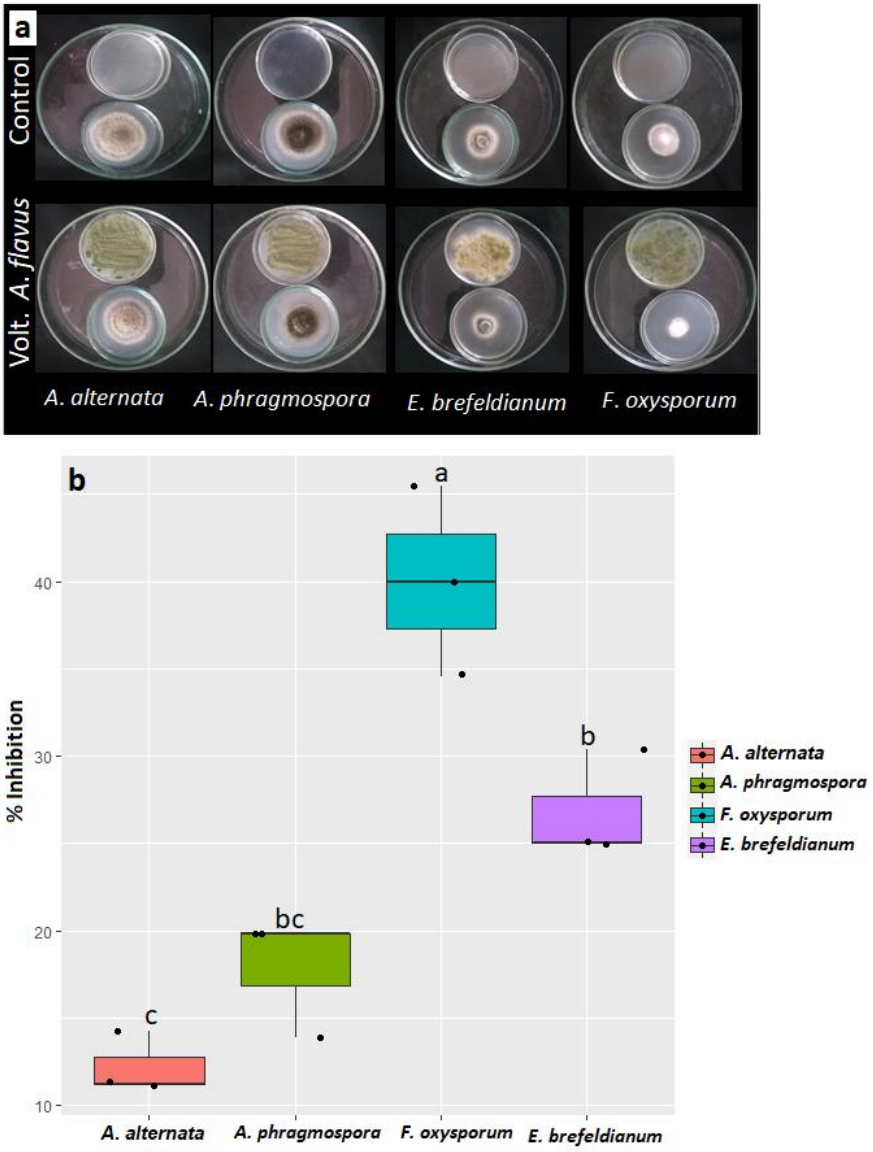


Figure 5. a) Antifungal Activities of AFEG-2017 Volatiles Against Pathogenic Fungi (*A. alternata*, *A. phragmospora*, *E. brefeldianum*, and *F. oxysporum*). The fungi in the first row were not exposed to fungal volatiles, while those in the second row, showing a significant inhibition, when exposed to AFEG-2017 VOCs. These pictures were taken at day 3 of the experiment; **b)** Box plot diagram showing the changes in inhibition percentage between the different pathogen, letters a, b, and c indicate significant differences $p<0.05$ (ANOVA after Tukeys test analysis).

using *in vitro* and *in vivo* tests.[35] The recent work of Zore et al., (2010) demonstrated that geranyl acetate exhibit excellent anti-candida activity.[36] Al-Marzoqi et al., (2015) reported that geranyl vinyl ether had antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerogenosa*, and *Klebsiella pneumonia*.[37] Lauric acid, 1-Hexadecanol and 9-octadecenoic acid was also reported as antimicrobial activity against pathogenic fungi and bacteria.[38,39]

The EOs is one of the main constituents of plants belonging to genus *Euphorbia*. There are many reports concerning the EOs of different members of this genus such as *E. macrorrhiza*, *E. hirta*, and *E. rigida*.[1] Elshamy et al.,

(2019) reported that the EOs of the aerial parts of *E. heterophylla* comprise 35 compounds, mainly monoterpenes; of them: 1,8-cineole, camphor, *β*-elemene, and *endo*-borneol,[40] while our results indicated that phytol is the major compound followed by 3,7,11,15-Tetramethyl-2-hexadecen-1-Ol and 1-Docosene.

Conclusion

This study proved that there is a harmony between VOCs produced by the medicinal plant *E. geniculata* and its endophyte *A. flavus*. These volatiles could successfully accelerate plant seedling and limit the growth of some important phytopathogens like *Alternaria phragmospora*, *Eupenicillium brefeldianum*, *A. alternate*, and *Fusarium oxysporum*. Thus, VOCs emitted by medicinal plant and its associated fungi have a promised role in crop growth promotion and protection from pathogens.

Authors' Contributions

FFA, NMK, SAE, AHM, and DBD conceived and designed the study; FFA, NMK, and SAE isolated and identified the endophytic fungus *A. flavus* associated with *Euphorpia* and screened the antifungal activity. NMK and AHM prepared the extracts, GC-MS analysis and identification of extract components. FFA, NMK, SAE, and DBD screened the role of VOCs on phytophatogens inhibition and promotion of plant seedling. All authors organized, analyzed the data, interpreted the data, wrote the paper and revised the final draft. Also, all authors read and approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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