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Antifungal volatiles from medicinal herbs suppress *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract
Medicinal herbs are the potential sources for antimicrobial compounds against soil-borne pathogens. The present investigation focuses on assessing the efficacy of antifungal activity of volatile organic compounds (VOCs) produced by seven different medicinal herbs in the suppression of *F. oxysporum* f.sp. *lycopercisi*, the causal agent of wilt disease in tomato plants. Among the seven samples tested *in-vitro*, the blend of VOCs emitted from mint (*Mentha spicata*) leaves inhibited the mycelial growth of *F. o.* f.sp. *lycopercisi* up to 41.88% followed by lemongrass (39.44%) and nochi leaves (34.66%). The important VOCs emitted by mint and lemongrass samples were identified through Headspace – Gas chromatography coupled with Mass Spectrophotometry (HS-GCMS) which revealed the presence of around 25 different compounds with varied functional groups. Among them -(-) carvone obtained from mint leaves had the highest relative abundance (2.08% peak area at 10.25 RT). In addition, geraniol and citronellol were also present with a peak area percentage of 15.85 at 10.37 RT and 5.27 at 9.97 RT, respectively. Pure compounds of carvone, geraniol and citronellol (Sigma Aldrich) along with plant samples were also tested *in vitro* for comparison on the inhibition of mycelial growth of *F. oxysporum* f. sp. *lycopercisi*. Among the samples, citronellol was found to be the most effective and suppressed the wilt pathogen up to 100% at 500 ppm, followed by carvone (up to 90% inhibition) in the partition plate method. The results indicated that VOCs produced from mint and lemongrass could be explored with an additive effect for the management of *F. oxysporum* f.sp. *lycopercisi* along with other Integrated Disease Management (IDM) practices.

Keywords: antifungal VOCs, citronellol, carvone, *F. oxysporum* f.sp. *lycopercisi*, HS-GCMS

Introduction
Tomato is a valued vegetable crop, well known for its taste and nutrition with high antioxidant and curative properties. However, at times the area and productivity in tomato become limited due to the occurrence of various biotic and abiotic stresses. A considerable yield loss have been recorded under greenhouse condition due to seedling blight, damping off and wilt diseases caused by *Rhizoctonia* spp, *Pythium* spp and *Fusarium* spp [14,32]. Huge loss of upto 45% in tomato crop grown under greenhouse condition was recorded in India [21, 31]. Among the biotic factors, *Fusarial* wilt incited by *Fusarium oxysporum* f. sp. *lycospersici* is the most prevalent soil-borne pathogen and causes significant damage to the crop. For several years, application of fungicides and soil fumigants such as methyl bromide were the most practices control measures against soil borne fungal pathogens. A constant application of chemical fungicides adversely affects the soil microbial community and crop productivity in addition to environment pollution and residual toxicity to human being.

Over the years, novel attempts such as soil solarisation, application of biocontrol agents, use of botanicals, application of safe biomolecule *etc.*, are extensively used to manage the soil borne fungal pathogens in and integrated way. Use of natural volatile organic compounds (VOCs) produced by microorganisms and plants will have a greater stakehold directly or indirectly for the management of soil borne plant pathogens [17, 19]. VOCs have been broadly demonstrated to induce defense mechanism against plant pathogens [4]. VOCs produced by various medicinal plants have been evaluated for controlling plant diseases. For instance, extracted VOCs from the leaves of *Lawsonia inermis*, *Achyranthes aspera* and *Mimosa pudica* [15, 2] have been explored for the management of fungal pathogens. VOCs of essential oil such as oregano (*Origanum syriacum* var. *bevanii*), thyme (*Thymbra spicata* sub sp. *spicata*), lavender

(*Lavandula stoechas* sub sp. *stoechas*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*), and laurel (*Laurus nobilis*) showed antifungal activities against *P. infestans* [26].
The present study was carried out to investigate the effect of volatile organic compounds produced by mint, coleus, lemon grass, tulasi, nochi, neem and vetiver leaves on the mycelial growth and sporulation of *F.* o. f.sp. *lycopersici*, infecting tomato plants. HS-GCMS analyses for profiling the VOCs and functional group identification through FT-IR were carried out. SEM studies were also conducted to reveal the morphometric changes brought out by the effective VOCs on the fungal pathogen.

Materials and Methods

Screening with medicinal plants

Fresh leaves of Mint (*Mentha spicata*), Lemon grass (*Cymbopogon citratus*), Coleus (*Coleus amboinicus*), Nochi (*Vitex negundo*), Thulasi (*Ocimum tenuiflorum*), Neem (*Azadirachta indica*) and Vetiver (*Vetiveria zizanioides*) were collected from medicinal garden, Tamil Nadu Agricultural University, Coimbatore and screened for *in-vitro* antagonism against the pathogen. The leaf samples (one gram) were crushed and placed on a sterile bottom glass Petri plate. A 5mm mycelial disc from 7 days old culture of *F. oxysporum* f. sp. *lycopersici* was placed at the centre of a sterile bottom petri plate containing PDA medium. The mycelial disc containing plate was placed over the other bottom that contained crushed leaf sample, sealed tightly as described by Dennis and Webster (1971). Then the plates were covered with aluminium foil and incubated for 7 days. Similarly, a control plate was maintained as described above without the leaf sample. The effect of the antagonistic activity of volatilomes against *F. oxysporum* f.sp. *lycopersici* was assessed based on the observation of mycelial growth inhibition. Each sample was replicated three times. The per cent growth reduction over control was calculated by using the formula:

$$\text{Per cent Inhibition (PI)} = \frac{\text{Mycelial growth on treated plate}}{\text{Mycelial growth on control plate}} \chi 100$$

Collection and characterization of VOC with antimicrobial action against *F. oxysporum* f.sp. *lycopersici*:

The volatiles from mint and lemongrass leaves were collected and analyzed using air - entrainment technique with slight modification [6]. The fresh leaf samples were collected and placed into the volatile chamber and closed with a double holed lid for inducing the volatile emission. One side of the lid was meant for passing moistened air inside the chamber as inlet and the other side for trapping volatiles from the chamber through the outlet. A motor pump was used to pass purified moistened air into the fish tank chamber through a water container *via* activated charcoal. Headspace volatiles were collected using a glass cartridge containing 'Porapak Q' volatile adsorbent material that was placed on the outlet hole (Fig 1). The experiment was performed for 48 h to trap the volatiles at 24 h interval. Then, the trapped volatiles were eluted with one ml of HPLC grade hexane for five times by adding 200µl to the column and collected in a vial for Headspace GC-MS analysis.
After that, the samples were immediately analyzed by HS-GCMS analysis using Thermo GC injector coupled with Mass spectrophotometer (Turbo Matrix 150, purchased from Perkin Elmer, USA). Helium gas was used as carrier gas (1.1 mL /min). The energy of electron impact was 70 eV, the ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. Electron impact (EI) of mass spectra was programmed from the range of 20-220 atomic mass unit at one sec intervals. One microlitre of the eluted volatile sample was injected in the GC column. Moreover, mass spectra data of each compound were compared with the NIST MS version 2.2 data library.

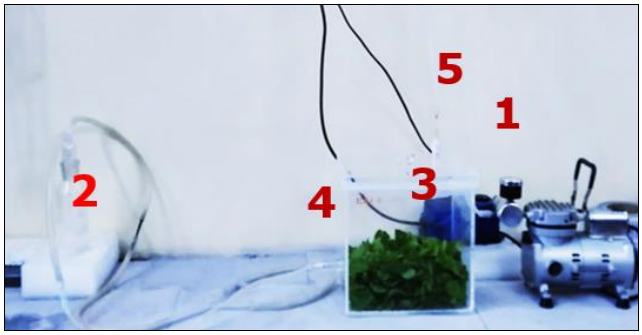


Fig 1: Trapping of volatiles through poropak Q by air entrainment technique
1. Motor pump 2. Water tank 3. Chamber 4. Inlet hole for passing moistened air 5. Outlet hole fitted with Poropak Q for trapping volatile emitted from leaves.

Confirmation of antimicrobial action with pure compounds

The effective VOCs released by mint and lemongrass leaves were identified by HS GC-MS analysis and the pure form of such compounds were purchased from Sigma Aldrich and tested separately, to assess the inhibition of mycelial growth of *F. oxysporum* f.sp. *lycopersici* by the individual compound. The suppression of fungal growth was tested by a bipartition plate assay method. The PDA medium was poured into one side of the plate, and the water agar medium on the other side. A 5mm mycelial disc of *F. oxysporum* f.sp. *lycopersici* from a four day old active growing hyphae was excised using a 5 mm cork borer and placed on one side of the PDA medium. Subsequently, the volatile compound was dotted on a 5mm sterile filter paper disc on the water agar medium. A filter paper disc alone was used as the control factor instead of adding the volatile compound. After inoculation, plates were wrapped and incubated for 7 days. The diametrical expansion of the mycelia of *F. oxysporum* f.sp. *lycopersici* was measured upon the interaction of volatile compounds. The experiment was repeated thrice with three replications, with five numbers of plates per replication.

Fourier transform-infrared spectroscopy (FTIR) analysis

The spectra of volatiles from leaves of *M. spicata* and *C. citratus* were obtained using an FTIR (FTIR–6800 JASCO, Japan) equipped with an Attenuated Total Reflectance (ATR) unit to identify the functional group. The absorbance spectra of each sample were recorded in a wavenumber range of 4000 to 400 cm^{-1} with a spectral resolution of 4 cm^{-1} and averaging of 64 scans per sample. The absorbance spectra was analysed using BioRadKnowItAll® 2017 software.

Scanning electron microscope analysis

A two mm mycelial disc of *F.oxysporum* f.sp. *lycopersici* from the VOCs treated plate, was cut off from the edge of the colonies. The mycelial samples were then coated with gold sputter and imagined using a scanning electron micrscope (FEI Quanta 250)

Statistical analysis

The experiment was performed in triplicate and analyzed using completely random design (CRD) and DMRT by using SPSS statistical software, as suggested by Gomez and Gomez (1984). The principle component analysis was made using XLSTAT.

Results

Isolation and molecular confirmation of *Fusarium* spp associated with tomato wilt

Fusarium sp. was isolated from wilt infected necrotic tissue of root. Phenotypic characterization of the isolated pathogen confirmed the presence of hyaline septate mycelia., Microconidia was small, oval in shape, hyaline single or bi-celled and are 9.07-10.96 µm in length and 3.42 -4.70 µm in width. Macroconidia were hyaline with 2-3 septations with a size of 22.21-28.01 µm (length) and 5.88-6.88 µm (width). Chlamydospore was either terminal or intercalary (Fig 2). The identity of *Fusarium* sp. was confirmed using ITS 1 and ITS 4 universal primer with amplicon size of ~ 560 bp and phylogenetic analyses showed 100 per cent homology with *Fusarium oxysporum* f.sp. *lycopersici.* The sequence was submitted at the NCBI database and assigned with the accession no: MW350042.

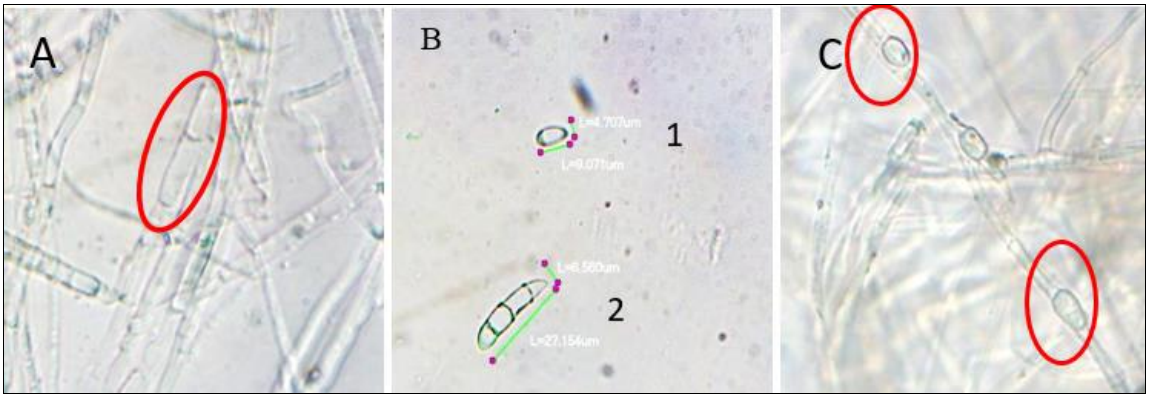


Fig 2: Morphological characteristics of *Fusarium* sp.
a. Hyaline and septate mycelium. b. Microconidia (1) and macroconidia (2) of *F. oxysporum* c. Chlamydospore produced either at terminal or intercalary.

***In vitro* screening with VOCs produced by *M. spicata* and *C. citratus* leaves**

The VOCs produced by the leaves of *M. spicata* inhibited mycelial growth of *F. oxysporum* f.sp. *lycopersici* to an extent of 41.88%, followed by the VOCs emitted by the leaves of *C. citratus* (39.66%) and *V. negundo* (34.44%) (Table 1). Regarding the colony growth of the pathogen, the control plate exhibited white-color at initial growth stage and turned to pink-colored mycelia; whereas the mint leaf (*M. spicata*) VOCs treated plate showed white mycelia with puffy appearance and with irregular growth. A similar observation was noticed with *C. citratus* and *V. negundo* leaf VOCs treated plates (Fig 3).

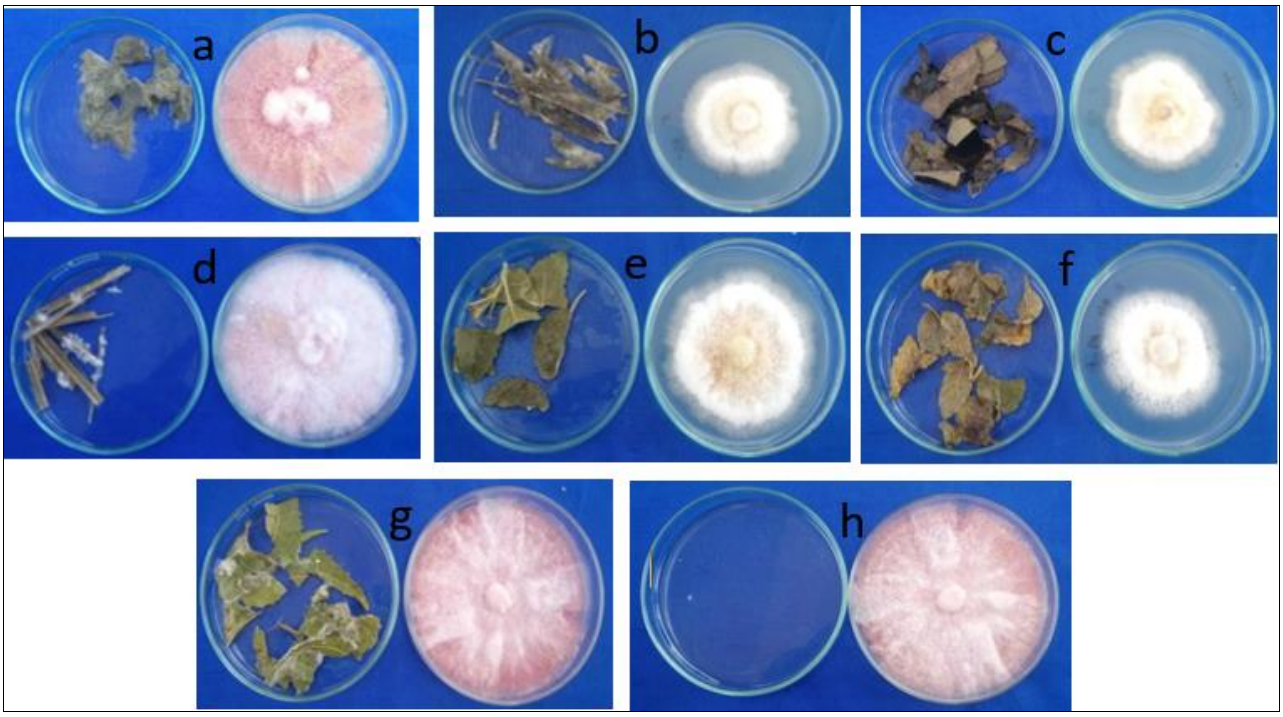


Fig 3: *In vitro* screening of plant volatiles against *F. oxysporum* f.sp. *lycopersici*
a. Coleus amboinicus, b. Cymbopogon citratus, c. Vitex negundo, d. Vetiveria zizanioides, e. Ocimum tenuiflorum, f. Mentha spicata, g. Azadirachta indica and h. Control

Table 1: *In vitro* screening of plant volatiles against *F. oxysporum* f.sp. *lycopersici*

Plant Sample	Average growth of mycelium (mm)	Per cent Inhibition over control (PI)
C. amboinicus	89.33[d]	0.77
C. citratus	54.30[a]	39.66
V. negundo	59.00[b]	34.44
V. zizanioides	90.00[d]	0.00
O. tuniflorum	72.33[c]	19.66
M. spicata	52.30[a]	41.88
A. indica	90.00[d]	0.00
Control	90.00[d]	0.00
CD (P=0.05)	4.55	-

Data are the mean of 3 replications and repeated thrice. Values with the same superscript letter in the same column are not significantly different (ANOVA).

Table 2: GC-MS analysis of VOCs from leaves of *M. Spicata*

S. No	RT	Compound	Molecular formula	Molecular weight	Relative abundance
1.	3.73	2-Penten-1-ol, 2-methyl-	$C_6H_{12}O$	100	0.1
2.	4.73	2,2,4-Trimethyl-3-pentanol	$C_8H_{17}N$	127	0.78
3.	5.52	Pentanoic acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	$C_{16}H_{30}O_4$	286	2.25
4.	6.79	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1à,2à,5á)-	$C_7H_{14}O$	114	0.2
5.	8.27	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	219	0
6.	9.86	trans-Carveol	$C_{10}H_{16}O$	152	0.12
7.	10.25	(-)-Carvone	$C_{10}H_{14}O$	150	2.08
8.	11.55	10,13-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	219	0.02
9.	11.55	Limonen-6-ol, pivalate	$C_{20}H_{32}O_3$	320	0.02
10.	11.92	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.01
11.	12.37	(-)-á-Bourbonene	$C_{15}H_{24}$	204	1.67
12.	12.9	Caryophyllene	$C_{15}H_{24}$	204.18	1.57
13.	13.31	alfa.-Copaene	$C_{15}H_{24}$	204	0.25
14.	13.63	(+)-epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$	204	0.84
15.	13.96	1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	$C_{15}H_{24}$	204	1.57
16.	14.67	Naphthalene,1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	$C_{10}H_4Cl_4$	264	0.51
17.	15.24	Rhodopin	$C_{40}H_{58}O$	554	0.01
18.	15.71	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240	0.13
19.	16.18	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.01
20.	17.46	Benzoic acid, 2-ethylhexyl ester	$C_{15}H_{22}O_2$	234	0.09
21.	18.54	Octadecane	$C_{18}H_{38}$	254	0.05
22.	18.89	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	0.06
23.	19.46	Phthalic acid, hept-4-yl isobutyl ester	$C_{31}H_{52}O_4$	488	0.1
24.	20.6	9,12,15-Octadecatrienoic acid,	$C_{18}H_{32}O$	264	0.12
25.	21.5	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	$C_{28}H_{40}O_{10}$	536	0.03

Table 3: GC-MS analysis of VOC from *C. citratus*

S. No	RT	Compound	Molecular formula	Molecular weight	Relative abundance
1.	2.89	Oxirane, 2-ethyl-2-methyl-	$C_5H_{10}O$	86	0.87
2.	4.75	2,2,4-Trimethyl-3-pentanol	$C_8H_{17}N$	127	0.38
3.	5.12	Hydroperoxide, 1-ethylbutyl	$C_6H_{14}O_2$	118	0.34
4.	5.54	Oxirane, butyl-	$C_6H_{12}O$	100	0.72
	7.03	3-Carene	$C_{10}H_{16}$	136	0.05
5.	7.95	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	154	0.07
6.	8.78	Isopulegol	$C_{10}H_{18}O$	154	0.16
7.	9.97	Citronellol	$C_{10}H_{20}O$	156	5.27
8.	10.37	Geraniol	$C_{10}H_{18}O$	154	15.85
9.	11.02	Geranyl vinyl ether	$C_{12}H_{20}O$	180	0
10.	12.13	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	$C_{10}H_{18}O$	154	1.2
11.	12.9	Caryophyllene	$C_{15}H_{24}$	204	0.13
12.	14.55	ç-Muurolene	$C_{15}H_{24}$	204	2.09
13.	15.32	à-acorenol	$C_{15}H_{26}O$	222	0
14.	15.59	4-epi-cubedol	$C_{22}H_{32}O_2$	328	0.63
15.	16.22	Cubedol	$C_{23}H_{22}O_6$	394	0.03
16.	16.79	à-Cadinol	$C_{15}H_{26}O$	222	0.13
17.	17.46	Benzoic acid, 2-ethylhexyl ester	$C_{15}H_{22}O_2$	234	0.18
18.	17.97	Geranyl isovalerate	$C_{15}H_{26}O_2$	238	0.04
19.	18.54	Octadecane	$C_{18}H_{38}$	254	0.04
20.	18.54	Heptacosane	$C_{20}H_{60}O_{10}Si_{10}$	740	0.04

21.	19.05	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.24
22.	19.46	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.16
23.	19.93	Betulin	$C_{30}H_{50}O_2$	442	0.03
24.	21.48	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.01
25.	22.62	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	0.03

HS GC-MS analysis of VOCs

The headspace analysis revealed that *M. spicata* and *C. citratus* leaves produced diverse volatile organic compounds than that of *V. negundo* leaves. The compound (-) -carvone was the most abundant VOC produced by mint leaves showing high relative abundance with 2.08% area at 10.25 RT (Fig 4). The compounds such as geraniol and citronellol were produced by lemongrass leaves with a high relative abundance area of 15.85% at 10.37 RT and 5.27% area at 9.97 RT, respectively (Fig 5).

The PCA analysis performed with different classes of VOCs such as terpenoids, alkaloids, benzinoids, alkanes, aldehydes, fatty acids *etc*., obtained from GC-MS analysis with different treatments and variables were correlated with PC 1 (principle component 1) value of 85.24% (Fig 6). The varaibles obtained from lemon grass leaves were located in the left end of scoring plot, showing positive correlation within the components followed by mint and nochi leaf volatiles. The benzinoids and unknown components from *V. negundo* leaves were negatively correlated with these treatments located in the right end of scoring plot.

These PCA results confirmed that most of VOCs from *C. citratus* leaves (Citronellol, geraniol, isopulegol, Murrolene, 3 carene, geranyl venyl ether) and *M. spicata* leaves (carvone, trans—carveol, caryophylene) belong to monoterpenoid class followed by sesquiterpenoids. The monoterpenoid class of VOCs could be responsible for the inhibition of *F.oxysporum* f.sp. *lycopercisi*.

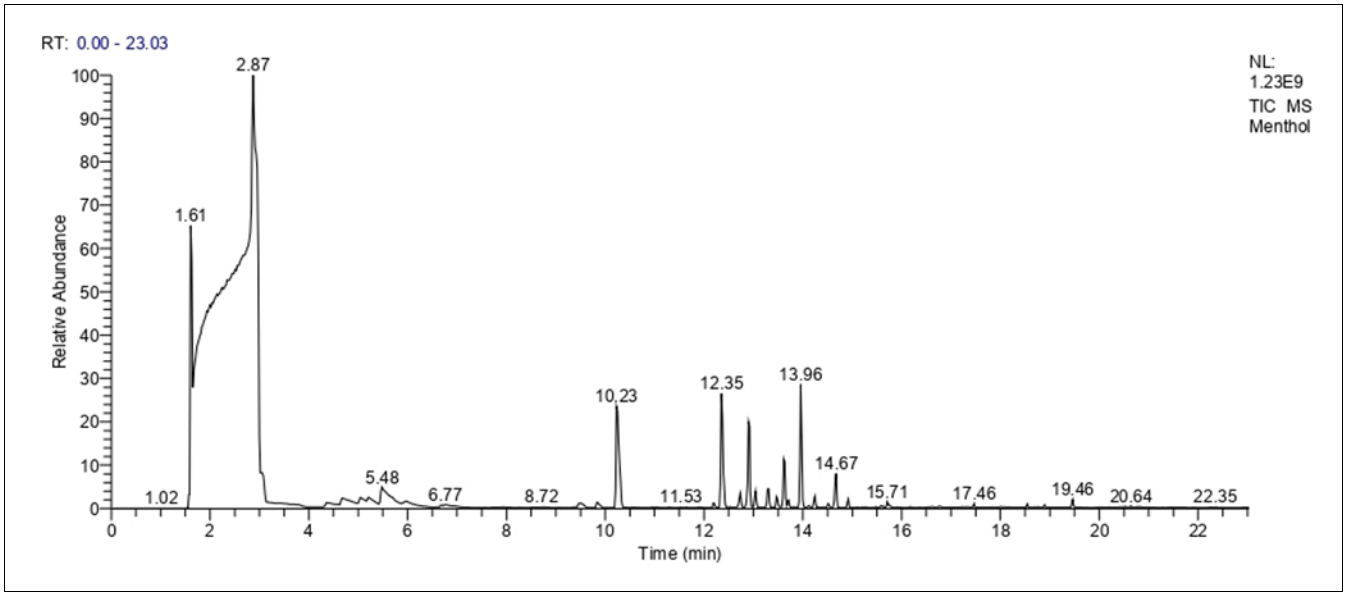


Fig 4: GC-MS chromatogram of volatile compounds from *M. Spicata*

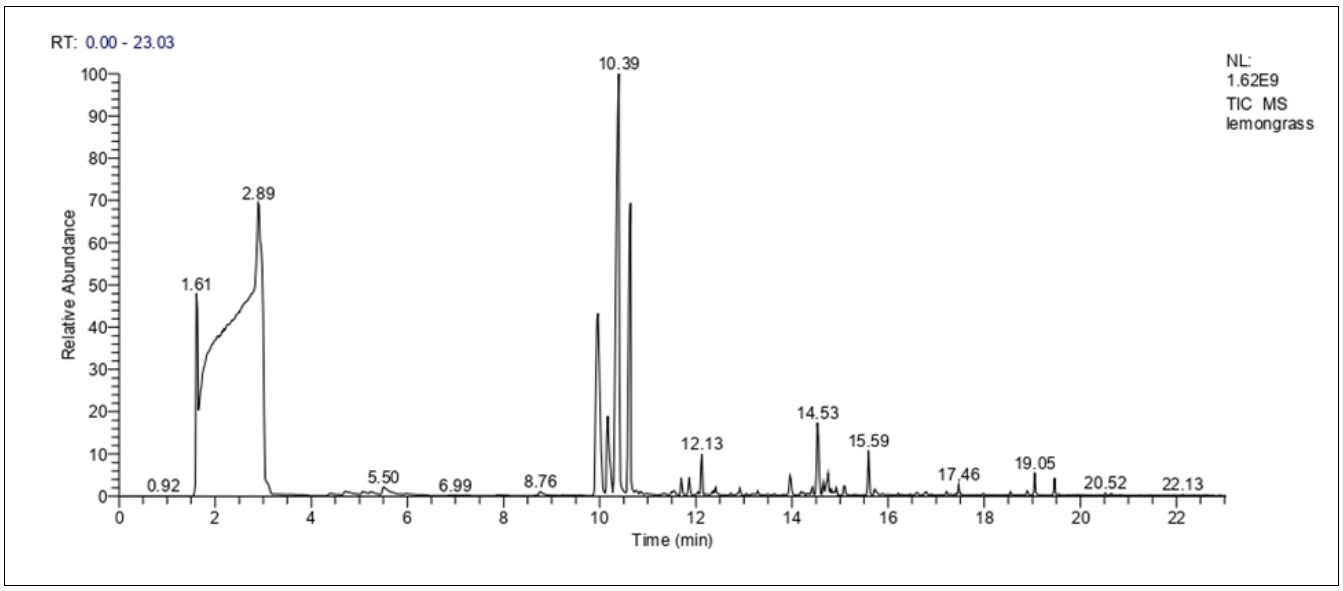


Fig 5: GC-MS chromatogram of volatile compounds from *C. Citratus*

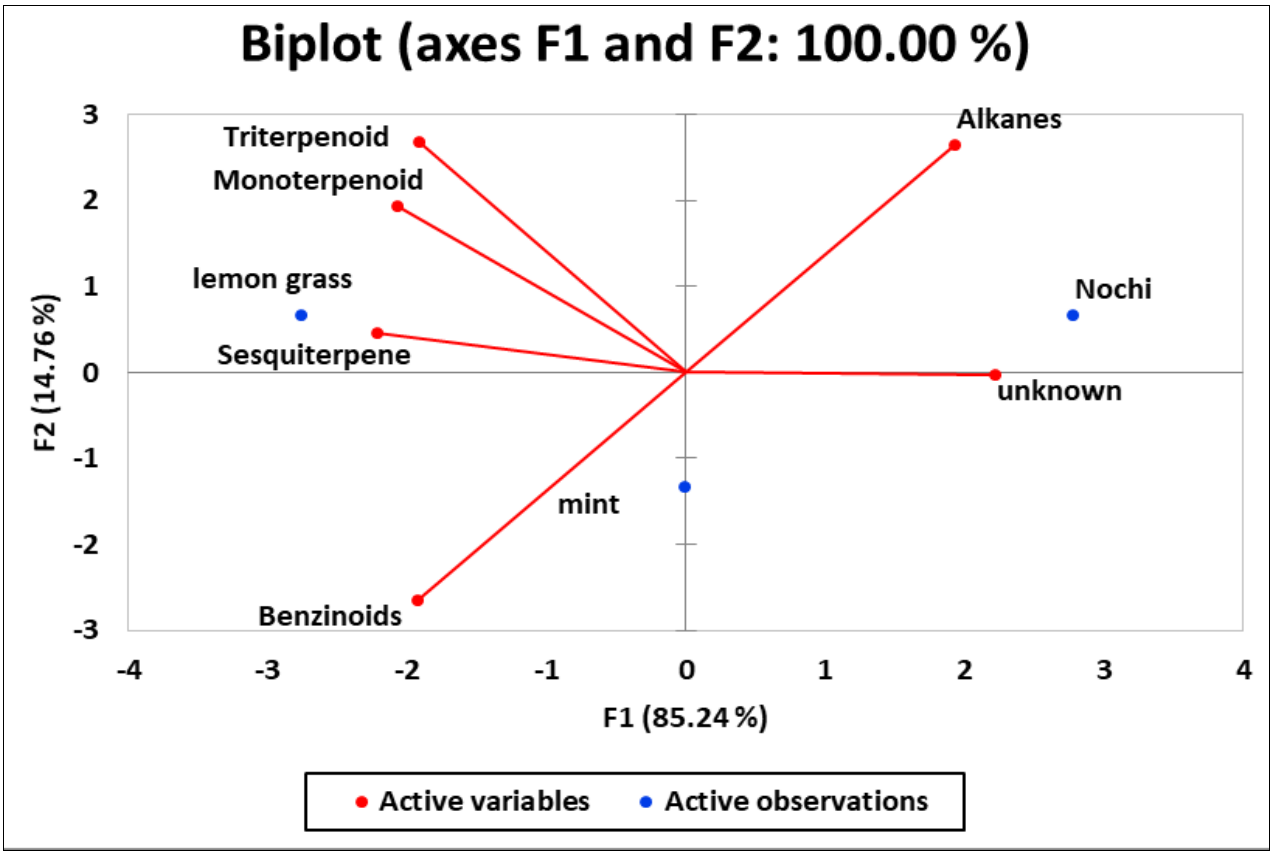


Fig 6: PCA (bi-plot) analysis on the distribution of different classes of volatile organic compounds among the effective treatment; Mint (*M. spicata*); Lemon grass (*C. citratus*); Nochi (*V. negundo*)

Antifungal activity of VOC

The pure VOCs carvone, citronellol, and geraniol (Sigma Aldrich) were evaluated for their antifungal activities against *F. oxysporum* f.sp. *lycopersici*. Citronellol completely suppressed the mycelial growth of *F.oxysporum* f.sp. *lycopersici* at the concentration of 500 ppm (Fig. 7). Carvone expressed 90% inhibition of mycelia at a concentration of 500 ppm, while the geraniol was comparatively less effective in reducing the mycelial growth of *F.oxysporum* f.sp. *lycopersici* (Table. 4). The mycelial agar plug of *F. oxysporum* f.sp. *lycopersici* retrieved from inhibited test plate was unable to grow on fresh PDA in the absence of the tested volatile compounds. The volatile of citronellol and carvone inhibited the growth of pathogen, causing abnormality in hyphal growth and conidial germination as compared to the mycelia in the control plate (Fig. 8).

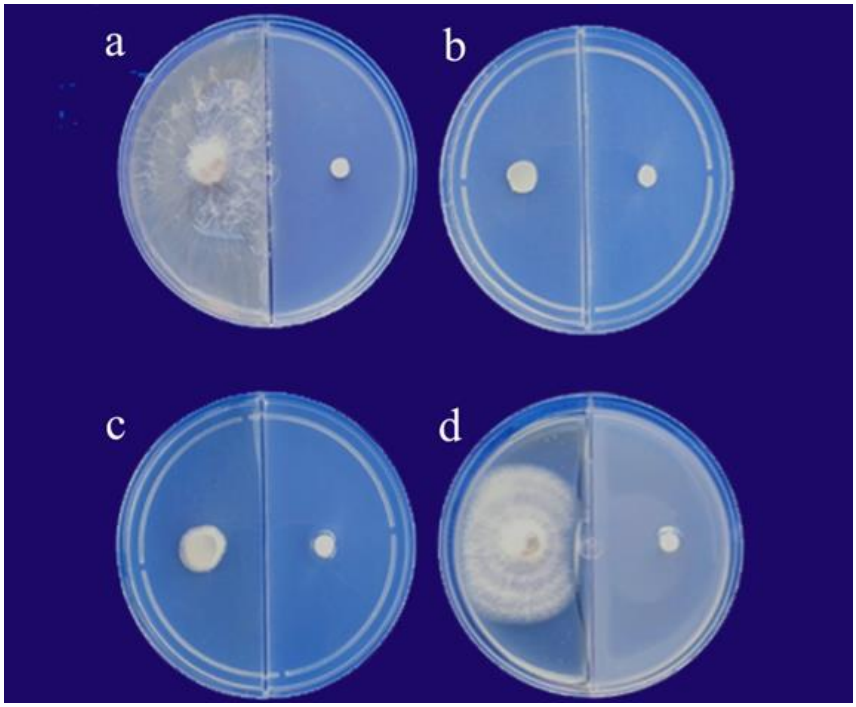


Fig 7: Effect of synthetic volatile compounds on the inhibition of mycelial growth of *F. oxysporum* f.sp. *lycopersici Control (F.o. f.sp. lycopersici), b. Citronellol treated plate (500 ppm), c. Carvone treated plate (500 ppm) and d. Geraniol treated plate (500 ppm).*

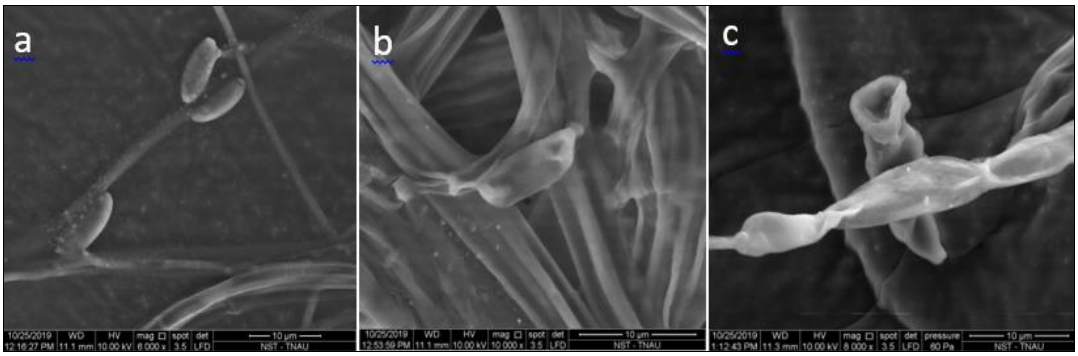


Fig 8: Scanning electron microscopy of *F. oxysporum* f.sp. *lycopersici* (a) on exposure to citronellol (b) and carvone (c)

Table 4: Effect of VOCs on the inhibition of mycelial growth of *F.o.* f.sp. *lycopersici*

Volatile compound	Mycelia growth inhibition (mm) at different concentration of volatiles				
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Carvone	45.49^{b}	44.71^{b}	57.65^{ab}	83.92^{ab}	90.98^{a}
Citronellol	22.75^{e}	35.69^{d}	72.16^{c}	87.45^{b}	100.00^{a}
Geraniol	0.00^{e}	3.14^{d}	9.02^{c}	35.69^{b}	41.96^{a}
Control	0.00	0.00	0.00	0.00	0.00

FT-IR analysis of *M. spicata* and *C. citratus* leaves

Transmission spectra were obtained from the volatiles of *M. spicata* and *C. citratus* leaves. The peaks assigned to the corresponding functional groups are shown in Fig. 9 & 10; Table. 5 & 6. The results of FT-IR analysis from the *M. spicata* leaves confirmed the presence of benzene derivative, alcohol (O-H) and alkane (C-H) in the regions between 726.06 cm^{-1} and 3742.19 cm^{-1}. The peak at 2927.41 cm^{-1} confirms the presence of alkane C-H stretching with medium intensity, which strongly matched with standard compound of carvone (Sigma Aldrich) at peak 2924.52 cm^{-1}. The spectra of volatiles from *C. citratus* leaves reveal the presence of primary alcohol (C-O), alkane (C-H) and alcohol (O-H) in the regions between 1051.01 cm^{-1} and 3734.48 cm^{-1}. The absorption peak at 1051.01 cm^{-1} confirmed as primary alcohol C-O stretching with strong intensity. The band at 1051.01 cm^{-1} from *C. citratus* leaves highly matched at the peak intensity (1055.84 cm^{-1}) of standard citronellol (Sigma Aldrich). The presence of exact functional group with standard compounds indicated the existence of wide range of potential phytomolecules in the leaves of *M. spicata* and *C. citratus.*

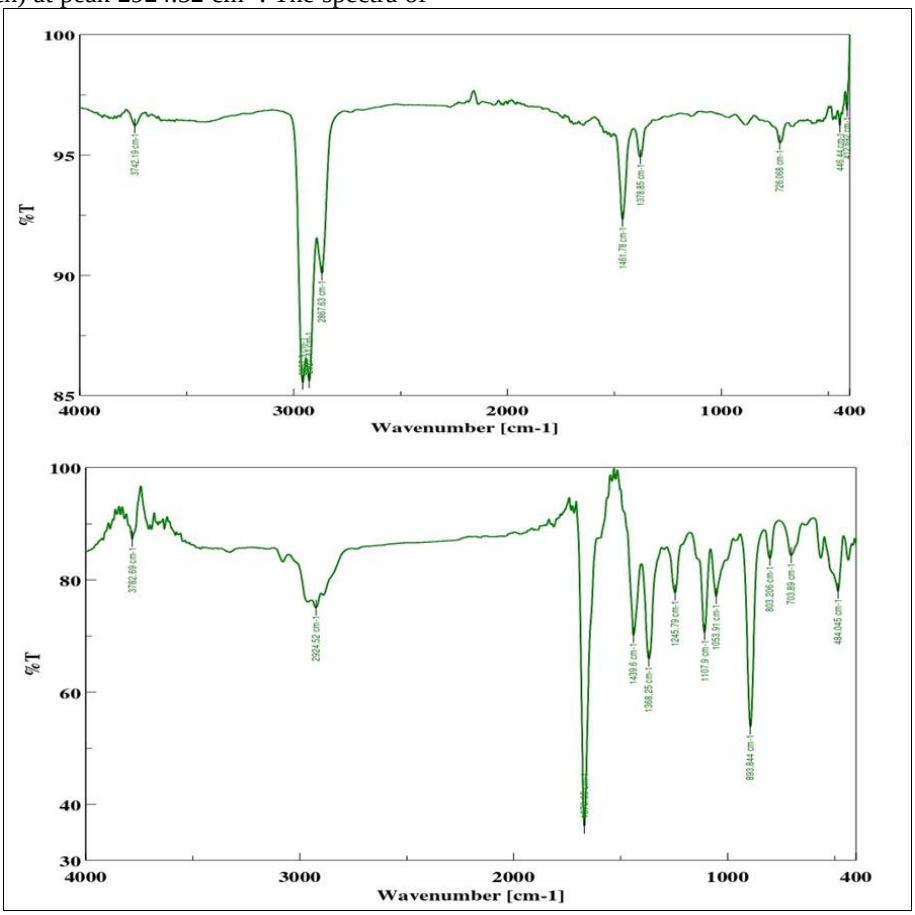


Fig 9: FT-IR analysis of *M. spicata*
FT-IR spectra of (a) *M. spicata* and (b) Standard compound Carvone from Sigma Aldrich

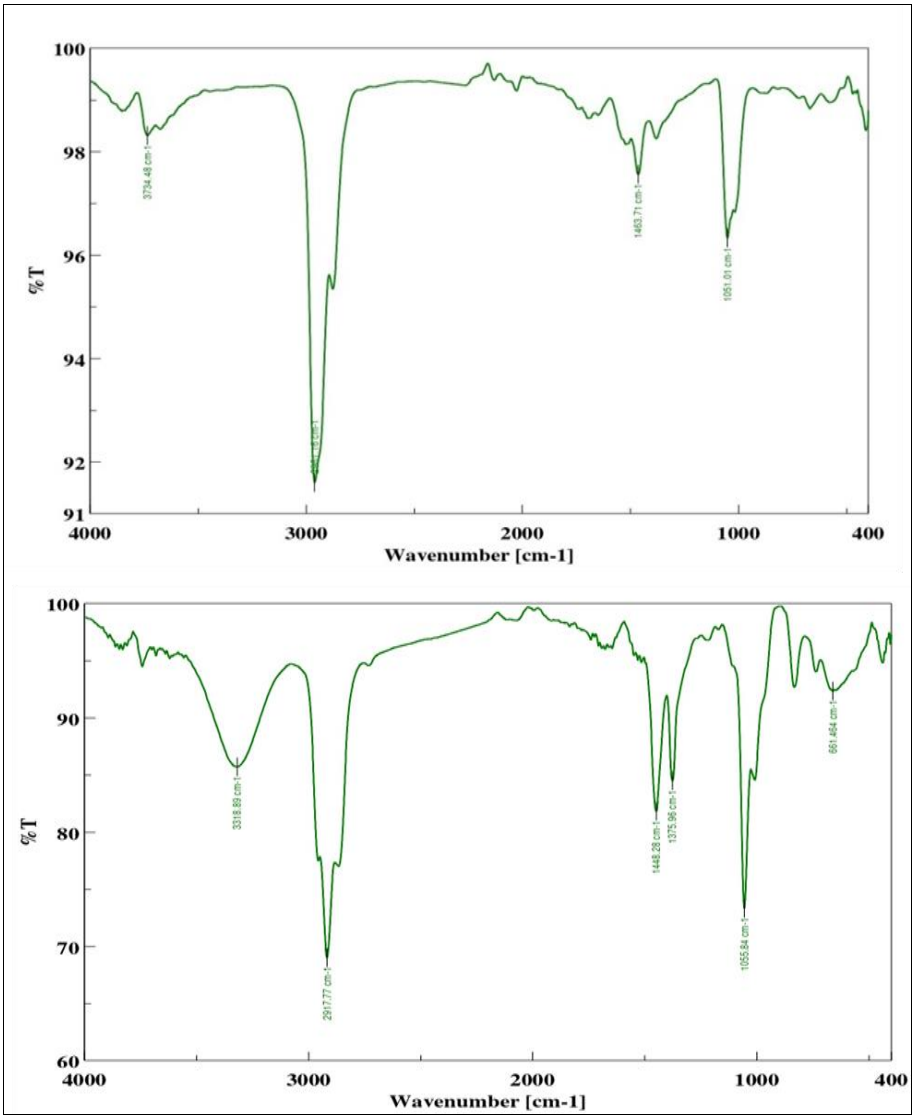


Fig 10: FT-IR analysis of *C. citratus* FT-IR spectra of (a) *C. citratus* and (b) Standard compound Citronellol from Sigma Aldrich

Table 5: FT-IR spectroscopy analysis of *M. Spicata*

S. No	Functional classification	Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
1.	Benzene derivative	-	-	726.06	-
2.	Alcohol	O-H	Bend	1378.85	Medium
3.	Alkane	C-H	Bend	1461.78	Medium
4.	Alkane	C-H	Stretch	2867.63	Medium
5.	Alkane	C-H	Stretch	2927.41	Medium
6.	Alkane	C-H	Stretch	3057.3	Medium
7.	Alcohol	O-H	Stretch	3742.19	Medium

Table 6: FT-IR spectroscopy analysis of *C. citratus*

S. No	Functional classification	Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
1.	Primary alcohol	C-O	Stretch	1051.01	Strong
2.	Alkane	C-H	Bend	1463.71	Medium
3.	Alkane	C-H	Stretch	2961.76	Medium
4.	Alcohol	O-H	Stretch	3734.48	Medium

Discussion

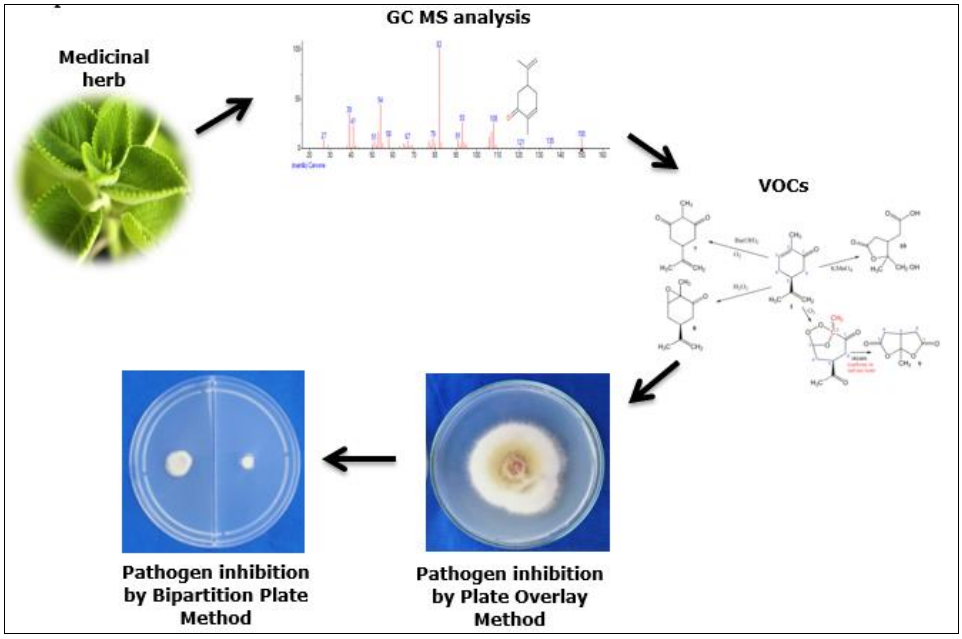
In this study, the antifungal nature of VOCs produced by seven medicinal herbs against *F. oxysporum* f.sp. *lycospercisi* were determined. The results of *in-vitro* screening displayed that VOCs emitted from *M. spicata* and *C. citratus* could inhibit the mycelial growth and bring out morphological changes in *F. oxysporum* f.sp. *lycospercisi.* Earlier author reported that the essential oils of medicinal plants caused certain morphological changes on the mycelial growth of *Penicillium digitatum*, *Didymella bryoniae*, *Colletotrichum lindemuthianum*, *Fusarium solani*, *Rhizoctonia solani* and *Pythium ultimum*[10]. The maximum mycelial growth inhibition in the present study was noticed with the VOCs of *C. citratus* (60 to 62 mm), followed by *M. spicata* (54 to 59 m) suggesting that the efficacy of VOCs of mint and lemongrass volatiles may be diverse and variable in nature. VOCs produced by the essential oils including *Citrus lemon*, *Olea europaea*, *Trachiyspirum ammi*, *Amygdalus communis*,

Psoralea corylifolia, *Lawsonia inermis*, *Mimosa pudica*, *Ocimum basilicum* and *Azadirachta indica* were harmful to a wide range of pathogenic fungi [25, 30, 24, 3, 5]. A scientist reported that certain VOCs such as isobutyric acid, 2-methyl-1-butanol and isobutanol released from *Muscodor albus* could suppress the mycelial growth of *Botrytis cinerea*, *Colletotrichum acutatum*, *C. coccodes*, *Geotrichum candidum*, *Monilinia fructicola*, *Penicillium digitatum* and *Rhizopus* spp[16]. In the present experiment also, a clear reduction in the linear mycelial growth was noticed when tested by plate overlay method suggesting that the volatiles emitted from *M. spicata* and *C. citratus* leaves had fungitoxic effect towards the target pathogen. This finding could be hypothetically considered as a useful tool for the suppression of wilt disease causing fungus through augmented application of soil mulch while designing Integrated Disease Management capsule.

In order to interpret the nature of VOCs produced by mint and lemongrass leaves, HS coupled with GC-MS was performed to trace VOCs even at a lower concentration through a headspace analysis. This method has been used to validate the VOCs emitted by fungi, bacteria, and yeasts by several workers [7, 9, 28]. The trapping of volatiles by air entrainment technique was followed as suggested by Sangeetha *et al.*, (2018). Stanley *et al.* (2018) also revealed on the use of air entrainment technique to trap volatiles emitted by the adult insect (*Conogethes punctiferalis*) from the sample chamber. The volatiles trapped from fresh leaves of *M. spicata* and *C. citratus* were further subjected to GC-MS analysis. The relative percentage of compounds present was detected in the headspace analysis. The most important volatile organic compounds produced by *M. spicata* and *C. citratus* leaves were identified as carvone, citronellol and geraniol. VOCs produced from leaves of *M. spicata* and *C. citratus* read through MS spectral library strongly supported the earlier results of authors [29, 23, 20] who have documented matching VOCs profile with various species of *M. spicata* and *C. citratus.* The FT-IR was performed to identify the functional group of organic components present in the leaves of *M. spicata* and *C. citratus* based on peaks shown in the region of IR radiation. The FTIR analysis from the leaves of *M. spicata* confirms the presence of alkane (C-H) functional group with medium intensity. Similarly, the leaves of *C. citratus* confirm the presence of primary alcohol (C-O) with strong intensity. This result highly matched with wavenumber frequency in standard compounds of carvone and citronellol.

Synthetic standard compounds emitting similar VOCs (Sigma Aldrich) were used to determine the best concentration against the target pathogen. *In-vitro* experiments revealed that citronellol could completely suppress the mycelial growth and bring out abnormalities in the mycelia of *F. oxysporum* f.sp. *lycopercisi* at the concentration of 500 ppm followed by carvone (over 90% suppression) at the same concentration. Aguiar *et al.*, (2014) observed that essential oils and citronellal has a strong effect on mycelial growth of *Pyricularia* (*Magnaporthe*) *grisea*, *Aspergillus* spp and *Colletotrichum musae* by volatile contact assay. [12] revealed that carvone has strong antifungal activities against mycelial growth of several phytopathogenic fungi *F. moniliforme*, *R. solani*, *S. sclerotiorum* and *Phytophthora capsici* by using filter paper disc sealed plate method this is in agreement with what was observed by [18, 13].

Use of VOCs having antimicrobial activity is the most important approach to be considered for effective designing of phyto-fumigation programmes for the management of *Fusarial* wilt pathogen infecting tomato in greenhouse cropping systems.



Graphical abstract

Conclusion

In this study, the experimental evidences clearly indicated the antifungal efficacy of volatile compounds produced by *M. spicata* and *C. citratus* leaves that could suppress the *F. oxysporum* f.sp. *lycopercisi* infecting tomato. In addition, such need to VOCs is formulated in a novel way so as to get slow release with effective dose for biofumigation of growth media or soil used in greenhouse cropping of tomato.

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