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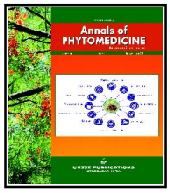

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Vapour phase mediated suppression of carvone and citronellol volatiles against *Fusarium oxysporum* f.sp. *lycospercisi*

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Abstract

Volatile organic compound (VOC) from plants known to have varied potential sources for antimicrobial activity against soil-borne pathogens. To evaluate *in vitro* activity of compounds, the antifungal activity of volatile organic compounds of carvone and citronellol (Sigma Aldrich) against spore germination of *F. oxysporum* f.sp. *lycopercisi* were explored. The minimum fungicidal concentration (MFC) was determined by vapour phase mediated antifungal assay (VMAA) using standard 96 well microtitreplates. We also investigated the nature of biological activity (Fungicidal or fungistatic) on spore germination of *F. oxysporum* f.sp. *lycopercisi*. VOCs of standard carvone inhibited the spore germination with MFC of 200 µl; which significantly correlated with volatile extract of *M. spicata* and effectively kills 80% of inoculum after 2 hours of exposure. A minimum colony of inoculum (CFU/200 µl) was observed on exposure to standard carvone, volatile extract of *M. spicata*; which showed fungicidal in nature than standard citronellol and *C. citratus* volatiles. This result indicated that VOCs of standard carvone could involve in suppression of *F. oxysporum* f.sp. *lycopercisi*. Furthermore, the study is needed to describe the development of antifungal volatile formulation as a new component of biocontrol strategy.

1. Introduction

Volatile organic compounds (VOCs) produced by odorous herb, plants and several microbial pathogens have significant role on control of phytopathogens.VOCs induced by plant herb as an eco-friendly acceptable method to enhance plant protection strategy. However, VOCs now mostly applied in the field of plant protection to repel or attract herbivores, parasitoids or predators (Moraes *et al.*, 1998; Dicke and Baldwin, 2010). VOCs are organic chemicals with low molecular weight and high vapour pressure on low boiling temperature (Vespermann *et al.*, 2007; Bennett *et al.*, 2012).

In current scenario, VOCs are produced by a wide range of plants, animals and microorganisms including fungi, bacteria, molds and yeasts. Such VOCs are called as biogenic volatile organic compounds (BVOCs), which are most commonly of alcohols, terpeniods and carbonyls. These compounds of volatiles may have potential antimicrobial activity or microbial interactions and may act as defense signalling mechanism (Bennett *et al.*, 2012). Several odorous herb inducing VOCs could be used as biofumigants or as phyto-fumigants or their mechanism can be used independently. Brilli *et al.* (2011) reported that there is evidence to suggest that green leaf volatiles (GLVs) from leaves of *Dactylis glomerata* and *Populus alba*, produce Z-3-hexenyl acetate, which is rapidly released after mechanical damage of leaf tissues and induced defense mechanism against fungal pathogen of *F. graminearum* in wheat (Ameye *et al.*, 2015). Some VOCs such as methyl salicylate (MeSA), camphene and pinene from *Arabidopsis*, actively involved in the defense mechanisms, leading to systemic acquired resistance (SAR) (Dempsey and Klessig, 2012; Riedlmeier *et al.*, 2017). Neri *et al.* (2007) reported that the plant volatiles of citral, carvacrol, and trans-2-hexenal were purchased from Sigma Aldrich and tested *in vitro*, found to be effective in mycelial growth and conidial germination of *Monilinialaxa*, the agent of brown rot of stone fruit. *In vitro* efficacy of VOCs from the flower of sweet orange (R-limonene and linalool) were moderately inhibited the mycelial growth of *Colletotrichum acutatum*, causing citrus post-bloom fruit drop. Similarly, Quintana-Rodriguez *et al.* (2018) reported that the premilinary screening of 22 different VOCs from the leaves inhibited the mycelial growth of fungal pathogens, *Colletotrichum lindemuthianum*, *Fusarium oxysporum* and *B. cinerea* when exposed to VOCs of nonanal, (+)-carvone, citral, trans-2-decenal, L-linalool, or nerolidol (Sigma Aldrich) by filter paper dish method.

Recently, Feyaerts *et al.* (2018) showed that EOs of *O. compactum*, *A. dracunculus* and *C. camphora* with their corresponding VOCs of carvcacrol, estragole, linalool, transcinnamaldehyde and citral were tested against *Candida* activities through vapour phase mediated assay and considered a novel class of antifungal compounds. In this context, use of VOCs producing medicinal herb could represents as a smart and attractive strategy for the control of soil borne disease of vegetable crops.

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In this present study, we introduce the vapour phase mediated assay to detect the antifungal activity of standard compounds carvone and citronellol (Sigma Aldrich) in comparison with volatile extract of medicinal herb produced by the leaves of *M. spicata* and *C. citratus.*

2. Materials and Methods

2.1 Materials

Pure standard VOCs, carvone and citronellol were purchased from Sigma Aldrich and stored at –20°C. These VOCs and volatile extract of *M. spicata, C. citratus* were used for testing the conidial inhibition of *F. oxysporum* f.sp. *lycopersici*. The volatile extract of *M. spicata* and *C. citratus* was done using air entrainment technique as described by Praveen *et al.* (2021) trapped volatiles were eluted with one ml of hexane for VMAA assay.

2.2 Methods

2.2.1 Isolation of *F. oxysporum*

F. oxysporum f.sp. *lycopersici* was isolated from wilt infected tomato plant and cultured on potato dextrose agar (PDA) medium at 25°C for 7 days. The conidial spores were obtained by flooding the cultures with sterile distilled water containing 0.05% (v/v) tween 80 and adjusted to 1×10^3 spores/ml.

2.2.2 Preparation of cell inoculum

A small loop of well grown conidia was collected from 7 days old culture, suspended in 1x phosphate buffered solution (8 g sodium chloride, 0.2 g potassium chloride, 1.44 g of disodium hydrogen phosphate, 0.24 g potassium dihydrogen phosphate in 1000 ml of sterile water). The cell density of suspended solution was measured by optical density at 600 nm (OD_{600}). Then, the suspended cell was mixed in Rosewell Park Memorial Institute-1640 medium (RPMI 1640, Sigma-Aldrich). The final cell inoculum of *F. oxysporum* f.sp. *lycopersici* was prepared as mentioned in the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.2.3 Vapour phase mediated antifungal susceptibility assay

The vapour phase mediated susceptibility assay (VMS) was performed in 96 well polystyrene microtiter plate. In the standard design, 200 µl ($5x10^3$ cfu) suspended inoculum of *F. oxysporum* f.sp. *lycopersici* was added to all 96 wells in microtiter plate except for well D/E 6-7. The volatile compound was added to well D/E 6-7 (500 µl) as volatility centre with slight modification as described by Feyaerts *et al.* (2018). Whereas, the blank was served without cell inocula to the well A-H, 1 and 12 or a separate microtiter plate was maintained to serve as blank. The microtiter plates were kept in the lid; covered with aluminium foil and incubated at room temperature. The periodical observation of cell density for each run of plate was performed at different hour *viz.*,0, 0:30, 1:00, 1:30, 2:00 and 2:30 h. After incubation, the plate was measured at OD_{600} with a multi-well plate reader. After reading at $OD_{600,}$ the suspended cell inoculum in the 96 well plates were pipetted out and resuspended in PDA medium for fungistatic and fungicidal activity.

2.3 Statistical analysis

The experimental result was performed in triplicate and analyzed using XLSTAT. The data were transformed and analyzed using completely random design (CRD). The DMRT test was performed by using SPSS statistical software, as suggested by Gomez and Gomez (1984).

3. Results

3.1 Vapour-phase mediated antifungal assay (VMAA)

Vapour phase mediated assay is a volatile assay, developed to characterize the behaviour and activity of volatile organic compounds using 96 well microlitre plates. VOCs of carvone and citronellol added to centre of four wells as volatility centre (Figure 1). Around these four wells, each well near to volatility centre makes up a new distance between them. The distance between the volatility centre and the other wells were designed from one cm to four cm, in which the VOCs (Volatility centre) could travel to the corresponding wells. The plate was kept incubated under different time period and predicted with spectrophotometric reader. In this, the conidial inhibition was signficantly increased in the nearest well (1 cm and 2 cm distance well) than the farthest well (4 cm) to the volatility centre and highly inhibited after one hour of treatment. Similarly, the volatile extract of *M. spicata* showed 80% similar result when compared with standard synthetic VOCs than volatiles of *C. citratus*. The growth of inhibition was visually observed in the petriplate by transferring the conidial suspension (200 µl) after different periods of spectrophotometric observation. A spectrophotometric reader showed that 95% reduction of conidial suspension of *F. oxysporum* f.sp. *lycopersici* compared to control suspension.

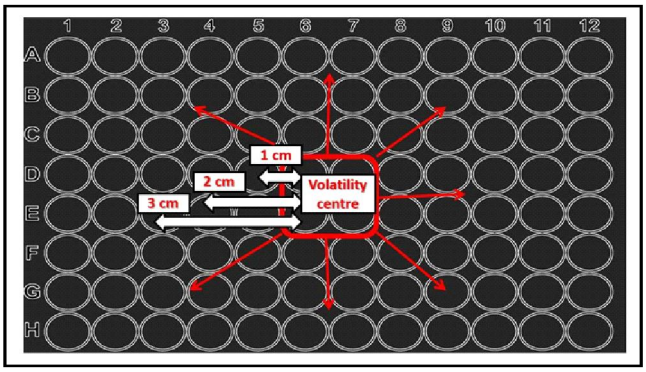

Figure 1: Illustration of vapour mediated antifungal assay in 96 well microtitre plate.

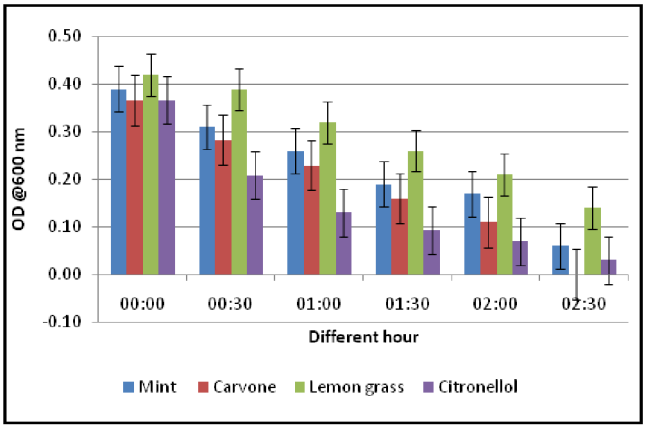

Figure 2: Graph illustrating the spectrophotometric reading of volatiles over the microtitre plate on different hours of incubation.

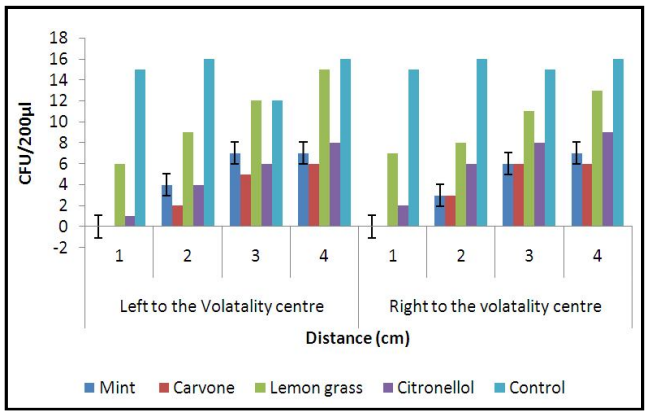

Figure 3: Graph representing the fungicidal or fungistatic nature of pathogen on culture media at different equidistant wells.

3.2 Fungistatic and fungicidal acitivity of *F. oxysporum* f.sp. *lycopersici*

The fungistatic and fungicidal activity of *F. oxysporum* f.sp. *lycopersici* was performed after periodical observation on spectrophotometric reader. The conidial suspension (200 µl) from 96 well microliter plates was transferred to the sterile plastic petriplate followed by adding the potato dextrose agar (PDA) medium and kept incubated at room temperature for 5 days. Each distance of conidial suspension from the volatility centre also followed with the same procedure. After incubation, the growth of conidial spores was counted and validated. In this, carvone treated plates were completely suppress spore germination in the nearest well (1 cm distance well) whereas, the conidial growth was observed in the 3 cm and 4 cm distance well accounting to average of 6 x10^3 cfu. Whereas, in *M. spicata* treated microtitre plate, the conidial count was completely inhibited in the nearest well (1 cm) and having 7 x 10^3 cfuat 4 cm well than in control plate (416 x 10^3 cfu) as presented in Table 1. Therefore, carvone treated plate and volatile extract of *M. spicata* was found to be fungicidal in nature at nearest well than farthest one. Volatile extract of *C. citratus* treated plate were observed as fungistatic activity.

4. Discussion

Over the years, antimicrobial screening of VOCs has shown as an interesting phenomenon in the field of crop protection and used as alternative biological control against wide range of phytopathogens. The selection of odorous plant volatiles represents an efficient method to identify the important VOCs for use in management of several phytopathogens. In this study, antifungal activity of carvone, citronellol, volatile extract of *M. spicata* and *C. citrates* were determined against spore germination of *F. oxysporum* f.sp. *lycopersici*. The result of VMA assay shown that the VOCs of carvone were found to be more effective in suppression of conidial spores of *F. oxysporum* f.sp. *lycopersici*, followed by citronellol with least inhibition. A similar strategy was previously reported by Feyearts *et al.* (2017); the strongest antimicrobial activity was observed near the centre of volatility and gradually decreased the spore inhibition of *C. albicans* and *C. glabrata* towards the end of microtitre plate on exposure to citronellol (Sigma Aldrich). In the present study, similar results were observed that the microtitre plate containing conidial suspension on exposure to volatile extract of *M. spicata*; minimum inhibition was displayed by volatile extract of *C. citratus*. In order to identify the nature of VOCs of carvone and citronellol, the fungistatic and fungicidal activity of *F. oxysporum* f.sp. *lycopersici* were determined. Our results showed that carvone and volatile extract of *M. spicata* had a fungicidal effect on conidial germination of *F. oxysporum* f.sp. *lycopersici*. VOCs of citronellol and volatile extract of *C. citratus* showed fungistatic activity. Kaddes *et al.* (2019) revealed that methyl prop-2-enoate and methyl propanoate has a fungicidal effect against *F. culmorum and C. sativus*. Zirihi *et al.* (2008) also revealed on the use of natural extract of *Combretum racemosum* had fungicidal effect towards the mycelial growth of fungal pathogens in tomato crops. The present study demonstrated, that the VOCs produced by carvone and citronellol could play a strong effect in managing *Fusarium* wilt of tomato. In this finding, the VOCs of carvone and citronellol could be effectively suppressing the wide range of phytopathogens. Over the past years, many authors interestingly contributed their finding due to antimicrobial potential and used as an alternative biocontrol strategy against several phytopathogens. Thus, the VOCs produced from *M. spicata* and *C. citratus* could develop suitable volatile based organic fumigants in preventing the soil borne pathogens. However, the new experimental analysis is necessarily required to evaluate the contribution of each VOC in the antagonistic activity against soil borne fungal pathogens. To our knowledge, vapour phase mediated antifungal assay (VMAA) is the first study that concentrated on biological activity of *F. oxysporum* f.sp. *lycopersici* infecting tomato crops.

Table 1: Effect of VOCs on spore inhibition of *F. oxysporum* f.sp. *lycospercisi*

	Left to the volatality centre (cm)			Right to the volatality centre (cm)		
Treatment	**1**	**2**	**3**	**1**	**2**	**3**
M. spicata	0.00^{a}(0.71)	4.00^{b}(2.12)	8.00^{c}(2.92)	0.00^{a}(0.71)	3.00^{a}(1.87)	6.00^{a}(2.55)
Carvone	0.00^{a}(0.71)	2.00^{a}(1.58)	5.00^{a}(2.34)	0.00^{a}(0.71)	3.00^{a}(1.87)	7 .00^{b}(2.74)
C. citratus	6.00^{c}(2.55)	9.00^{c}(3.08)	12.00^{d}(3.54)	7.00^{c}(2.74)	8.00^{c}(2.92)	11.00^{d}(3.39)
Citronellol	1.00^{b}(1.22)	4.00^{b}(2.12)	6.00^{b}(2.55)	2.00^{b}(1.58)	6.00^{b}(2.55)	8.00^{c}(2.92)
Control	15.00^{d} (3.94)	16.00^{d}(4.06)	12.00^{d}(3.54)	15.00^{d}(3.94)	16.00^{d}(4.06)	15.00^{e}(3.94)
SED	0.01	0.02	0.03	0.02	0.03	0.03
CD	0.03	0.07	0.09	0.06	0.10	0.10

5. Conclusion

Research on plant disease management triggered by diverse VOC is highly expanding. In recent years, many VOC producing plant herbs are able to induce defense mechanism against pathogens. The response in plants to VOC is regulated, *via*, different biosynthetic pathways. More research is needed to optimize VOC based formulation to defend plant pathogens. In this study, it is strongly confirmed that volatilomes of mint (*M. spicata*) could have antimicrobial activity to fight against several plant pathogens for sustainable farming.

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Conflicts of interest

The authors declare that there are no conflicts of interest relevant to this article.

References

Ameye, M.; Audenaert, K.; De Zutter, N.; Steppe, K.; Van Meulebroek, L.; Vanhaecke, L.; Vleesschauwer, D.; Haesaert, G. and Smagghe, G. (2015). Priming of wheat with the green leaf volatile Z-3-hexenyl acetate enhances defense against *Fusarium graminearum* but boosts deoxynivalenol production. Plant Physiol., **167**:1671-1684.

Bennett, J.W.; Hung, R.; Lee, S. and Padhi, S. (2012). Fungal and bacterial volatile organic compounds: An overview and their role as ecological signaling agents. In: Hock (Ed.), Fungal Associations, The Mycota IXB. Springer-Verlag, Berlin Heidelberg.

Brilli, F.; Ruuskanen, T. M.; Schnitzhofer, R.; Müller, M.; Breitenlechner, M.; Bittneret, V.; Georg Wohlfahrt, G.; Loreto, F. and Hanse, A.(2011). Detection of plant volatiles after leaf wounding and darkening by proton transfer reaction "Time-of-Flight" mass spectrometry (PTR-TOF). PLoS One, **6**:e20419.

CLSI. (2008). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts - Third Edition: Approved Standard M27 A3. Wayne, PA, USA.

De Moraes, C. M.; Lewis, W. J.; Pare, P. W.; Alborn, H. T. and Tumlinson, J. H. (1998). Herbivore-infested plants selectively attract parasitoids. Nature, **393**:570-573.

Dempsey, D.A. and Klessig, D.F. (2011). SOS - too many signals for systemic acquired resistance? Trends Plant Sci., **17**:538-545.

Dicke, M. and Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: Beyond the 'cry for help'. Trends Plant Sci., **15**:167-175.

Feyaerts, A. F.; Mathe, L.; Luyten, W.; De Graeve, S.; Van Dyck, K.; Broekx, L. and Van Dijck, P. (2018). Essential oils and their components are a class of antifungals with potent vapour-phase-mediated anti-Candida activity. Scientific Reports, **8**(1):39-58.

Feyaerts, A. F.; Mathé, L.; Luyten, W.; Tournu, H.; Van Dyck, K.; Broekx, L. and Van Dijck, P. (2017). Assay and recommendations for the detection of vapour-phase-mediated antimicrobial activities. Flavour and Fragrance Journal, **32**(5):347-353.

Kaddes, A.; Fauconnier, M.L.; Sassi, K.; Nasraoui, B. and Jijakli, M.H. (2019). Endophytic fungal volatile compounds as solution for sustainable agriculture. Molecules, **24**:10-65.

Neri, F.; Mari, M.; Brigati, S. and Bertolini, P. (2007). Fungicidal activity of plant volatile compounds for controlling *Monilinia laxa* in stone fruit. Plant Dis**., 91**:30-35.

Praveen, T.; Krishnamoorthy, A.S.; Sivakumar, U. and Amirtham, D. (2021). Antifungal volatiles from medicinal herbs suppress *Fusarium oxysporum* f.sp. *lycopersici*. J. Entomol. Zool. Stud., **9**(2):1083-1093.

Quintana-Rodriguez, E.; Rivera-Macias, L. E.; Adame-Alvarez, R. M.; Torres, J. M. and Heil, M. (2018). Shared weapons in fungus-fungus and fungus-plant interactions? Volatile organic compounds of plant or fungal origin exert direct antifungal activity *in vitro*. Fungal Ecol., **33**:115-121.

Riedlmeier, M.; Ghirardo, A.; Wenig, M.; Knappe, C.; Koch, K. and Georgii, E.; (2017). Monoterpenes support systemic acquired resistance within and between plants. Plant Cell, **29**:1440-1459.

Vespermann, A.; Kai, M. and Piechulla, B. (2007). Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. Appl. Environ. Microbiol., **17**:5639-5641.

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