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RESEARCH ARTICLE

Biocontrol of *Colletotrichum falcatum* with volatile metabolites produced by endophytic bacteria and profiling VOCs by headspace SPME coupled with GC–MS

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Abstract A total of 49 endophytic bacteria isolated from sugarcane were screened in vitro for antagonistic property against *C. falcatum* through production of volatile organic compounds (VOCs). Among them, 27 bacteria produced volatiles with moderate inhibitory level, i.e. 30 to 50% and 9 produced volatiles with strong inhibitory properties, i.e. > 50% mycelial growth inhibition over control. The volatile compounds produced by *B. axarquiensis*—ESR 7 inhibited *C. falcatum* mycelia growth to the tune of 59.2% followed by *B. licheniformis*—ESR 26 (57.8%) and *B. subtilis*—ESR 24 (54.8%), respectively. The volatiles produced by bacteria not only inhibited the radial growth of mycelium but also suppressed the vertical expansion of mycelia and caused deformation in mycelia growth. The VOCs produced by 24 endophytic bacteria completely inhibited spore formation in *C. falcatum* culture. Profiling of antagonistic VOCs produced by bacterial strains ESR 7, ESR 24 and ESR 26 was done by head space-solid phase microextraction (SPME) coupled with gas chromatography mass spectral analysis. The analysis showed the presence of 63 compounds belonging to chemical groups of alcohols, esters, hydrocarbons, ketones, acids, amino acid, carbohydrates, ethers, aldehydes, amines and amides. Among the identified microbial volatiles, 6 compounds viz., acetic acid, methoxy-phenyl-oxime, octamethyl-cyclotetrasiloxane, 5,7-dimethyl-undecane, hexamethyl-cyclotrisiloxane and dodecane were reported in VOCs produced by all three bacteria. However, among 63 volatiles, only 31 were already reported to be produced by many bacteria and fungi and 11 compounds viz., acetic acid, hexanal, 2-ethyl-1-hexanol, undecane 5,7-dimethyl, undecane 3,7-dimethyl, 2-decanone, dodecane, 2-undecanone, 2-dodecanone, 1,2-Benzenedicarboxylic acid, diisooctyl ester and 2-methyl-hexadecanol were reported with antagonistic property against many plant pathogens. The study revealed that many VOCs produced by *B. axarquiensis*—ESR 7, *B. subtilis*—ESR 24 and *B. licheniformis*—ESR 26 play role in mediating antagonism against *C. falcatum*.

Keywords Sugarcane · Endophytic bacteria · Red rot · Biocontrol · VOCs · SPME · GC–MS

Introduction

Red rot caused by *Colletotrichum falcatum* Went. is regarded as disease of major importance in all the sugarcane growing countries (Chona 1980; Singh 2008). In India, red rot was first noticed in Godavari delta of Madras province (Barber 1901) after that a series of epidemics were reported in many parts of country (Chona and Padwick 1942; Satyavir 2003; Viswanathan and Samiyappan 2000). The pathogen is primarily sett borne and causes disease in all stages of crop; however, the more pronounced symptom and loss is noticed in stalk (Viswanathan and Rao 2011). Red rot infection on sugarcane reduces cane yield drastically and also affects the juice quality parameters such as brix value, sucrose content, purity and commercial cane sugar (Kumar et al. 2000; Satyavir 2003; Sharma et al. 2017). Yield loss ranging from 28 to 82 per cent has been reported in sugarcane due to red rot disease (Kirtikar and Verma 1962; Ahmad et al. 1986), and in few sugar factory

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areas, 100 per cent loss of crop due to red rot has also been reported (Viswanathan and Samiyappan 2000). In red rot affected canes, increase in total soluble salts, acidity, reducing sugars and reduction in sucrose and purity of cane juice was recorded (Singh and Waraitch 1977). The pathogen reduced 32.5% extraction, 39% commercial cane sugar (Satyavir et al. 2002) and caused 25–75% reduction in sucrose content (Viswanathan and Samiyappan 1999). Till date, red rot remains as century old unresolved problem in sugarcane cultivation affecting both livelihood of farmers and also causing economic loss to sugarcane-based industries in the country.

Among different practices followed for red rot management, planting of resistant variety was found to be the best way to overcome disease problem (Viswanathan and Alexander 1997). However, due to the development of new variant of this fungus, newly released resistant variety often becomes susceptible after some years of cultivation (Padmanaban et al. 1996; Yadav 2006). Application of fungicide also failed in field due to impervious nature of hard rind of sugarcane seed material (sett), bulky nature of sett required for planting, difficulty in application of fungicide to the grown-up crop, lack of sustainable protection by virtue of the long duration nature of crop, etc. (Kumar and Satyavir 1998). The biocontrol agent such as *Trichoderma harzianum*, *T. viride*, *Chaetomium globosum*, *Bacillus* spp., *Pseudomonas fluorescens*, *P. putida* were found to be strong competitors against *C. falcatum* in vitro, and among them, few were moderately effective in field (Kumar and Satyavir 1998; Viswanathan and Samiyappan 2002; Jayakumar et al. 2007; Singh et al. 2008; Hassan et al. 2011, 2012; Joshi et al. 2019b; Patel et al. 2019). Availability of completely effective biocontrol agents against *C. falcatum* under field condition is lacking due to the deep seated nature of pathogen (Viswanathan and Samiyappan 2000). Hence, there is a need for mechanism for enhanced permeation of the biocontrol agent in the hard rind and fibrous inner tissue of sugarcane and attack the pathogen in "pathogen zone" and provide sustainable protection to crop. In this direction, the choice of endophytes as disease management tool is thought to be promising.

Endophytic bacteria occupy internal tissues of plants and colonize an ecological niche similar to that of phytopathogens, which makes them as suitable biocontrol agents (Berg et al. 2005). Several endophytic bacteria were reported with antagonistic potential against pathogens of many crops such as wheat (Herrera et al. 2016), rice (Nagendran et al. 2014), cotton (Selim et al. 2017), potato (Berg et al. 2005), vegetable crops (Xia et al. 2015), fruit crops (Daungfu et al. 2019). Among many mechanisms of antagonism, the phenomenon known as induced systemic resistance (ISR) was reported in many endophytic bacteria (Kloepper and Ryu 2006). Bacterial endophytes also prevent disease in plant through synthesis of many novel antibiotics (Compant et al. 2010). Another possible less understood mechanism is production of volatile organic compounds (VOCs) by endophytic bacteria that are inhibitory to pathogens. The recent developments in solid phase microextraction (SPME) can extract the volatile metabolites produced by microorganisms in a short period of time, and gas chromatography–mass spectral (GC–MS) technique can analyse the complete composition of VOCs produced by microbes (Jeleń 2003). Profiling microbial VOCs (mVOCs) will provide insight into role and mechanisms of volatiles in disease control.

The literatures reveal many mechanisms of action of mVOCs on plant pathogens. The VOCs produced by *B. velezensis* caused morphological changes in the ultrastructure and organelle membranes of *Sclerotinia sclerotiorum* and also reduced lesions produced by pathogen on host plant (Massawe et al. 2018). The bacteria *B. subtilis* isolated from soil showed production of volatiles with antifungal property up to 93% against soil-borne pathogens, and these volatiles controlled even overwintered sclerotium of *S. sclerotiorum* (Liu et al. 2008). The volatiles produced by endophytic *P. putida* isolated from black pepper inhibited broad range of pathogens such as *Phytophthora capsici, Pythium myriotylum, Gibberella moniliformis, Rhizoctonia solani, Athelia rolfsii* and *C. gloeosporioides* (Sheoran et al. 2015). Few of the volatiles such as 2, 3-butanediol and acetoin produced by endophytes triggered the plant growth promotion and also involved in inducing resistance of host plant against pathogens (Ryu et al. 2004). Several such results highlight the importance of VOCs in plant disease management. Profiling of such volatiles produced by bacteria showed production of wide variety of compounds comparable to those of plants and fungi (Farag et al. 2006; Schulz and Dickschat 2007); however, their ecological function is largely unknown. Hence, profiling mVOCs and identification of active compounds will serve as important source through which disease management can be addressed. Considering the importance of volatiles produced by microbes, the present experiment was undertaken to assess the in vitro fungistatic property of VOCs produced by endophytic bacteria and to analyse their complete composition.

Materials and Methods

Endophytic bacteria

A total of 49 endophytic bacteria isolated from root, stem (cane) and buds of healthy sugarcane varieties viz., Co 86032 and BO 91 and maintained in the culture collection

of Plant Pathology section, ICAR-Sugarcane Breeding Institute (ICAR-SBI), Coimbatore were utilized for the present experiment. All these bacteria were cultured on nutrient agar (NA) medium and maintained on NA slants.

Pathogen isolate

The virulent isolate of *C. falcatum* Cf671 available in the culture collection of Plant Pathology section, ICAR-SBI, Coimbatore was used throughout the experiment. The fungus was plated on oat meal agar (OMA) medium and sub-cultured on slants.

Assessing antagonistic properties of VOCs

The antagonistic potential of VOCs produced by endophytic bacteria was tested in vitro against *C. falcatum* by following the sealed plate method (Fernando et al. 2005). Each bacterium was streaked onto NA medium in the bottom of Petri dish. From actively growing culture of *C. falcatum*, 8 mm mycelia plug was cut and placed in the centre of the bottom dish of a second Petri plate containing OMA. The dish containing the fungal mycelial disc was inverted over the bacterial plate, and both the dishes were sealed with parafilm to prevent the escape of volatiles. In the same set-up, the plates with *C. falcatum* on OMA and NA medium without any bacterium served as control. Three replicates were maintained for each bacterium, and the plates were incubated at room temperature (28 ± 2 °C), and the radial growth of the fungus was measured at the time when the fungal growth in the control plates reached full plate. The antagonistic property was calculated in terms of inhibition of radial and vertical growth of *C. falcatum* mycelia and inhibition of sporulation. The mycelia radial growth inhibition was calculated using the following formula,

$$I = (C - T) \times 100/C$$

where C = Mycelia growth of *C. falcatum* in control plate, T = Mycelia growth of *C. falcatum* in sealed plate with bacteria, I = Inhibition of mycelia growth (%).

Inhibition of vertical growth of mycelium and sporulation in volatile exposed plates was recorded qualitatively by comparing the growth and sporulation in control plate. In case of mycelia growth, the deviation from normal growth, i.e. suppression of mycelia growth and any other visual changes were recorded, while the occurrence of orange colour sporulation were compared with control plate and recorded as concentrated, normal, sparse and no sporulation.

Analysis of VOCs by headspace SPME-GC–MS

Three endophytic bacteria viz., ESR 7 (*B. axarquiensis*), ESR 24 (*B. subtilis*) and ESR 26 (*B. licheniformis*) those showed efficient antagonistic properties through production of VOCs were selected for analysis.

Collection of VOCs

Headspace volatiles produced by the bacterial endophytes were collected as per the method described by Crespo et al. (2008). The NA medium was poured into the 20 ml head space vials, closed with cotton plug, sterilized, and NA slants were prepared. The caps of vials were sterilized separately. Each bacterial culture was streaked onto separate NA slants in headspace vials, closed with cap, and maintained at room temperature (28 ± 2 °C) for 3 days for the bacteria to grow. The bacterial cultures with trapped volatiles inside the headspace of vials were used for analysis.

Volatile analysis by GC–MS

The volatiles produced by endophytic bacteria were collected using the technique of SPME. The SPME syringe equipped with fibre (50/30 divinylbenzene/carburen on polydimethylsiloxane) inserted directly into the head space of vial by auto-sampler combined with agitator and exposed to the volatiles for 40 min to entrap the volatile compounds. The fibre containing the volatiles was then automatically injected into Agilent 7890A GC–MS equipped with silicon capillary column (30 m × 0.25 mm × 0.25 µm) for analysis. After desorption, the oven temperature was initially held at 40 °C for 2 min, increased to 150 °C at a rate of 2 °C min^{-1}, further increased to 280 °C at the rate of 10 °C min^{-1} and held for 2 min at 280 °C. The carrier gas used was helium with flow velocity of 1.0 ml min^{-1}, and ionization voltage was 70 eV. Mass spectra were scanned from 35 to 350 amu, and identification of volatile compounds produced by bacteria was made using spectral matches of National Institute of Standards and Technology (NIST) library.

Statistical analysis

Data representing radial growth of mycelia were assessed by Kolmogorov–Smirnov test, Shapiro–Wilk's test and Levene's test to check the assumptions of normality and homogeneity of variance (Thode 2002). The data were then analysed by one-way ANOVA, and the significant differences between the means were compared by Duncan test at the significance level of $P \leq 0.05$ (Hsu 1996; Sileshi 2012). All statistical analyses were performed using the

software—IBM SPSS statistics 21.0 (SPSS, Chicago, USA).

Results

Antagonistic potential of VOCs produced by endophytic bacteria

The volatiles produced by all 49 endophytic bacteria showed fungistatic effect on *C. falcatum*. The antagonistic properties were exhibited either on mycelia growth or sporulation of *C. falcatum* and sometimes both (Table 1). Among 49 endophytes tested, the volatiles produced by 9 bacteria exhibited high (> 50%) inhibitory action on radial growth of *C. falcatum* mycelia, 27 showed moderate inhibition (30 to 50%) and the remaining 13 bacteria produced volatiles with low inhibitory level (< 30%). In sealed plate method of testing, significantly ($P < 0.05$) lowest mean mycelia radial growth of 3.67 cm was recorded in *C. falcatum* co-cultured with ESR 7, followed by 3.8 cm in ESR 26 and 4.07 cm in ESR 24 co-cultured plates, while it was 9.0 cm in control (Fig. 1). In other words, the endophytic *B. axarquiensis*—ESR 7 produced volatile that inhibited *C. falcatum* mycelia growth to the tune of 59.2% followed by *B. licheniformis*—ESR 26 (57.8%) and *B. subtilis*—ESR 24 (54.8%). Among 9 efficient strains of bacteria 6 viz., ESR 7, ESR 14, ESR 21, ESR 24, ESR 26 and ESR 30 were endophytes isolated from roots and 3 viz., ESB 3, ESB 6 and ESB 7 were isolated from bud. The shoot isolates of endophytic bacteria were identified as producer of volatiles with poor antagonistic potential.

Qualitative assessment of vertical growth of *C. falcatum* in co-culture plates showed that except 8 bacteria the VOCs produced by all other bacteria suppressed the vertical expansion of mycelia. In the remaining 41 mVOCs producers two viz., ESR 30 and ESB 24 suppressed the mycelia growth of *C. falcatum* into very thin layer. The VOCs produced by few bacteria strains such as ESS 6, ESR 7, ESR 9, ESR 17, ESR 19, ESR 21, ESR 24, ESR 26, ESR 28, ESB 6 and ESB 16 caused deformation in mycelia, i.e. the volatiles caused production of fragmented and powdery mycelia. Overall, VOCs produced by various endophytic bacteria caused different effect on *C. falcatum* mycelia such as suppression of radial and vertical growth of mycelia, fragmented and patchy mycelia growth, restricted radial growth of mycelia with fluffy appearance in centre, deformation in mycelia, i.e. culture appearing as powdery growth and variation in colouration of mycelia. Assessing the effect of VOCs exposure on sporulation of *C. falcatum* culture showed that volatiles of 24 bacteria completely inhibited sporulation and 21 bacteria reduced spore formation of *C. falcatum* in culture plates. In contrary, in the co-cultured plates of ESS 3, ESS 16, ESS 35, ESR 22, ESR 29, ESB 5 and ESB 10 the suppression of growth of mycelium was noticed along with production of concentrated spores in the middle of radial growth.

Composition of VOCs produced by endophytic bacteria

The HS-SPME coupled GC–MS analysis of VOCs produced by bacteria isolates *B. axarquiensis*—ESR 7, *B. subtilis*—ESR 24 and *B. licheniformis*—ESR 26 showed the presence of 63 compounds (Table 2). The TIC of VOCs produced by ESR 7 showed the presence of 23 compounds with 12 sharp peaks (Fig. 2a). Analysis of mass spectrum revealed that the compound Silanediol, Dimethyl-($C_2H_8O_2Si$) corresponding to RT 5.21 was most abundant followed by compound Oxime-, methoxy-phenyl-($C_8H_9NO_2$) at RT 9.33 and Benzeneethanamine, N-[(pentafluorophenyl) methylene]-,beta.,4-bis[(trimethylsilyl)oxy]-($C_{21}H_{26}F_5NO_2Si_2$) at RT 13.61. The other prominent peaks were identified at RT 8.35, 11.37, 12.71, 14.15, 14.86, 16.42, 16.98 and 23.42. The total identified VOCs belonged to five major group viz., alcohols (4), acids (2), esters (2), hydrocarbons (15) and ketones (6) as per the matching compound in NIST database. The TIC presented in Fig. 2b identified 30 compounds in volatiles of ESR 24 strain. The six most abundant compounds identified in descending order were Oxime-, methoxy-phenyl-(RT 9.94), Pentane, 3-ethyl-2-methyl-(RT 20.80), Cyclotrisiloxane, hexamethyl-(RT 20.48), Cyclotetrasiloxane, octamethyl-(RT 14.67), Pentanoic acid (RT 15.34) and Benzeneethanamine, N-[(pentafluorophenyl) methylene]-.beta.,4-bis[(trimethylsilyl)oxy]-(RT 25.06). The bacteria isolate ESR 24 produced a wide group of compounds belonging to amino acid (1), alcohol (1), acids (4), esters (2), hydrocarbons (13), aldehydes (3), ketones (4) and amines (2). The TIC of volatile compounds produced by ESR 26 identified 29 compounds (Fig. 2c), and among them, two viz., Oxime-, methoxy-phenyl-(RT 10.14) and Cyclotrisiloxane, hexamethyl-(RT 20.52) were most abundant. The other six prominent peaks were identified at RT 1.62, 1.98, 6.07, 14.81, 25.07 and 27.42. The identified 29 compounds belonged to wide group of chemicals viz., amino acid (1), ester (1), ether (1), hydrocarbons (8), carbohydrate (1), hydrogen cyanide (1), acids (2), alcohol (1), ketones (6), aldehyde (1), amine (4) and amides (2).

Among the identified 63 compounds, 4 viz., ethyl benzene, 2-ethyl-1-hexanol, 1-ethynyl-4-methyl-benzene and benzeneethanamine, N-[(pentafluorophenyl)methylene]-, beta.,4-bis[(trimethylsilyl)oxy]- were identified in VOCs produced by both ESR 7 and ESR 24. The volatile compound 2-Decanone was produced by both ESR 7 and ESR

Table 1 Effect of VOCs produced by endophytic bacteria on the growth and sporulation of *C. falcatum*

S. no.	Isolate name	Radial growth of *C. falcatum* mycelia (cm)*	Mycelia growth inhibition over control (%)	Vertical growth of mycelia and appearance	Sporulation
Shoot isolates					
1	ESS 3	$6.27 \pm 0.15^{n-p}$	30.3	Suppressed growth	Sporulation concentrated at centre of radial growth
2	ESS 6	6.97 ± 0.06^{st}	22.6	Suppressed mycelia with powdery appearance	No sporulation
3	ESS 9	$6.70 \pm 0.10^{q-s}$	25.6	Normal growth	No sporulation
4	ESS 11	$5.93 \pm 0.15^{k-o}$	34.1	Suppressed growth	No sporulation
5	ESS 13	7.67 ± 0.06^{u}	14.8	Normal growth	Sporulation sparse
6	ESS 14	$6.50 \pm 0.30^{o-r}$	27.8	Suppressed growth	No sporulation
7	ESS 16	$6.37 \pm 0.15^{o-q}$	29.2	Suppressed growth	Sporulation concentrated at centre of radial growth
8	ESS 35	7.10 ± 0.20^{t}	21.1	Normal growth	Sporulation concentrated at centre of radial growth
9	ESS 40	$6.53 \pm 0.06^{p-r}$	27.4	Suppressed and white mycelia.	Sparse sporulation concentrated at centre of radial growth
Root isolates					
10	ESR 2	7.53 ± 0.06^{u}	16.3	Suppressed growth	No sporulation
11	ESR 3	5.07 ± 0.25^{h}	43.7	Suppressed growth	Sparse sporulation concentrated at centre of radial growth
12	ESR 4	$5.57 \pm 0.06^{i-k}$	38.1	Suppressed growth	Normal sporulation
13	ESR 7	3.67 ± 0.15^{a}	59.2	Suppressed and fragmented mycelia growth	No sporulation
14	ESR 8	$4.67 \pm 0.25^{d-g}$	48.1	Suppressed growth	No sporulation
15	ESR 9	5.10 ± 0.10^{h}	43.3	Suppressed & fragmented growth	No sporulation
16	ESR 10	$5.67 \pm 0.12^{i-k}$	37.0	Suppressed growth	No sporulation
17	ESR 11	$5.90 \pm 0.27^{j-n}$	34.4	Suppressed growth	No sporulation
18	ESR 12	$6.20 \pm 0.27^{m-p}$	31.1	Suppressed growth	No sporulation
19	ESR 13	5.50 ± 0.17^{ij}	38.9	Suppressed growth	No sporulation
20	ESR 14	$4.43 \pm 0.15^{c-e}$	50.8	Suppressed and white mycelia.	No sporulation
21	ESR 17	$4.67 \pm 0.15^{d-g}$	48.1	Fragmented mycelia growth	Sparse sporulation
22	ESR 18	$4.53 \pm 0.06^{d-f}$	49.7	Suppressed growth	Sparse sporulation
23	ESR 19	$6.50 \pm 0.36^{o-r}$	27.8	Suppressed and powdery mycelia growth	Sparse sporulation
24	ESR 21	4.10 ± 0.20^{bc}	54.4	Suppressed and fragmented mycelia growth	Sparse sporulation concentrated at centre of radial growth
25	ESR 22	$6.10 \pm 0.27^{l-o}$	32.2	Fluffy ash colour mycelia growth	Sporulation concentrated at centre of radial growth
26	ESR 23	$4.80 \pm 0.27^{e-h}$	46.7	Suppressed growth	No sporulation
27	ESR 24	4.07 ± 0.06^{bc}	54.8	Suppressed and fragmented mycelia growth	No sporulation
28	ESR 26	3.80 ± 0.17^{ab}	57.8	Suppressed, fragmented powdery mycelia growth	No sporulation
29	ESR 28	$4.90 \pm 0.20^{f-h}$	45.6	Suppressed & fragmented mycelia growth	No sporulation
30	ESR 29	5.50 ± 0.36^{ij}	38.9	Normal growth	Sporulation concentrated at centre of radial growth
31	ESR 30	4.50 ± 0.17^{de}	50.0	Highly suppressed growth	Very sparse sporulation.
32	ESR 31	$6.50 \pm 0.44^{o-r}$	27.8	Suppressed growth	Sparse sporulation
33	ESR 32	$6.50 \pm 0.10^{o-r}$	27.8	Suppressed growth	No sporulation
34	ESR 34	$6.83 \pm 0.15^{r-t}$	24.1	Suppressed growth	Sparse sporulation

Table 1 (continued)

S. no.	Isolate name	Radial growth of *C. falcatum* mycelia (cm)*	Mycelia growth inhibition over control (%)	Vertical growth of mycelia and appearance	Sporulation
Bud isolates					
35	ESB 1	$5.60 \pm 0.20^{i\text{-}k}$	37.8	Suppressed growth	No sporulation
36	ESB 3	$4.43 \pm 0.06^{c\text{-}e}$	50.8	Suppressed growth	Sporulation throughout the mycelia
37	ESB 5	$5.60 \pm 0.40^{i\text{-}k}$	37.8	Normal growth	Sporulation concentrated at centre of radial growth
38	ESB 6	4.10 ± 0.17^{bc}	54.4	Suppressed, fragmented white mycelia growth	No sporulation
39	ESB 7	4.30 ± 0.20^{cd}	52.2	Suppressed & fragmented mycelia growth	No sporulation
40	ESB 10	$5.83 \pm 0.06^{i\text{-}m}$	35.2	Suppressed growth	Sporulation concentrated at centre of radial growth
41	ESB 11	$5.97 \pm 0.06^{k\text{-}o}$	33.7	Suppressed growth	No sporulation
42	ESB 16	5.00 ± 0.27^{gh}	44.4	Suppressed, fragmented white mycelia growth	No sporulation
43	ESB 17	5.47 ± 0.12^{i}	39.2	Normal growth	Very sparse sporulation
44	ESB 19	$6.40 \pm 0.36^{o\text{-}q}$	28.9	Suppressed growth	Sporulation throughout the mycelia
45	ESB 20	$5.80 \pm 0.10^{i\text{-}l}$	35.6	Suppressed growth	Sporulation throughout the mycelia
46	ESB 22	$6.10 \pm 0.36^{l\text{-}o}$	32.2	Normal growth	Sparse sporulation
47	ESB 23	5.10 ± 0.17^{h}	43.3	Suppressed growth	Sparse sporulation
48	ESB 24	$4.63 \pm 0.06^{d\text{-}g}$	48.6	Highly suppressed growth	No sporulation
49	ESB 36	$5.70 \pm 0.44^{i\text{-}k}$	36.7	Normal growth	Sparse sporulation
50	Control	9.00 ± 0^{v}	–	Normal growth	Profuse sporulation throughout mycelia

*Values given are mean of 3 replications ± standard deviation

Different letters in the same column indicate significant difference at 5% level ($P < 0.05$) by Duncan's multiple range test

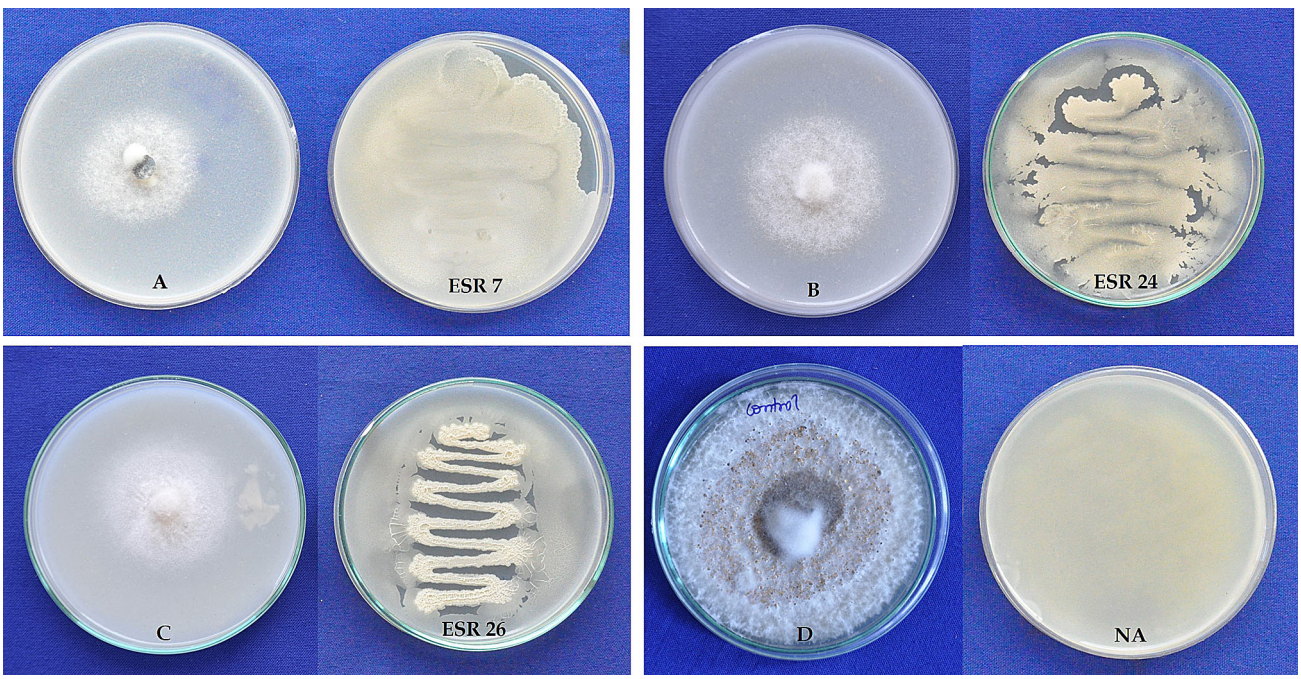

Fig. 1 Inhibitory effect of VOCs produced by endophytic bacteria on mycelia growth and sporulation of *C. falcatum*. **a** *C. falcatum* exposed to VOCs of ESR 7; **b** *C. falcatum* exposed to VOCs of ESR 24; **c** *C. falcatum* exposed to VOCs of ESR 26; **d** Control—*C. falcatum* exposed to NA medium

Table 2 Volatile organic compounds produced by *B. axarquiensis*—ESR 7, *B. subtilis*—ESR 24 and *B. licheniformis*—ESR 26 and their antifungal property

S. no.	Volatile compounds	Chemical formula	Presence of volatile compound and its RT*			Antifungal property	References
			ESR 7	ESR 24	ESR 26		
1	Alanine	$C_3H_7NO_2$	×	√ (0.17)	√ (0.19)	Unknown	–
2	Dimethyl ether	C_2H_6O	×	×	√ (1.62)	Unknown	–
3	4-Penten-1-yl acetate	$C_7H_{12}O_2$	×	√ (1.72)	×	Unknown	–
4	n-Hexylmethylamine	$C_7H_{17}N$	×	×	√ (1.98)	Unknown	–
5	1-Tetrazol-2-ylethanone	$C_3H_4N_4O$	×	×	√ (2.16)	Unknown	–
6	Cyclobutanol	C_4H_8O	×	×	√ (2.54)	Unknown	–
7	N-Chlorodimethylamine	C_2H_6ClN	×	√ (2.64)	×	Unknown	–
8	Silanediol, dimethyl	$C_2H_8O_2Si$	√ (2.85; 5.21)	×	×	No	Tahir et al. (2017)
9	1-Methyldodecylamine	$C_{13}H_{29}N$	×	×	√ (3.07)	Unknown	–
10	Benzene	C_6H_6	√ (3.28)	×	×	Unknown	–
11	(2-Aziridinylethyl) amine	$C_4H_{10}N_2$	√ (1.79; 3.91)	×	×	Unknown	–
12	Acetic acid	$C_2H_4O_2$	√ (4.42)	√ (3.10)	√ (3.53)	Yes	Farag et al. (2006), Giorgio et al. (2015), Lee et al. (2016)
13	Propane, 2-chloro-2-nitro-	$C_3H_6ClNO_2$	×	√ (3.95)	×	No	Heenan-Daly et al. (2019)
14	Butanoic acid, 1-methylethyl ester	$C_7H_{14}O_2$	×	×	√ (3.99)	No	Tait et al. (2014)
15	Toluene	C_7H_8	×	√ (4.11)	×	Unknown	–
16	Hexanal	$C_6H_{12}O$	×	√ (4.98)	×	Yes	Katoch et al. (2017)
17	Hydroxylamine, O-(phenylmethyl)-	C_7H_9NO	√ (5.03)	×	×	Unknown	–
18	2-Butanone, 3-methoxy-3-methyl-	$C_6H_{12}O_2$	×	×	√ (5.27)	Unknown	–
19	4-Methylbenzylidene-4-methylaniline	$C_{15}H_{15}N$	×	×	√ (6.07)	Unknown	–
20	Hexane, 3,3-dimethyl-	C_8H_{18}	√ (6.33)	×	×	No	Ajilogba and Babalola (2019)
21	2-Fluoro-3-trifluoromethyl benzoic acid, nonyl ester	$C_{17}H_{22}F_4O_2$	√ (6.90)	×	×	Unknown	–
22	Ethyl benzene	C_8H_{10}	√ (7.48)	√ (7.01)	×	No	Banerjee et al. (2010), Ajilogba and Babalola (2019)
23	*N*,*N*-Dimethylformamide diethylacetal	$C_7H_{17}NO_2$	×	√ (7.63)	×	Unknown	–
24	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	√ (8.04)	×	×	No	Tait et al. (2014)
25	Butanedioic acid, phenyl-	$C_{10}H_{10}O_4$	×	√ (8.21)		Unknown	–
26	Styrene	C_8H_8	√ (8.35)	×	×	No	Kanchiswamy et al. (2015), Lee et al. (2016)
27	2-Heptanone	$C_7H_{14}O$	×	√ (8.46)	×	No	Liu et al. (2008)
28	Butanimidamide	$C_4H_{10}N_2$	×	×	√ (8.47)	Unknown	–
29	(4H-[1,2,4]Triazol-3-yl) acetonitrile	$C_4H_4N_4$	×	×	√ (9.87)	Unknown	–
30	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	√ (9.33)	√ (9.94)	√ (10.14)	No	Tahir et al. (2017), Guneser et al. (2017), Gao et al. (2018)
31	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	√ (11.37)	√ (14.67)	√ (30.42)	No	Rath et al. (2018)
32	2-Heptanone, 6-methyl-	$C_8H_{16}O$	×	√ (11.74; 12.26)	√ (11.75)	No	Liu et al. (2008)
33	1-Hexanol, 2-ethyl-	$C_8H_{18}O$	√ (11.95)	√ (16.19)	×	Yes	Fernando et al. (2005), Chen et al. (2008), Liu et al. (2008), Che et al. (2017)
34	Benzene, 1-ethynyl-4-methyl-	C_9H_8	√ (12.22)	√ (16.56)	×	Unknown	–

Table 2 (continued)

S. no.	Volatile compounds	Chemical formula	Presence of volatile compound and its RT*			Antifungal property	References
			ESR 7	ESR 24	ESR 26		
35	Pentane, 2,3,4-trimethyl-	C_8H_{18}	×	×	√ (12.29)	Unknown	–
36	5-Chloro-1,3-dimethyl-1H-pyrazole-4-sulfonic acid, 2-methyl-5-trifluoromethyl-2H-pyrazol-3-yl ester	$C_{10}H_{10}ClF_3N_4O_3S$	×	×	√ (12.38)	Unknown	–
37	Undecane, 5,7-dimethyl-	$C_{13}H_{28}$	√ (12.39)	√ (17.86)	√ (21.21)	Yes	Fernando et al. (2005)
38	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	√ (12.71)	√ (20.48)	√ (20.52)	No	Rath et al. (2018)
39	Undecane, 3,7-dimethyl-	$C_{13}H_{28}$	√ (13.01)	×	×	Yes	Fernando et al. (2005)
40	Carbonic acid, butyl phenyl	$C_{11}H_{14}O_3$	×	√ (13.47)	×	Unknown	–
41	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-,beta.,4-bis[(trimethylsilyl)oxy]-	$C_{21}H_{26}F_5NO_2Si_2$	√ (13.61)	√ (25.06)	×	Unknown	–
42	2-Decanone	$C_{10}H_{20}O$	√ (13.68; 13.76)	×	√ (24.64)	Yes	Yuan et al. (2012), Che et al. (2017)
43	Furan, 2-pentyl-	$C_9H_{14}O$	×	√ (13.68)	×	No	Farag et al. (2006)
44	Napthalene	$C_{10}H_8$	√ (14.09)	×	×	No	Che et al. (2017)
45	Dodecane	$C_{12}H_{26}$	√ (14.15)	√ (27.42)	√ (27.42)	Yes	Fernando et al. (2005), Farag et al. (2006), Zou et al. (2007), Massawe et al. (2018)
46	2,2-Dimethyl-3-heptanone	$C_9H_{18}O$	×	×	√ (14.21)	Unknown	–
47	Decane, 2,4-dimethyl-	$C_{12}H_{26}$	×	√ (14.23)	×	No	Zhou et al. (2014)
48	Xylose	$C_5H_{10}O_5$	×	×	√ (14.81)	Unknown	–
49	2-Undecanone	$C_{11}H_{22}O$	√ (14.86)	×	×	Yes	Giorgio et al. (2015), Vallejo et al. (2020)
50	4-Pyridinamine, 2,6-dimethyl-	$C_7H_{10}N_2$	×	×	√ (15.08)	Unknown	–
51	Pentanoic acid	$C_5H_{10}O_2$	×	√ (15.34)	×	–	–
52	2-Dodecanone	$C_{12}H_{24}O$	√ (16.42)	×	×	Yes	Yuan et al. (2012), Tait et al. (2014), Guevara-Avendaño et al. (2019)
53	Tetredecane	$C_{14}H_{30}$	√ (16.98)	×	×	No	Fernando et al. (2005), Chen et al. (2008)
54	Nonane, 4,5-dimethyl-	$C_{11}H_{24}$	×	×	√ (17.87; 20.84)	Unknown	–
55	Undecane, 4,7-dimethyl-	$C_{13}H_{28}$	×	×	√ (18.22)	No	Giorgio et al. (2015)
56	Sulfurous acid, dipentyl ester	$C_{10}H_{22}O_3S$	×	√ (19.37)	×	Unknown	–
57	Pentane, 3-ethyl-2-methyl-	C_8H_{18}	×	√ (20.80)	×	Unknown	–
58	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	√ (22.57)	×	×	Yes	Kudalkar et al. (2012), Mallaiah et al. (2016)
59	Benzaldehyde, 2,5-bis[(trimethylsilyl)oxy]-	$C_{13}H_{22}O_3Si_2$	×	×	√ (22.60)	No	Hanif et al. (2019)
60	Squalene	$C_{30}H_{50}$	√ (23.42)	×	×	No	Bojke et al. (2018)
61	Hexadecanal, 2-methyl-	$C_{17}H_{34}O$	×	√ (24.96)	×	Yes	Raza et al. (2015)
62	p-Trimethyl silyloxy phenyl-bis (trimethylsilyloxy) ethane	$C_{17}H_{34}O_3Si_3$	×	×	√ (25.07)	Unknown	–
63	Azulene	$C_{10}H_8$	×	√ (25.56)	×	No	Strobel (2006)

*Retention time (min:s); √- detected, × - not detected

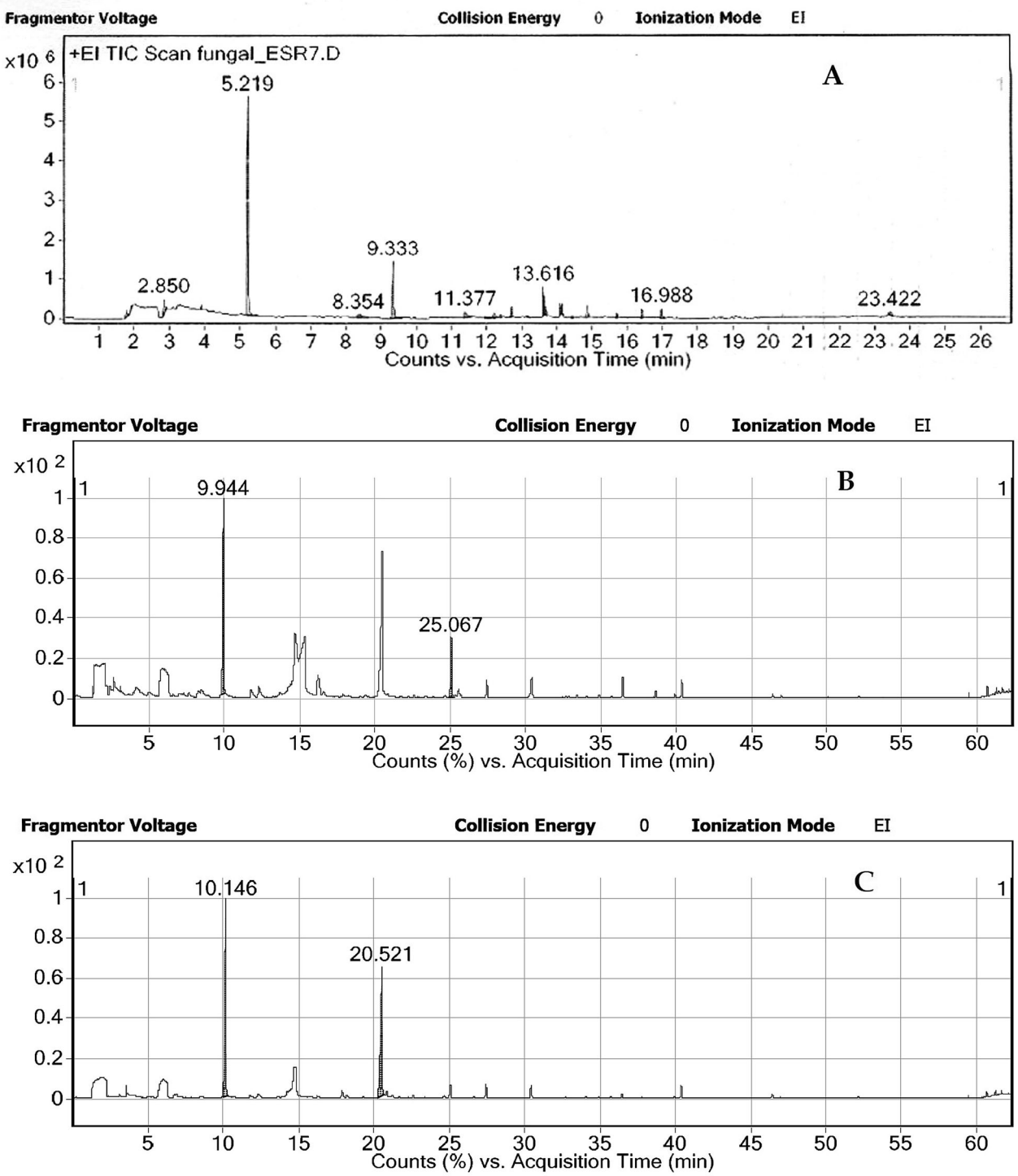

Fig. 2 Total ion chromatogram of VOCs produced by endophytic bacteria. **a** *B. axarquiensis*—ESR 7; **b** *B. subtilis*—ESR 24; **c** *B. licheniformis*—ESR 26

26, while two compounds viz., alanine and 6-methyl-2-Heptanone were found in VOCs produced by ESR 24 and ESR 26. The chromatogram analysis further revealed that 6 compounds viz., acetic acid, methoxy-phenyl-oxime, octamethyl-cyclotetrasiloxane, 5,7-dimethyl-undecane, hexamethyl-cyclotrisiloxane and dodecane were identified in VOCs produced by all three bacteria strains.

Discussion

Utilization of endophytic bacteria for biological suppression of diseases of many crops including sugarcane was successfully demonstrated (Hoon et al. 2007; Amaresan et al. 2019; Jayakumar et al. 2019). Such endophytic bacteria produce VOCs in nature and chemical profiling of such volatiles will throw light on the mechanisms of antagonism by endophytic bacteria, which can be aptly utilized for plant disease management. In the present study, the endophytic bacteria isolated from sugarcane were capable of producing VOCs that are inhibitory to sugarcane red rot pathogen. Among the tested bacterial strains, 9 were capable of producing volatiles that can inhibit > 50% mycelial radial growth of *C. falcatum*. Earlier, the endophytic *Trichoderma* spp. isolated from sugarcane were also reported to produce volatiles with antagonistic property against red rot pathogen (Joshi et al. 2019a, 2019b). The literature has showed that inhibitory activity of volatiles varied with bacteria and pathogens. Ting et al. (2009) found that endophytic bacteria were capable of producing inhibitory volatiles towards the wilt pathogen *F. oxysporum* f.sp. *cubense* rece 4 (FocR4) to the tune of 20.3%. The endophyte *B. velezensis* isolated from maize produced volatiles with inhibitory activity on the growth of *S. sclerotiorum* up to 86.7% (Massawe et al. 2018). In few instance, the volatiles produced by endophytic bacteria were completely inhibitory to the pathogen. Che et al. (2017) reported that the volatiles produced by a strain of *Lysinibacillus* sp were inhibitory to the mycelial growth of *C. acutatum* to the tune of 100%. In terms of inhibiting radial growth of *C. falcatum*, the potential three VOCs producers were of *Bacillus* spp. *Bacillus* spp. as a common soil bacteria and endophytic bacteria were known for production of their diverse range of secondary metabolic products including antibiotics, volatile organic compounds, antifungal agents, etc. (Lodewyckx et al. 2002). *Bacillus subtilis* isolated from soil produced antifungal volatile organic compounds and that even controlled the over wintered sclerotoid germination of *S. sclerotiorum* (Liu et al. 2008).

Among the tested bacteria volatiles produced by 45% of endophytes completely inhibited the sporulation of *C. falcatum*, while 47% bacteria reduced spore formation substantially. In many of the sporulated culture, the volatiles altered the pattern of spore formation. Xie et al. (2020) reported that among VOCs produced by *B. subtilis* two compounds viz., 2-heptanone, and isopentyl acetate strongly inhibited both sporulation and germination of *C. lunata* and 2-methylbutyric acid inhibited sporulation. Chen et al. (2008) reported that the volatiles generated by *B. subtilis* inhibited the spore germination and elongation of germ tubes of *Botrytis cinerea*. In addition, the culture of *C. falcatum* exposed to VOCs of endophytes showed varied patterns of mycelia growth (Table 1) due to deformation and disintegration of fungal mycelia by volatiles. Xing et al. (2018) also reported similar such result, i.e. VOCs produced by actinobacteria *Streptomyces fimicarius* inhibited the growth and development of *Peronophythora litchii* by destroying the integrity of the cell wall. Yuan et al. (2012) reported that some specific volatiles viz., benzothiazoles phenol and 2,3,6-trimethyl-phenol produced by *B. amyloliquefaciens* could affect the growth and spore germination of phytopathogen. The earlier findings reveal that kind of volatiles produced by each bacterium decides the effect on fungus. Hence, the functions of each volatile can be elucidated only by complete profiling of mVOCs.

In the present study, we analysed the complete composition of VOCs produced by 3 bacteria those showed effective inhibitory properties on mycelia growth and sporulation of *C. falcatum*. The literature study showed that among 63 identified compounds in the present study 31 were already reported to be produced by either bacteria or fungi. The composition of VOCs varied among endophytic bacteria ESR 7, ESR 24 and ESR 26, and only six compounds were reported in volatiles produced by all three. In these 6 compounds, methoxy-phenyl-oxime was not only common but also produced in abundance in VOCs of all three bacteria. It is a ketone compound reported naturally in the secondary metabolites of bacteria and fungi (Xu et al. 2011). The abundant presence of this compound was also reported in VOCs produced by *B. subtilis* and *B. amyloliquefaciens* (Gao et al. 2018; Tahir et al. 2017); however, no antifungal properties were reported for this compound. Acetic acid was another compound produced in considerable quantity by all three bacteria. It was reported to be produced by bacteria strains of *Bacillus* sp from rhizosphere (Farag et al. 2006). Acetic acid produced by certain bacteria was reported to play a role in induction of biofilms formation by bacteria. The biofilms contain exopolysaccharides as major constituents, which indirectly increase the stress tolerance of plant (Chen et al. 2015; Liu and Zhang 2015). The volatiles of rhizosphere bacteria *Pseudomonas* spp. USB2104 contained acetic acid in considerable quantity. Analysis of antifungal activity of VOCs against *S. sclerotiorum* showed that acetic acid was among 2 most active compounds in reducing the mycelia growth and the fungi exposed to this volatile caused variation in hyphal structure and cytoplasm abnormalities (Giorgio et al. 2015). This report corroborates the present findings, in which exposure of *C. falcatum* culture to VOCs of ESR 7, ESR 24 and ESR 26 caused production of fragmented and powdery mycelia growth. Undecane 5,7-dimethyl and dodecane were two hydrocarbons reported in volatiles of all three bacteria. They were occasionally

reported in the volatiles produced by microorganisms (Farag et al. 2006; Schulz and Dickschat 2007). While assessing the antifungal activity of VOCs against *S. sclerotiorum*, it was found that in the plates exposed to undecane and dodecane produced abnormally shaped and spongy sclerotia (Fernando et al. 2005). Few *Bacillus* spp. were reported to produce dodecane in VOCs, but it possessed least inhibitory action on pathogens (Gu et al. 2007; Zou et al. 2007; Massawe et al. 2018). Ryu et al. (2004) reported the presence of dodecane in *B. subtilis* GB03 strain was capable of inducing systemic resistance (ISR) in host plant. The other two hydrocarbons reported in VOCs of all three bacteria were viz., Octamethyl-cyclotetrasiloxane and hexamethyl-cyclotrisiloxane. Earlier studies have shown the presence of this compound in volatile profile of *B. mojavensis* an endophytic bacterium isolated from maize, but no antagonistic properties were identified (Rath et al. 2018).

The endophyte ESR 24 produced active compound hexanal in its volatile composition. Katoch et al. (2017) reported large hexanal production (43.9% of total VOCs) by an endophytic fungus *Fusarium* sp isolated from a medicinal herb and that inhibited the phytopathogens such as *Sclerotinia* sp and *A. flavus*. In the present study, both ESR 7 and ESR 24 produced alcohol 2-ethyl-1-hexanol and the presence of this compound was reported in volatiles of *Pseudomonas* spp. (Fernando et al. 2005) and *B. subtilis* (Chen et al. 2008; Liu et al. 2008) with antifungal activity against *S. sclerotiana*, *B. cinerea* and *S. sclerotiana*, respectively. The active ketone compound 2-decanone was reported in ESR 7 and ESR 26 and that was earlier reported in volatiles of *Bacillus* spp. and *Pseudomonas* spp. (Fernando et al. 2005; Liu et al. 2008). GC–MS analysis of VOCs produced by *B. amyloliquefaciens* NJN-6 strain detected 36 compounds and analysis of individual compound against *F. oxysporum* showed that 2-decanone could exhibit 100% inhibition of this pathogen (Yuan et al. 2012). One more active compound produced by ESR 7 was 2-undecanone. The findings of Vallejo et al. (2020) showed the abundant presence of 2-undecanone in volatile produced by two bacteria *Bacillus* sp and *Pseudomonas* sp with effective antagonistic properties against *Fusarium* spp. causing dieback disease. Another ketone compound 2-dodecanone produced in considerable quantity in ESR 7 was also reported in VOCs of many microbes with antifungal properties. Production of this compound was reported in *Bacillus* sp and *Pseudomonas* sp and when synthetic form of volatile 2-dodecanone was tested for antifungal property, it could reduce mycelial growth of *F. solani* by 38.5% (Guevara-Avendaño et al. 2019). The ester compound 1,2-Benzenedicarboxylic acid, diisooctyl ester was reported in ESR 7 and that compound was earlier reported with antagonistic property against *F. incarnatum* (Mallaiah et al. 2016). One alcohol compound hexadecanol produced by strain ESR 24 was also earlier reported in volatiles of *P. polymyxa* with 60% antagonistic potential against *F. oxysporum* f.sp. *niveum* (Raza et al. 2015). Overall, among 63 identified compounds 11 were reported with antifungal properties, 20 were already reported in VOCs of many bacteria and fungi; however, their antifungal properties were not established and functions of remaining 32 compounds are not known.

Among 49 endophytic bacteria tested against *C. falcatum* for antagonistic VOCs production, the strain ESR 7 showed highest efficacy and that result corroborates with identified active volatiles from the literature, i.e. among 11 identified active VOCs 9 were present in ESR 7. Hence, the present study reveals the evidence that VOCs produced by *B. axarquiensis*—ESR 7, *B. subtilis*—ESR 24 and *B. licheniformis*—ESR 26 play key roles in mediating antagonism against *C. falcatum*. Further analysis of functionally known and unknown VOCs may result in identification of new natural compounds that can be utilized for the management of red rot disease.

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Compliance with ethical standards

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

References

Ahmad, M., R. Ali, and S. Fasihi. 1986. Effect of different infection levels of red rot of sugarcane on cane weight and juice quality. *Journal of Agricultural Research (Lahore)* 24: 129–131.

Ajilogba, C.F., and O.O. Babalola. 2019. GC–MS analysis of volatile organic compounds from Bambara groundnut rhizobacteria and their antibacterial properties. *World Journal of Microbiology & Biotechnology* 35: 83. https://doi.org/10.1007/s11274-019-2660-7.

Amaresan, N., V. Jayakumar, Krishna kumar, and N. Thajuddin. 2019. Biocontrol and plant growth-promoting ability of plant-associated bacteria from tomato (*Lycopersicum esculentum*) under field condition. *Microbial Pathogenesis* 136: 103713. https://doi.org/10.1016/j.micpath.2019.103713.

Banerjee, D., G. Strobel, B. Geary, J. Sears, D. Ezra, O. Liarzi, and J. Coombs. 2010. *Muscodor albus* strain GBA, an endophytic fungus of Ginkgobiloba from United States of America, produces volatile antimicrobials. *Mycology* 1(3): 179–186. https://doi.org/10.1080/21501203.2010.506204.

Barber, C.A. 1901. Sugarcane disease in Godawari and Ganjam districts. *Madras Department Land Records and Agricultural Bulletin* 512(43): 181–194.

Berg, G., A. Krechel, M. Ditz, R.A. Sikora, A. Ulrich, and J. Hallmann. 2005. Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic

function against plant pathogenic fungi. *FEMS Microbiology Ecology* 51: 215–229.

Bojke, A., C. Tkaczuk, P. Stepnowski, and M. Gołębiowski. 2018. Comparison of volatile compounds released by entomopathogenic fungi. *Microbiological Research* 214: 129–136. https://doi.org/10.1016/j.micres.2018.06.011.

Che, J., B. Liu, G. Liu, Q. Chen, and J. Lan. 2017. Volatile organic compounds produced by *Lysinibacillus* sp. FJAT-4748 possess antifungal activity against *Colletotrichum acutatum*. *Biocontrol Science and Technology* 27: 1349–1362. https://doi.org/10.1080/09583157.2017.1397600.

Chen, H., X. Xiang, W. Jun, W. Lijun, Z. Zheng, and Y. Zengliang. 2008. Antagonistic effects of volatiles generated by *Bacillus subtilis* on spore germination and hyphal growth of the plant pathogen, *Botrytis cinerea*. *Biotechnology Letters* 30: 919–923. https://doi.org/10.1007/s10529-007-9626-9.

Chen, Y., K. Gozzi, F. Yan, and Y. Chai. 2015. Acetic acid acts as a volatile signal to stimulate bacterial biofilm formation. *mBio* 6(3): e00392. https://doi.org/10.1128/mBio.00392-15.

Chona, B.L. 1980. Red rot of sugarcane and sugar industry- a review. *Indian Phytopathology* 33: 191–206.

Chona, B.L., and G.W. Padwick. 1942. More light on the red rot epidemic. *Indian Farming* 3: 70–73.

Compant, S., C. Clément, and A. Sessitsch. 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology & Biochemistry* 42: 669–678.

Crespo, R., N. Pedrini, Juárez, and G.M. Dal Bello. 2008. Volatile organic compounds released by the entomopathogenic fungus *Beauveria bassiana*. *Microbiological Research* 163: 148–151. https://doi.org/10.1016/j.micres.2006.03.013.

Daungfu, O., S. Youpensuk, and S. Lumyong. 2019. Endophytic bacteria isolated from citrus plants for biological control of citrus canker in lime plants. *Tropical Life Sciences Research* 30(1): 73–88. https://doi.org/10.21315/tlsr2019.30.1.5.

Farag, M.A., C.M. Ryu, L.W. Sumner, and P.W. Paré. 2006. GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. *Phytochemistry* 67: 2262–2268. https://doi.org/10.1016/j.phytochem.2006.07.021.

Fernando, W.G.D., R. Ramarathnam, A.S. Krishnamoorthy, and S.C. Savchuk. 2005. Identification and use of potential bacterial organic antifungal volatiles in biocontrol. *Soil Biology & Biochemistry* 37: 955–964.

Gao, H., P. Li, X. Xu, Q. Zeng, and W. Guan. 2018. Research on volatile organic compounds from *Bacillus subtilis* cf-3: Biocontrol effects on fruit fungal pathogens and dynamic changes during fermentation. *Frontiers in Microbiology* 9: 456. https://doi.org/10.3389/fmicb.2018.00456.

Giorgio, A., A. De Stradis, P. Lo Cantore, and N.S. Iacobellis. 2015. Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on *Sclerotinia sclerotiorum*. *Frontiers in Microbiology* 6: 1056. https://doi.org/10.3389/fmicb.2015.01056.

Gu, Y.-Q., M.-H. Mo, J.P. Zhou, C.-S. Zou, and K.-Q. Zhang. 2007. Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. *Soil Biology & Biochemistry* 39: 2567–2575.

Guevara-Avendaño, E., A.A. Bejarano-Bolívar, A.L. Kiel-Martínez, M. Ramírez-Vázquez, A. Méndez-Bravo, E.A. von Wobeser, D. Sánchez-Rangel, J.A. Guerrero-Analco, A. Eskalen, and F. Reverchon. 2019. Avocado rhizobacteria emit volatile organic compounds with antifungal activity against *Fusarium solani*, *Fusarium* sp. associated with Kuroshio shot hole borer, and *Colletotrichum gloeosporioides*. *Microbiological Research* 219: 74–83. https://doi.org/10.1016/j.micres.2018.11.009.

Guneser, O., A. Demirkol, Y.K. Yuceer, S.O. Togayc, M.I. Hosoglub, and M. Elibol. 2017. Production of flavor compounds from olive mill waste by *Rhizopus oryzae* and *Candida tropicalis*. *Brazilian Journal of Microbiology* 48: 275–285.

Hanif, S., B. Stodart, S. Savocchia, and G. Ash. 2019. *Profiling volatile organic compounds produced by Bacillus species with biocontrol properties against Leptosphaeria maculans*. pp. 265. Abstract from Australasian Plant Pathology Society Conference, Melbourne, Australia.

Hassan, M.N., S. Afghan, and F.Y. Hafeez. 2011. Biological control of red rot in sugarcane by native pyoluteorin-producing *Pseudomonas putida* strain NH-50 under field conditions and its potential modes of action. *Pest Management Science* 67(9): 1147–1154. https://doi.org/10.1002/ps.2165.

Hassan, M.N., S. Afghan, and F.Y. Hafeez. 2012. Biological suppression of sugarcane red rot by *Bacillus* spp. under field conditions. *Journal of Plant Pathology* 94(2): 325–329.

Heenan-Daly, D., S.L.S. Velivelli, and B.D. Prestwich. 2019. The role of rhizobacterial volatile organic compounds in a second green revolution—the story so far. In *Field crops: Sustainable management by PGPR. Sustainable development and biodiversity 23*, ed. D.K. Maheshwari and S. Dheeman, 191–220. Cham: Springer. https://doi.org/10.1007/978-3-030-30926-8_8.

Herrera, S.D., C. Grossi, M. Zawoznik, and M.D. Groppa. 2016. Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. *Microbiological Research* 186–187: 37–43. https://doi.org/10.1016/j.micres.2016.03.002.

Hoon, K.S., H.S. Cho, H. Cheong, C.M. Ryu, J.F. Kim, and S.H. Park. 2007. Two bacterial entophytes eliciting both plant growth promotion and plant defense on pepper (*Capsicum annuum* L.). *Journal of Microbial Biotechnology* 17(1): 96–103.

Hsu, J.C. 1996. *Multiple comparisons: Theory and methods*. London: Chapman & Hall.

Jayakumar, V., A. Ramesh Sundar, and R. Viswanathan. 2019. Biological suppression of sugarcane smut with endophytic bacteria. *Sugar Tech* 21(4): 653–660. https://doi.org/10.1007/s12355-018-0684-1.

Jayakumar, V., R. Bhaskaran, and S. Tsushima. 2007. Potential of plant extracts in combination with bacterial antagonist treatment as biocontrol agent of red rot of sugarcane. *Canadian Journal of Microbiology* 53(2): 196–206.

Jeleń, H.H. 2003. Use of solid phase microextraction (SPME) for profiling fungal volatile metabolites. *Letters in Applied Microbiology* 36: 263–267.

Joshi, D., J. Gupta, A. Mishra, M. Upadhyaya, S.K. Holkar, and P. Singh. 2019a. Distribution, composition and bioactivity of endophytic *Trichoderma* spp. associated with Sugarcane. *Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences* 89: 1189–1200. https://doi.org/10.1007/s40011-018-1036-3.

Joshi, D., P. Singh, S.K. Holkar, and S. Kumar. 2019b. *Trichoderma*-mediated suppression of red rot of sugarcane under field conditions in subtropical India. *Sugar Tech* 21(3): 496–504. https://doi.org/10.1007/s12355-018-0624-0.

Kanchiswamy, C.N., M. Malnoy, and M.E. Maffei. 2015. Chemical diversity of microbial volatiles and their potential for plant growth and productivity. *Frontiers in Plant Science* 6: 151. https://doi.org/10.3389/fpls.2015.00151.

Katoch, M., K. Bindu, S. Phull, and M.K. Verma. 2017. An endophytic *Fusarium* sp. isolated from *Monarda citriodora* produces the industrially important plant-like volatile organic compound hexanal. *Microbiology* 163: 840–847. https://doi.org/10.1099/mic.0.000479.

Kirtikar, and H.S. Verma. 1962. A review on effect of sugarcane diseases on yield and juice qualities in Uttar Pradesh. *Indian Sugar* 12: 103–108.

Kloepper, J.W., and C.M. Ryu. 2006. Bacterial endophytes as elicitors of induced systemic resistance. In *Microbial root endophytes. Soil Biology*, vol. 9, ed. B.J.E. Schulz, C.J.C. Boyle and T.N. Sieber, 33–52. Berlin, Heidelberg: Springer. https://doi.org/10.1007/3-540-33526-9_3.

Kudalkar, P., G. Strobel, S. Riyaz-Ul-Hassan, B. Geary, and J. Sears. 2012. *Muscodor sutura*, a novel endophytic fungus with volatile antibiotic activities. *Mycoscience* 53: 319–325. https://doi.org/10.1007/s10267-011-0165-9.

Kumar, A., and Satyavir. 1998. Evaluation of biological control agents against red rot (*Colletotrichum falcatum*) of sugarcane. *Tests of Agrochemicals and Cultivars* 19: 72–73.

Kumar, S., V. Kumar, and V. Kumar. 2000. Deterioration in juice quality of sugarcane due to pathotypes of red rot pathogen. *Annals of Agri-Bio Research* 5: 31–35.

Lee, S., M. Yap, G. Behringer, R. Hung, and J.W. Bennett. 2016. Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. *Fungal Biology and Biotechnology* 3: 7. https://doi.org/10.1186/s40694-016-0025-7.

Liu, W., W. Mu, Z. Bingyu, and L. Feng. 2008. Antifungal activities and components of VOCs produced by *Bacillus subtilis* G8. *Current Research in Bacteriology* 1(1): 28–34. https://doi.org/10.3923/crb.2008.28.34.

Liu, X.-M., and H. Zhang. 2015. The effects of bacterial volatile emissions on plant abiotic stress tolerance. *Frontiers in Plant Science* 6: 774. https://doi.org/10.3389/fpls.2015.00774.

Lodewyckx, C., J. Vangronsveld, F. Porteous, E.R.B. Moore, S. Taghavi, M. Mezgeay, and D.V.D. Lelie. 2002. Endophytic bacteria and their potential applications. *Critical Reviews in Plant Sciences* 21(6): 583–606. https://doi.org/10.1080/0735-260291044377.

Mallaiah, B., E. Rajinikanth, and M. Muthamilan. 2016. Isolation and identification of secondary metabolites produced by *Trichoderma viride* inhibiting the growth of *Fusarium* in *Carnatum* (desm.) sacc. incitant of crossandra wilt. *The Bioscan* 11(3): 1525–1529.

Massawe, V.C., A. Hanif, A. Farzand, D.K. Mburu, S.O. Ochola, L. Wu, H.A.S. Tahir, Q. Gu, H. Wu, and X. Gao. 2018. Compounds of endophytic *Bacillus* spp. have biocontrol activity against *Sclerotiana sclerotiorum*. *Phytopathology* 108: 1373–1385.

Nagendran, K., G. Karthikeyan, P. Mohammed Faisal, P. Kalaiselvi, M. Raveendran, K. Prabakar, and T. Raguchander. 2014. Exploiting endophytic bacteria for the management of sheath blight disease in rice. *Biological Agriculture & Horticulture* 30(1): 8–23. https://doi.org/10.1080/01448765.2013.841099.

Padmanaban, P., D. Mohanraj, R. Viswanathan, M.M. Rao, N. Prakasam, R. Jothi, and K.C. Alexander. 1996. Differential interaction of sugarcane clones to pathotypes of *Colletotrichum falcatum* Went. *Sugar Cane* 4: 16–20.

Patel, P., R. Shah, B. Joshi, K. Ramar, and N. Amaresan. 2019. Molecular identification and biocontrol activity of sugarcane rhizosphere bacteria against red rot pathogen *Colletotrichum falcatum*. *Biotechnology Reports* 21: e00317. https://doi.org/10.1016/j.btre.2019.e00317.

Rath, M., T.R. Mitchell, and S.E. Gold. 2018. Volatiles produced by *Bacillus mojavensis* RRC101 act as plant growth modulators and are strongly culture-dependent. *Microbiological Research* 208: 76–84.

Raza, W., J. Yuan, N. Ling, Q. Huang, and Q. Shen. 2015. Production of volatile organic compounds by an antagonistic strain *Paenibacillus polymyxa* WR-2 in the presence of root exudates and organic fertilizer and their antifungal activity against *Fusarium oxysporum* f. sp. *niveum*. *Biological Control* 80: 89–95. https://doi.org/10.1016/j.biocontrol.2014.09.004.

Ryu, C.M., M.A. Farag, C.H. Hu, M.S. Reddy, J.W. Kloepper, and P.W. Pare. 2004. Bacterial volatiles induce systemic resistance in arabidopsis. *Plant Physiology* 134: 1017–1026.

Satyavir, A. Kumar, K. Raj, and K.S. Virk. 2002. Red rot of sugarcane: The research scene in Haryana. In *Sugarcane crop management*, ed. S.B. Singh, G.P. Rao and S. Eswaramoorthy, 109–126. Houston: SCI TECH Publishing, LLC.

Satyavir, 2003. Red rot of sugarcane current scenario. *Indian Phytopathology* 56: 245–254.

Schulz, S., and J.S. Dickschat. 2007. Bacterial volatiles: The smell of small organism. *Natural Product Reports* 24: 814–842.

Selim, H.M.M., N.M. Gomaa, and A.M.M. Essa. 2017. Application of endophytic bacteria for the biocontrol of *Rhizoctonia solani* (Cantharellales: Ceratobasidiaceae) damping-off disease in cotton seedlings. *Biocontrol Science and Technology* 27: 81–95. https://doi.org/10.1080/09583157.2016.1258452.

Sharma, G., J. Singh, A. Arya, and S.R. Sharma. 2017. Biology and management of sugarcane red rot: A review. *Plant Archives* 17: 775–784.

Sheoran, N., A.V. Nadakkakath, V. Munjal, A. Kundu, K. Subaharan, V. Venugopal, S. Rajamma, S.J. Eapen, and A. Kumar. 2015. Genetic analysis of plant endophytic *Pseudomonas putida* BP25 and chemo-profiling of its antimicrobial volatile organic compounds. *Microbiological Research* 173: 66–78.

Sileshi, G.W. 2012. A critique of current trends in the statistical analysis of seed germination and viability data. *Seed Science Research* 22: 145–159.

Singh, N. 2008. Sustainable management of red rot disease of sugarcane. *Indian Sugar* 8: 21–30.

Singh, O.N., and K.S. Waraitch. 1977. Metabolic changes induced by *Colletotrichum falcatum* Went. in sugarcane. *Sugarcane Pathologists' Newsletter* 19: 7–9.

Singh, V., R.L. Srivastava, S.K. Awasthi, and B.B. Joshi. 2008. Biological control of red rot disease of sugarcane through *Trichoderma harzianum* and *Trichoderma viride*. *Indian Phytopathology* 61: 486–491.

Strobel, G. 2006. *Muscodor albus* and its biological promise. *Journal of Industrial Microbiology and Biotechnology* 33: 514–522. https://doi.org/10.1007/s10295-006-0090-7.

Tahir, H.A.S., Q. Gu, H. Wu, Y. Niu, R. Huo, and X. Gao. 2017. *Bacillus* volatiles adversely affect the physiology and ultrastructure of *Ralstonia solanacearum* and induce systemic resistance in tobacco against bacterial wilt. *Scientific Reports* 7: 40481. https://doi.org/10.1038/srep4048.

Tait, E., J.D. Perry, S.P. Stanforth, and J.R. Dean. 2014. Identification of volatile organic compounds produced by bacteria using HS-SPME-GC–MS. *Journal of Chromatographic Science* 52: 363–373.

Thode, H.C. 2002. *Testing for normality*. New York: Marcel Dekkers.

Ting, A.S.Y., S.W. Mah, and C.S. Tee. 2009. Prevalence of endophytes antagonistic towards *Fusarium oxysporum* f. sp. *cubense* race 4 in various plants. *European Journal of Sustainable Agriculture* 3: 399–406.

Vallejo, N.B., D.A.C. Pozosb, J.L.M. Villanuevaa, M.R. Vázqueza, G.L.C. Villarnovoc, J.A.G. Analcoa, L.P.P. Martínezb, and F. Reverchon. 2020. Forest tree associated bacteria for potential biological control of *Fusarium solani* and of *Fusarium kuroshium*, causal agent of *Fusarium* dieback. *Microbiological Research* 235: 126440. https://doi.org/10.1016/j.micres.2020.126440.

Viswanathan, R., and G.P. Rao. 2011. Disease scenario and management of major sugarcane diseases in India. *Sugar Tech* 13: 336–353. https://doi.org/10.1007/s12355-011-0102-4.

Viswanathan, R., and K.C. Alexander. 1997. Management of sugarcane diseases. *Indian Journal of Sugarcane Technology* 12: 37–48.

Viswanathan, R., and R. Samiyappan. 1999. Red rot disease of sugarcane: Major constraint for Indian sugar industry. *Sugar Cane* 5: 9–15.

Viswanathan, R., and R. Samiyappan. 2000. Red rot disease in sugarcane: Challenges and prospects. *Madras Agricultural Journal* 87(10–12): 549–559.

Viswanathan, R., and R. Samiyappan. 2002. Induced systemic resistance by fluorescent pseudomonads against red rot disease of sugarcane caused by *Colletotrichum falcatum*. *Crop Protection* 21: 1–10.

Xia, Y., S. DeBolt, J. Dreyer, D. Scott, and M.A. Williams. 2015. Characterization of culturable bacterial endophytes and their capacity to promote plant growth from plants grown using organic or conventional practices. *Frontiers in Plant Science* 6: 490. https://doi.org/10.3389/fpls.2015.00490.

Xie, S., J. Liu, S. Gu, X. Chen, H. Jiang, and T. Ding. 2020. Antifungal activity of volatile compounds produced by endophytic *Bacillus subtilis* DZSY21 against *Curvularia lunata*. *Annals of Microbiology* 70: 2. https://doi.org/10.1186/s13213-020-01553-0.

Xing, M., L. Zheng, Y. Deng, D. Xu, P. Xi, M. Li, G. Kong, and Z. Jiang. 2018. Antifungal activity of natural volatile organic compounds against Litchi downy blight pathogen *Peronophythora litchii*. *Molecules* 23: 358. https://doi.org/10.3390/molecules23020358.

Xu, F., W. Tao, and J. Sun. 2011. Identification of volatile compounds released by myxobacteria *Sorangium cellulosum* AHB103-1. *African Journal of Microbiological Research* 5: 353–358.

Yadav, R.I. 2006. Research vision to manage red-rot disease of sugarcane in India. *Sugar Tech* 8: 99–100.

Yuan, J., W. Raza, Q. Shen, and Q. Huang. 2012. Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. *cubense*. *Applied and Environmental Microbiology* 78: 5942–5944.

Zhou, J.Y., X.Y. Zhao, and C.C. Dai. 2014. Antagonistic mechanisms of endophytic *Pseudomonas fluorescens* against *Athelia rolfsii*. *Journal of Applied Microbiology* 117: 1144–1158.

Zou, C.S., M.H. Mo, Y.Q. Gu, J.P. Zhou, and K.Q. Zhang. 2007. Possible contributions of volatile-producing bacteria to soil fungistasis. *Soil Biology & Biochemistry* 39: 2371–2379.