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Applying Wheat-Associated Bacteria's Volatile Organic Compounds (VOCs) as Antifungal Agents to Treat Fungal Diseases

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Abstract

Wheat, the first crop cultivated on earth, is a crucial staple food that must be grown in larger quantities to meet the needs of the expanding global population. Fusarium head blight (FHB), caused by destructive plant pathogenic fungi, harms wheat and other cereal crops. The degradation of fresh produce by fungi results in significant global economic losses. Despite the boost in agricultural productivity from chemical fertilizers and pesticides, there are notable environmental consequences. Researchers stress the importance of microbial VOCs in medicinal and agricultural biotechnology. In sustainable agriculture, microbial volatile organic compounds (MVOCs) are potential eco-friendly substitutes for conventional pesticides. Hence, the objective of this study was to assess the effectiveness of volatile organic compounds, produced by bacteria isolated from Egyptian wheat plants, in inhibiting the growth of *Fusarium graminearum.* Two hundred bacterial isolates collected from various parts of wheat plants and soil samples were evaluated for their ability to produce volatile organic compounds with antifungal properties in vitro. The findings revealed that the VOCs inhibited the growth of *Fusarium graminearum* by 50.1 ± 2.1% after ten days, indicating a significant inhibitory effect on fungal mycelial growth. In this work, bioactive secondary metabolites were chemically identified using a *GC-MS* spectrometer for VOCs released by three tested antifungal bacterial isolates: *Bacillus paramycoides*, *Achromobacter denitrificans* and *Alcaligenes faecalis. Bacillus paramycoides* has been identified as the producer of 9 bioactive substances, which have known antibacterial and some anticancer properties, along with previous recognition for their effectiveness as antifungal agents. *Achromobacter denitrificans* also produces 3 volatile organic compounds with documented antifungal capabilities. The research emphasizes the antifungal properties of these volatile organic compounds (VOCs) and highlights the discovery of 3 VOCs produced by the *Alcaligenes faecalis* strain, all showing antifungal activity in prior studies. This underscores the potential of these bioactive substances and VOCs as effective antifungal agents. According to these findings, these substances can be employed as antagonists to develop a cutting-edge method of preventing fungal infections.

Keywords: Antagonistic effect, VOC, Wheat, *Fusarium graminearum*, GC-MS

1. Introduction

Wheat (*Triticum aestivum L.*) is one of the most important grain crops worldwide as a food for humans and animals [1], The crucial commodity for food security, wheat is a source of grain and energy as well as vitamins (particularly B vitamins), phytochemicals, and dietary fiber [2] . Since Egypt is one of the world's largest wheat importers, wheat is the country's main food grain crop. In Egypt**,** wheat is used to make pasta and bread, and straw is used to feed animals [3]. Egypt is experiencing an escalating wheat gap, despite producing approximately 9.7 million tonnes ($6x10^7$ ardeb) ($0.9 x10^9$ Kg) from 3,320,477 fed (1,394,588 he) according to FAOSTAT, 2023[4] . Egypt's rapidly expanding population faces hurdles due to a lack of wheat output. Significantly quick costs of food, fuel, and fertilizer have increased globally as a result of export sanctions imposed by states in reaction to the ongoing conflict between Russia and Ukraine[5]. Numerous biotic (microbial illnesses) and abiotic (water, temperature, and climate change) limiting variables must be overcome to increase wheat output. The main threat is also the existence of a variety of pathogenic fungi [6], that affect seed germination, plant health and yield, grain nutritional content, and marketing value [7], as well as lower yield and quality and jeopardize food security [8]. In 2019, a 22% yield loss was a result of the major destructive loss brought on by rusts, smuts, Fusarium head blight, Septoria leaf blotch, tan spot, and powdery mildew. The mutation and diversity of virulence contribute to the rise in this percentage[9] The development of various fungicide substances and resistant cultivars and Variations in

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pathogen virulence cause cultivars to become more vulnerable to them [10]. One of the most significant diseases affecting wheat is Fusarium Head Blight (FHB), which is brought on by a variety of fungi including *Fusarium culmorum*, *Fusarium graminearum*, and *Fusarium avenaceum* [9] because they create mycotoxins that infect grains of cereal [11]. Grain pests decrease grain productivity and quality[12] and endanger people, animals, and plants[11,13]. *F. graminearum* is the one that is spread the most [14], and it is also said to be the predominant FHB pathogen in Egypt [15]. The primary toxin made by *F. graminearum* is deoxynivalenol[16].There are no real management practices for the disease, and it is demanded new strategies to control it [15]. Excessive use of pesticides pollutes the environment, deteriorates the ecosystem, and pathogens to become resistant [13,17]. For instance, using Carbendazim to stop FHB incidence and DON accumulation in the wheat field[10,18]. Carbendazim has been banned in the EU and the United States [19]. Crop residue removal, crop rotation, pathogen-free seed, and cultivation are not compatible with cereal-intensive areas[20–22], in addition, encourage Fusarium growth or DON production under favorable environmental conditions [23,24]. The potential replacement for synthetic fungicides by biocontrol microorganisms is considerable [25,26]. Through parasitism, antibiosis, host resistance induction, volatile organic compound (VOC) emission, and biofilm formation, they prevent the growth of pathogenic organisms[27]. One prominent antifungal method used by hostile microorganisms is VOC generation [28]The antifungal properties of microbial VOCs must be discussed for commercial purposes[29].MVOCs can enhance plant physiological and hormonal pathways to boost biomass and yield production[20,23], as well as in plant health via antifungal, antibacterial, oomyceticidal, and nematicidal activity. They can also stimulate plant immunity via salicylic acid (SA) and jasmonic acid (JA) routes[30,31]. Gas chromatography mass spectrometry (GC-MS) with direct headspace injection is applied to the analysis of bacterial VOCs to determine the impact of experimental parameters on the generated VOC profiles [32]. The Current study aimed to evaluate the antagonistic activity of bacterial isolates collected from Egyptian wheat (*Triticum aestivum* L.) against *F. graminearum* by producing a volatile organic compound (VOC). The released VOCs emitted by bacterial isolates were analyzed by using a gas chromatography-mass spectrometry (GC-MS) with direct headspace injection

2. Materials and Methods.

2-1. Wheat samples collection and preparation.

Samples of Egyptian wheat (variety Giza155) have be en obtained from Cairo University's Faculty of Agricu lture in Giza, Egypt. Five grams of bulk soil and wheat rhizosphere samples after removing the loosely attached soil, were collected and vigorously mixed for 1 min at maximum speed using vortex in sterile 45 ml 0.85% NaCl saline solution [33] .Tenfold serial dilutions of the obtained microbial suspensions were prepared and 100 µl from each dilution were spread on the surface of rich nutrient agar medium (NA)[34]. The fungal plant pathogen utilized in this study was *Fusarium graminearum*, procured from Environmentally friendly compounds Lab (307) at the National Research Centre (NRC), Dokki, Giza, Egypt. Total bacteria counts were determined by estimating colony-forming units (CFU) following a 3-day incubation at 30°C. All plates were subsequently incubated at 28 ± 2°C for 4 to 10 days, and morphologically different colonies were isolated and purified.

2-2. Bacterial isolation and screening for VOC-mediated antifungal Activity.

Plates containing 50 to 200 colonies were examined to identify morphologically distinct bacterial colonies from various plant environments. These isolates were purified through re-streaking on the same medium used for their initial isolation and stored in a -20°C freezer following inoculation on NB broth medium supplemented with 20% glycerol. To assess the impact of bacterial volatile organic compounds (VOCs) on the growth of *Fusarium graminearum*, Plastic Petri dishes measuring 90mm in diameter were utilized to physically separate the bacteria from the fungi. One dish housed the bacterial isolates grown on NA medium after 24 hours of incubation at 30°C, while the other contained a piece of *Fusarium graminearum* mycelium on Potato Dextrose Agar (PDA). These dishes were sealed opposite each other and incubated at 25°C, with the fungal plate positioned underneath to prevent spores from reaching the bacterial plate. The setup was then incubated for 7 days at 30°C, with daily observation for growth indications. As a control, the fungal dish was only exposed to a Petri dish containing NA medium [35]. The diameter of fungal growth was determined after seven days of incubation. To determine the percentage of inhibition, we used the formula [(diameter of fungus in control—diameter of fungus exposed to VOCs) *100/diameter of fungus in control]. The minimum level of fungal growth served as an indicator of the bacterial antifungal activity of *F. graminearum*[36].

2-3. Collection and Analysis of VOCs

For trapping the VOCs, the bacterial isolates were inoculated individually in 10ml sterile glass vials containing 2.5ml of nutrient agar medium with three replicates each, un-inoculated medium served as controls. All vials were closed and incubated at 30°C. After 3 days, VOCs from the headspace of each vial were collected by headspace with a 65-mm

polydimethylsiloxane-divinylbenzene Fiber (Supel co., Bellefonte, USA)[32].

2-4. Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

The GC-MS analysis was conducted at the Department of Medicinal and Aromatic Plants Research, National Research Centre, using a gas chromatography-mass spectrometry instrument with the following specifications: a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA) connected to an ISQ Single Quadrupole Mass Spectrometer detector. The GC-MS system featured a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness) and utilized helium as the carrier gas at a flow rate of 1.0 ml/min with a split ratio of 1:10. The temperature program involved starting at 40^{o}C, increasing at a rate of 5^{o}C/min up to 240^{o}C, and holding for 1 minute. The injector and detector temperatures were maintained at 220^{o}C. Electron ionization (EI) at 70 eV was used to obtain mass spectra in the range of m/z 35-400. Compound identification was primarily achieved through the analysis of mass spectra using authentic chemicals, the Wiley spectral library collection, and the NSIT library[32].

2-5. Genomic DNA extraction from Bacterial isolates.

The bacterial isolates were cultured in nutrient broth (NB) for 24 hours and then collected by centrifugation at 12,000 g for 5 minutes after being washed three times by resuspending in 0.85% NaCl and centrifugation. Genomic DNA extraction was carried out using the GeneJET Genomic DNA purification kit (Thermo Fisher Scientific, Lithuania) following the manufacturer's instructions. The extracted DNA was assessed for quantity and purity through agarose gel electrophoresis and ethidium bromide staining under UV light, as well as with a NanoDrop spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, Germany). The DNA was stored at −20°C[37].

2-6. Identification of VOC producing bacterial isolates using 16S rRNA gene sequencing

PCR amplification was carried out on the 16S rRNA gene fragment of three bacterial isolates known for their antifungal activities mediated by volatile organic compounds (VOCs). The amplification was performed using specific bacterial primers, namely F-27 [38] and R1494-1514 [39] on a Bio-Rad T100 thermal cycler-USA. The reaction mixture was composed of Taq buffer, dNTPs, $MgCl_2$, DMSO, forward and reverse primers, DNA template, GoTaq enzyme, and water to make a final volume of 25 µl. The PCR procedure included an initial denaturation step at 94°C for 5 minutes followed by 30 cycles of 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes, ending with a final extension at 72°C for 10 minutes. The resulting PCR products underwent analysis through 1% agarose gel electrophoresis, were subsequently purified, and sequenced by Macrogen in Seoul, Republic of Korea. The partial sequences of the 16S rRNA genes were then compared for similarity matches in the GenBank database using BLASTn. The 16S rRNA gene sequences of the isolates were recorded in the NCBI GenBank database under the accession numbers (PP227470-PP227472) .Typically, PCR amplification was conducted with a fragment of the 16S rRNA gene to determine the bacterial strains [40,41] .

2-7. Phylogenetic analysis of bacterial strains

The evolutionary lineage of the bacterial isolates was determined through the Neighbor Joining method. The phylogenetic tree was constructed using nucleotide sequences from bacterial isolates in the study, including three sequences of the 16S rRNA gene, along with five sequences from the closest matches found in the NCBI GenBank database. The tree was generated using the Maximum Composite Likelihood method, with evolutionary assessments performed using MEGA version 5 software [42], The accuracy of the phylogenetic tree structure was validated through bootstrap analysis, consisting of 1000 iterations.

3-Results & Discussion:

3-1. Isolation and purification of bacteria from wheat plants and its soil.

Colony-forming units (CFU) were estimated to determine the total counts of bacteria**.** The results indicated that the 357 bacterial pure colonies were isolated from different sphere of two Egyptian wheat plant, the large number was isolated from root and soil compared with leaves and shoot (Table 1).

Table 1: The total number of isolates obtained from different spheres of wheat using the nutrient agar media to evaluate their antifungal activity.

Sphere	Plant A	Plant B
Root	50	43
Soil	45	40
Rhizosphere	37	38
Leaves	21	23
Shoot	28	32

3-2. The Effect of bacterial isolates VOCs on Fungal growth.

The results showed that the bacterial isolates from wheat plants could produce volatile organic

compounds. The inhibition was indicated by the colony diameter of *Fusarium* sp. in the plate that had been inoculated and the presence of a small diameter for fungus growth in the plate parallel to the plates in which the bacteria grow. These results showed that they could produce secondary metabolites as volatile compounds that function as antifungals. To obtain the desired samples, we meticulously handpicked all the colonies that exhibited distinct shapes on each Petri dish. We then proceeded to carry out a preliminary screening of the obtained isolates to conclusively determine their in vitro Anti-toxical activities. A collective of 200 out of the 357 bacterial isolates sourced from various areas of wheat plants and soil types underwent screening for their capacity to produce volatile organic compounds with in vitro antifungal properties. A selection of 146 bacterial isolates that demonstrate antifungal activity have been identified. Shoot samples yielded the greatest number of antifungal bacteria, with 121 samples, closely followed by root endo-sphere and leaf endo-sphere samples (11 and 9 bacterial isolates, respectively) Three bacterial isolates were derived from the rhizosphere, while two bacterial isolates were obtained from the root endophytic compartments. Over three weeks of sample monitoring, it was observed that fungal growth in the dishes remained fixed in front of bacterial isolates. Nonetheless, there was a noticeable uptick in fungus growth in some samples after a certain period. Over three weeks of sample monitoring, it was observed that fungal growth in the dishes remained fixed in front of bacterial isolates. Nonetheless, there was a noticeable uptick in fungus growth in some samples after a certain period. After that, 44 pure bacterial colonies were selected for their antifungal activity, and 16 bacterial isolates could inhibit the fungal growth up to 50%–88%. The largest percentage of VOC-producing bacteria affecting fungus growth in spheres of wheat were isolated from the shoot, followed by the root, then rhizosphere, leaves, and finally in the soil.

3-3. Identification of bacterial isolates using 16S rRNA gene sequencing

The 16S rRNA gene sequence analysis was performed on three bacterial isolates as shown in Table (3) and Figure (1). Their 16S rRNA gene sequence was affiliated with *Bacillus paramycoides* 3E, *Achromobacter denitrificans* 7E and *Alcaligenes faecalis* 10E.

The bacterial phylogenetic studies are often used to investigate the evolutionary relationships between different bacterial species and genera using their 16S rRNA gene sequences. The results in Figure 1 show that the tree sheds light on the evolutionary links between different bacterial species. This indicates that *Alcaligenes and Achromobacter* are closely linked, whereas *Bacillus* is distantly related.

3-4. The identification of the VOCs responsible for the antifungal properties of *Bacillus paramycoides, Achromobacter denitrificans,* and *Alcaligenes Faecalis* was carried out through GC-MS analysis using direct headspace injection.

The GC-MS technique was utilized to identify total VOCs, considering factors such as retention time, peak areas, molecular weight, and formula. The results of the analysis revealed the main chemicals present. Based on their retention time, molecular weight, and known biocontrol properties from previous research, volatile compounds were selected as the by-product of the evaluated bacterial strains. These compounds have shown potential to inhibit microbial growth, along with other important health and environmental benefits. *Bacillus paramycoides* has been identified as the producer of nine bioactive substances, detailed in Table (4). Apart from their known antibacterial and, in some cases, anticancer properties, previous studies have recognized their effectiveness as antifungal agents. The antifungal capabilities of three volatile organic compounds produced by *Achromobacter denitrificans* have also been documented. The research highlights the antifungal properties as a key aspect of these VOCs, alongside the discovery of three volatile organic compounds generated by the *Alcaligenes faecalis* strain sample, all of which have demonstrated antifungal activity in prior studies. This suggests the potential of these bioactive substances and VOCs as effective antifungal agents. *Bacillus paramycoides* is known for producing nine bioactive compounds, listed in Table (4). These bioactive compounds namely: 3-Decyn-2-ol, 3-Trifluoroacetoxypentadecane, Ethyl iso-allocholate, Oleic Acid, trans-13-Octadecenoic acid, cis-11-Eicosenoic acid, cis-Vaccenic acid, Octadecanal, 2-bromo and cis-13-Octadecenoic acid. Apart from their antibacterial and even anticancer properties, previous studies have praised these compounds for their antifungal effectiveness. This antifungal capability has also been highlighted in the research. Additionally, *Achromobacter denitrificans* produces three volatile organic compounds with documented antifungal properties.

These substances that are bioactive are specifically, Butanal, 3-methyl, Octanal and Hexane. Several studies attribute the antifungal activity of these VOCs to be a key function.

Table 2: Evaluation of antifungal activity (inhibition %) of 44 bacterial isolates against *F. graminearum* by exposure to Volatile Organic Compounds (VOCs).

Code	Sphere*	Mycelial growth diameter (cm)**	%inhibition***
R182	Rhizosphere	1	88.88%
L4	Endo leaves	1	88.88%
S330	Soil	1	88.88%
R145	Endo Root	1.5	83.3%
Sh125	Endo Shoot	1.5	83.3%
S32	Soil	2	77.77%
Sh1	Endo Shoot	2.2	75.55%
RH 64	Rhizosphere	2.8	68.88%
R168	Endo Root	3	66.66%
R166	Endo Shoot	3.3	63.33%
RH 559	Rhizosphere	3.5	61.11%
L127	Endo Leaves	4	55.55%
Sh 96	Endo Shoot	4.3	52.22%
PF1	Endo Root	4.3	52.22%
R178	Endo Root	4.4	51.11%
R176	Endo Root	4.5	50%
R175	Endo Root	4.8	46.66%
R27	Endo Root	4.9	45.55%
Sh 100	Endo Shoot	5	44.44%
Sh 183	Endo Shoot	5.1	43.33%
RH 2	Rhizosphere	5.5	38.88%
RH148	Rhizosphere	6	33.33%
Rh165	Rhizosphere	6	33.33%
L123	Endo leaves	6.5	27.77%
Sh84	Endo Shoot	6.6	26.66%
L138	Endo leaves	7	22.22%
L13	Endo Leaves	7	22.22%
L9	Endo Leaves	7	22.22%
Rh19	Rhizosphere	7	22.22%
Sh87	Endo Shoot	7	22.22%
Sh100	Endo Shoot	7.1	21.11%
RH88	Rhizosphere	7.3	18.88%
Sh99	Endo Shoot	7.5	16.66%
S186	Soil	8	11.11%
Sh80	Endo Shoot	8	11.11%
Sh94	Endo Shoot	8	11.11%
Sh124	Endo Shoot	8	11.11%
R164 B	Endo Root	8	11.11%
Sh98	Endo Shoot	8	11.11%
L126	Endo Leaves	8.3	7.77%
R181	Endo Root	8.5	5.5%
RH 66B	Rhizosphere	8.5	5.5%
S167	Soil	8.5	5.5%
Sh84	Endo Shoot	8.5	5.5%

*Source of bacterial isolates from different spheres.

**Diameter of mycelial growth (DCM)

**% inhibition (Highest antifungal activity against Mycelial growth & Smallest Fungal Growth Diameter).

Table (3): Sequence analysis of 16S rRNA gene of three promising bacteria isolated from different wheat plant spheres and soil types.

Identification	%identity	Accession number
Bacillus paramycoides-3E (S330)	99.6	PP227470
Achromobacter denitrificans-7E (R182)	99.93	PP227472
Alcaligenes faecalis-10E (L4)	99.86	PP227471

Furthermore, three volatile organic compounds have been identified in the sample from the *Alcaligenes faecalis* strain these bioactive compounds include , butanal, 3-methyl, octanal, and 2-Heptanamine-(cas), all of which have demonstrated antifungal activity in previous studies.

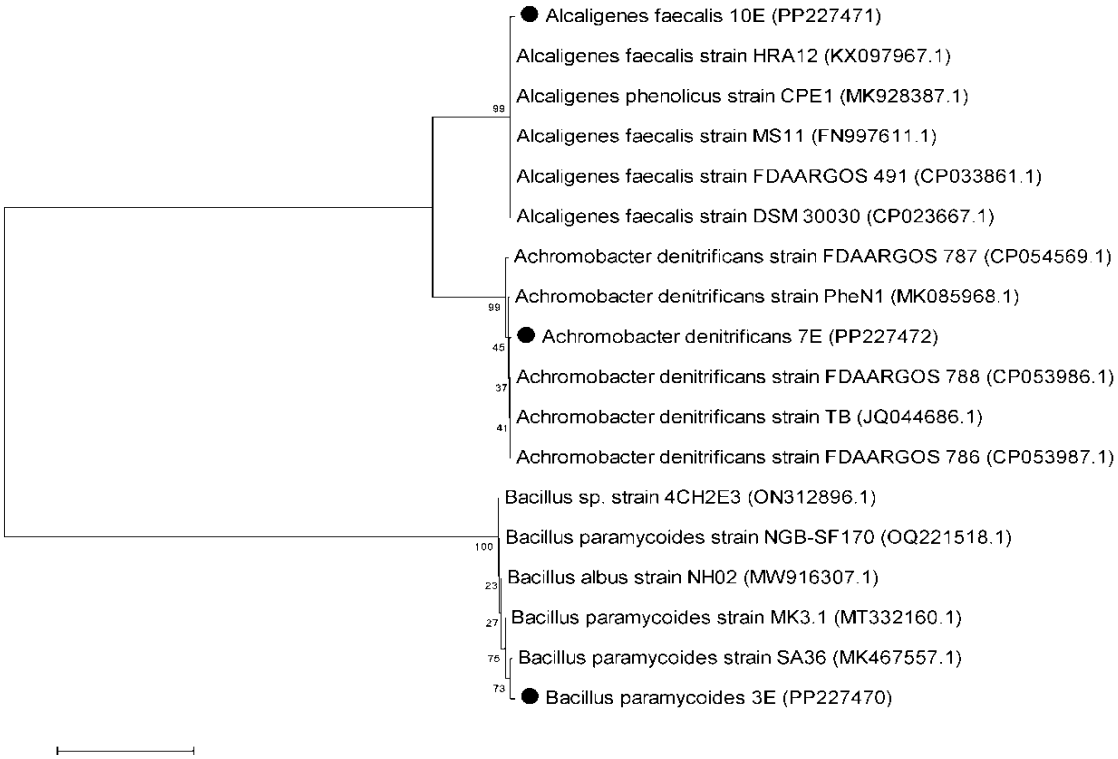

Fig. 1. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences

4-Disscusion

Several Microbial volatile organic compounds (MVOCs) are produced exceptionally by microorganisms during their metabolism for many purposes. Studies revealed the vital role of MVOC in plant interactions and microbial communication. They
enhance plant growth and production through plant's physiological and hormonal pathways modification. Additionally, MVOCs have been proven to reduce stress and increase plant immunity. Thus, MVOCs act as effective biocontrol instead of various chemical fertilizers, pesticides [66], and Fungicides[67]. The predominant microorganism in MVOCs production and characterization is *Bacillus subtilis*[68] and their antagonistic effect against fusarium species in wheat [69]. In this study, we found various bacterial from different spheres in two wheat plant samples produce microbial volatile organic compounds that perform antifungal activity against *Fusarium graminearum*: *Bacillus paramycoides, Achromobacter denitrificans*, and *Alcaligenes faecalis.* Studies indicate the potential of bioactive compounds produced by co-culture *Bacillus paramycoides* LBKURCC218 with *Aspergillus fumigatus* LBKURCC269 fermentation as antifungal agents [70]. *Bacillus paramycoides* was shown mycelial growth inhibition through volatile compound production against *Fusarium oxysporum* and *Botrytis cinerea* [71] . We found that *Bacillus paramycoides* produced majority of bioactive compound inhibit the mycelial growth analyzed by GC-mass spectrum: 3-Decyn-2-Ol, 3- Trifluoroacetoxypentadecane , 3-ethyl-5- Octadecane (2-ethylbutyl)-, Ethyl iso-allocholate, Oleic Acid, trans-13-Octadecenoic acid, cis-11-Eicosenoic acid, cis- Vaccenic acid, trans-13-Octadecenoic acid , 2-bromo Octadecanal and cis-13-Octadecenoic acid. a previous study found that 3-Decyn-2-Ol was from major chemical components in *Chlorella vulgaris* extracts that showed 10.98 % antifungal activity against six plant pathogenic fungi (in vitro) (*Fusarium oxysporum*, *Fusarium* sp., *Fusarium solani*, *A. flavus*, *A. niger*, and *A. alternate* [43].3-Decyn-2-Ol was analyzed by GC-MS results as VOC produced by the bacterial and viral groups [72]. Yousef et al. (2018) reported that Trifluoroacetoxy-pentadecane as volatile compound produced by modified *(Trichoderma harzianum*) inhibit the growth of *R. solan* [73] . Oleic Acid from *Trichoderma* spp. extracts with antifungal activity against Cocoa pathogens, which was identified by gas chromatography–mass spectrometry (GC-MS)[74]. Oleic acid and cis-Vaccenic acid were reported as volatile organic compound from *Camellia oleifera* seed cake extraction [75]. Awonyemi *et al*. (2020) found that Cis-13-Octadecenoic acid, Cis-Vaccenic acid, and Trans-13- Octadecenoic acid were bioactive compounds extracted from *Raphia taedigera*, these compounds identified by chromatography-mass spectrometry [76]. In this research, Awonyemi *et al*. indicated from other references the biological importance of these compounds, Oleic Acid was reported for its antifungal, anti-inflammatory, antioxidants, antibacterial [77].

Table: (4) The chemical identification of the bioactive compounds by GC-MS using Direct Head space injection from *Bacillus paramycoides* 3E, *Alcaligenes Faecalis*10E and *Achromobacter denitrificans* 7E

Retention time	Percentage Area			Compound Name	Molecular Formula/MW	Proposed Function
	*Bacillus paramycoids*3E(S330)	*Alcaligenes Faecalis* 10E(R182)	*Achromobacter denitrificans* 7E (L4)			
2.01	7.51	-	-	3-Decyn-2-ol	C10H18O/154	Antifungal activity[43] Antibacterial activity [44 45]
2.14	38.77	-	-	3-Trifluoroacetoxypentadecane	C17H31F3O2/282	Anti- nephrotoxicand antioxidantactivities [46]
46.77	6.65	-	-	Ethyl iso-allocholate	C26H44O5/436	Antifungal Activity [47]
51.92	5.84	-	-	Oleic Acid	C18H34O2/282	Antifungal Activity [48–50] Anti-microbial Activity [51]
53.39	5.68	_	_	trans-13-Octadecenoic acid	C18H34O2/282	Good anti-inflammatory Antifungal and antimicrobial agent [52]Antifungal Activity [48]Anti-cancer, Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, antileukotriene- D4, hypocholesterolemic,5-alpha reductase inhibitor, anemiagenic [53]
54.03	6.45	_	_	cis-11-Eicosenoic acid	C20H38O2/310	Antimicrobial and antifungal Activity [54]
56.62	6.64	_	_	cis-Vaccenic acid	C18H34O2/282	Antifungal Activity [55] antibacterial activity and hypolipidemic effect[56 57]
57.54	10.12	_	_	Octadecanal, 2-bromo	C18H35BrO/346	Antibacterial and antifungal activity[58 59 60]
58.42	6.09	_	_	cis-13-Octadecenoic acid	C18H34O2	Antifungal[61]
1.18	_	11.16	11.17	Butanal, 3-methyl	C5H10O/86	Antifungal Activity [62]
1.47	_	72.75	_	Octanal	C8H16O/128	Antifungal Activity [63]
2.37	_	0.97	1.17	Hexane	C6H14/86	Antifungal activity and antibiofilm [64]
1.72	_	_	1.35	2-Heptanamine-(cas).	C7H17N/115	Antimicrobial activity [65]

Oleic acid, cis-13-Octadecenoic acid and cis - Vaccenic acid were from volatile organic compounds produced by *T. asperellum* AU131 and *T. longibrachiatum* AU158 identified by GC-MS, they were examined their antagonist mechanism against *F. xylarioides* [61] .Whereas ethyl iso-allocholate was a major volatile organic compound of *Pleurotus ostreatus* Polar Extract (PoPE) that identified by GC-MS technique and its biological relevance was observed in their inhibitory effect on radial mycelial growth. The percentage of inhibitory effect on radial mycelial growth (PMGI) of three plant fungal pathogens (*Fusarium oxysporum*, *Fusarium solani*, and *Rhizoctonia solani* was used to assess PoPE's antifungal activity in addition to antimicrobial, cytotoxic, immunomodulating, and antioxidant properties[78]. Octadecane and Ethyl iso-allocholate were revealed as antibiotic metabolites isolated from *Actinomycetes* species in the soil of Rijal Alma, Saudi Arabia, GC-mass and their potential antibacterial properties analysed these bioactive compounds were assessed [47]. Al- Jassani *et al*. (2016) mentioned Ethyl iso-allocholate was a secondary metabolite of *Saccharomyces cerevisiae*, in addition to their antibacterial and antifungal ability investigated [79]. Cis-11-Eicosenoic, its production has been mentioned from *Mortierella* fungi [80], also it is exhibited as antibacterial bioactive extract of *Petroselinum crispum* leaves that is analysed using GC-MS analysis [81]. It is indicated that cis-Vaccenic acid, trans-13-Octadecenoic acid, and cis-11-Eicosenoic acid are Phyto-compounds present in the extract of the *Camellia sinensis* as determined by

GC–MS analysis, where they proved effective antimicrobial activity (antifungal and antibacterial) [82].Cis-11-Eicosenoic is found in component of *Halomonas alkaliphila* [83]. Octadecanal, 2–bromo, was found in *Paecilomyces lilacinus* acetone extract, showed the highest antimicrobial activity against the tested bacterial strains and *Candida albicans* in addition to Anti-cancer activity and Total Antioxidant Capacity[84]. Octadecanal, 2–bromo is mentioned as chemical element of *Cladosporium spongiosus* ethanol extract that exhibited antifungal activity against the selected *Candida* species [85]. GC-MS analysis of an ethyl acetate extract from endophytes *Bacillus* species identified Octadecanal, 2- bromo- as a bioactive molecule, also it confirmed its antifungal, antibacterial and antioxidant activity [58]

In un-contacted face-to-face dual culture testing, *Alcaligenes faecalis* N1-4 isolated from tea rhizosphere soil produced abundant antifungal volatiles and greatly inhibited the growth of *Aspergillus flavus* [86]. Symbiotic bacteria (*Alcaligenes faecalis*) were isolated from entomopathogenic nematodes EPN *Oscheius* species. Bioassays were conducted against entomopathogenic fungi (EPFs) and plant pathogenic fungi (PPFs) to determine infectivity. Both volatile and non-volatile symbiotic bacterial exudations negatively impacted EPF and PPF. It suggests that has a common mode of action by deterring fungal strains of functionally different origin. Bacterial exudates from *E. faecalis* could create a soil microbial community that is more resistant to fungal pathogens and could be integrated into pest management programs to protect crops [87]. From volatile organic compounds profiles of EPB *Alcaligenes faecalis* were butanoic acid, 2- methyl-, 3-methyl butyl ester, butanoic acid, 3-methyl, pentyl ester. In the present study, *Alcaligenes faecalis* produced 3-methyl-butanal which was indicated as an antifungal VOC [62]. Butanal,3-methyl- are VOCs produced by *Bacillus* strains displayed striking inhibitory activities to the tested soil-borne pathogenic fungus *F. oxysporum* [88]. Butanal, 3-methyl- are volatile compounds produced by endophytic bacteria with antifungal activity against *Aspergillus flavus*, *Penicillium citrinum*, *Aspergillus fumigatus*, *Fusarium oxysporum*, and *Rhizopus stolonifera* [89].

Octanal are from aldehydes that is determined using HS-SPME and GC-MS, and it found their antifungal activity against selected fungi strains[90].Octanal is one of volatile compound produced by Soybean homogenates showed strong fungal inhibition of *Aspergillus flavus* growth [91] Octanal as VOC produced by *Aspergillus oryzae* that impacted *Aspergillus flavus* growth [92]. On the other hand, a number of fungi have the ability to produce volatile compounds that have an antimicrobial effect and also play an important role in the bioremediation of environmental pollutants [93–95].

5-Conclusions

GC–MS analysis of three Bacterial isolates from various wheat sphere plants confirmed the presence of volatile organic compounds. These compounds have proven in vitro antifungal activity on *F. graminearum.* These compounds can be used as antagonists to create a novel strategy to prevent fungal diseases

6-Conflicts of interest

The authors declare that there is no conflict of interests.

7.Formatting of funding sources

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