SECONDARY COMPOUNDS RELEASED BY RHIZOSPHERIC BACTERIA EXHIBIT FUNGISTIC ACTIVITY AGAINST PHYTOPATHOGENIC FUNGUS.

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ABSTRACT
The aim of conducted research was to devaluate the inhibition effect of metabolites secreted by some Rhizospheric bacteria on the growth of two pathogenic fungi: Rhizoctonia solani and Fusarium solani, 330 bacteria isolates were obtained from the rhizosphere of the wheat, tomato, cowpea and Mt. Atlas mastic tree grown in the Sulaimani. Thirty-five isolates were screened for suppression of phytopathogenic fungus. Results showed that most of these rhizospheric bacteria were have fungistic potential in different degrees. The highest inhibition of the linear growth of fungi was noted for F. solani and R. solani, by Bacillus cereus LXJ73 (90.41%), Bacillus atropheaus SM-1 (75%) respectively. Six bacterial isolates were selected which have potential effect on the growth of the two studied fungi, five isolates belonging to the Bacillus genus and one isolate belonging to the genus Klebsiella, these isolates were molecularly confirmed by 16SrRNA, and their activity for inhibiting fungal growth were studied. The extracted metabolities analysed by Gas chromatography-Mass spectrometry (GC-MS), showing that extracts contain different volatile organic compounds as Pyrrol[1,2-a]pyrazine-1,4-dione, hexahydro-, 2-Pentanone, 4-hydroxy-4-methyl-, Isososteviol methyl ester,2-methyl-, Butanoic acid, 3-methyl-, 3-Pyrrolidin-2-yl-propionic acid, 9-Octadecenamide, (Z), Hexadecane, Tridecane, 2,5-Piperazinedione, 3,6-bis(2-methyl propyl)-, 1,2-Benzenedicarboxylic acid, diisooctoyl ester, Acetic acid, Dodecane, beta.-D-Glucopyranose, 1,6-anhydro-, Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester and cis-Vaccenic acid. The presence of these compounds indicate that all studied Bacillus species and Klebsiella michiganensis M1-3-11having antagonistic activity and they can be used for development of biocontrol agents to reducing the phytopathogenic problems and decreasing mycotoxine in contaminated crops.

Keywords: biocontrol, rhizospheric bacteria, fungistic activity, volatile organic compounds.

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INTRODUCTION

Disease control methods include the elimination of infected plants with subsequent chopping or use of systemic pesticides as part of integrated pest control strategies. However, given the need to consider restrictions on using agrochemicals for plant export and consumption, further research should focus on environmentally friendly alternatives for plant disease management. One of these alternatives is biological control, in which naturally occurring beneficial bacteria with antagonistic activity are used against the causes of disease. Bacterial or fungal strains which isolated from the endosphere or rhizosphere usually having the biocontrol agents concerning with plant diseases (18). Various bioactive compounds are produce by rhizobacteria as a particular interest, and they play an important function in supporting plant growth, productivity, and phytopathogens fungi protection. (4) Reports of rhizobacteria with antagonistic activity against fungal pathogens are widespread in the literature and Bacillus spp. and Pseudomonas spp. in especial are commonly cited among the most effective biocontrol agents. Multiple mechanisms may be implicated in the antifungal activity of rhizobacteria. Bacteria can outgrow fungi by competing for space or resources, or actively inhibit fungal development by emitting antibiotics in diffusible components (2). It has been shown the bacterial species secrete cyclo lipopeptides with antimicrobial effects, principally from the families of iturine, fengycin, or surfactant. (15) The antifungal effects of iturine and fengycin from Bacillus subtilis/B. amyloliquefy ancient was studies against Fusarium oxysporum and F. solani (6,17). The genus Bacillus has been shown to emit compounds, including aliphatic ketones that can impede the growth of several species of fusarium (8). The aim of the current study were (1) to isolate and identify the rhizospheric organisms from the rhizosphere of four plants species, wheat, cowpea, tomato, and Mt. Atlas mastic tree; (2) to assess the inhibitory impact of rhizospheric microorganisms from chose plants against the two chosen phaytopathoginic fungus; and (3) to analyze the antifungal characteristics of their organic extracts, and to expand the results for environmentally friendly biocontrol agents.

MATERIALS AND METHODS

Soil sampling: During the planting period between April 2017 and July 2018, soil samples with plant roots were collected from field trials of four different species: tomato (Solanum lycopersicum L.), cowpea (Vigna unguiculata L.), bread wheat (Triticum sativum L.), and Mt. Atlas mastic tree (Pistacia atlantica). The samples of the soil taken in triplicate in 20 cm depth and stored at 4 °C.

Bacterial isolation and growth condition

The adhering soil of the roots zone in 3-6 mm thick were taken by sterile forceps from each studied plants. From this, 1gm of the soil was taken into the test tube with 9 ml of sterile distilled water then shaken in a rotary shaker at 150 rpm for 30 minutes before dil ution (11). To determine the colony-forming units (CFU), bacterial cultures were double diluted in a 96-well microplate by transferring 20 µl of bacterial culture into 180 µl Phosphate buffered saline with (dilutions 10⁻¹,10⁻²,10⁻³ and 10⁻⁴) (16). Then, from each dilution, 20 µl were placed onto the surface of LB agar. Plates were inverted and incubated at 30°C for 24h. For calculate the CFU per milliliter, the average number of bacterial colonies in 20 µl was multiplied by 50, and the dilution factor using the following formula:

\[ \text{CFU mL}^{-1} = \text{Average number of colonies for a dilution} \times 50 \times \text{dilution factor} \]

For long-term storage, the bacterial isolates were grown in nutrient broth medium until they reached 10⁸ and the cells were agitated well then frozen at -80°C in 30% glycerol and 70% nutrient broth.

Microscopic and biochemical identification of the bacterial isolates

Microscopic examination was performed by preparing a thin smear, then gram stained with standard gram staining procedures as described by(22) and examined under oil lens(x100 power). Soil rhizosphere bacteria cells appeared in different sizes, shapes, and colors. The pink to red colors are gram-negative and pale purple to dark purple colors are gram-positive. Using VITEK® 2 Systems (bioMérieux, USA) for biochemical identification as the manufacturer's instruct-
tions, the stock culture isolates were sub cultured and the fresh Gram-negative isolate was subcultured on MacConkey agar plates and the Gram-positive isolates recultured on nutrient agar plates for 18 to 24 h at 30°C for activation. A bacterial suspension prepared by mixing a small bacterial colony with 3 ml of a 0.45% sodium chloride solution, and it was adjusted in a range between 0.5-0.63 for Gram-negative and 1.8-2.20 for Gram-positive spore-forming bacilli according to McFarland standard. The prepared test cards and specimen test tubes Placed in the cassette, then within 10 minutes the cassette transferred to the cassette loading station in the Vitek 2 system (23).

Molecular identification of selected isolates and Sequencing
The evaluating 16S rRNA sequences was performed for molecular Identification of bacterial strain by purifying the DNA genome of the bacterial strain through using the DNA purification kit for the bacterial genomic (Geneaid, Taiwan). The16SrRNA amplified by the PCR reaction, using forward BF27 (5'-AGAGTTTGATCCTGG CTCAG-3') and U1492R(5'-GGTTACCTT GTTACGACTT-3'). The PCR condition was performed in 3 stages , 1st stage initial denaturation at 94 °C for 5 min ; 2nd stage in 30 cycles denaturation at 94 °C for 1min, annealing at 52 °C for 1min and extension at 72 °C for 2 min; 3rd stage final extension at 72 °C for 5 min. Then using Easy pure®Quick Gel Extraction kit (Beijing, China) for purification and sequenced (Sangar sequencing, Macrogen South Korea). Then blasted with sequences related species from the NCBI GenBank database.

Antifungal activities of the Isolates
The dual culture assay performed for the antifungal activities of 35 isolates against two phytopathogenic fungi (R. solani and F. solani). Using potato dextrose agar (PDA) medium, for carrying out this a 6 mm plug from the edge of a old culture (5 day) of the pathogenic fungi inoculated at the center of PDA medium and then the fungus growth was stressed by inoculating the isolates in a long streak of 2 cm on the medium surface and 3 cm away from the fungi. Inhibition growth of the fungal was measured after incubation for 7 days at temperature 28-30°C and compared with that of the control (i.e., without the bacterial isolate). Isolates that showed significant antifungal activity against the phytopathogens in this tests were repeated in three replicates, thus, the inhibition rate was measured in percentage according to the formula of (9).

Isolate cultivation and crude extraction of bioactive compounds
Based on their antagonistic activities the most active isolates against the two selected fungi were cultivated for crude extraction of their active compounds. The selected isolates were cultured on a Luria Bertani plate (LB). The cultured plates were incubated at 30°C for 24hr (with 3 replicate). Each actively growing pure culture of the isolates was used to inoculate 100 ml of Luria Bertani broth (1). The extraction were performed by using the method (1) with some modifications, 10% of the cultured in LB broth were taken in to be used as a seed culture for fermentation medium in three separate 150 ml of (yeast extract, glucose, NaCl, oatmeal, CaCO₃ , at pH 7.0). Then the cultures were incubated at 30 °C for 10 days inshaking incubators of 180 rpm. Then the medium was centrifuged for 30 min at 6000 × g to separate the bacterial cells. The supernatant was mixed with unequal volumes of n-hexane as an organic solvent, then shacked for 30 min. and separated in a separating funnel. The solvent was removed by a rotary evaporator.

Analysing of crude cell extract by GC-MS
Using GC-MS- QP2010 Ultra, (Shimadzu Co., Japan) for Identification and analysing of the bacterial metabolites. the helium flowed at 1 ml/min through an instrument supported with an Rtx-5ms column (30 m × 0.25 mm ID, 0.25 μm film thickness). The diluted sample was heated at 60°C for 30 min and 1 ml of the headspace was injected using a gas syringe (21). the temperature had been increased at 6°C per min, then the column was saved at 40°C for 2 min, then at 250°C for 13 min. The injection port was at 200°C and interface at 250°C. The chromatogram and mass spectra were recorded and analyzed. The m/z peaks representing mass to charge ratio charac teristic of the antimicrobial fractions were compared with those in the mass spectrum of NIST (National Institute for Standards and
Technology) library of the corresponding organic compound.

**Statistical analyses**
All data were statistically analyzed by using one-way ANOVA with 0.05 probability. Applying the Duncan test for comparing the means of the values among the treatments with one another.

**RESULTS AND DISCUSSION**

**Characterization of rhizosphere bacteria**

Plant growth promoting rhizobacteria (PGPR) were isolated from the rhizosphere of tomato (*Solanum lycopersicum* L.), cowpea (*Vigna unguiculata* L.), bread wheat (*Triticum sativum* L.), and Mt. Atlas mastic tree (*Pistacia atlantica*). Pure colonies were identified by morphological characteristics and biochemical methods. One hundred fourteen, 89, and 127 isolates are Gram-positive, Gram-negative, and bacilli spore, respectively, as shown in Figure (1).

![Figure 1. Frequency of bacterial isolates from the rhizosphere of selected plants represented by percent for each family](image)

Bacterial isolates with antifungal activity were confirmed their identification molecularly by sequencing their 16S rRNA gene for more confirmation. The partial sequences were ranged between 1430-1545bp, with a similarity of 98%-100% of the blasted sequence.

**Antagonistic activity against the studied pathogenic fungi**

The inhibition of pathogen mycelial growth and suppression of spore germination considered as a results of antagonistic activities of the rhizosphere bacteria against phytopathogens fungi. These 330 bacterial isolates are distinguished into 17 groups according to its response to the Gram-stain, and microscopic characteristics like size and cell shape of the bacterial cells were also done. 35 isolates from the total bacterial isolates were selected randomly that obtained from four plant species, they were checked for their antagonistic activity by growing each isolates with *R. solani*, and *F. solani* pathogens in one plates. The results of inhibition percentages of fungal growth by the 35 bacterial isolates are appeared in (Table-1). A significant difference in the growth inhibition was observed among the isolates. The radial growth of mycelial of the *R. solani* and *F. solani* pathogens were inhibited by all studied isolates, with inhibition percentages ranges from 20.83 to 90.42% and 41.25 to 75.00% against *R. solani*, and *F. solani*, respectively. Bacterial isolates with the local number 9, 20, and 6 from 35 isolates showed the value of growth suppression between 10-50, 50-80, and 80-100%, respectively, in *R. solani*. Whereas, 14, 13 and 8 isolates of 35 bacterial isolates exhibited a range of inhibition of growth from 30 to 50, 50 to 60, and 60 to 80% against *F. solani*. The highest inhibition of growth (90.42%) against *R. solani* was showed by *Bacillus cereus* LXJ73 followed by *Bacillus licheniforms* B27 (86.66%) and *Bacillus licheniformis* 1 (86.66%), while the maximum value of the growth inhibition (75%) in *F. solani* was displayed by *Bacillus atrophaeus* SM-1 followed by *Bacillus licheniformis* KUBOT-AB1 (66.87%) and *Klebsiella michiganensis* M1-3-11 (65.41%).
pesticides have helped farmers reduce crop losses caused by microbial phytopathogens, but they have also been linked to deforestation, the evolution of resistancy of the pathogens, and threats to validity of the human. Several studies have suggested the use of useful rhizospher bacteria as a biological control agents to solve the problems caused by phytopathogenic fungi and to reduce the harmful belongings of pesticide or a chemical fertilizer (18,6,2). (11) also showed growth inhibition of R. solani by B. licheniforms and B. pumillus. recently discovered that Bacillus VOCs inhibited many fungal growths in different degrees. In another investigation by(12) found that the severity of the pathogens was significantly reduced. Furthermore, our findings are came in agreement with the reporting of (14), who found B. Pumilus SMH101 has a strong antifungal spectrum against F. solani, R. solani, and C. albicans.

**Extract analysis by GC-MS**
The GC–MS analysis indicated that out of the six strains found, K. michiganensis M1-3-11, B. licheniforms KUBOTAB1, B. licheniforms B27, B. cereus LXJ73, B. sonorensis KW50P, and B. atrouphes SM1, B. atrouphes SM1 had the most metabolites with 27, B. licheniforms B27 had the fewest metabolites, with just five, while isolates K. michiganensis M1-3-11, B. licheniforms KUBOTAB1, B. cereus LXJ73, and B. sonorensis KW50P had 20, 14, 24, and 26 metabolites, respectively. some organic compounds were repeteadly detected by the four isolates, Klebsiella michiganensis M1-3-11, B. licheniforms B27, B. sonorensis KW50P and B. atrouphes SM1 is pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-1,4-dione. Others compound repeted to B. licheniforms KUBOTAB1, B. cereus LXJ73 undecane. Those common to K. michiganensis M1-3-11, B. licheniforms KUBOTAB1is Tridecane. The predominated components in different isolates extracts were: 2-Pentanone, 4-hydr oxy-4-methyl- and Pyrrolo[1,2 alpyra zine-1,4-dione, hexahydro- in K. michiganensis M1-3-11, undecane, Bis-(3,5,5-trimethyl hexyl) phthalate, Butanoic acid, 3-methyl- andPyrrolo[1,2-a]pyrazine-1,4-dione, hexa hydro- in B. licheniforms KUBOTAB1, 3-Octanone, 8-(6-tricosyl-2-methyl,1,3-dioxan -4-yl)-4-methyl-[4R-[4.alpha. (R®),6.beta. ]] and Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propylester in B. licheniforms B27, pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro- and 9-Octadecenamide, (Z)- in B. cereus LXJ73, Butanoic acid, 3-methyl- and pyrrolo[1,2-a]pyrazine-1,4-dione, hexa hydro- in B. sonorensis KW50P, and Butanoic acid, 3-methyl-, Butanoic acid, 2-methyl- and pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- in B. atrouphes SM1. A variety of compounds have several biological pro perties including cytotoxicity, antioxidant, anti inflammatory, antimicro bial, and anti viral activities were observed (25). Isolated 3- Pyrrolidin-2-yl-propionic acid as a bioactive organic compound from Klebsiella michiganensis M1-3-11, B. sonorensis KW50P, and B. atrouphes SM1 showed its antifungal activity against aflatoxin producing Aspergillus by (5). B. cereus LXJ73 formed hexadecane, which had antimicrobial, antifungal, and antioxi dant properties (26). B. licheniforms B27 and B. cereus LXJ73 produced the saturated fatty acid lauric acid, which repoted that suppressing the growth of R. solani (24). Dodecane, which was found in B. cereus LXJ73, had antifungal properties (19).Penta decane was detected in B. cereus LXJ73 displayed for F. oxysporum growth according to (27). In a study by (12) showed significant antimicrobial activity of B. licheniforms.
Table 1. The inhibition zone (%) caused by PGPR against some phytopathogens fungus

| Name                                      | Inhibition of Growth % | Rhizoctonia solani | Fusarium solani |
|-------------------------------------------|------------------------|--------------------|-----------------|
| Bacillus cereus LX73                      | 90.417 a               | 62.500 abcd        |                 |
| Bacillus licheniformis B27                | 86.667 ab              | 61.250 abded       |                 |
| Bacillus licheniformis1                   | 86.667 ab              | 54.167 bcdef       |                 |
| Bacillus atrophaeus SM-1                  | 83.333 ab              | 75.000 a           |                 |
| Bacillus sonorenseis Kw50p                | 81.250 ab              | 57.500 abdef       |                 |
| Bacillus zhangzhouensis Qi_110           | 80.000 ab              | 65.000 abc         |                 |
| Klebsiella michiganensis M1-3-11          | 79.167 ab              | 65.417 abc         |                 |
| Bacillus safensis JS5                     | 77.083 ab              | 47.917 bedefgh      |                 |
| Bacillus pumilus 3-19                     | 75.000 abc             | 56.250 abdefd      |                 |
| Bacillus licheniforms KUBOTAB1            | 75.000 abc             | 66.875 ab          |                 |
| Paenibacillus fuchanolyticus              | 65.417 abdefg          | 40.417 fgh         |                 |
| Pseudomonas putida                        | 64.583 abdefg          | 46.667 dcfgh       |                 |
| Klebsiella pneumonia                      | 60.417 abdefgh         | 53.333 bedf         |                 |
| Bacillus coagulans                        | 60.417 abdefgh         | 49.167 bedfg        |                 |
| Bacillus megatherium                      | 60.417 abdefgh         | 40.147 abe          |                 |
| Pseudomonas fluorescens                   | 60.417 abdefgh         | 58.333 babedef      |                 |
| Rhizobium radiobacter                     | 56.250 abdefg          | 33.750 gb          |                 |
| Burkholderia giadioli                     | 52.083 abdefg          | 47.917 bedefgh      |                 |
| Coronobacter sakazakii /                 | 47.917 bedefgh         | 45.417 defgh        |                 |
| Geobacillus toebii                        | 36.250 cdefgh          | 44.167 dfg          |                 |
| Brevibacillus laterosporus                | 34.167 dfg             | 31.250 h           |                 |
| Moganella morganii                        | 30.417 efgh            | 47.917 bedefgh      |                 |
| Alloioococcus otitis                      | 30.000 fgh             | 34.167 gh          |                 |
| Pantoea spp                               | 29.167 fgh             | 57.292 abefgh       |                 |
| Bacillus gelatinii                        | 27.917 gh              | 47.917 bedefgh      |                 |
| Virgibacillus pantothenticus              | 27.083 gh              | 56.667 abedefg      |                 |
| Streptococcus suis L                      | 20.833 h               | 42.500 efgh         |                 |
| Pr > F (Strains)                          | < 0.0001               | < 0.0001           |                 |
| Significant                               | Yes                    | Yes                |                 |

Table 2. Characterization of VOCs emitted by *Klebsiella michiganensis* M1-3-11, analyzed by –GC-MS

| No. peak | Compound                                                        | RT(min) | RA(%) |
|----------|-----------------------------------------------------------------|---------|-------|
| 1        | 2-Pentanone, 4-hydroxy-4-methyl-                                | 3.187   | 37.6  |
| 2        | Oxime-, methoxy-phenyl_                                         | 3.582   | 0.33  |
| 3        | 2-[4-Chloro-trans-styril]-6-chloro-5-[4-chlorophenyl]-4-[3,5    | 4.148   | 0.41  |
| 4        | bis[pyrrolidinomethy]-4-hydroxyanilin]                         |         |       |
| 5        | Rhodium, di.-mu.-chlorobis[1,2,5,6,-eta.-]-1,5-cyclooctadiene]dih- | 4.262   | 0.42  |
| 6        | N-Benzyl-2-[(benzyl-phenyl-carbamoyl)-methoxy]-1,2-diphenyl-ethoxy]-N-phenyl-acetamide | 4.61 | 0.44  |
| 7        | Phenylethyl Alcohol                                              | 5.249   | 0.83  |
| 8        | 1,4,3,6-Dianhydro-alpha.-d-glucopyranose                        | 6.026   | 1.01  |
| 9        | 2,4,4-Trimethylbut-2-enolide                                    | 6.557   | 0.78  |
| 10       | Tridecane                                                       | 7.209   | 0.56  |
| 11       | Tetradecane                                                     | 8.055   | 1.32  |
| 12       | 1-Oxaspiro[4,5]decane 4-carbonitrile, 2-oxo-                    | 8.497   | 3.83  |
| 13       | 3-Pyrrolidin-2-y1-propionic acid                                | 9.351   | 0.42  |
| 14       | 3-Pyrrolidin-2-y1-propionic acid                                | 9.56    | 1.79  |
| 15       | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydroro                  | 9.793   | 1.21  |
| 16       | 2-(E)-Heptenoic acid, (4S)-4-[(R)-alanyl]amino]-6-methyl-      | 10.011  | 33.19 |
| 17       | Piperazine, 1,4-bis(1-oxoacetadecyl)-                           | 12.175  | 3.14  |
| 18       | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-                  | 12.275  | 0.95  |
| 19       | Ketone, vinyl-pyrrolinidyl-                                     | 14.786  | 7.3   |
| 20       | Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'- (phenylmethyl)-, (5',alpha,10.alpha)- | 15.076 | 2.37  |
|          |                                                                  | 17.645  | 2.09  |
Table 3. Characterization of VOCs emitted by *B. licheniforms* KUBOTAB1, analyzed by –GC-MS

| No. peak | Compound | RT(min) | RA(%) |
|---------|----------|---------|-------|
| 1       | Butanoic acid, 3-methyl- | 3.052   | 7.73  |
| 2       | 1-Pentacontanol          | 3.293   | 1.45  |
| 3       | 1-Pentanol, 4-methyl-2-propyl- | 3.593   | 2.95  |
| 4       | 1-Decanol, 2-ethyl-      | 3.836   | 4.72  |
| 5       | .beta.-Thionaphthol maltung heptaacetate | 3.925   | 1.7   |
| 6       | Nonane, 2-methyl-       | 4.175   | 10.17 |
| 7       | Heptadecane, 2,6-dimethyl- | 4.72    | 2.25  |
| 8       | Undecane               | 5.01    | 11.09 |
| 9       | 1,4:3,6-Dianhydro-alpha.-d-glucopyranose | 6.025   | 3.66  |
| 10      | Naphthalene, decahydro-2,6-dimethyl- | 6.071   | 4.09  |
| 11      | Naphthalene, decahydro-1,5-dimethyl- | 6.112   | 4.67  |
| 12      | Tridecane              | 7.209   | 6.91  |
| 13      | Bis-(3,5,5-trimethylhexyl) phthalate | 8.493   | 8.31  |
| 14      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- | 10.013  | 30.29 |

Table 4. Characterization of VOCs emitted by *B. licheniforms* B27, analyzed by –GC-MS

| No. peak | Compound | RT(min) | RA(%) |
|---------|----------|---------|-------|
| 1       | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- | 10.01   | 3.1   |
| 2       | 3-Octanone, 8-(6-tricosyl-2-methyl-1,3-dioxan-4-yl)-4-methyl- | [4R-[4 alpha.-R],6 beta.]]- | 25.094  | 8.15  |
| 3       | Cholestanol[3,2-c]isocoumarin-1'-(2'H)-one, 3',4'-dihydro-6',7'-dimethoxy- | 25.85   | 2.22  |
| 4       | 3-Octanone, 8-(6-tricosyl-2-methyl-1,3-dioxan-4-yl)-4-methyl- | [4R-[4 alpha.-R],6 beta.]]- | 26.981  | 13.56 |
| 5       | Lauric acid, 2-(hexadecyloxy)-3-(octadecyloxy)propyl ester | 27.935  | 72.96 |

Table 5. Characterization of VOCs emitted by *B. cereus* LXJ73, analyzed by –GC-MS

| No. peak | Compound | RT(min) | RA(%) |
|---------|----------|---------|-------|
| 1       | Butanoic acid, 2-methyl- | 3.159   | 6.3   |
| 2       | Octane, 4-ethyl-         | 3.595   | 1.29  |
| 3       | 3-Carbethoxy-6-n-butyl-7-octadecylmercapto-4-quinoline | 4.363   | 3.64  |
| 4       | Undecane               | 5.01    | 3.96  |
| 5       | Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl- | 5.442   | 9.63  |
| 6       | Undecane               | 5.791   | 6.93  |
| 7       | 1,4:3,6-Dianhydro-alpha.-d-glucopyranose | 6.019   | 2.18  |
| 8       | Bicyclo[2.2.2]octane, 1,2,3,6-tetramethyl- | 6.075   | 2.15  |
| 9       | Naphthalene, decahydro-1,5-dimethyl- | 6.111   | 2.37  |
| 10      | Pentadecane            | 7.208   | 2.59  |
| 11      | .beta.-D-Glucopyranose, 1,6-anhydro- | 8.049   | 2.81  |
| 12      | Hexadecane             | 8.497   | 2.83  |
| 13      | 1,3,5-Cycloheptatriene, 2,5-dihexyl-7,7-dimethyl- | 8.769   | 1.93  |
| 14      | 1,3,5-Cycloheptatriene, 2,4-dihexyl-7,7-dimethyl- | 8.93    | 2.16  |
| 15      | Dodecane, 1',thiobis- | 9.827   | 3.14  |
| 16      | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- | 10.008  | 21    |
| 17      | Phthalic acid, butyl undecyl ester | 11.785  | 4.01  |
| 18      | N-Isosetylidene-2-butylamine | 15.087  | 2.72  |
| 19      | 9-Octadecenamide, (Z)- | 17.294  | 11.24 |
| 20      | Hexanedioic acid, mono(2-ethylhexyl)ester | 17.657  | 1.72  |
| 21      | Isosteviol methyl ester | 18.679  | 0.32  |
| 22      | Methyl steviol         | 18.877  | 0.93  |
| 23      | Lauric acid, 2-methylbutyl ester | 19.037  | 1.14  |
| 24      | 1,2-Benzenedicarboxylic acid, diisooctyl ester | 19.703  | 3.01  |
Table 6. Characterization of VOCs emitted by *B. sonorensis* KW50P, analyzed by –GC-MS:

| No. Peak | Compound                                      | RT(min) | RA(%)  |
|----------|-----------------------------------------------|---------|--------|
| 1        | Butanoic acid, 3-methyl                        | 3.197   | 28.36  |
| 2        | Butanoic acid, 2-methyl                        | 3.248   | 8.03   |
| 3        | Evonine                                        | 3.483   | 0.51   |
| 4        | Oxime-, methoxy-phenyl-                        | 3.525   | 0.44   |
| 5        | 1,4:3,6-Dianhydro-alpha-d-glucopyranose         | 6.023   | 1.8    |
| 6        | Naphthalene, decahydro-1,5-dimethyl            | 6.17    | 1.03   |
| 7        | Bicyclo[2.2.2]oct-5-ene-2-carbonitrile, 2-chloro- | 7.568   | 0.73   |
| 8        | Phenol, 2,4-bis(1,1-dimethylthyl)-              | 7.947   | 0.59   |
| 9        | .beta.-D-Glucopyranose, 1,6-anhydro-            | 8.042   | 3.67   |
| 10       | Benzaldehyde, 4-(dimethylamino)-                | 8.292   | 1.62   |
| 11       | d-Arabinohexonic acid, 2-deoxy-3,4,5-tris-O-(trimethylsilyl)-, trimethylsilyl ester, bis(trimethylsilyl) | 8.533   | 1.1    |
| 12       | o-Acetyl-N,N'-carbonyl-tetrahydro-solasodine    | 9.348   | 0.67   |
| 13       | Docriacontyl trifluoroacetate                   | 9.566   | 1.97   |
| 14       | 3-Pyrroldin-2-yl-propionic acid                | 9.796   | 2.24   |
| 15       | Ppyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-   | 10.005  | 24.68  |
| 16       | Pentadecanoic acid                             | 10.378  | 1.2    |
| 17       | 6-Oxabicyclo[3.1.0]hexan-3-one, 2,2,4,4-tetramethyl- | 10.447  | 1.36   |
| 18       | n-Hexadecanoic acid                            | 11.862  | 2.36   |
| 19       | Nitro-1-arginine                               | 12.192  | 2.82   |
| 20       | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-  | 14.792  | 2.21   |
| 21       | 2-Decene, 3-methyl-, (Z)-                      | 15.075  | 5.61   |
| 22       | 9-Octadecenamide, (Z)-                         | 17.294  | 1.81   |
| 23       | 5-Ethyl-1-nonene                               | 17.656  | 1.11   |
| 24       | Iseosteviol methyl ester                       | 18.683  | 1.59   |
| 25       | Methyl steviol                                 | 18.875  | 0.62   |
| 26       | 1,2-Benzenedicarboxylic acid, diisooctyl ester | 19.705  | 1.86   |

Table 7. Characterization of VOCs emitted by *B. atrouphes* SM1, analyzed by –GC-MS

| No. peak | Compound                                      | RT(min) | RA(%)  |
|----------|-----------------------------------------------|---------|--------|
| 1        | Butanoic acid, 3-methyl                        | 3.225   | 18.01  |
| 2        | Butanoic acid, 2-methyl                        | 3.309   | 10.76  |
| 3        | 3-Pyrindinemethamine                           | 7.566   | 0.17   |
| 4        | 3,6-Dimethylpyrazine-2,5-dione                 | 8.237   | 1.41   |
| 5        | Diethyl Phthalate                              | 8.469   | 0.84   |
| 6        | (1,3)Diazepan-2,4-dione                        | 8.558   | 1.65   |
| 7        | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-  | 8.897   | 2.96   |
| 8        | 5-Methoxypyrroldin-2-one                       | 9.358   | 4.1    |
| 9        | 3-Pyrroldin-2-yl-propionic acid                | 9.569   | 3.47   |
| 10       | 3-Pyrroldin-2-yl-propionic acid                | 9.808   | 1.42   |
| 11       | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-   | 10.08   | 29.21  |
| 12       | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- | 11.645  | 1.3    |
| 13       | N(1)-(3-Methyl-1,2,4-oxadiazol-5-y1)-pyrrolidine carboxamidine | 11.791  | 2.35   |
| 14       | Nitro-1-arginine                               | 12.223  | 2.96   |
| 15       | Hexahydro-2(1H)azocinone                       | 13.004  | 1.98   |
| 16       | 9-Octadecenamide, (Z)-                         | 13.398  | 0.91   |
| 17       | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- | 14.062  | 1.22   |
| 18       | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-  | 14.821  | 1.89   |
| 19       | Hexanal, 3-(hydroxymethyl)-4-methyl            | 15.103  | 2.06   |
| 20       | 1,9-Dioxacyclohexadeca-4,13-diene-2-10-dione, 7,8,15,16-tetrahydro- | 15.667  | 0.62   |
| 21       | Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5' | 17.024  | 0.45   |
| 22       | 7(8H)-Pteridinone, 6-methyl                    | 17.142  | 3.4    |
| 23       | 9-Octadecenamide, (Z)-                         | 17.1301 | 0.88   |
| 24       | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- | 17.66   | 0.58   |
| 25       | 7(8H)-Pteridinone, 6-methyl                    | 19.676  | 2.05   |
| 26       | Lupeol                                         | 26.494  | 0.65   |
| 27       | Lup-20(29)-en-3-ol, acetate, (3.beta.)-        | 28.651  | 2.71   |

**Conclusion**

The determination of different biological active compounds qualitatively from crude extracts of 6 selected rhizospheric bacteria by using GC-MS indicated different chemical compounds with high molecular weight and low molecular weight in varying amounts revealed in each of the bacterial extracts. 6 identical VOCs are detected in both *Bacillus* and *Klebsiella*. These active compounds are important biologically as antibiotic, antifungal compounds by inducing systematic resistance of plants important. The inhibition of pathogen mycelial growth and suppression of spore production.
germination conceded as a result of antagonistic activities of the rhizosphere bacteria against phytopathogens fungi. These results are a best example of the bioc...er bacteria. 

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