



Separation and Identification of Volatile Compounds from Liquid Cultures of *Pseudomonas aeruginosa* by GC-MS and its Effect on Resistance to *Rhizoctonia solani*

Ola Hadi Jaafar¹, Aymen Jasim Mahdi², Sayed Saad El-Din Aboshosha³,
Alia Abed Elbaky Shoeib³, Amany Hassan Shams³

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ABSTRACT

Background: This research focused on isolating the fungus *Rhizoctonia solani* from potato tubers afflicted by black scurf disease and assessing its pathogenicity and resistance when exposed to *Pseudomonas aeruginosa* bacteria. The objective of this research was to separate and identify the volatile compounds from liquid cultures of *Pseudomonas aeruginosa* by gas chromatography-mass spectrometry (GC-MS) and to evaluate their effect on resistance to *Rhizoctonia solani*.

Methods: The pathogenicity of *Rhizoctonia solani* was examined using radish seeds. Laboratory experiments assessed the inhibitory effect of *Pseudomonas aeruginosa* on PDA medium. The chemical profile of bioactive substances present in the methanolic extract of *Pseudomonas aeruginosa* was analyzed through GC-MS and greenhouse trials evaluated the effect of the combined treatment with *Pseudomonas aeruginosa* and *Rhizoctonia solani* on infection severity and potato plant growth indicators.

Result: The findings revealed a germination rate of 12% in the pathogenicity test, in contrast to the control treatment, which achieved 100%. Laboratory experiments indicated that *Pseudomonas aeruginosa* exhibited significant inhibitory effects, with an inhibition rate of *Rhizoctonia solani* on PDA medium reaching 69.22%, compared with 0% in the control treatment. GC-MS analysis identified a total of 12 compounds within the bacterial extract. Greenhouse trials demonstrated that the combined treatment with *Pseudomonas aeruginosa* and *Rhizoctonia solani* resulted in a disease severity of 18.75% compared to 75% in the control. Furthermore, all growth indicators of potato plants were enhanced in the presence of *Pseudomonas aeruginosa* alongside *Rhizoctonia solani*.

Key words: GC-MS, Potato, *Pseudomonas aeruginosa*, *Rhizoctonia solani*.

INTRODUCTION

Rhizoctonia is a genus of soil-borne, multi-host fungi, comprising species that attack the roots, stems and leaves of plants, causing diseases such as root rot, damping-off and petiole and crown diseases. *Rhizoctonia* problems have a significant economic impact on global agriculture, due to their ability to remain in the soil for long periods through structures such as sclerotia, making complete eradication or isolation difficult. Causes losses in important crops such as rice, beans, potatoes, coffee, legumes and pasture crops such as alfalfa (Akber and Fang, 2024) among others. Biological control has proven to be very successful in combating many plant diseases using biological agents that have a great ability to limit the growth of pathogenic fungi (Abdullah *et al.*, 2019; Mahmood and Al-Abedy, 2021). Among these agents are the bacteria *Pseudomonas* spp, which have proven their resistance to fungi lurking in the soil through multiple mechanisms, such as their high competitive ability, their stimulation of systemic resistance and the enhancement of plant root growth. This is considered one of the methods followed and an alternative to chemical pesticides due to their harmful effects on the environment (Hend *et al.*, 2017). The results of the seed treatment experiment with a mixture of the biological control agent *T. herzanium* + *Pseudomonas fluorescens* bacteria, along with the addition of the same mixture to the

¹Department of Plant Protection, College of Agriculture, University of Kerbala, Iraq.

²College of Pharmacy, University of Kerbala, Kerbala, Iraq.

³Department of Plant Pathology, College of Agriculture, University of Alexandria, Alexandria, Egypt.

Corresponding Author: Ola Hadi Jaafar, Department of Plant Protection, College of Agriculture, University of Kerbala, Iraq.
Email: ola.jaafar@uokerbala.edu.iq

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soil, showed a 63.93% reduction in root rot infection and an increase in yield (Singh *et al.*, 2023). In a field study on mothbean to evaluate environmentally friendly biological control strategies against *R. solani*, the results showed that treating seeds with *Pseudomonas fluorescens* bacteria and *T. herzanium* reduced infection to 21.78% with a significant increase in yield (Godara and Singh, 2021). *Pseudomonas aeruginosa* is known to enhance plant growth and suppress many fungal diseases

(Audenaert *et al.*, 2002). Resistance can be induced on the aerial parts of plants by pre-inoculation with PGPR in the root system (Van Loon *et al.*, 1998) *P. aeruginosa* produces a range of antifungal compounds (e.g., pyocyanin, pyrrolnitrin, phenazines, HCN, rhamnolipids, etc.) and can inhibit *R. solani* in in vitro tests and in some field/greenhouse experiments (Emad and Alsalam, 2017). The *Pseudomonas aeruginosa* has shown a significant role in resisting the *Rhizoctonia solani*, which causes rice blight (Thammasittirong *et al.*, 2025).

MATERIALS AND METHODS

This study was conducted at the Plant Pathology Department, College of Agriculture, University of Alexandria, Egypt, for the 2022-2023 agricultural season.

Isolation and identification of pathogenic fungi

Potato plants affected by black scurf disease were gathered for analysis. The tubers underwent a gentle washing with tap water to eliminate soil and debris, followed by cutting them into small segments measuring 0.5 to 1 cm. These segments were then sterilized in a 1% sodium hypochlorite solution for three minutes and rinsed in sterile distilled water for an additional two minutes to ensure the removal of any leftover disinfectant. After drying on sterile filter paper, the pieces were placed into Petri dishes (9 cm in diameter) filled with sterile potato dextrose agar (PDA) medium, which had been autoclaved at 121°C under 1.5 kg/cm² pressure for 15 minutes and enriched with tetracycline antibiotic at a concentration of 200 mg/L. The plates were incubated at a temperature of 25±1°C for three days, during which fungal colonies were isolated from the PDA medium using the hyphal tip technique. The fungal species linked to the infected tubers was initially identified through its morphological features. Additionally, molecular identification and the characterization of genetic diversity among fungal and virus plant pathogens have been extensively established utilizing PCR-based sequencing techniques (Abdullah *et al.*, 2019; Al-Abedy *et al.*, 2019; Al-Shujairi *et al.*, 2022). To perform this on our isolates, was conducted by extracting genomic DNA from pure fungal hyphae using the DNeasy Plant Mini Kit following the manufacturer's guidelines. To amplify the ITS (internal transcribed spacer) region of the ribosomal DNA (rDNA), ITS1 and ITS4 primers were employed. The resulting sequence was submitted to the GenBank database and analyzed against other sequences utilizing the BLAST program.

Pathogenicity assessment

The pathogenicity of the *R. solani* fungus was assessed using a plate method (Bolkan and Butler, 1974). This procedure involved petri dishes measuring 9 cm in diameter, which contained 15-20 ml of water agar medium. The water agar was prepared by dissolving 20 g of agar in one liter of distilled water and sterilized in an autoclave for 15 minutes under

previously specified conditions. Once the medium solidified, the center of each dish was inoculated with a 0.5 cm diameter disc taken from the periphery of pure colonies of the fungus that were five days old. Control dishes containing the same medium were left uninoculated. All dishes, both inoculated and non-inoculated, were incubated at a temperature of 25±1°C for three days. Local radish seeds underwent sterilization using a 1% sodium hypochlorite solution for two minutes, followed by three washes with distilled water and were then arranged circularly near the edge of both the inoculated and non-inoculated dishes at a density of 15 seeds per plate. Three dishes were allocated to each fungal isolate along with control treatments. The dishes were subsequently placed in an incubator set to maintain a temperature of ±25°C. After seven days, germination percentages were calculated using the following equation:

The percentage of germination =

$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Testing the effect of *P. aeruginosa* on *R. solani* on nutrient agar medium

Two complete loops of bacterial suspension were applied as parallel lines on nutrient agar medium for each strain, followed by incubation at 28°C for 24 hours. A disc of *R. solani* was positioned centrally between the two bacterial growth lines. Each isolate of *R. solani* underwent five replicates. The inoculated Petri dishes were then incubated at 28°C for a duration of five days, with daily monitoring of fungal growth. Measurements of growth were recorded. In the streaking assay, the bacteria's inhibitory effect on fungal development was visually evaluated by contrasting the treated plates with their controls (which lacked bacteria) (Matar *et al.*, 2009). The percentage inhibition of fungal growth was determined using the equation below:

% of inhibition =

$$\frac{\text{The growth rate of the colony in control} - \text{The growth rate of the colony in the treatment}}{\text{The growth rate of the colony in the control}} \times 100$$

Evaluation of the biological agents *P. aeruginosa* in protection of potato plant in plastic house

The effectiveness of biological agents in safeguarding potato plants from black scurf disease was examined. 'Spunta' potato tubers were placed in plastic bags with a diameter of 25 cm (one tuber per bag) that could hold up to 2 kg. Each bag was filled one-third of the way with a uniform blend of sand and dry clay and the plants were watered as necessary. The inoculum for the fungus *Rhizoctonia solani* was prepared following the method outlined in reference (Dewan and Sivasithamparam, 1989). This fungus was cultivated using local maize seeds; specifically, 50 g of seeds were soaked in 150 ml of water within 250 ml bottles for a duration of six hours. After soaking, the excess water was removed and the bottles were autoclaved at 121°C

and 15 psi for one hour. Following this, five discs measuring 5 mm in diameter from a pure culture of *R. solani* grown on PDA medium were used to inoculate the seeds. The bottles were then incubated at a temperature of $25 \pm 1^\circ\text{C}$ for two weeks, with manual shaking performed every three days to promote aeration and ensure uniform fungal distribution among the seeds. Additionally, bacterial inoculum was cultured in 250 ml glass bottles containing 100 ml of nutrient medium; each bottle received an inoculation from the bacterial culture after one day and was incubated at $28 \pm 1^\circ\text{C}$ for one to two days. Prior to planting, bacterial density measurements were taken. The experiment included the following treatments:

- 1- The pathogen *R. solani* only.
- 2- Potato plants only.
- 3- *P. aeruginosa* only.
- 4- *P. aeruginosa* + *R. solani*.

An extract from the pathogenic fungus *R. solani* was incorporated into local maize seeds in all relevant treatments, at a concentration of 1% (w/w). A solution containing *P. aeruginosa* bacteria, cultured for three days and reaching a concentration of 5×10^9 colony-forming units/mL, was introduced into the soil at a dosage of 7.5 mL per pot (Larkin, 2004). Following this, three days later, the fungal extract was applied. Data collection occurred two months post-planting. The severity of the disease was assessed using the following scale:

- 0- Healthy plant.
- 1- One spot on the stem with a diameter less than 25 mm.
- 2- One spot on the stem with a diameter between 26-50 mm.
- 3- Presence of spots measuring between 51-75 mm in diameter.
- 4- Spots exceeding 75 mm in diameter that completely encircle the stem.

The percentage of disease severity was determined according to a specific formula (McKinney, 1923).

% of disease severity =

$$\frac{(\text{Number of plants in degree } 0 \times 0) + \dots + (\text{Number of plants in degree } 4 \times 4)}{\text{Number of plants studied} \times 4} \times 100$$

Root dry weight and shoot and root lengths of the potato plants were measured.

Analysis of bioactive compounds in methanol extract of *P. aeruginosa* bacteria using GC-MS technology

Subcultures of *Pseudomonas aeruginosa* isolates were cultivated in nutrient broth (NB) for a period of 48 hours at temperatures ranging from 27 to 29°C . Following this, the metabolites were extracted from the liquid culture and concentrated to dryness utilizing a rotary evaporator set at 45°C . The resulting residue was reconstituted in 1 mL of methanol, filtered through a $0.2 \mu\text{m}$ syringe filter and subsequently stored at 4°C for 24 hours prior to being analyzed by mass spectrometry in gas chromatography (Hameed *et al.*, 2018).

Statistical analysis

The data were analyzed using a randomized complete block design (RCBD) according to the program SPSS. The means were compared using the least significant difference between the means at a (LSD) least significant difference probability level of 0.05.

RESULTS AND DISCUSSION

The fungus *R. solani* was extracted from potato tubers affected by black scurf disease and categorized according to its morphological traits (Ajayi-Oyetunde and Bradley, 2018). PCR analysis verified the existence of the ITS region in *R. solani*, which was subsequently sequenced and submitted to the NCBI GenBank database with accession number OP787992. Regarding *P. aeruginosa* bacteria (Fig 1), it had previously been utilized in a study and is cataloged in the gene bank under accession number KF922508 (Abo-Zaid *et al.*, 2015). It is important to note that this bacterium is an opportunistic pathogen to humans, therefore biosafety regulations must be observed when using it in agriculture.

The pathogenicity assessment

The results showed that the fungus *R. solani* caused a significant decrease in radish seed germination, with a germination rate of only 12% compared to 100% in the control.

In vitro inhibitory effects of *P. aeruginosa* on *R. solani*

The results of this test indicate that the use of *P. aeruginosa* bacteria inhibited the growth of the *R. solani* fungus isolate, with a growth rate of (2.77 cm) compared to (9 cm) for the *R. solani* fungus alone (Fig 2). The *P. aeruginosa* bacteria treatment achieved an inhibition rate of 69.22%, compared to 0% for the control treatment. These results were similar to those of another study (Shaheen *et al.*, 2025) which demonstrated the ability of *P. aeruginosa* to inhibit the *R. solani*.

Identification of bioactive compounds presented in *Pseudomonas aeruginosa* extract

In the current study examining the presence of volatile and semi-volatile bioactive compounds in methanolic extracts of *Pseudomonas aeruginosa*, a total of twelve bioactive constituents were identified (Table 1) (Fig 3). The compounds detected, along with their respective area percentages, are



Fig 1: *P. aeruginosa* bacteria.

as follows: Oleic acid (40.46%), hexadecanoic acid (20.63%); 10-octadecenoic acid, methyl ester (8.59%); hexadecanoic acid, methyl ester (7.87%); 9-octadecenoic acid (5.63%); 7,10-octadecadienoic acid, methyl ester (5.24%); octadecanoic acid, methyl ester (2.22%); 16-octadecenoic acid, methyl ester (2.17%); cyclotrisiloxane, hexamethyl (1.94%); cyclotrisiloxane, hexamethyl- (1.86%); 4-Hexyl-1-(7-Methoxycarbonylheptyl) (1.77%) and 1,4-Diphenylbut-3-ene-2-ol (1.62%).

In this study, the effect of several fatty acids (including oleic acid) on several fungi was studied and their effect on *R. solani* was proven (Liu *et al.*, 2008). Another study demonstrated that *Pseudomonas* P2 bacteria produce

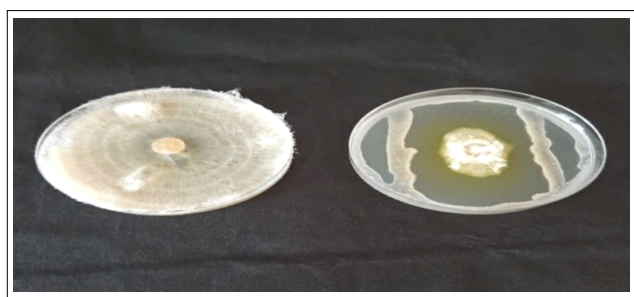


Fig 2: Antifungal effects of *P. aeruginosa* on *R. solani*.

volatile compounds that exert strong control over *R. solani*. It demonstrated their effect on fungal morphology and the structure of fungal cells (Elkahoui *et al.*, 2015). Fatty acids such as oleic acid and hexadecanoic acid, identified in our *P. aeruginosa* extract, have been recognized for their potent antifungal and antioxidant activities in previous GC-MS profiling studies (Kavitha *et al.*, 2023).

Evaluation of the effectiveness of *P. aeruginosa* bacteria in protecting potato plants from *R. solani* fungal infection in plastic house

This research examined the impact of *P. aeruginosa* bacteria on mitigating black scurf disease, which is induced by the fungus *R. solani*. As illustrated in Table 2, *P. aeruginosa* demonstrated a beneficial effect by reducing the disease incidence by 18.75% compared to the control group treated solely with the pathogen, which exhibited a disease incidence of 75%. Additionally, the presence of these bacteria led to an increase in the dry weight of potato roots, measuring at 2.57 grams, as opposed to 1.03 grams observed in the control treatment with just the pathogen. The bacterial treatment also resulted in enhanced lengths for both shoot and root systems, measuring 26.62 cm and 27.11 cm respectively, when compared to control lengths of 17.41 cm for shoots and 18.37 cm for roots.

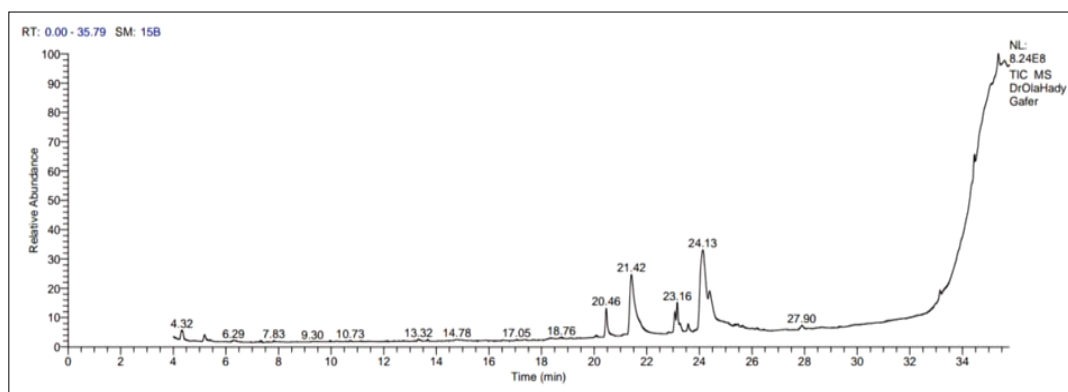


Fig 3: Gas chromatography profile of crude extract of *Pseudomonas aeruginosa*.

Table 1: Peak number, retention time and area% of the detected compounds detected in *Pseudomonas aeruginosa* extract.

Peak (No.)	Retention time (min.)	Area %	Molecular formula	Compound name
1	24.11	40.46	$C_{18}H_{34}O_2$	Oleic acid
2	21.41	20.63	$C_{16}H_{32}O_2$	Hexadecanoic acid
3	23.16	8.59	$C_{19}H_{36}O_2$	10-Octadecenoic acid, methyl ester
4	20.46	7.87	$C_{17}H_{34}O_2$	Hexadecanoicacid, methyl ester
5	24.40	5.63	$C_{18}H_{34}O_2$	9-Octadecenoic acid
6	23.07	5.24	$C_{19}H_{34}O_2$	7,10-Octadecadienoic acid, methyl ester
7	23.58	2.22	$C_{19}H_{38}O_2$	Octadecanoicacid, methyl ester
8	23.27	2.17	$C_{19}H_{36}O_2$	16-Octadecenoic acid, methyl ester
9	5.19	1.94	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, hexamethyl
10	4.35	1.86	$C_6H_{18}O_3Si_3$	Cyclotrisiloxane, hexamethyl-
11	27.89	1.77	$C_{25}H_{40}O_2$	4-Hexyl-1-(7-methoxycarbonylheptyl)
12	4.31	1.62	$C_{16}H_{16}O$	1,4-Diphenylbut-3-ene-2-ol

Table 2: Assessment of the efficacy of *Pseudomonas aeruginosa* bacteria in safeguarding potato plants against *Rhizoctonia solani* fungal infection within a greenhouse setting.

*Treatments	Disease severity %	Root dry weight (gm)	Plant length (cm)	
			Shoot	Root
<i>R. solani</i> only	75	1.03	17.41	18.37
Potato plants only	0	2.98	26.42	26.88
<i>P. aeruginosa</i> only	0	3.88	32.22	33.13
<i>P. aeruginosa</i> + <i>R. solani</i>	18.75	2.57	26.62	27.11
LSD 0.05	3.994	0.056	0.056	0.056

*Each value represents the mean of three replicates.

These improvements are believed to stem from competition for nutrients and space as well as the activation of plant immune responses (ISR), which bolster resistance against soil-borne fungi such as *R. solani* (Aiattas *et al.*, 2024). The antagonistic potential of pseudomonas strains against *R. solani* has been previously documented in similar soil ecosystems (Rani *et al.*, 2016).

CONCLUSION

The study demonstrated that *Pseudomonas aeruginosa* has a clear inhibitory effect against *Rhizoctonia solani*, as it reduced fungal growth under laboratory conditions and decreased disease severity in potato plants under greenhouse conditions. GC-MS analysis revealed the presence of twelve bioactive compounds in the bacterial extract, mainly fatty acids and their derivatives, which may contribute to antifungal activity. The treatment with *P. aeruginosa* also improved root dry weight and shoot and root lengths, indicating its potential use as a biological control agent against black scurf disease in potato.

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Author's contribution

Dr. Ola handled data preparation and research writing, Dr. Amani provided the bacteria and prepared the bacterial extract for use in the GC-MS instrument and Dr. Sayed and Dr. Alia supervised the research process, Assistant Lecturer Ayman in Statistical Analysis of Research Data.

Novelty statement

GC-MS technology was used to identify the components of *Pseudomonas argonosa* bacteria and to determine the role of some of these components in resisting the fungus *R. solani* and their effect on certain potato plant growth indicators.

Multiple tifs and other monovalent isolates were used to detect resistance to *R. solani*, the causative agent of black scurf disease in potatoes.

Conflict of interest

The absence of conflicting information in the research is what the authors claim.

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