Egyptian Journal of Phytopathology, Vol. 53, No.2, pp 90 - 110 (2025)
DOI: 10.21608/EJP.2025.406064.1150

ORIGINAL ARTICLE

Harnessing Antimicrobial Activities of *Azotobacter* and *Bacillus* Species for Suppression of Root Rot Disease in Sugar-beet

Fouad M. S.[1*], Ahmed S. T. [2] , Abd El-Megeed F. H.[3]

Received: 20 July 2025 / **Accepted**: 21 August 2025 / **Published online**: 27 August 2025

ABSTRACT

Plant growth promoting bacteria (PGPB), *Azotobacter chroococum* (B1), *Bacillus megaterium* (B2) and *B. velezenesis* (B3) were evaluated for their potential against *Rhizoctonia solani* which is the causal agent of root rot in sugar-beet. Samples collected from three Egyptian governorates (Sharqia, Kafr El-Sheikh and Ismailia) showed the distinctive symptoms of Rhizoctonia root rot. Moreover, the *in vitro* pathogenicity test revealed that the most virulent isolate (MFRh, GenBank accession number LC819603) was isolated from Sakha, Kafer-El-Sheikh governorate (31.0894° N, 30.9444° E). Bacterial strains inhibited fungal growth with values ranging from 42.96±1.67% to 44.81±0.98%. They also verified for hydrolytic enzymatic activity and were positive for cellulase, chitinase and pectinase. Strain B3 has the highest enzyme index for cellulase activity with enzymatic index, 1.98±0.09. GC/MS profile analyses for the bacterial extract of the B1, B2 and B3 recorded about 13, 15 and 13 certain volatile major compounds, respectively that recognized for their antimicrobial activity. Acetonitrile, oleic acid and heptaethylene gave the highest ratio in B1, B2 and B3 extracts respectively as a major compound. Additionally, B1 extract contained 2, 3-Butanediol which is recognized as plant growth promoter. *In vivo* experiments indicated significant decreases in disease severity as 25.4±1.88, 27.4±1.16 and 25.8%±1.83 due to seed treatment with B1, B2 and B3 suspensions, respectively compared to positive control, being 75.2%. Metabolic performance of sugar-beet seedlings was assessed providing the highest significant values in total phenols; proline content and peroxidase activity in leaves under the infection stress upon the application of *B. velezenesis* as 19.82±2.05 mg/g, 0.33±0.01mg/g and 3447.00±39.17 U/g, respectively. However, the total chlorophyll content exhibited the maximum value when seeds were treated with *A. chroococum* alone (38.03±0.78 mg/g).

Keywords: *Rhizoctonia solani*, *Beta vulgaris* L, root rot, *A. chroococum*, *B. megaterium*, *B. velezenesis*

[*]**Correspondence : Marwa S. Fouad**
E-mail: msfouad2020@yahoo.com

Marwa S. Fouad
https://orcid.org/0009-0008-5374-8772
1- Plant Pathology Research Institute, Agricultural Research center, Giza 12619, Egypt.

Shadia T. Ahmed
2- Sugar Crops Research Institute, Agricultural Research Centre, Giza, Egypt.

Fayrouz H. Abd El-Megeed
3- Department of Microbial Genetic Resources, National Gene Bank (NGB), Agricultural Research center, 12619, Giza, Egypt.

INTRODUCTION

Sugar-beet (*Beta vulgaris* L.) an annual vegetable crop is widely distributed in many temperate regions including South America, Europe, North America, North Africa and Asia. It has been developed as a key source of sugar manufacture worldwide. Different varieties of sugar-beet can adapt to environmental changes and growing conditions **(**Haque and Parvin 2020). Due to the great consumption of sugar in Egypt, sugar-beet production should be increased to encounter the human demands for sugar as an alternative to sugar cane (Ibrahim et al. 2021).

Losses in crop yield and sugar content were mainly related to soil-borne pathogens at all stages of growth. Some soil associated microbiomes such as *Rhizoctonia solani*, are described as the main cause of damping-off and root-rot disease (Afifi et al. 2018). Rhizoctonia disease has challenged to be managed because it is soil- and seed-borne, and easy to spread through different plant organs like tubers. So, the main challenge that faces farmers is how to manage the disease and decrease disease severity in a

safe way that doesn't disturb ecological balance.

Several approaches have been employed to control *R. solani* including physical and chemical methods, and crop rotation. As the limited potential of those methods, a need for alternative strategies has been generated. Biological control is considered to be the most efficient and eco-friendly way to protect plants from soil-borne pathogens. Different studies have established that certain antagonistic organisms are used as bio-agents and can be effectively suppress Rhizoctonia disease (Aydın, 2022).

Various microorganisms can produce lytic enzymes and have gained a significant interest as biocontrol agents against fungal pathogens (Collinge et al. 2022). These enzymes, also called cell-wall-degrading enzymes, are capable of breaking down the fungal cell wall structural components as chitin and glucan (Yarullina et al. 2016; Siddiqui and Khan 2017). By disrupting the integrity of the fungal cell wall, lytic enzymes can effectively hinder the growth and spread of pathogenic fungi **(**Jadhav et al. 2017**).** The use of lytic enzymes-producing microorganisms provides a natural and eco-friendly alternative to traditional chemical fungicides which in turn reduces the risk of pathogen resistance development and decreases the impact on non-target organisms (Ramankutty et al. 2018)

Several species belonging to the genus Bacillus is the most extensively considered for their production of extracellular lytic enzymes (Sadhu and Maiti 2013)**.** Different species within this genus have been isolated from a wide range of habitats allowing them to struggle physico-chemical stresses and thus making them potential sources of alkalophilic, psychrophilic, acidophilic, thermophilic and halophilic cellulolytic enzymes (Malik and Javed 2021).

Bacillus velezensis has been recognized for its significant antimicrobial activity producing several antifungal compounds like lipopeptides, volatile compounds, stable extracellular metabolites and enzymes. Meanwhile, it acts as plant growth-promoting bacteria (PGPB). So, *B. velezensis* was suggested as a promising biocontrol agent for suppressing the plant pathogenic fungi in the bio-industry (Baptista et al. 2022).

Azotobacter bacteria had been classified as plant growth-promoting rhizobacteria (PGPR) where it capable of improving soil fertility and promoting plant growth (Katiyar et al. 2016). It benefits plant health and can restrain the growth of different plant pathogens as an antagonistic bacterium (Zian and Aly 2020).

The present work aims to evaluate and compare the antagonistic influence of some bacterial strains (PGPB) against *Rhizoctonia solani* infecting sugar beet in Egypt and their performance in reducing disease severity with improvement of plant resistance in an environmentally safe manner.

MATERIAL AND METHODS

Sugar-beet seeds, biocontrol agents and the fungicide

Sugar-beet seeds

Danish cultivar, Matros, was kindly provided by the Pest and Diseases Research Department, Sugar Crops Research Institute, Agricultural Research Centre, Giza, Egypt.

Biocontrol agents

Three bacterial strains were used as biocontrol agents in this work. *Azotobacter chroococum* (B1) and *Bacillus megaterium* (B2) were kindly obtained from the Microbiology Department, Faculty of Science, Ain-Shams University and they were deposited in NCBI GenBank with accession numbers of (EMCCN1004 and EMCN1029, respectively). *Bacillus velezensis* (B3) was isolated from common bean seeds in previous study as mentioned by Fouad et al. 2024, and deposited in NCBI GenBank with accession number of (ON000400) . Bacterial strains were cultured on Nutrient Agar medium (BD, Difico™ Georgia, GA, and USA).

Fungicide

Rhizolex-T 50% (WP) (Sumi Agro Company, Japan) was applied as seed

treatment with the manufacturer's recommended dose (3g/kg seed).

Isolation and morphological identification of the associated fungi

Out of twelve Egyptian governorates cultivating sugar beet (Dakahlia, Gharbia, Sharqia, Kafr El-Sheikh, Matrouh, Minya, Menofia, New Valley, Damietta, Ismailia, Beheira, and Port Said), samples were collected only from three governorates including Sharqia (30.7327° N, 31.7195° E), Kafr El-Sheikh (31.1431° N, 30.8039° E), and Ismailia (30.5831° N, 32.2654° E) that have the naturally infected plants with typical disease symptoms. Infected roots were washed with tap water to get rid of any soil particles from the sample and chopped into small pieces. Chopped parts were surface sterilized with NaOCl (2%) solution for 2 min. The sterilized pieces were washed well with sterile distilled water to remove any remnants of NaOCl (Al-Fadhal et al. 2019). The dried pieces were transferred to Petri dishes containing Czapek's yeast agar (CYA) medium, (Shi, 1996): (g/l) 1.0 of K_2HPO_4, 3.0 of $NaNO_3$, 0.5 of $MgSO_4.7H_2O$, 0.01 of $FeSO_4$, 0.5 of KCl, 30.0 of Sucrose, 5.0 of Yeast extract, 15.0 of Agar at neutral pH. CYA was supplemented with 20 mg/L antibiotic, chloramphenicol. At 25 ± 2 °C, all Petri dishes were incubated for almost 5 days. To obtain a pure culture, a hyphal tip of the concerned pathogen was removed using sterile glass needle and put onto the solid CYA media plate and incubated at 25± 2 °C for 12- 15 days with daily examination. The developing fungal pathogen was identified and characterized in Plant Pathology Research Institute, ARC, Giza, Egypt, based on the culture properties, morphological and microscopic characteristics (Farhaoui et al. 2024).

Pathogenicity test

The isolated fungus was evaluated for pathogenicity to the corresponding host in petri dishes under *in vitro* conditions. A disc (0.5cm) of 5-days old CYA growth culture medium was placed in the center of a petri dish that contained 2% water agar and then incubated at 25±2°C for 48 h. As the incubation period completed, eight surface sterilized sugar-beet seeds were arranged peripherally around the fungus growing piece. Surface sterilization of seeds was performed using NaOCl (2%) solution for 5 min followed by washing with sterile H_2O and dried on sterilized blotter paper. In control plates, seeds were set around disc of the sterile CYA medium. All petri plates were incubated at 25±2°C with 12 h photoperiod for 5-day with daily follow-up (Basbagci et al. 2019).

Molecular identification and phylogenetic analysis of the isolated fungus

DNA Extraction and Polymerase chain reaction (PCR) Amplification

The genomic DNA of the fungal isolate was extracted from a one-week-old CYA growth culture using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of the ribosomal internal transcribed spacer (ITS) rRNA region was done using ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS4 (5-TCCTCCGCTTATTGATATGC-3) primers, as described by Ortiz et al. (2019). PCR was performed using the standard reaction mixture (50 µL): 1× PCR buffer, 1.5 mM of $MgCl_2$, 15 pmol of each primer, 200 mM of each dNTP, 1U of Taq polymerase enzyme (Promega Corporation, WI, USA), and 50 ng of the DNA template. PCR reaction was designed as follows: Primary denaturation was achieved at 94 ◦C for 3 min; 30 cycles of denaturation were attained at 94 ◦C for 30 s; annealing stage was performed at 58 ◦C for 30 s; extension step was done at 72◦C for 90 s; final extension was completed at 72 ◦C for 10 min. The PCR product was detected on 1.5% agarose gel electrophoresis and then it was purified using the PureLink PCR Purification Kit (Thermo Fisher Scientific).

Sequencing and phylogenetic analysis

PCR product was sequenced at Macrogen, Inc. (Seoul, Korea) and the obtained sequence was compared with the other related sequences using the BLAST function in GenBank (https://blast.ncbi.nlm.nih.gov/). The retrieved sequence was aligned using Clustal W version 1.8 (Altschul et al. 1997)

and subjected to phylogenetic analyses. The phylogenetic tree was constructed using the maximum likelihood (Saitou and Nei 1987) in MEGA X version 10 (Kumar et al. 2018) with the Tamura-Nei model. Bootstrap support for each node was evaluated with 1000 replicates. The average nucleotide identity was calculated between straind fungi and closely related reference strains using MEGA X software.

In Vitro* antagonistic activity of bacterial strains against *R. solani

Dual culture plate method was used to determine the antagonistic potential of the bacterial strains against *R. solani* (Mohamadpoor et al. 2020). A disc (0.5 cm), taken from 5-days old culture of the tested fungus was put 1 cm apart from the edge of CYA growth medium plate and a looped swab of each bacterial bioagent strain was streaked individually at the opposite side of the plate, 1 cm apart from edge. For control, only a disc of *R. solani* was set peripherally in a CYA growth medium plate. Incubate the plates at 25 ± 2◦C for 5 days. When the pathogen growth reached the entire surface of the plate in the control treatment, the percentage of growth inhibition was measured as the formula stated by Zhou et al. (2022).

Evaluation of extracellular enzyme activities of bacterial strains

Bacterial ability to produce hydrolytic enzymes was examined on a solid basal medium amended separately with (1% *w/v*) different sole carbon sources (carboxymethyl cellulose [CMC], and colloidal chitin, as well as pectin of citrus peel) for detection of the production of cellulase, chitinase, and pectinase, respectively, (Mohamed et al. 2022). 10 µL of the bacterial culture, 10^6cfu/ml were spotted on the surface of specific agar plates and incubated at 30 °C/72 h (Rawat and Tewari 2012). At the end of the incubation period, the plates were flooded with Gram's iodine solution (Kasana et al. 2008) for 5 min and the plates were then screened out for halozone formation. The diameter of clear zones around colonies refered to the qualitative enzyme activity (Arora and Verma 2017). The enzyme index (EI) was determined as mentioned by Ferbiyanto et al. (2015)

Gas chromatography mass spectrometry (GC/MS)

At Egypt-Japan University of Science and Technology (E-JUST), gas chromatography of (GC/MS-QP2010 Ultra) system (Shimadzu, Japan) was used to explore the chemical composition of the culture filtrates. Column, Teknokroma TRB-WAX.DBL 30m x ID 0.25mm x DF 0.25 µm (P/N:TR-930232). Injection volume was 0.4 µL (split ratio 99:1). Oven temperature was 60 ◦C for 3 min and increased to 180 ◦C for 1 min; increased to 260 ◦C and left for 5 min. Carrier Gas: He, constant flow rate 1.5mL/min. Mass detector: MS-QP Transfer Line. The temperature of the ion source was 265 ◦C. The scanning of the mass analyzer was set at 300 ◦C. Identification of each compound was by comparing the retention time and mass spectra with libraries of NIST 11 Spectral Library (Gaithersburg, MD, USA).

***In vivo* experiments**

Two greenhouse experiments were conducted at the same time with the same design and identical conditions (temp., 23±2°C; a daily light duration of 10-12 hours and humidity between 60-70%) at Plant Pathology Research Institute and Sugar Crops Research Institute, Agricultural Research Centre, Giza, Egypt (. Pots (25 cm in diameter) were filled with clay-sand soil (clay: sand, 2:1 v/v) and arranged as ten replicates for each treatment with ten seeds per pot. Negative control pots were prepared devoid of pathogen. Experimental pots were infested with *R. solani* inoculum that previously prepared by inoculating discs of CYA growing culture plates of 5 days old culture (25 ±2 °C), into sterilized medium of sorghum: coarse sand: water (2:1:2 v/v) and incubated at 25 °C for 15 days. *R. solani* sand corn inoculum was incorporated into soil at the rate of 3 % w/w in each pot and mixed thoroughly. Pots were irrigated regularly for one week to allow the spread of pathogen prior to seeding. Seeds were

surface sterilized as described in pathogenicity test. Then, seeds were soaked separately, for 1 h, in the bacterial suspensions of B1, B2 and B3 that were adjusted to 10^6cfu/ml for each.

Experimental design was arranged as nine treatments; (1) a negative control devoid of any treatment and (2) positive control that involved pathogen inoculum only (MFRh). Single application of each bacterial suspension of B1, B2 and B3 were considered as treatments (3), (4) and (5) respectively where the pots were devoid of pathogen inoculum. (6) Pathogen plus the chemical fungicide (Rhizolex-T 50% WP) that was applied as seed treatment at 3 g/kg seed (F+MFRh). (7) Pathogen plus B1 (B1+ MFRh), (8) Pathogen plus B2 (B2+ MFRh) and (9) Pathogen plus B3 (B3+ MFRh). Experiment was kept for 45 days under greenhouse conditions with regular irrigation.

Disease assessment

Disease parameters were recorded as pre-emergence damping off (%) and post-emergence damping off (%) at 15 and 30 days, respectively after sowing for all treatments. The percentage of survived plants was assessed at 45 days after sowing according to El-Rayes et al. (2022). Disease severity was calculated as follows:

$$\text{Disease severity (\%)} = \frac{\sum (n \times v)}{5\,N} \times 100$$

Where: n= number of seedlings in each category, v= numerical values of symptoms category N = total number of seedlings, (5) = the maximum numerical value of the symptom category (Zanella et al. 2020).

Metabolic changes in sugar-beet leaves

Changes in metabolic activity in seedlings leaves were estimated after 30 days of planting. Phenolic content was determined using a spectrophotometer (UV-Vis Spectronic 601). At 520 nm, density of the blue color developed was measured using catechol as standard (Snell and Snell 1953). For proline measurement, colorimetric ninhydrin method was utilized as mentioned by Bates et al. (1973). Total chlorophyll content was evaluated according to Dere et al. (1998). Peroxidase (POX) activity was assessed as a defense related enzyme as described by Fouad et al. (2024).

Statistical analyses

Data were analyzed using a one-way analysis of variance (ANOVA). Tukey–Kramer test for multiple comparisons with $p \leq 0.05$ level of probability as the significance level. The statistical software MINITAB (Minitab® 19.2020.1 version, Minitab Inc., State College, PA, USA) was used.

Map creation

The map was created by Arc/GIS, (version 10.3).

RESULTS

Morphological identification of the isolated fungus and pathogenicity test

The isolated fungus gave the characteristic macroscopic and microscopic view that was characteristic to *Rhizoctonia solani*. It formed off-white colonies and produced abundant brown irregular sclerotia. Mycelium composed of hyphae with a right-angled branching pattern and had constriction at the origin of branching. Collected diseased samples from three Egyptian governorates, Sharqia, Kafr El-Sheikh and Ismailia (Fig. 1), showed the typical symptoms of Rhizoctonia root rot. According to the *in vitro* pathogenicity test, the most virulent tested fungal isolate was obtained from Sakha, Kafer-El Shaikh governorate (31.0894° N, 30.9444° E) and generated distinctive symptoms in diseased seedlings meanwhile, healthy seedlings in the control plates did not develop any disease symptoms (Fig. 2).

Molecular identification of the isolated pathogenic fungus

Based on the ITS sequence homology, fungal isolate was identified as *Rhizoctonia solani* with high similarity of 98% and named as MFRh. The retrieved ITS sequence has been deposited in GenBank under accession number LC819603. A whole of 13 sequences of close relatives were downloaded from the National Center for Biotechnology Information and combined

with sequence in this study for phylogenetic tree construction (Fig. 3). According to the ITS phylogenetic tree, strain MFRh had an ITS sequence similarity of 98.3% to the *Rhizoctonia solani* AG-4 HGIII strain (Access. No. KF881897) which was isolated from infected plant in India, supported by high bootstrapping value (98%).

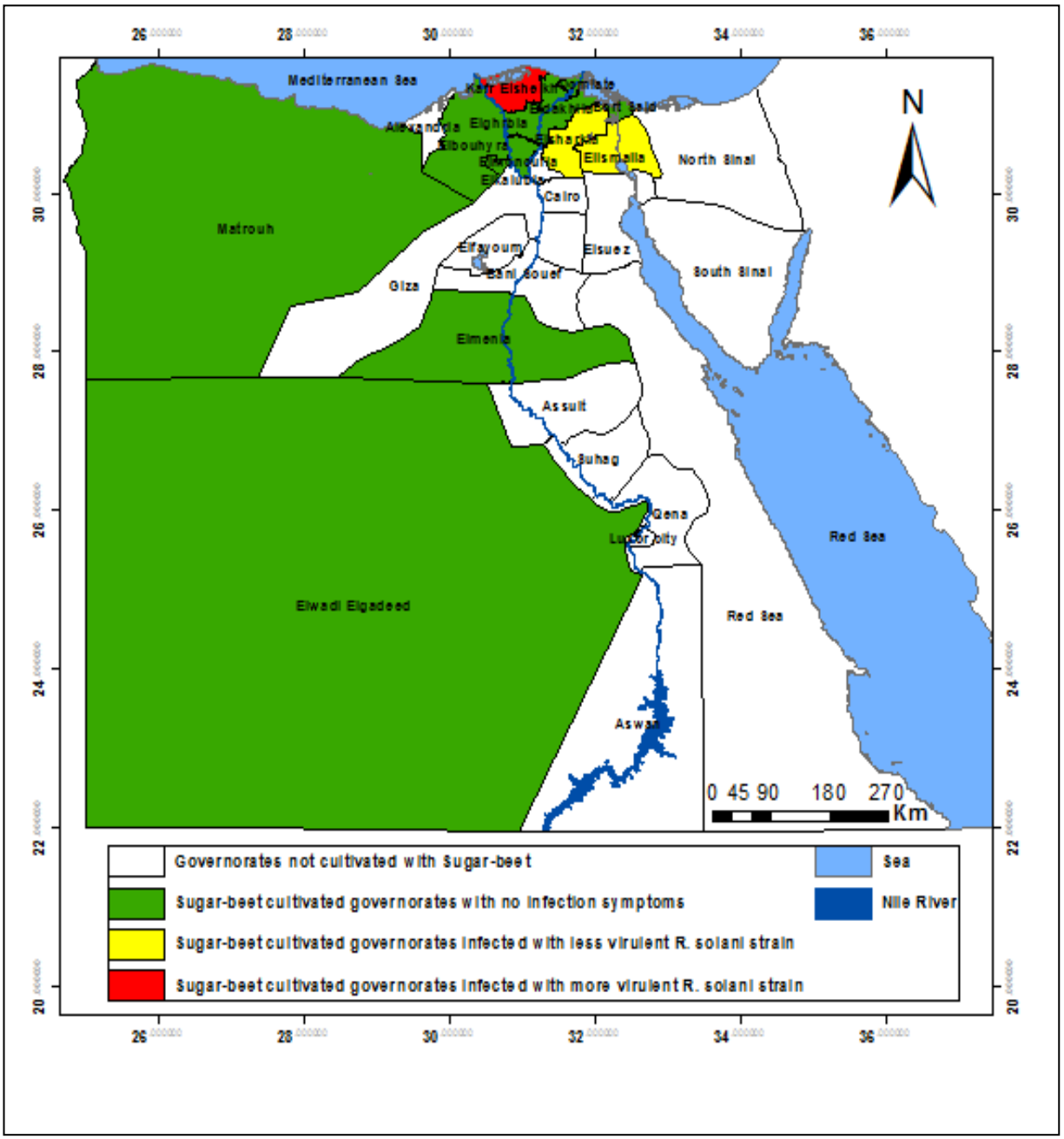

Fig. 1 Locations of governorates well-known by sugar-beet cultivation and demonstration of *R. solani* root rot incidence.

Fig. 2 Pathogenicity test: Healthy sugar-beet seedlings (cv. Matros) without any symptoms (a), infected seedlings showing typical disease symptoms (b).

In Vitro antagonistic activity of Bacterial strains against R. solani (MFRh)

The represented data in (Table 1 and Fig. 4) showed almost similar apparent activity of the bacterial strains toward the pathogen without statistically significant difference in fungal growth inhibition (%).

Table 1. The antifungal activity of the bacterial strains against *R. solani*

Bacterial strain	Fungal growth inhibition (%)
B1	43.32±0.73 [a]
B2	42.96±1.67 [a]
B3	44.81±0.98 [a]

Data were represented as mean ± SE . Mean values with the same letters are not significantly different (Tukey test at $P \leq 0.05$), n=3

Extracellular enzymatic activity of bacterial strains

Three antagonistic bacterial strains were tested for cellulase, chitinase, and pectinase activity on a basal salt medium supplemented separately with different carbon sources (CMC, colloidal chitin, and pectin) for their hydrolytic enzyme production. Bacterial strains were assumed to be potential secretors for cellulase, chitinase, and pectinase with development of a halo colorless zone indicating enzyme production (Fig. 5).

All the bacterial strains were positive for cellulase, chitinase and pectinase enzymes production. For cellulase activity, strain B3 has the highest enzymatic index (EI) at 1.98±0.09 which is significantly greater than

the values for B1 and B2 (1.76±0.03 and 1.43±0.04). B1, B2 and B3 exhibit very high chitinase activity without significant difference recording 5.35±0.17, 5.45±0.16 and 5.70±0.14 respectively. The pectinase enzymatic indices are similar across the three strains reaching 1.59±0.04, 1.62±0.08 and 1.39±0.03 for B1, B2 and B3, respectively without significant difference.

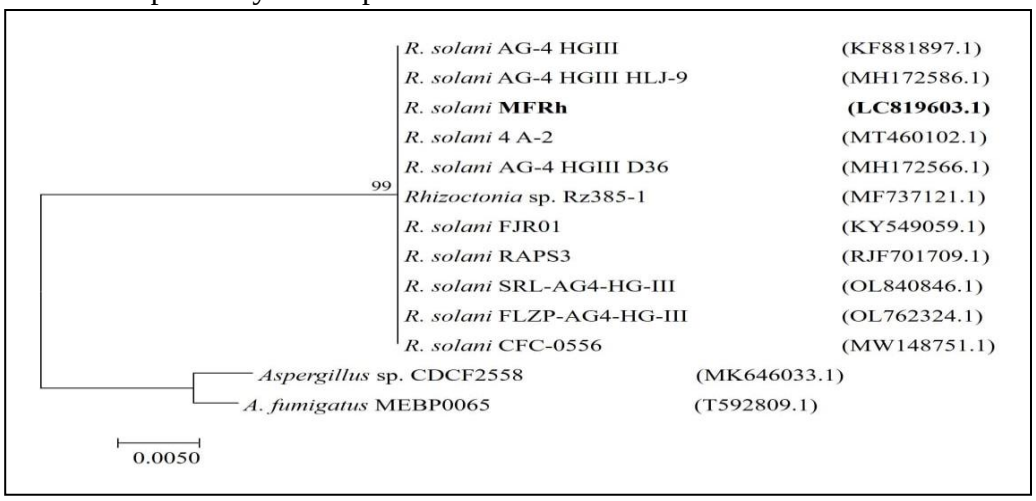

Fig. 3 Maximum likelihood (ML) phylogenetic tree based on ITS rDNA sequences of fungal strain (in bold) and fungal ITS sequences from the GenBank. GenBank accessions are in parentheses. Bootstrap values are indicated for each nod (1000 replicates).

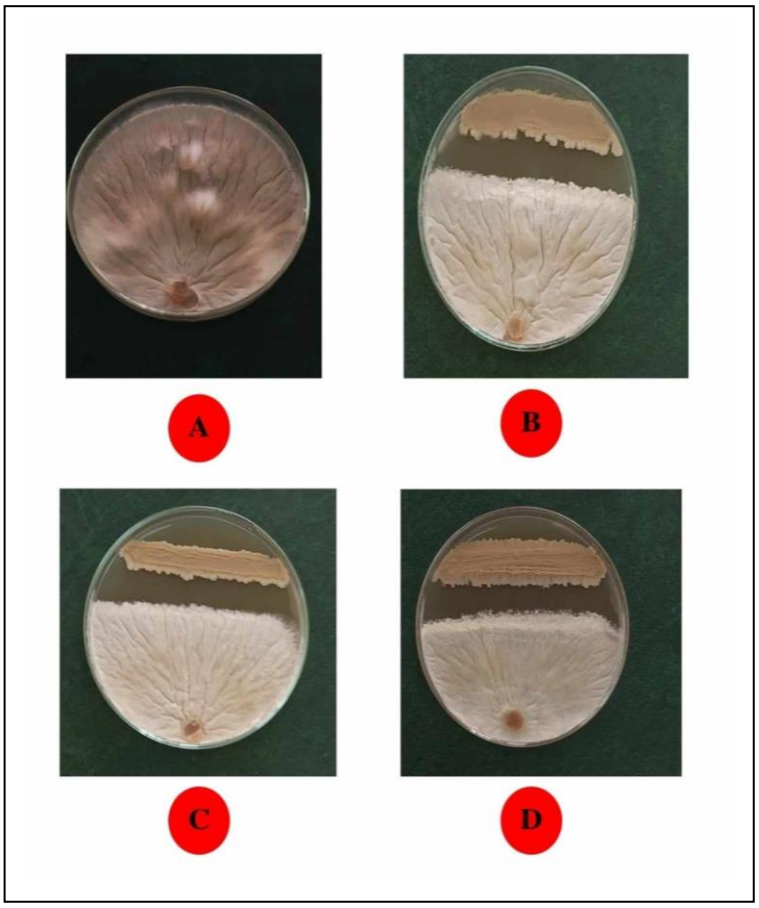

Fig. 4 *In vitro* antagonistic activity of three different bacterial strains. Growth culture pattern of *R. solani* **(**MFRh) strain on CYA plate (A), Dual culture of MFRh and *Azotobacter chroococum* (B1) strain (B), Dual cultured MFRh and *Bacillus velezenesis* (B3) strain (C) and Dual culture of MFRh and *Bacillus megaterium* (B2) strain (D)

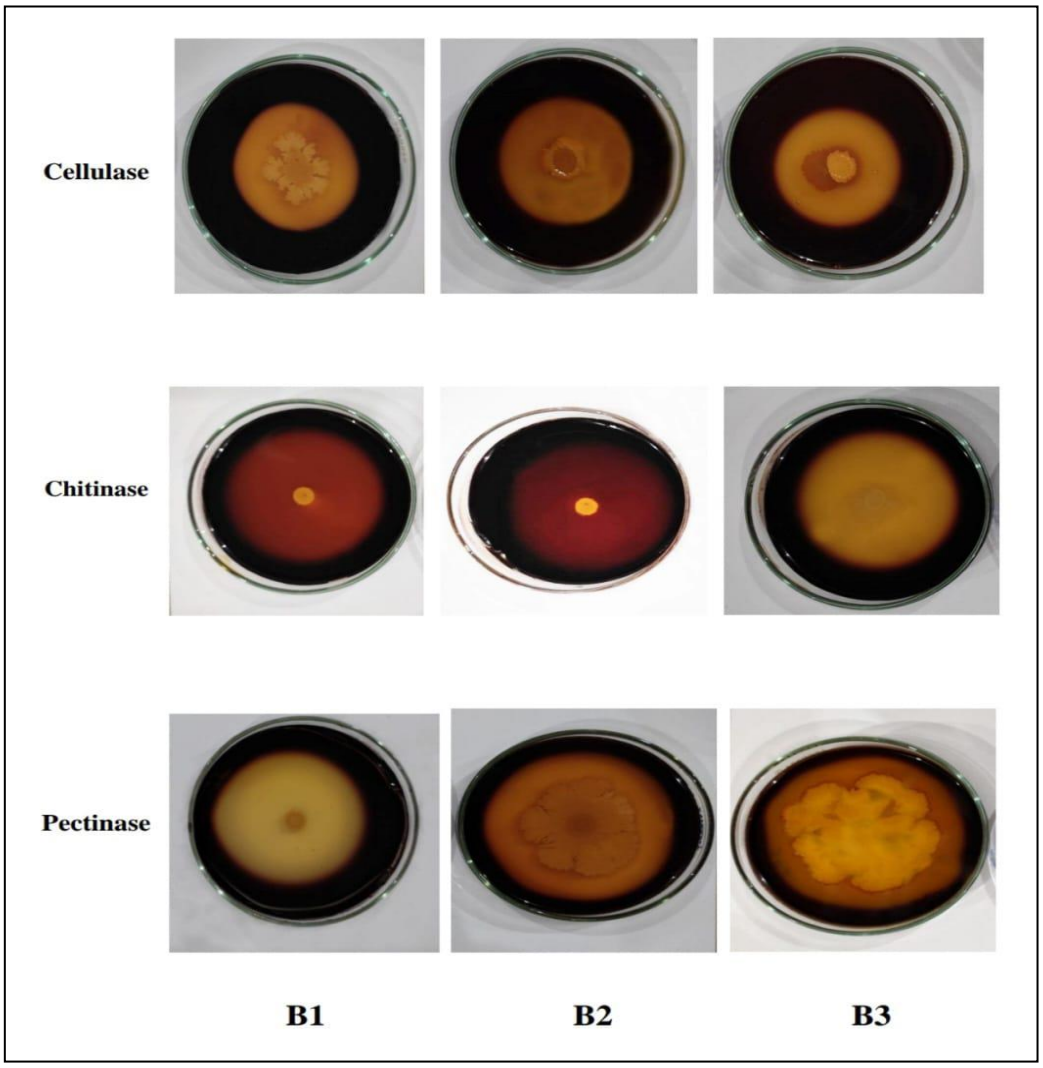

Fig. 5 Enzymes production by bacterial strains on agar plates showing a clear zone on specific medium after staining with Gram iodine stain: cellulase, Chitinase and Pectinase

Gas chromatography mass spectrometry (GC/MS) analysis

GC/MS analyses of the chemical composition of bacterial extracts of certain strains (B1, B2 and B3) recorded about 50, 50 and 26 certain volatile compounds, respectively **(**Tables S1, S2, and S3) and (Figs. S1, S2, and S3). They were identified as secondary metabolites as shown in figures (6, 7 & 8). *A. chroococum* (B1) extract involved almost (14) major compounds as shown in Table (2) where acetonitrile gave the highest ratio but glycerol 1-palmitate exhibited the lowest one.

Meanwhile, *B. megaterium* (B2) extract included about (15) major compounds represented in Table (3) as oleic acid which recorded the highest proportion but Propane, 1-(1-ethoxyethoxy)-gave the lowest. Likewise, *B. velezenesis* (B3) extract provided (13) significant compounds displayed in Table (4) where heptaethylene possessed the highest ratio, nevertheless the 2-Butanol, 3-methyl- provided the lowest one.

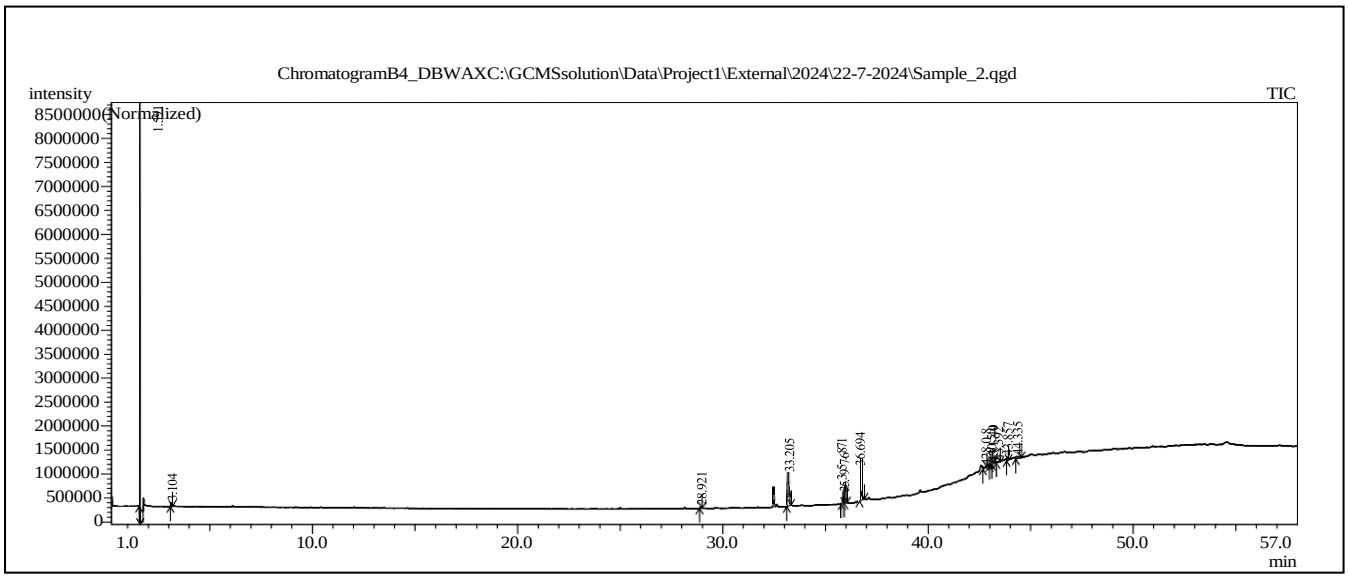

Fig. 6 GC/MS chromatogram showing the major bioactive compounds of strain, *Azotobacter chroococum* (B1)

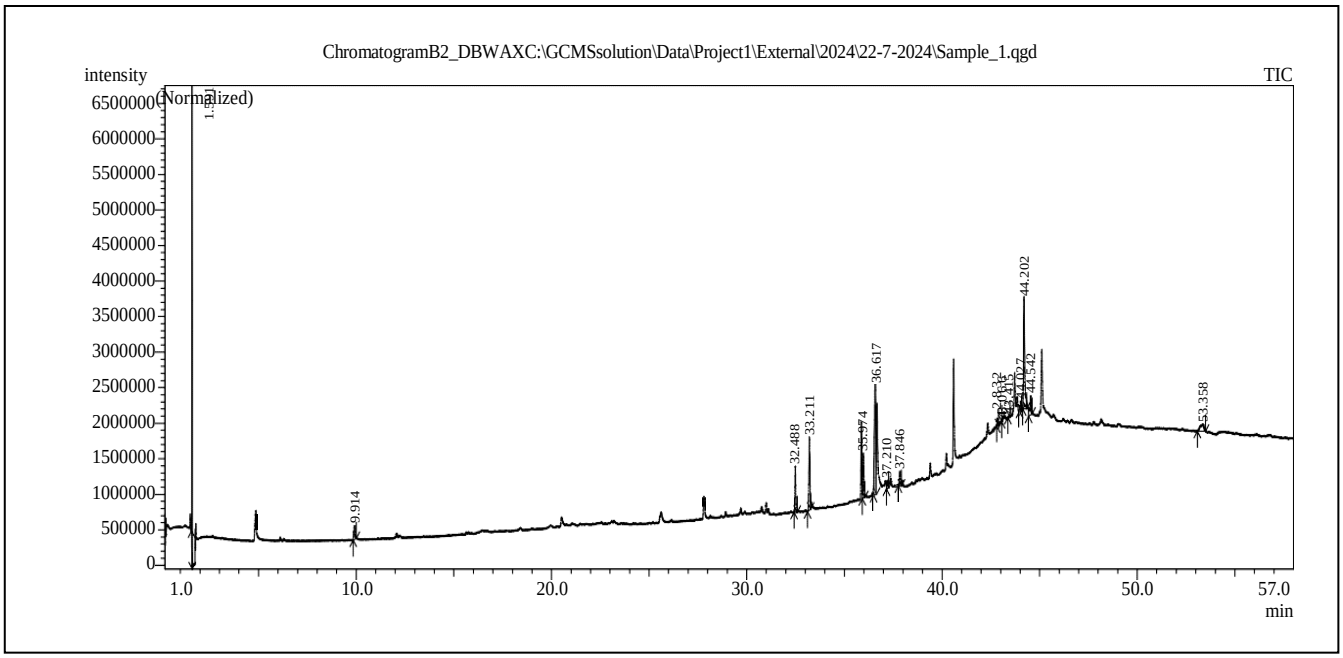

Fig. 7 GC/MS chromatogram showing the major bioactive compounds of strain, *Bacillus megaterium* (B2)

Table 2. GC/MS quantitative analysis of the *Azotobacter chroococum* (B1) extract as major bioactive metabolites.

Peak	R.Time	Area%	Height%	Compound Name
1	1.591	28.27	60.40	Acetonitrile
13	35.871	7.08	5.00	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
14	35.976	3.91	2.69	9-Octadecenoic acid (Z)-, methyl ester
16	36.694	10.11	4.93	Oleic Acid
24	42.808	0.81	0.17	Tetraethylene glycol
28	43.392	0.54	0.13	15-crown-5
32	43.857	1.19	0.52	Diisooctyl phthalate
34	44.335	1.74	0.34	Heptaethylene glycol
9	28.921	0.37	0.23	Tetradecanoic acid
12	33.205	7.61	4.73	n-Hexadecanoic acid
26	43.050	0.33	0.14	Ethanol, 2-(vinyloxy)-
27	43.140	0.30	0.21	Glycerol 1-palmitate
18	39.607	0.46	0.32	Stearic acid, 2-hydroxy-1-methylpropyl
5	3.104	0.69	0.59	2,3-Butanediol, [S-(R*,R*)]-

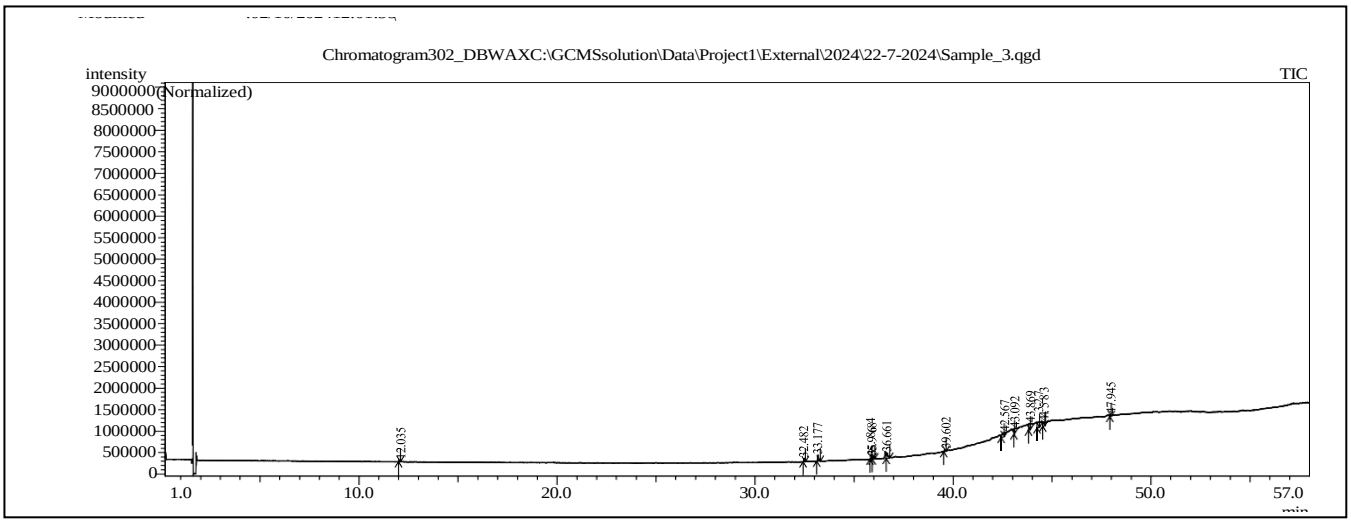

Fig. 8 GC/MS chromatogram showing the major bioactive compounds of strain, *Bacillus velezenesis* (B3)

Table 3. GC/MS quantitative analysis of the *B. megaterium* extract as major bioactive metabolites.

Peak	R.Time	Area %	Height%	Compound Name
4	1.591	7.29	26.48	Acetonitrile
17	33.211	4.05	4.12	n-Hexadecanoic acid
20	36.617	6.91	6.19	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
19	35.947	2.21	2.53	9-Octadecenoic acid (Z)-, methyl ester
47	44.202	10.03	6.91	Oleic Acid
25	37.846	0.93	0.82	Tetraethylene glycol
39	43.023	1.67	0.71	12-crown-4
42	43.408	3.35	0.71	15-crown-5
45	44.027	1.89	1.14	Pentaethylene glycol
48	44.542	1.45	1.09	Diisooctyl phthalate
50	53.358	1.37	0.39	heptaethylene glycol
15	32.488	2.12	2.57	Hexadecanoic acid, methyl ester
37	42.750	0.38	0.56	Propane, 1-(1-ethoxyethoxy)-
23	37.210	0.61	0.45	2-Ethyl-n-butyric acid ethyl ester
8	9.914	0.80	0.77	1,1-Ethanediol, diacetate

Table 4. GC/MS quantitative analysis of the *B. velezenesis* extract as major bioactive metabolites.

Peak	R.Time	Area%	Height%	Compound Name
3	12.035	1.04	1.50	2-Butanol, 3-methyl-
4	32.482	1.99	2.93	Hexadecanoic acid, methyl ester
5	33.177	4.71	5.40	n-Hexadecanoic acid
6	35.864	2.90	3.88	9,12-Octadecadienoic acid, methyl ester
7	35.968	1.70	2.31	9-Octadecenoic acid (Z)-, methyl ester
15	43.092	2.17	4.57	2-Propanol, 1,1'-oxybis-
9	36.661	6.04	4.79	Oleic Acid
17	43.869	7.07	5.61	12-crown-4
25	47.945	1.55	1.63	Pentaethylene glycol
19	44.327	8.70	4.79	heptaethylene glycol
12	42.567	6.61	3.74	Linoleic acid ethyl ester
20	44.583	5.63	3.52	3-Bromobutyric acid
11	39.602	1.61	1.48	Stearic acid, 2-hydroxy-1-methylpropyl

Disease assessment and Metabolic changes in sugar-beet seedlings

Data obtained from the two *in vivo* greenhouse experiments were almost similar without significant variations (Table 5). It was obvious that the treatment of sugar-beet seeds with bacterial suspensions of strains (B1, B2 and B3), each alone, significantly decreased disease severity compared to positive control. The potential of B1 and B3 was similar and higher than B2 in reduction of disease influence. The metabolic performance of sugar-beet seedling leaves was assessed through the measurement of total phenols, proline content, total chlorophyll and peroxidase activity. Data in table (6) show that seed treatment with bacterial suspensions lead to significant increase in the measured metabolic parameters levels in seedlings leaves under the infection stress. Treatment with B3 gave the highest values for total phenols, proline content and peroxidase activity. However, total chlorophyll content exhibited the maximum value upon treatment with *A. chroococum* alone.

Table 5. Rhizoctonia root rot development on sugar-beet seedlings upon the application of bacterial strains (B1, B2 and B3) treatments under greenhouse conditions

Treatment	Disease Assessment			
	Pre-emergence damping Off (%)	Post- emergence damping Off (%)	Survival (%)	Disease severity (DS %)
-ve control	3±0.18 ^{c}	2±0.15 ^{b}	95±4.81 ^{a}	0±0.00 ^{d}
+ve control (MFRh)	37±2.09 ^{a}	28±1.63 ^{a}	35±0.97 ^{c}	75.2±3.06 ^{a}
B1	2±0.01 ^{c}	2±0.02 ^{b}	96±2.10 ^{a}	0±0.00 ^{d}
B2	3±0.14 ^{c}	2±0.09 ^{b}	95±3.01 ^{a}	0±0.00 ^{d}
B3	3±1.04 ^{c}	3±0.26 ^{b}	94±1.88 ^{a}	0±0.00 ^{d}
Fungicide + MFRh	15±0.92 ^{b}	8±1.10 ^{b}	77±2.06 ^{b}	30.6±2.56 ^{b}
B1 + MFRh	13±0.16 ^{bc}	7±0.08 ^{b}	80±2.24 ^{b}	25.4±1.88 ^{c}
B2 + MFRh	15±0.07 ^{b}	7±0.88 ^{b}	78±1.31 ^{b}	27.4±1.16 ^{bc}
B3 + MFRh	11±1.04 ^{bc}	6±0.17 ^{b}	83±3.74 ^{ab}	25.8±1.83 ^{c}

Data were represented as mean±SE, n=10

Means of the same parameter with different letters are significantly different (Tukey test at $P \leq 0.05$)

Rhizoctonia solani isolated strain (MFRh *), Azotobacter chroococum* (B1), *Bacillus megaterium* (B2) and *Bacillus velezensis* (B3)

Table 6. Physiological changes in sugar-beet seedlings leaves as affected by bacterial strains (B1, B2 and B3) treatments under infection stress

Treatment	Total phenols (mg/g)	Proline (mg/g)	Total chlorophyl (mg/g)	Peroxidase (U/g)
-ve control	9.15±0.87 ^{e}	0.06±0.01 ^{e}	27.24±2.04 ^{bc}	988.33±12.14^{d}
+ ve control (MFRh)	11.41±1.56^{d}	0.11±0.01^{d}	9.54±0.98 ^{d}	2561.00±25.86 ^{c}
B1	13.05±0.61 ^{cd}	0.28±0.03 ^{ab}	38.03±0.78 ^{a}	3205.33±42.00 ^{ab}
B2	11.87±1.83 ^{d}	0.21±0.03^{c}	35.68±1.08 ^{a}	2986.00±15.68 ^{abc}
B3	14.65±2.07 ^{bc}	0.27±0.09 ^{ab}	29.88±1.11 ^{b}	3110.00±21.09 ^{ab}
Fungicide + MFRh	12.32±1.82 ^{cd}	0.19±0.01 ^{c}	24.82±2.30 ^{c}	2906.67±22.18^{bc}
B1+ MFRh	15.90±1.98 ^{b}	0.31±0.04 ^{ab}	34.36±1.88 ^{a}	3287.33±18.76 ^{ab}
B2 + MFRh	13.66 ±0.88^{bc}	0.29±0.02 ^{ab}	29.77±2.19 ^{b}	3058.00±31.08 ^{ab}
B3 + MFRh	19.82±2.05 ^{a}	0.33±0.01^{a}	27.74± 0.95^{bc}	3447.00±39.17 ^{a}

Data were represented as mean±SE, n=3

Means of the same parameter with different letters are significantly different (Tukey test at $P \leq 0.05$) *Rhizoctonia solani* isolated strain (MFRh *), Azotobacter chroococum* (B1), *Bacillus megaterium* (B2) and *Bacillus velezensis* (B3)

DISCUSSION

Rhizoctonia root rot on sugar-beet is a devastating problem that resulted in significant crop losses worldwide ranges from 50-60% (Mirsa et al. 2023). Conventional approaches, including chemical and physical methods, have been employed to manage that disease. Nonetheless the chemical treatments with fungicides or plant extract may be toxic for the non-targeted organisms and pose threats to human health through skin contact or inhalation (Ahmad et al. 2024). Likewise, physical methods such as heat and radiation may not eliminate all life stages of the pathogen and can damage soil structure in addition to their expensive costs. In contrast, biological control was more sustainable to disease management and safe for human health with fewer costs (Cucu et al. 2025).

Therefore, our objective was to find the mechanisms involved in basal resistance of sugar-beet and biocontrol agents, bacteria (PGPB) against this pathogen to plan better strategies for disease management.

Plant growth promoting bacteria (PGPB) have a prolonged consideration when applied to seeds, roots, and soils to help the plant to grow and develop where it was required for N_2 fixation, plant growth promotion, and management of plant pathogenic

microorganisms. *Azotobacter* and *Bacillus* species have been described to manage pathogenic fungi (El-Saadony et al. 2022).

The tested bacterial strains were examined for their antagonistic competences to manage *R. solani.* As fungal cell wall consisted of polymeric compounds like chitin, proteins, cellulose, hemicellulose and others (Heydari and Pessarakli 2010), the bacterial lytic activity was evaluated through their production of cellulase, pectinase and chitinase. B1, B2 and B3 strains where mainly explored to excrete these hydrolytic enzymes with the dominance of B3 (*Bacillus velezenesis*) in cellulase production. These findings suggest that the bacterial strains, particularly B3, could be valuable for biological control applications. Our findings were consistent with those noticed by Khan et al., (2018) and Wang et al., (2024).

Likewise, GC-MS profile in the present work exhibited the presence of common bioactive metabolites that announced for their antimicrobial activity and produced with different concentrations. Those compounds involved acetonitrile which displayed higher biological activities being antimicrobial and antioxidant bioactive molecule (Dulay et al. 2017). Hexadecanoic acid and its derivatives have the ability to supress fungal growth by forming complex compounds and bind to the active groups of fungal cell wall altering its integrity (Johannes 2013). Also, oleic acid targets proteins involved in biosynthetic pathways of nucleic acid, amino acid and vitamins that in turn threats fungal virulence (Muthamil et al. 2020). Moreover, alcohols possess an antimicrobial activity by denaturing and coagulating the proteins present in the microbial cell wall (Kubo et al. 2003). Stearic acid and its derivatives targeted enzymes involved in mycotoxins production and disrupt fungal activity (Guimarães and Venâncio 2022). Meanwhile, Diisooctyl phthalate was recommended as potent antimicrobial agent where it inhibits the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GSH) leading to increase of reactive oxygen species (ROS) production and generation of oxidative stress in the cytoplasm of microbial cells, resulting in cell death (Premjanu and Jaynthy 2014). Polyethylene glycol and its derivatives possessed an antimicrobial activity related to their hydrophilic property and the ability to remove water preventing microbial growth and development (Munir et al. 2022). Furthermore, 12-crown-4 was able to chelate metals surrounding the microbe, interrupting the required flow of nutrients leading to metabolism disruption (Edis et al. 2017). Similarly, 15-crown-5 also exhibited an antifungal activity as mentioned by Gül et al. (2019). Butyric acid and its derivatives were found to be antifungal compounds as reported by (Nguyen et al. 2011).

Finally, GC-MS profile of B1 strain includes palmitate which announced for its antifungal activity (Walters et al. 2004) as well as 2, 3-Butanediol that being described as plant growth promoter (Wu et al. 2019). For strain B3, linolenic acid exists in its extracts and it was identified for its antimicrobial activity (Walters et al. 2004). In spite of the little concentration of the most produced bioactive compounds in the tested bacterial strains, the relative homogeneity of those metabolites provokes the antimicrobial influence with popular decrease in disease severity ratio.

Biopriming of seeds with PGPB triggered a phenomenon known as induced systemic resistance (ISR). Positive role of PGPB was strengthens plant cell wall, modulate plant metabolism and enhanced synthesis of plant defense compounds such as phytohormones that increase plant resistance upon challenge by pathogens. In addition, PGPB enhanced the aggregation of phenolic compounds and defense-related enzymes like peroxidase (El-Saadony et al. 2022).

Elicitation of induced systemic resistance was related to rapid activation of multiple defense mechanisms involve the accumulation of some 2^{ry} metabolites, such

as proline. Proline accumulation role was to eliminate the excessive ROS and decrease oxidative stress in plant cells. Oxidative stress is an imbalance state between the production of ROS and the neutralization of free radicals by antioxidants causing destruction of cellular components including lipids, proteins, metabolites and nucleic acids which finally lead to cell death (Hossain et al. 2014).

In photosynthetic tissues, chlorophyll content decreased significantly under infection stress compared to the healthy plant (Mibei et al. 2016). PGPR are known to stimulate plant growth through different mechanisms, such as raising the availability of nutrients uptake, increasing root biomass and area which in turn improves the plant's nutrient absorption capacity for photosynthetic apparatus that could lead to enriched photosynthesis (de Andrade et al. 2023). Application of *A. chroococum* increases total chlorophyll content with the highest ratio as it was recognized to its role in N_2 fixation which is associated with increase in chlorophyll level (Wang et al. 2021).

CONCLUSION

A. chroococum, B. megaterium and *B. velezenesis* displayed a potent antifungal influence toward Rhizoctonia root rot as they decreases disease severity being 25.4±1.88, 27.4±1.16 and 25.8%±1.83, respectively. This observation was related to their ability to produce hydrolytic enzymes such as cellulase, chitinase and pectinase which are capable of breaking down the structural components of fungal cell wall. Moreover, they were able to excrete certain volatile metabolites involved in their GC-MS profile known to their antimicrobial activity. They also elicited the induced-systemic resistance in the plant by enhancing the accumulation of proline and phenolics as well as increase peroxidase activity. It was obvious that *B. velezenesis* displayed superiority in cellulase production and improvement of seedling induced systemic resistance. Additionally, it possessed a significant potential in reduction of disease severity which is promising in disease management and being placed in the ranks of microorganisms used in manufacture of protective bio-fertilizers.

SUPPLEMENTARY DATA

All unpublished data are available at the Egyptian Journal of Phytopathology cite or by the direct contact with authors.

AUTHOR CONTRIBUTIONS

Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests. The contents of the manuscript have neither been published nor under consideration for publication elsewhere.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (Chat GPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

STATEMENT AND ETHICS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this article. Ethical approval was not required for this study.

REFERENCES

Afify AH, El-Sayed AB and El-Pana SEM (2018) Biological control of *Rhizoctonia solani* causing sugar beet damping off. J. Food and Dairy Sci., 3rd Mansoura International Food Congress (MIFC) October: 123 – 127. DOI: 10.21608/jfds.2018.77768

Ahmad, M. F., Ahmad, F. A., Alsayegh, A. A., Zeyaullah, M., AlShahrani, A. M., Muzammil and K., Hussain, S. (2024). Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. Heliyon, 10(7).

Al-Fadhal FA, AL-Abedy AN and Alkhafije DU (2019) Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. Egyptian Journal of Biological Pest Control 29(47):1-11. https://doi.org/10.1186/s41938-019-0145-5.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Research. 25:3389–3402.https://doi.org/10.1093/nar/25.17.3389

Arora NK and Verma M (2017) Modified microplate method for rapid and efficient estimation of siderophore produced by bacteria. 3 Biotech 7: 1–9.DOI: 10.1007/s13205-017-1008-y

Aydın, M. H. (2022). *Rhizoctonia solani* and its biological control. Türkiye Tarımsal Araştırmalar Dergisi, 9(1), 118-135.

Baptista JP, Teixeira GM, de Jesus MLA, Bertê R, Higashi A, Mosela M, da Silva1 DV, de Oliveira JP, Sanches DS, Brancher JD, Balbi-Peña MI, Pereira UP and de Oliveira AG (2022) Antifungal activity and genomic characterization of the biocontrol agent *Bacillus velezensis* CMRP 4489. Scientific Reports. 12:17401. doi: 10.1038/s41598-022-22380-0

Basbagci G, Unal F, Uysal A and Dolar FS (2019) Identification and pathogenicity of *Rhizoctonia solani* AG-4 causing root rot on chickpea in Turkey. Spanish J. of Agri Research 17(2) e1007. 10.5424/sjar/2019172-13789

Bates L, Waldren RP and Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil 39: 205-207. https://www.jstor.org/stable/42932378

Collinge D, Jensen D, Rabiey M, Sarrocco S, Shaw M and Shaw R (2022) Biological control of plant diseases – what has been achieved and what is the direction? Plant Pathol. 71 (5): 1024-1047, 10.1111/ppa.13555

Cucu, M. A., Choudhary, R., Trkulja, V., Garg, S., and Matić, S. (2025). Utilizing environmentally friendly techniques for the sustainable control of plant pathogens: A Review. Agronomy, 15(7), 1551.

de Andrade LA, Santos CHB, Frezarin ET, Sales LR and Rigobelo EC (2023) Plant growth-promoting rhizobacteria for sustainable agricultural production. Microorganisms 11(4): 1088.https://doi.org/10.3390/microorganisms11041088

Dere Ş, Güneş T and Sivaci R (1998) Spectrophotometric determination of chlorophyll - a, b and total carotenoid contents of some algae species using different solvents. Turkish J. of Botany 22: 13–16. https://journals.tubitak.gov.tr/botany/vol22/iss1/3

Dulay RMR, Miranda LA, Malasaga JS, Kalaw SP, Reyes RG and Hou CT (2017) Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamour*. Biocatalysis and Agri Biotechnology 9: 141-144. https://doi.org/10.1016/j.bcab.2016.12.003

Edis Z, Bloukh SH and Sara HA (2017) Antibacterial and antifungal activity of the triiodide [Na (12-Crown-4) 2] I3 on the pathogens *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus faecalis*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella ndpneumoniae* and *Candida albicans*.Preprints https://doi.org/10.20944/

El-Rayes MM, Ali INM, Abd El-Nabi HM, Morsy KMM and Khalil MII (2022)

Induction of systemic resistance in cluster bean against damping-off and root rot diseases. Egyptian J of phytopathology 50(2):33-43. DOI: 10.21608/ejp.2022.160238.1068

El-Saadony MT, Saad AM, Soliman SM, Salem HM, Ahmed AI, Mahmood M, El-Tahan, AM, Ebrahim, AA, Abd El-Mageed, TA, Negm, SH and Selim, S (2022) Plant growth-promoting microorganisms as biocontrol agents of plant diseases: mechanisms, challenges and future perspectives. Frontiers in Plant Science 13: 923880. doi: 10.3389/fpls.2022.923880

Farhaoui A, Tahiri A, Khadiri M, El Alami N and Lahlali R (2024). Assessing management strategies for mitigating Rhizoctonia damping-off in sugar beet cultivation. The Microbe, 5, 100164.

Ferbiyanto A, Rusmana I and Raffiudin R (2015) Characterization and identification of cellulolytic bacteria from gut of worker Macrotermes gilvus. Hayati J. Biosci., 22: 197–200. DOI: 10.1016/j.hjb.2015.07.001

Fouad MS, Saber WI, Badr HH, Mohamed HA, Farroh KY and Gomah AA (2024) Metabolic and molecular evidence for the detection of the pathogenic *Pseudomonas aeruginosa* on common bean seeds and its control via chitosan-silver nanocomposite. Egyptian Journal of Botany. 64(1), pp. DOI: 10.21608/ejbo.2023.212107.2337

Guimarães A and Venâncio A (2022) The potential of fatty acids and their derivatives as antifungal agents: A review. Toxins *14*(3), 188. DOI: 10.3390/toxins14030188

DŞ, Ogutcu H and Hayvalı Z (2019) Investigation of photophysical behaviours and antimicrobial activity of novel benzo-15-crown-5 substituted coumarin and chromone derivatives. Journal of Molecular Structure 1204, 127569 DOI: 10.1016/j.molstruc.2019.127569

Haque ME and Parvin MS (2020) Sugar beet, its disease rhizoctonia root rot, and potential biological agents. Annals of Agri Bio Research. 37(1):96-101. https://www.researchgate.net/publication/349944382

Heydari A and Pessarakli M (2010) A review on biological control of fungal plant pathogens using microbial antagonists. Journal Biological Science, 10:273–290. DOI: 10.3923/jbs.2010.273.290

Hossain MA, Hoque MA, Burritt DJ and Fujita M (2014) Proline protects plants against abiotic oxidative stress: biochemical and molecular mechanisms. Oxidative damage to plants (pp. 477-522). DOI: 10.1016/B978-0-12-799963-0.00016-2

Ibrahim DS, Wahdan RH and El-Sagheer AM (2021) Effects of roots-applied resistance inducers on penetration and development of root-knot nematode in sugar-beet. Egyptian Academic Journal of Biological Sciences B. Zoology. 13(1): 159-171. http://eajbsz.journals.ekb.eg/

Jadhav H, Shaikh S and Sayyed R (2017) Role of hydrolytic enzymes of rhizo flora in biocontrol of fungal phytopathogens: an overview. Rhizotrophs*:* Plant Growth Promotion to Bioremediation Springer, Singapore, 183–203. DOI:10.1007/978-981-10-4862-3_9

Johannes E (2013) Pemanfaatan Senyawa Bioaktif Hasil Isolasi Hydroid Aglaophenia cupressina Lamoureoux sebagai Bahan Sanitizer pada Buah dan Sayuran Segar (Doctoral dissertation, Universitas Hasanuddin).

http://repository.unhas.ac.id/handle/123456789/1246

Kasana RC, Salwan R, Dhar H, Dutt S and Gulati A (2008) A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. Curr Microbiol 57: 503-507. DOI: 10.1007/s00284-008-9276-8

Katiyar D, Hemantaranjan A and Singh, B (2016) Plant growth promoting Rhizobacteria-an efficient tool for agriculture promotion. Adv Plants Agric Res. 2016;4(6):426-434. DOI: 10.15406/apar.2016.04.00163

Khan N, Martínez-Hidalgo P, Ice TA, Maymon M, Humm EA, Nejat N and Hirsch AM (2018) Antifungal activity of Bacillus species against Fusarium and analysis of the potential mechanisms used in biocontrol. Frontiers in microbiology 9: 2363. https://doi.org/10.3389/fmicb.2018.02363

Kubo I, Fujita T, Kubo A and Fujita KI (2003) Modes of antifungal action of alkanols against *Saccharomyces cerevisiae*. Bioorganic & medicinal chemistry 11(6): 1117-1122. DOI: 10.1016/s0968-0896(02)00453-4

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35,1547. DOI: 10.1093/molbev/msy096

Malik WA and Javed S (2021) Biochemical characterization of cellulase from *Bacillus subtilis* strain and its effect on digestibility and structural modifications of lignocellulose rich biomass. Front. Bioeng. Biotechnol, 9: 800265. doi: 10.3389/fbioe.2021.800265

Mibei EK, Ambuko J, Giovannoni JJ, Onyango AN and Owino WO (2016) Carotenoid profiling of the leaves of selected African eggplant accessions subjected to drought stress. Food Science and Nutrition, 5(1), 113–122. doi: 10.1002/fsn3.370

Misra, V., Mall, A. K. and Singh, D. (2023). Rhizoctonia root-rot diseases in sugar beet: Pathogen diversity, pathogenesis and cutting-edge advancements in management research. The Microbe, 1,pp, 100011. https://doi.org/10.1016/j.microb.2023.100011

Mohamed AH, Abd El-Megeed FH, Hassanein NM, Youseif SH, Farag PF, Saleh SA, Abdel-Wahab BA, Alsuhaibani AM, Helmy YA and Abdel-Azeem AM (2022) Native Rhizospheric and Endophytic Fungi as Sustainable Sources of Plant Growth Promoting Traits to Improve Wheat Growth under Low Nitrogen Input. J. Fungi, 8, 94. https://doi.org/10.3390/jof8020094

Mohamadpoor M, Amini J, Ashengroph M and Azizi A (2020) Evaluation of biocontrol potential of *Achromobacter xylosoxidans* strain CTA8689 against common bean root rot. Physiological and Molecular Plant Pathology 117, 101769. DOI: 10.1016/j.pmpp.2021.101769

Munir T, Imran M, Muzammil S, Hussain AA, Alam MFE, Mahmood A and Afzal M (2022) Antimicrobial activities of polyethylene glycol and citric acid coated graphene oxide-NPs synthesized via Hummer's method. Arabian Journal of Chemistry 15(9): 104075. https://doi.org/10.1016/j.arabjc.2022.104075

Muthamil S, Prasath KG, Priya A, Precilla P. and Pandian SK (2020) Global proteomic analysis deciphers the mechanism of action of plant derived oleic acid against *Candida albicans* virulence and biofilm formation. Scientific Reports, 10(1), 5113. DOI: 10.1038/s41598-020-61918-y

Nguyen LN, Lopes LCL, Cordero RJB and Nosanchuk JD (2011) Sodium butyrate inhibits pathogenic yeast

growth and enhances the functions of macrophages. J. Antimicrob. Chemother 66: 2573–2580. https://doi.org/10.1093/jac/dkr358.

Ortiz, J.; Soto, J.; Almonacid, L.; Fuentes, A.; Campos-Vargas, R. and Arriagada, C.(2019) Alleviation of metal stress by *Pseudomonas orientalis* and *Chaetomium cupreum* strains and their effects on *Eucalyptus globulus* growth promotion. Plant Soil, 436, 449–461.

Premjanu N and Jaynthy C (2014) Antimicrobial activity of diethyl phthalate: An insilico approach. Asian J Pharm Clin Res. 7(4): 141-142. https://journals.innovareacademics.in/index.php/ajpcr/article/view/1446.

Ramankutty N, Mehrabi Z, Waha K, Jarvis L, Kremen C, Herrero M and Rieseberg LH (2018) Trends in global agricultural land use: implications for environmental health and food security. Annu Rev. Plant Biol. 69 :789–815, https://doi.org/10.1146/annurev-arplant-042817-040256.

Rawat R and Tewari L (2012) Purification and characterization of an acidothermophilic cellulase enzyme produced by *Bacillus subtilis* strain LFS3. Extremophiles 16: 637–644. DOI: 10.1007/s00792-012-0463-y

Sadhu S and Maiti TK (2013) Cellulase production by bacteria: A review. Microbiology Research Journal International 3:235-58. DOI: 10.9734/BMRJ/2013/2367

Saitou N and Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4, 406–425 DOI: 10.1093/oxfordjournals.molbev.a040454

Shi YY (1996) Isolation and screening of cellulosed decomposing microorganisms. Journal of Najing Agricultural University, 19(3):51-57. DOI: 10.1007/978-1-4939-7877-9_4

Siddiqui ZA and Khan M (2017) Biofilm formation by *pseudomonas* spp. and their significance as a biocontrol agent. Biofilms in Plant and Soil Health 14, https://doi.org/10.1002/9781119246329.ch5.

Snell FD and Snell CT (1953) Colorimetric methods of analysis including some turbidimetric and naphelometric methods.3, D. Van Nostrand Co., Inc., Princeton, Jersey, Toronto, New York and London, 606p.

Walters D, Raynor L, Mitchell A, Walker R and Walker K (2004) Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia 157: 87–90. DOI: 10.1023/b:myco.0000012222.68156.2c

Wang N, Fu F, Wang H, Wang P, He S, Shao H and Zhang X (2021) Effects of irrigation and nitrogen on chlorophyll content, dry matter and nitrogen accumulation in sugar beet (*Beta vulgaris* L.). Scientific Reports 11(1): 16651. doi: 10.1038/s41598-021-95792-z

Wang M, Zhang Y, Cai H, Zhao X, Zhu Z, Yan Y and Tu M (2024) A new biocontrol agent *Bacillus velezensis* SF334 against rubber tree fungal leaf Anthracnose and its genome analysis of versatile plant probiotic traits. Journal of Fungi, 10(2): 158. https://doi.org/10.3390/jof10020158

Wu Y, Zhou J, Li C and Ma Y (2019) Antifungal and plant growth promotion activity of volatile organic compounds produced by *Bacillus amyloliquefaciens*. MicrobiologyOpen 8, e00813.. https://doi.org/10.1002/mbo3.813

Yarullina L, Akhatova A and Kasimova R (2016) Hydrolytic enzymes and their proteinaceous inhibitors in regulation of plant-pathogen interactions. Russian Journal of Plant Physiology. 63(2): 193–

203, https://doi.org/10.1134/S1021443716020151,

Zanella EJ, Berghetti J, Scheidt BT, Casa RT, Bogo A, Gonçalves MJ, Berghetti J and Martins FC (2020) Charcoal rot severity and yield components of common bean cultivars inoculated with *Macrophomina phaseolina*. Summa Phytopathologica *46*(4): 299–304. https://doi.org/10.1590/0100-5405/240745

Zhou A, Wang F, Yin J, Peng R, Deng J, Shen D a M and H (2022) Antifungal action and induction of resistance by *Bacillus* sp. strain YYC 155 against *Colletotrichum fructicola* for control of anthracnose disease in *Camellia oleifera*. Frontiers in Microbiology 13:956642.https://doi.org/10.3389/fmicb.2022.956642

Zian AH and Aly MM (2020) Impact of Co-Inoculation with *Rhizobium leguminosarum* and some plant growth promoting *Rihzobacteria* against *Rhizoctonia solani* and *Fusarium oxysporum* infected *Faba Bean*. Journal of Plant Protection and Pathology 11 (9):441-453.DOI: 10.21608/jppp.2020.117990