Egypt. J. Microbiol. **59,** pp.29 - 49 (2024)

EgyptianJournalofMicrobiology
http://ejm.journals.ekb.eg/

Methylotrophic Bacteria and Rhizobium as Biofertilizers and Biocontrol Agents against Rhizoctonia Root Rot of Faba Bean

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THE impact of a combination of methylotrophic bacteria either as seed treatment (*Methylobacterium nodulans*) or foliar application (*Methylobacterium mesophilicum*) and *Rhizobium leguminosarum* biovar *viciae* for control of Rhizoctonia root rot under greenhouse and field conditions was studied. A mix of rhizobial inoculation (RI) + ⅓ N + *M. nodulans* significantly decreased the percentage of pre, and post-emergence damping off and caused a significant increase of survived plants, also enhanced crop parameters and yield followed by (a mix of RI + ⅓ N + *M. mesophilicum*), and Rizolex-T treatments. Meanwhile, the same treatments resulted in increases in NPK contents. All treatments showed an excess in peroxidase and polyphenol oxidase activities as well as for phenolic contents, the considerable increase was related to a (mix of rhizobial inoculation (RI) + ⅓ N + *M. nodulans*). All tested rhizobial and methylobacterial strains produce IAA, HCN, and ammonia, they also, were resistant to Ampicillin, and Colistin antibiotics and susceptible to Tetracycline. Eventually, results indicated that the integration of methylotrophic bacteria and Rhizobium in addition to a one-third amount dose of nitrogen fertilization decreased the occurrence of Rhizoctonia root rot as well as the remarkable increase of faba bean yield.

Introduction

The faba bean (*Vicia faba* L.) is considered one of the most globally significant legume crops in the world for use as a vegetable and pulse crop. Both dry and fresh seeds are recommended due to their benefits for human nutrition as a dietary source of fiber and protein. Furthermore, incorporating faba beans in crop rotation schemes enhances soil quality since they can fix atmospheric N_2 to levels up to 200 kg N ha^{-1}, as well as increasing soil organic matter (Karkanis, et al., 2018). The area under cultivation of faba bean in Egypt dropped from 35024 hectares in 2016 to 25105 hectares in 2022, and the imported value of faba bean in 2022 was 286,453,000 $ (FAOSTAT, 2024).

Soil-borne fungi caused considerable yield loss in faba bean production. Rhizoctonia root rot caused by *Rhizoctonia solani* is one of the most common root diseases of the faba bean in Egypt (Atwa, 2016). Seed and root rot, hypocotyl canker, as well as damping-off, are the disease's distinctive symptoms (Lamari & Bernier, 1985; Omar, 1986). Due to its soil-dwelling lifestyle, high saprophytic competitiveness, and extensive host range, control of *R. solani* is very challenging (Ogoshi, 1987). Fungicides have been primarily used to control faba bean root rot diseases. Fungicides are always effective when applied, but because of their non-target environmental effects and the resulting pathogen's resistance, researchers are now looking for alternatives.

However, chemical fertilizers are critical in meeting the world population's ever-increasing demand for food. Excessive usage of chemical fertilizers and inadequate plant uptake of these fertilizers can induce various harmful effects on soils and cause harmful impacts on ecosystems (Ongley et al.,2010). So,neither chemical fertil-

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Received 9/12/2023 , accepted 10/7/2024
DOI: 10.21608/EJM.2024.250351.1237

izers nor chemical fungicides are environmentally friendly or ecologically sustainable. According to Ramakrishna et al., (2019), the world's public health and agricultural production have been significantly impacted by the extensive and continuous use of chemical fertilizers as well as pesticides over the past 50 years.

Plant-growth-promoting rhizobacteria (PGPR) now in use is one of the alternatives either as biofertilizers or biocontrol agents. They facilitate plant growth directly by either assisting in resource receive (nutrients and essential salts) or indirectly by reducing the infections' negative effects of various pathogens on plant growth (Glick, 2012).

Rhizobia are significant as biocontrol agents and biofertilizers because they stimulate plant growth. These bacteria either directly or indirectly act as a biocontrol agent and promote plant growth through processes such as N_2 fixation, siderophore production, nutrient supply, phytohormone synthesis, and mineral solubilization. They also inhibit pathogen growth by influencing the production of cellulase, protease, lipase, and β-1,3 glucanase, and they improve plant defense by inducing systemic resistance (Deshwal et al*.,* 2003 b; Gopalakrishnan et al., 2015).

Methylobacterium spp. have shown great promise as a microbial agent in agriculture and this genus is a key component of plant microbiomes. They can grow using only organic one-carbon molecules such as formate, formaldehyde, methanol, and methylamine as their carbon and energy source. The majority of *Methylobacterium* species are classified as "pink-pigmented facultative methylotrophs" (PPFM), that are known to be highly beneficial to their hosts (Jorge et al., 2019; Zhang et al., 2021). The use of methylotrophs, both rhizospheric and non-rhizospheric, as bioinoculants is widespread, and their use increasing as a substitute for chemical fertilizers. Their correlation with plant growth can be used to support environmentally friendly sustainable and cost-effective agricultural operations (Ahlawat et al., 2018). Moreover, *Methylobacterium* species can produce a wide range of antimicrobial compounds (Ryan et al., 2008; Poorniammal et al., 2009), competing for nutrients with pathogens, or inducing systemic resistance (ISR) that protect host plants from infection (Indiragandhi et al., 2008; Berg, 2009).

This work aims to use *Methylobacterium* and rhizobium as biocontrol agents for controlling Rhizoctonia root rot disease, and also as a biofertilizer.

Material and Methods

Plant material

Faba bean seeds (*Vicia faba* L.), cultivar Giza 843 were obtained from the Legume Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt

Pathogen

Isolation and morphological identification of the pathogen

The fungus *Rhizoctonia solani* Kühn (Isolate SKS 39) was isolated from naturally infected faba bean plants, showing damping off and root rot symptoms, cultivated in Sakhaa, Kafr El-Sheikh Governorate. Its pathogenicity was confirmed and identified based on cultural properties and microscopic morphological characters according to (Sneh et al., 1991; Seema et al., 2012). The cultures were maintained on malt extract agar slants under a phosphate buffer (pH 6.5) at 4± 0.5°C (Boeswinkel, 1976).

Molecular identification of the pathogen

The grown mycelium on malt extract broth was filtrated through sterile Whatman filter paper No. 1. and stored at -70°C in Eppendorf tubes until use (Yin et al., 2017). DNA was extracted by using a modified method of CTAB (cetyltrimethylammonium bromide) as described by Munir et al., (2020). The obtained DNA samples were kept at -20°C until used later and to check the integrity of the DNA, about 5 µl of the isolate was loaded into 1.5 % agarose gel. DNA appears as a sharp band when viewed using the UV transilluminator. Extracted genomic DNA of the isolate was amplified according to White et al., (1990) by using the universal primer pair ITS1 (´5TCCGTAGGTGAACCTGCGG-3´) as forward and ITS4 (´5TCCTCCGCTTATTGATATGC-3´) as reverse, and the PCR products were electrophoresed on agarose gel (1.5%).

The ITS fragments were extracted and purified from agarose gel for sequence preparation by using a PCR purification kit (Trans Biotech, Beijing, China). PCR products of the targeted band were sent to the sequence service (Macrogene, Netherlands) for sequencing. The Sequences were assembled with BioEdit software (Hall, 2005) and Basic Local Alignment Search Tool BLASTn searched for the nearest matches in the NCBI (National Centre for Biotechnology Information) GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) . The GenBank database provided

us with the fungal ITS-related sequences for phylogenetic analysis using MEGA11 software (Tamura et al., 2021). The phylogenetic tree was constructed to illustrate the relationships that existed among the homologous fungi by using the Maximum Likelihood method and 1000 bootstrap replicates based on the Jukes-Cantor model (Jukes & Cantor 1969). The ITS sequences were aligned using CLUSTAL W (Thompson et al., 1994; Larkin et al., 2007). The tree was drawn to scale, with lengths measured in the number of substitutions per site. Twenty-one nucleotide sequences were involved in the analysis.

Preparation of pathogen inoculum

Inoculum of *R. solani* (isolate SKS 39) was prepared by growing the fungus in glass bottles 500 cc containing sterilized sorghum medium (100 g of sorghum grains and 90 mL of water). The bottles were inoculated with equal disks (0.5 cm) of four days old *R. solani* culture and incubated at 24 ± 1°C for 21 days, during this period the incubated bottles were vigorously shaken daily for the first 4 days to encourage more rapid and uniform colonization of the sorghum grains then shaken every three days to ensure uniform distribution of the fungal growth. After the incubation period, the glass bottles were then evacuated, and their content was air dried at room temperature and crushed in a mill to pass through a 3-mm sieve. The dried crushed inoculum was stored in paper bags at 4 ± 1°C until added to the soil within one week (Gaskill, 1968).

Seed and soil treatments

Healthy uniformity seeds of faba bean were surface disinfected by submerging in sodium hypochlorite (1%) for 2 min, washed numerous times with sterilized water, then left to dry on a screen cloth with a paper towel underneath to absorb the excess water at room temperature for about two hours.

A) *Plant growth promoting Rhizobacteria (PGPR)*

Two bacterial species of methylotrophs (PPFMs) namely *Methylobacterium mesophilicum* and *Methylobacterium nodulans* were previously isolated and identified (Orf et al., 2005; Orf et al., 2014) using Bergey's Manual of Systematic Bacteriology, 2nd edition (Garrity et al.,2005), and random amplified polymorphism DNA (RAPD) analysis according to Welliams et al., (1990). Methanol Mineral Salts (MMS) medium was used for cultivation and maintenance of *Methylobacterium* species (Holland & Polacco, 1992). Isolates were activated on fresh slants of MMS-agar medium and after 72 hr. transferred to flasks containing 50 mL of MMS- broth medium. The flasks were placed to grow on a rotary shaker at one hundred twenty rpm for 3 days at 28±1°C.

B) *Root-nodule bacteria*

Two isolates of *Rhizobium leguminosarum* biovar *viciae* (Faba bean) namely El-Khattaba (Ali & Orf, 2022) and 481 (Ali & Hafez, 2018) specifics to faba bean kindly acquired from the biofertilizers Production Unit, Agricultural Microbiol. Res. Dept., Soils Water and Environment Res. Inst., (SWERI), Agric. Res. Centre (ARC), Giza, Egypt. Strains were grown on yeast extract Mannitol (YEM) broth medium on a rotary shaker at one hundred twenty rpm for 24 hr. at 28±1°C. (Vincent, 1970).

C) *Seed biopriming*

For single treatment, 720 mL broth ($4x10^9$ cfu/mL) of *M. nodulans*, or *R. leguminosarum* biovar *viciae* El-Khattaba or 481 was used individually per one Kg of the carrier material (peat moss and vermiculite-based formulation). For the mixed treatment, 360 mL broth of *M. nodulans* and 360 mL of *R. leguminosarum* biovar *viciae* El-Khattaba or 481 were mixed with one Kg of the carrier. Healthy faba bean seeds were coated with the tested bacterial formulation either solely or in different combinations, using the sucrose-saturated solution to cover the seeds as adhesive 15 hr. before sowing time. The coated seeds were left to air-dry on a screen cloth.

D) *Fungicidal treatment*

Seed dressing was carried out to the disinfected faba bean seeds by applying the Rizolex-T 50% WP (20% Tolclophos-methyl and 30% Thiram), Sumitomo Chemical Company Ltd. at the recommended dose (3 g/kg) to the 1% methylcellulose (as a sticker) moistened seeds in polyethylene bags and shaking well to ensure even distribution of the fungicide.

E) *Control*

The disinfected faba bean seeds were coated with the peat moss and vermiculite-based formulation and the coated seeds were left to air-dry on a screen cloth 15 hr. before sowing time.

Greenhouse experiment

The trials were carried out in the greenhouse of Plant Pathology Research Institute, Agricultural Research Centre, Giza. Pots (30 cm in diameter) with a bottom drainage hole were sterilized by dipping in 5% formalin solution for 15 minutes and left for one week until complete formalin evaporation. Sand clay soil 1:2 (V/V) that was disinfested with steam was used to fill the pots. Soil infestation was achieved by mixing the inoculum of *R. solani* with the soil at 2% of soil weight (Papavizas & Davey, 1962). Sterilized uninoculated crushed sorghum grains were added to the disinfested soil at the same rate for use as healthy control. The infested soil was mixed thoroughly and watered every 2 days for a week before planting to stimulate the fungal growth and ensure its distribution in the soil. Five seeds of treated faba bean seeds, as mentioned before, were sown in each pot and pots were irrigated immediately. Twelve replicated pots were used for each treatment. The recommended doses of N.P.K. fertilizers were used as follows: Superphosphate (1.5 g pot^{-1}) was added before plantation, potassium sulfate (0.5 g pot^{-1}) was applied at 10 days after plantation, and N-Fertilization as ammonium sulfate was used at rates of 1.5 g N/ pot^{-1} (⅓ N) as a starter dose 10 days after plantation or 4.5 g pot^{-1} (Full N) in two equal split doses at 10 and 25 days after plantation. The treatments were as follows:

1) Infested soil + Rhizobial inoculation (481) +1.5 g N/pot^{-1} (⅓ N).
2) Infested soil +Rhizobial inoculation (El Khatattba) +1.5 g N/pot^{-1} (⅓ N).
3) Infested soil +a mix of rhizobial inoculation+1.5 g N/pot^{-1} (⅓ N).
4) Infested soil +a mix of rhizobial inoculation + foliar application with (*M. mesophilicum* 4.3 mL at $4x10^7$ cfu/ mL /plant, 30 days after planting) + 1.5 g N/pot^{-1} (⅓ N).
5) Infested soil +a mix of rhizobial inoculation + seed coating with (*M. nodulans*) +1.5 g N/ pot^{-1} (⅓ N).
6) Infested soil +Fungicide treatment + 4.5 g N/ pot^{-1} (Full N).
7) Infested soil + 4.5 g N/pot^{-1} (Full N).
8) Un infested soil + 4.5 g N/ pot^{-1} (Full N).

Twelve plants (four replicates each of three plants) were uprooted 60 days after sowing; plant height (cm) was measured, and the stem was cut at the soil line. Soil particles were eliminated by running water over the roots. Numbers of nodules were recorded. Shoots, roots, and nodules were placed in paper bags and oven-dried at 70°C for 48h, then weighed. The dry plant samples of the shoots were ground and prepared for wet digestion using H_2SO_4 and H_2O_2 methods as described by Lim & Jackson, (1982). The digests were then subjected to the measurement of nutrients i.e., nitrogen, phosphorus, and potassium (Cottenie et al., 1982).

Disease assessment

The disease incidence (DI) % was measured by recording pre-emergence damping-off, post-emergence damping-off, and the percentage of survived plants 15, 30, and 45 days after sowing, respectively according to the following formulas:
Pre-emergence % = number of un-germinated seeds x 100 / number of planted seeds.
Post-emergence % = number of dead seedlings x 100 / number of planted seeds.
Survived plants % = number of survived plants x100 / number of planted seeds.
Reduction or increasing % over the infected control was calculated according to the following formula: Reduction or Increasing % = Disease incidence (DI) of infected control - DI of treatment x100 /DI of infected control.

The plants were scored for disease severity through a 0–5 numerical rating scale for the degree of damage (Ondřej et al., 2008) and the disease index of root rot was calculated using the following equation:
Disease Index = Σ (FV) / NX X100
Where: F=number of roots tested in each grade; V= degree of damage (0-5); N=total number of tested plants, and X= the highest degree of infection (5).

Field experiments:

The field experiments were carried out during the winter growing seasons of 2020 - 2021 and 2021-2022 at Giza Agricultural Research Station, Giza Governorate, Egypt, in a field known to have a root rot history. The disinfected faba bean seeds were treated in the same manner as a greenhouse experiment. The treated faba bean seeds were sown in the field on 28 October 2020 and on 27 October 2021. The field trial (28 plots) was designed in a complete randomized block with four replicates. The area of each plot was 10.5 m^2 and consisted of five rows each row was 3.5 m in length and 0.6 m in width. All treatments were sown in hills 20 cm apart on both sides of the row ridge, with two seeds per hill, plant population = 140,000 plants/ feddan (fed=4200 m^2). All other recommended agricultural practices were followed according to

the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation except for the fertilization treatment, the dose of P fertilizer: 100 Kg fed^{-1} superphosphate (15.5 % P_2O_5) was added during soil preparation. Potassium sulfate (48% K_2O) at 50 Kg fed^{-1} was applied as soil application before the first irrigation. For N-fertilization, ammonium sulfate (20.5% N) was applied at the rates of 15 and 50 kg N fed^{-1} in two equal doses before the first and second irrigation. The treatments were as follows:

1) Rhizobial inoculation (481) +15 Kg N/Fed^{-1} (⅓ N).
2) Rhizobial inoculation (El Khatattba) +15 Kg N/Fed^{-1} (⅓ N).
3) A mix of rhizobial inoculation+15 Kg N/ Fed^{-1} (⅓ N).
4) A mix of rhizobial inoculation + foliar application with *M. mesophilicum* (6 L at 4x10^9 cfu/mL /fed^{-1}, 30 days after planting) +15 Kg N/fed^{-1} (⅓ N).
5) A mix of rhizobial inoculation + seed coating with *M. nodulans* +15 Kg N/fed^{-1} (⅓ N).
6) Fungicide treatment +50 Kg N/fed^{-1} (Full N).
7) Full nitrogen 50 Kg N/fed^{-1} (Full N).

The disease incidence (DI) % was determined as mentioned before. Random samples of ten faba bean plants were collected (from the inner rows) at the harvest stage from each plot. Plant growth parameters of plant height (cm), number of branches, number of pods per plant, weight of hundred seeds, and seed weight per plant were recorded as well as seed yield ton/ feddan was calculated.

Some physical and chemical properties of the soil in field experiments were carried out according to Jackson (1973) at the soil analysis Lab., Soils, Water and Environmental Research Institute, ARC, Giza, Table (1).

PGPR activities in vitro:

Indole acetic acid (IAA) detection:

The detection of IAA was done by using Salkowski reagent according to Khamna et al., (2010). The color developed by the positive reaction, the appearance of a pink color, indicated the presence of an indole compound.

Hydrogen cyanide (HCN) detection:

The HCN produced by strains under study was detected according to Bakker & Schippers, (1987). The discoloration of the filter paper to orange-brown after incubation indicates microbial production of cyanide.

Ammonia (NH_3) detection:

The ammonia production was revealed by the addition of Nessler's reagent giving a yellow-to-brown color of peptone water inoculated by bacterial cultures (Cappuccino & Sherman 1992).

Antibiotic resistance test:

Three antibiotics namely ampicillin, colistin, and tetracycline were used to estimate the antibiotic resistance of the two rhizobial isolates and two *Methylobacterium* species using the standard disk diffusion method as described by EUCAST (2021), and antibiotics resistance standard range of Gram-negative bacteria (Inhibition zone diameter, mm) as reported by Bauer et al., (1966).

Effect of faba bean treatments on activity of oxidative enzymes and phenol content.

Faba bean plants were grown as mentioned before in the greenhouse experiment. Fifteen days after sowing, the activity of peroxidase (PO), polyphenol oxidase (PPO), and phenol contents were determined in tissue extracts of six faba bean plants from each treatment mentioned before in the greenhouse treatment.

TABLE 1. Some Physical and Chemical properties of the used soil.

Physical properties	Value	Chemical properties	Value
Sand %	31.70	pH	7.87
Silt %	30.85	E.C (ds m1 25^{o}C)	1.32
Clay%	37.45	Saturation percent (S.P) %	47%
Texture grade	Clay loam	Organic matter %	1.74
		Total nitrogen %	0.074

A) *Assay of peroxidase (PO):*

Extraction and assay of peroxidase (PO) activity were carried out according to Chakraborty & Chatterjee (2007).

B) *Assay of polyphenol oxidase (PPO):*

Extraction and assay of polyphenol oxidase enzyme (PPO) were carried out according to Rocha & Morais (2001).

C) *Determination of phenolic compounds:*

The extraction of phenolic compounds was carried out according to Sutha et al*.,* (1998). Phenolic compounds were determined using methods of analysis described by Snell & Snell (1953).

Statistical analysis

Completely randomized design (CRD) and randomized block design (RBD) were implemented in the greenhouse experiment and field experiment, respectively. The obtained data were subjected to computer statistical software (ASSISTAT) originated by Silva & Azevedo (2009). Data were analyzed using analysis of variance (ANOVA), and mean values were compared using Duncan's multiple range test at a significance level of $P \leq 0.05$.

Results

1-Evaluation of the rhizobial and methylobacterial strains for excreting ammonia, HCN, and IAA.

The visual examination revealed that all rhizobial and methylobacterial strains could produce IAA, HCN, and ammonia with different capabilities, by altering the color, a pink color for IAA; orange-brown for HCN; and yellow-to-brown color for NH_3.

Antibiotic resistance studies

Results showed that all tested bacterial strains are resistant to ampicillin and colistin because there was no inhibition zone and they are sensitive to tetracycline because the zone of inhibition exceeds 19 mm.

3-Morphological and molecular identification of the pathogen

Using microscopic and morphological features like the number of nuclei (more than two), production of sclerotia, brown pigmentation, and the branch base constriction of hyphae, the isolate SKS 39 was identified according to (Sneh et al*.,* 1991; Seema et al., 2012) as *Rhizoctonia solani*. BLAST analysis revealed that the ITS sequence of isolate SKS 39 is *R. solani* with accession number OR538875.1. From the phylogenetic analysis, our isolate showed 99.82% identity with other *R. solani* isolates with GenBank accession numbers MH025376.1 from Oman, MW498395.1 from Iraq, and OR074128.1 from Egypt. In addition, our isolate showed 95.28% identity with other *R. solan*i isolates with GenBank accession numbers FJ746974.1 and FJ746973.1 from the USA, OP612668.1 from Saudi Arabia, and OM918223.1 from Egypt, Fig. (1).

4-Greenhouse experiments

4-1-Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on the occurrence of damping-off *disease of faba bean plants grown in artificially contaminated soil by R. solani*

According to Table (2), all treatments significantly reduced the percentage of pre- and post-emergence damping off and increased the number of survived plants as compared to the control grown in artificially infested soil with *R. solani*. The most effective treatments in decreasing pre-emergence damping off were (a mix of rhizobial inoculation + ⅓ N + seed treatment with *M. nodulans*) and Rizolex-T. This curative impact was extended to post-emergence damping off, all treatments were effective in increasing the surviving plants compared with the control. Meanwhile, results indicate that all treatments reduced the disease index of root rot of *R.solani*. Rizolex-T and (a mix of rhizobial inoculation + ⅓ N + *M. nodulans*) recorded the lowest values as compared with the control grown in artificially infested soil.

4-2- Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on some crop parameters of faba bean plants grown in artificially contaminated soil by R. solani

The findings shown in Table (3) demonstrated that there were remarkable increases in the crop parameters of the faba bean plants. There are no significant differences in plant height between treatments except for the Rizolex-T treatment. On the other hand, (a mix of rhizobial inoculation + ⅓ N + seed treatment with *M. nodulans*) treatment and (a mix of rhizobial inoculation + ⅓ N + foliar application with *M. mesophilicum*) followed by (a mix of RI + ⅓ N) treatment scored the high value of shoot dry weight, root dry weight, nodule dry weight as well as nodules number compared to control plants grown in Infested soil (Full N).

The levels of N, P, and K increased in all

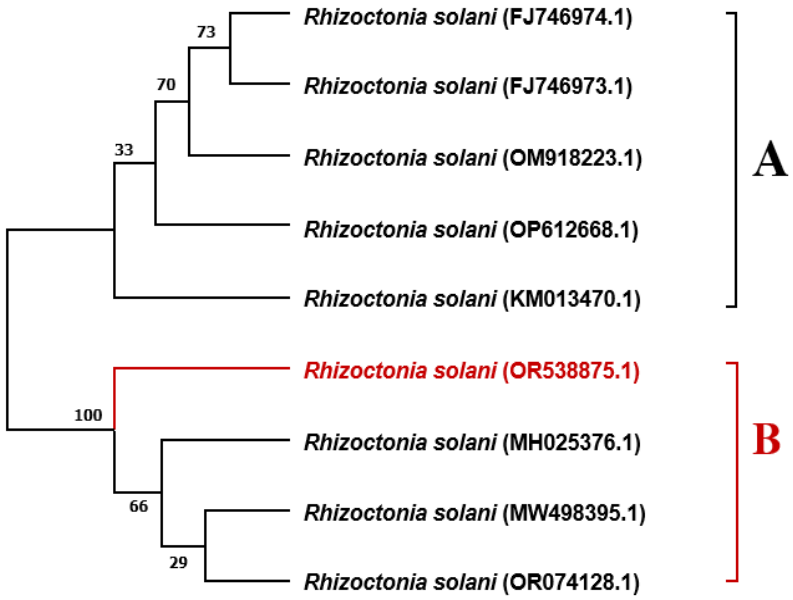

Fig.1. Phylogenetic tree based upon CLUSTAL W alignment of the ITS region of rDNA nucleotide sequences of 9 *Rhizoctonia solani* isolates. A maximum likelihood was used, with bootstrap values after 1000 replications of calculated run by using MEGA11 software. The branch numbers indicate bootstrap values. Our own sequenced *R. solani* isolate (ITS: OR538875.1). is shown in red color and the tree shows its identity with the most similar *R. solani*, GenBank accession numbers.

TABLE 2. Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on the occurrence of damping-off disease of faba bean plants grown in artificially contaminated soil by *R. solani*

Treatments	Damping- off				Survived plants %	Increasing over infected control %	Disease Index
	Pre-emergence		Post-emergence				
	Incidence %	Reduction %	Incidence %	Reduction %			
RI[1](481) + (⅓ N)[2]	16.0 b	66.7	4.0 b	60.0	80.0 b	90.5	47.1
RI (El Khatattba) + (⅓ N)	20.2 b	58.3	4.0 b	60.0	76.0 bc	81.0	48.0
Mix of RI + (⅓ N)	20.0 b	58.3	4.0 b	60.0	76.0 bc	81.0	48.7
Mix of RI + *M. mesophilicum*[3] + (⅓ N)	20.0 b	58.3	4.0 b	60.0	76.0 bc	81.0	45.3
Mix of RI+ *M. nodulans* + (⅓ N)	8.0 c	83.3	4.0 b	60.0	88.0 ab	100.1	30.4
Rizolex –T (Full N)[4]	8.0 c	83.3	4.0 b	60.0	88.0 ab	100.1	24.8
Control (*R. solani*) (Full N)	48.0 a	0.0	10.0 a	0.0	42.0 d	0.0	62.7
Control healthy (non-infested soil) (Full N)	4.0 d	-	0.0 c	-	96.0 a	-	0.0

1) (RI)= Rhizobial inoculation
2) (⅓ N) = 1.5 g N/pot^{-1} as a starter dose.
3) Foliar application (4.3 mL at 4x10^{7} cfu/ mL /plant, 30 days after planting)
4) (Full N) = 4.5 g pot^{-1} in two equal split doses were added at 10 and 25 days after plantation.
Means in each column followed by the same letters are not significantly different using Duncan's multiple range test, (p = 0.05).

TABLE 3. Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on some crop parameters of faba bean plants grown in artificially contaminated soil by R. solani after 60 days of planting.

Treatments	Plant height (cm)	Shoot dry weight (g/ plant)	Root dry weight (g/plant)	Nodules number /plant	Nodules dry weight (mg/ plant)	N mg/g	P mg/g	K mg/g
RI[1](481) + (⅓ N)[2]	60.25 ab	7.61 c	1.75c	23 d	246.65 c	3.24	0.313	1.71
RI (El Khatattba) + (⅓ N)	61.75 a	6.93 d	1.76 c	21 e	233.98 d	3.31	0.321	1.87
Mix of RI + (⅓ N)	60.75 a	7.7 bc	1.82 b	25 c	271.33 b	3.04	0.318	1.76
Mix of RI + *M. mesophilicum*[3] + (⅓ N)	61.25 a	7.99 ab	1.86 b	27 b	293.73 a	3.91	0.332	2.01
Mix of RI+ *M. nodulans* + (⅓ N)	61.5 a	8.05 a	1.95 a	31 a	296.13 a	3.94	0.351	2.21
Rizolex –T (Full N)[4]	57.0 c	7.06 d	1.64 d	0.00	0.00	2.73	0.172	1.42
Control (*R. solani*) (Full N)	28.75 d	2.92 e	0.65 e	0.00	0.00	2.31	0.134	1.21
Control healthy (non-infested soil) (Full N)	57.75 bc	7.09 d	1.70 c	0.00	0.00	3.23	0.235	1.51

1) (RI)= Rhizobial inoculation
2) (⅓ N) = 1.5 g N/pot^{-1} as a starter dose.
3)Foliar application (4.3 mL at 4x10^7 cfu/mL /plant, 30 days after planting).
4) (Full N) = 4.5 g N/ pot^{-1} in two equal split doses were added at 10 and 25 days after plantation, and without Rhizobial inoculation
Means in each column followed by the same letters are not significantly different using Duncan's multiple range test, (p = 0.05).

treatments; the largest rise occurred with (a mix of RI + ⅓ N + *M.nodulans*) treatment and (a mix of RI +⅓ N+ *M. mesophilicum*) treatment in comparison to the control grown in artificially contaminated soil.

It is observed that a combination of rhizobial inoculation with ⅓ N (1.5 g N/pot^{-1}) increases the value of crop parameters of faba bean plants over full nitrogen fertilization treatment. Also, the addition of methylotrophic bacteria as a seed treatment or foliar application to the mix of rhizobial inoculation plus ⅓ N (1.5 g N/pot^{-1}) improves the crop parameters over full nitrogen (4.5 g pot^{-1}) alone treatment which can decrease the amount of fertilization to the one-third amount.

5-Field experiments

5-1-Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on the occurrence of damping-off disease of faba bean plants under natural infection

As compared to the untreated control in two seasons, the results shown in Table (4) demonstrate that all treatments considerably reduced the percentage of pre- and post-emergence damping-off disease and raised the percentage of plants that survived. The maximum decrease of pre-emergence damping off was recorded with (a mix of RI + ⅓ N + *M.nodulans)* treatment followed by Rizolex-T treatment in two seasons. In the first season, the maximum reduction of post-emergence damping off was observed with (a mix of RI + ⅓ N + *M. nodulans*), (a mix of RI + ⅓ N + *M. mesophilicum*) and Rizolex-T treatments. In the second season, the maximum reduction was observed with (a mix of RI + ⅓ N + *M. nodulans*) and Rizolex-T treatments. On the other hand, the maximum increase of survived plants was recorded with (a mix of RI + ⅓ N + *M. nodulans*), Rizolex-T treatments, and (a mix of RI + ⅓ N + *M. mesophilicum*) respectively in the two seasons.

5-2- Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on some growth parameters of faba bean plants under natural infection

Under natural infection in the field, Data in Table (5) revealed that all treatments separately or in combination significantly stimulated the growth parameters of faba bean plants in comparison with the untreated control treatment in the two seasons.

TABLE 4. Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on the occurrence of damping-off disease of faba bean plants under natural infection at Giza Agricultural Research Station during winter growing seasons 2020-2021 and 2021-2022.

(A): First season

Treatments	Damping- off				Survived plants %	Increasing over infected control %
	Pre-emergence		Post-emergence			
	Incidence%	Reduction%	Incidence%	Reduction%		
RI[1](481) + (⅓ N)[2]	12.1 b	65.72	2.0 c	60.0	85.9 c	43.88
RI (El Khatattba) + (⅓ N)	12.5 b	64.58	2.3 c	54.0	85.2 c	42.71
Mix of RI + (⅓ N)	12.6 b	64.30	3.8 b	24.0	83.6 c	40.36
Mix of RI + *M. mesophilicum*[3] + (⅓ N)	9.3 b	73.65	1.2 d	76.0	89.5 b	50.25
Mix of RI+ *M. nodulans* + (⅓ N)	5.6 c	84.13	1.2 d	76.0	93.2 a	56.11
Rizolex –T (Full N)[4]	5.8 c	83.56	1.3 d	74.0	92.9 a	55.61
Control (Full N)	35.3 a	-	5.0 a	-	59.7 d	-

(B): second season

Treatments	Damping- off				Survived plants %	Increasing over infected control %
	Pre-emergence		Post-emergence			
	Incidence%	Reduction%	Incidence%	Reduction%		
RI[1](481) + (⅓ N)[2]	11.0 c	70.1	3.0 b	42.30	84.0 c	44.82
RI (El Khatattba) + (⅓ N)	12.5 c	66.03	2.2 c	57.69	85.3 bc	47.06
Mix of RI + (⅓ N)	15.0 b	59.23	2.0 c	61.53	83.0 c	43.1
Mix of RI + *M. mesophilicum*[3] + (⅓ N)	10.3 c	72.01	1.8 c	65.38	87.9 ab	51.55
Mix of RI+ *M. nodulans* + (⅓ N)	6.6 d	82.06	1.3 d	75.0	92.1 a	58.79
Rizolex –T (Full N)[4]	7.2 d	80.43	1.6 d	69.23	91.2 a	57.24
Control (Full N)	36.8 a	-	5.2 a	-	58.0 d	-

1) (RI)= Rhizobial inoculation

2) (1⁄3 N) = (15) Kg N fed $^{-1}$as a starter dose.

3)*M. mesophilicum* as a foliar application (6 L at 4x10^{9}cfu/mL /fed^{-1}, 30 days after planting)

4)(Full N) = 50 Kg N/fed in two equal doses were added before the first and second irrigation

Means in each column followed by the same letters are not significantly different using Duncan's multiple range test, (p = 0.05).

TABLE 5. Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on some growth parameters of faba bean plants under natural infection at Giza Agricultural Research Station during winter growing seasons 2020-2021 and 2021-2022 after 60 days of planting.

(A): First season

Treatments	Shoot dry weight (g/ plant)	Nodules number / plant	Nodules dry weight (mg/ plant)	N mg/g	P mg/g	K mg/g
RI[1](481) + (⅓ N)[2]	9.36 d	44 c	331.66 d	3.12	0.684	2.28
RI (El Khatattba) + (⅓ N)	12.00 c	28 d	314.34 e	2.62	0.411	1.67
Mix of RI + (⅓ N)	10.20 d	54 b	354.32 c	2.85	0.469	1.83
Mix of RI + *M. mesophilicum*[3] + (⅓N)	14.60 b	57 b	390.37 b	4.08	0.715	2.77
Mix of RI+ *M. nodulans* + (⅓ N)	17.13 a	65 a	423.40 a	4.79	0.839	3.25
Rizolex –T (Full N)[4]	8.86 d	22 d	250.33 f	2.74	0.428	1.68
Control (Full N)	6.50 e	11 e	136.38 g	1.88	0.307	1.43

(B): Second season

Treatments	Shoot dry weight (g/ plant)	Nodules number / plant	Nodules dry weight (mg/ plant)	N mg/g	P mg/g	K mg/g
RI[1](481) + (⅓ N)[2]	13.56 d	73 c	436.00 b	3.22	0.624	2. 57
RI (El Khatattba) + (⅓ N)	12.90 e	53 d	366.00 c	3.53	0.567	2. 45
Mix of RI + (⅓ N)	15.10 c	76 c	437.00 b	3.77	0.709	2.86
Mix of RI + *M. mesophilicum*[3] + (⅓ N)	16.36 b	121 b	475.33 ab	4.25	0.719	2.94
Mix of RI+ *M. nodulans* + (⅓ N)	17.37 a	149 a	491.00 a	5.38	0.764	3.30
Rizolex –T (Full N)[4]	10.53 f	36 e	282.00 d	3.61	0.537	1.89
Control (Full N)	8.66 g	26 f	214.00 e	2.31	0.392	1.83

1) (RI)= Rhizobial inoculation
2) (1⁄3 N) = (15) Kg N fed^{-1} as a starter dose.
3)*M. mesophilicum* as a foliar application (6 L at 4x10^{9}cfu/mL /fed^{-1}, 30 days after planting)
4)(Full N) = 50 Kg N/fed in two equal doses were added before the first and second irrigation
Means in each column followed by the same letters are not significantly different using Duncan's multiple ran test, (p = 0.05).

The treatment (a mix of RI + ⅓ N + *M. nodulans*) showed maximal effect at all tested growth parameters, the dry weight of shoot, nodules number, and dry weight of nodules in the two successive seasons followed by (a mix of RI + ⅓ N + *M. mesophilicum*), whereas control treatment (full nitrogen) recorded the lowest values of all growth parameters as compared with other treatments.

Meantime in the two seasons, (a mix of RI + ⅓ N + *M. nodulans*) and (a mix of RI + ⅓ N + *M. mesophilicum*) treatments resulted in worthy increases of nitrogen, phosphorus, and potassium contents in comparison with control treatment.

5-3- Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on some crop parameters and yield of faba bean plants under natural infection

All treatments singly or in combination under natural infection significantly showed stimulatory effects for crop parameters of faba bean plants and yield in comparison with control in the two seasons (Table 6).

Plant height

In two seasons, the treatments with the greatest effects were a combination of (a mix of RI + ⅓ N + *M. nodulans*) and a combination of(a mix of RI + ⅓ N + *M. mesophilicum)* followed by (RI 481+⅓ N)) and (a mix of RI +⅓ N)) in the first season while followed by Rizolex-T, (a mix of RI +⅓ N) and (RI 481+⅓ N)) in the second season.

The branches number

In comparison with untreated control, all treatments considerably increased the number of branches.

Number of pods per plant

The result revealed an increase in the number of pods with all treatments over the untreated control in two seasons. In the first season, the (mix of RI + ⅓ N + *M. nodulans*) treatment showed the highest value followed by the (mix of RI + ⅓ N + *M. mesophilicum*). As for the second season, the maximum increases were recorded for the (mix of RI + ⅓ N + *M. nodulans*) and the(mix of RI + ⅓ N + *M. mesophilicum*) followed by the (mix of RI +⅓ N) and (RI El-Khatattba+⅓ N).

Seed weight (g)/plant

In two seasons the (mix of RI + ⅓ N + *M. nodulans*) and the (mix of RI + ⅓ N + *M. mesophilicum*) treatments showed the highest value followed by the (mix of RI +⅓ N) and the (RI 481+⅓ N) in the first season, and in the second season followed by (RI 481+⅓ N),the (mix of RI +⅓ N), (RI El-Khatattba+⅓ N) and Rizolex-T.

TABLE 6. Impact of Rizolex-T, methylotrophic bacteria, and Rhizobium strains on some crop parameters of faba bean plants under natural infection at Giza Agricultural Research Station during winter growing seasons 2020-2021 and 2021-2022

(A): First season

Treatments	Plant height (cm)	Number of branches/ plants	Number of pods/ plant	Seed weight / Plant (g)	100-seed weight (g)	Seed yield (ton/fed)
RI[1] (481) + (⅓ N)[2]	99.0 ab	3.9 ab	17.5 d	40.4 b	74.2	1.365 c
RI (El Khatattba) + (⅓ N)	93.4 c	4.1 a	18.7 c	38.8 c	72.1 d	1.351 c
Mix of RI + (⅓ N)	95.0 b	3.8 ab	19.1 c	40.9 b	73.2 c	1.372 c
Mix of RI + *M. mesophilicum*[3] + (⅓ N)	100.9 a	4.1 a	20.5 b	42.6 a	78.8 a	1.557 b
Mix of RI+ *M. nodulans* + (⅓ N)	102.4 a	4.0 a	22.4 a	42.7 a	78.3 a	1.669 a
Rizolex –T (Full N)[4]	88.4 c	3.5 b	18.9 c	38.0 c	75.7 b	1.525 b
Control (Full N)	62.0 d	2.2 c	11.8 e	21.6 e	64.5 e	0.920 d

(B): Second season

Treatments	Plant height (cm)	Number of branches/ plant	Number of pods/ plant	Seed weight/ Plant (g)	100-seed weight (g)	Seed yield (Ton/fed)
RI[1] (481) + (⅓ N)[2]	104.8 c	3.5 a	19.2 d	41.1 a	76.6 b	1.397 d
RI (El Khatattba) + (⅓ N)	101.3 d	3.8 a	20.3 bc	38.9 b	75.6 b	1.373 d
Mix of RI + (⅓ N)	105.0 c	3.3 a	20.6 b	41.0 ab	77.4 ab	1.409 d
Mix of RI + *M. mesophilicum*[3] + (⅓ N)	109.0 ab	3.8 a	21.9 a	42.0 a	78.0 a	1.586 b
Mix of RI+ *M. nodulans* + (⅓ N)	111.0 a	3.8 a	22.2 a	42.8 a	79.2 a	1.638 a
Rizolex –T (Full N)[4]	106.0 bc	3.0 a	19.4 cd	38.4 b	75.7 b	1.475 c
Control (Full N)	73.3 e	2.0 b	12.0 e	21.9 c	64.1 c	1.934 e

1) (RI)= Rhizobial inoculation

2)(1⁄3 N) = (15) Kg N fed $^{-1}$as a starter dose.

3)*M. mesophilicum* as a foliar application (6 L at 4x10^{9}cfu/mL /fed^{-1}, 30 days after planting)

4)(Full N) = 50 Kg N/fed in two equal doses were added before the first and second irrigation

Means in each column followed by the same letters are not significantly different using Duncan's multiple range test, (p = 0.05).

The weight of hundred seeds

In the first season, the (mix of RI + ⅓ N + *M. mesophilicum*) and the (mix of RI + ⅓ N + *M. nodulans*) showed the highest value followed by Rizolex -T, while in the second season, followed by the (mix of RI +⅓ N), (RI 481+⅓ N), Rizolex-T and (RI El-Khatattba+ ⅓ N).

Seed yield

All treatments considerably enhanced seed yield during both seasons in comparison with the control. The maximum values were recorded with the (mix of RI + (⅓ N) + *M. nodulans*) followed by the (mix of RI + ⅓ N) + *M. mesophilicum*) and Rizolex-T respectively. Meantime, the remaining treatments did not have any statistically significant differences.

6-Impact of the methylotrophic bacteria and Rhizobium strains on the activity of peroxidase, polyphenol oxidase enzymes, and content of phenol

6-1-The activity of peroxidase and polyphenol oxidase enzymes

According to Table (7), all treatments significantly increased peroxidase and polyphenol oxidase activities in comparison to untreated control, the considerable increase was related to (a mix of RI + ⅓ N + *M. nodulans*) treatment in both enzymes.

6-2-Phenolic contents

In comparison with untreated controls, the content of total phenols has been significantly increased in all treatments (Table 8). The highest increase was recorded with (a mix of RI + ⅓ N + *M. nodulans*) treatment as 89.08% over the untreated control with full nitrogen fertilization followed by (RI El-Khatattba+⅓ N) and the (a mix of RI +⅓ N) treatments as 79.08 and 75.6% over untreated control respectively.

For free phenol content, the maximum increase was shown with a (mix of RI + ⅓ N + *M. nodulans*) treatment (197.08 % increase over control) followed by (a mix of RI +⅓ N)and (RI El-Khatattba+(⅓ N) treatments (126.1 % and 106.79 % increase over control respectively). On the other hand, the maximum increase of conjugated phenol was observed with (RI El-Khatattba+⅓ N) treatment (65.23% increase over control) followed by (RI 481+⅓ N) treatment (60.38% increase over control).

TABLE 7. Impact of the methylotrophic bacteria and Rhizobium strains on the activity of oxidative enzymes of faba bean plants grown in artificially contaminated soil by *R. solani*

Treatments	activity of Peroxidase (Absorbance at 430 nm)		activity of Polyphenol oxidase (Absorbance at 495 nm)	
	Activity	Increasing over infected cotrol%	Activity	Increasing over infected control%
RI (481) + (⅓ N)	0.282 c	58.42	0.201 c	107.2
RI (El Khatattba) + (⅓ N)	0.279 c	56.74	0.210 c	116.49
Mix of RI + (⅓ N)	0.271 c	52.24	0.211 c	117.5
Mix of RI+ *M. nodulans* + (⅓ N)	0.301 d	69.1	0.231 d	138.1
Control (*R. solani*) (Full N)	0.178 b	-	0.097 b	-
Control healthy (non-infested soil) (Full N)	0.091 a		1.79 a	

Means in each column followed by the same letters are not significantly different using Duncan's multiple range test, (p0.05).

TABLE 8. Impact of the methylotrophic bacteria and Rhizobium strains on phenolic contents of faba bean plants grown in artificially contaminated soil by *R. solani*

Treatments	Phenolic contents (mg/g fresh weight)					
	Total phenols	Increasing over infected control %	Free phenols	Increasing over infected control %	Conjugated phenols	Increasing over infected control %
RI (481) + (⅓ N)	6.701 c	66.65	2.401 b	79.18	4.300 de	60.38
RI (El Khatattba) + (⅓ N)	7.201 d	79.08	2.771 bc	106.79	4.430 e	65.23
Mix of RI + (⅓ N)	7.061 d	75.60	3.031 c	126.19	4.030 d	50.31
Mix of RI+ *M. nodulans* + (⅓ N)	7.603 e	89.08	3.981 d	197.08	3.622 c	35.1
Control (*R. solani*) (Full N)	4.021 b	-	1.340 a	-	2.681 b	-
Control healthy (non-infested soil) (Full N)	1.791 a		1.101 a		0.690 a	

Means in each column followed by the same letters are not significantly different using Duncan's multiplerange test, (p = 0.05).

Discussion

In Egypt, the faba bean, or *Vicia faba* L., is a significant crop grown for food, vital for soil fertility, and human nutrition as a good source of vegetarian protein, and animal nourishment. The yield production was significantly lowered as a result of the significant decrease in the cultivated area (Omar, 2021). However, Rhizoctonia damping-off and root rot diseases are the most common cause of yield instability and losses in faba bean production in most parts of the world, resulting in a significant decline in crop throughput and seed quality (Mazen et al., 2008). To meet the enormous demand for food from expanding populations, there is an overreliance on chemical pesticides and fertilizers. Unfortunately, the excessive utilization of such fertilizer's outcomes in human health problems, toxicity in plants, and environmental contaminants (Lassaletta et al., 2014). Therefore, significant effort is being made to reduce the usage of synthetic fungicides and maximize the use of alternative management measures to manage plant pathogens in soil.

The use of biofertilizers as biocontrol agents can be a powerful means to ensure that the growing population is not only provided with food but also agricultural production is protected from the effects of various environmental pressures (Mahanty et al., 2017).

Plant-beneficial microbes, also referred to as plant growth-promoting microbes (PGPMs), which play a major role in improving crop productivity and providing resistance to stress conditions have recently received more attention (Yadav and Yadav, 2018 a). It is well recognized that some strains of *Methylobacterium* have an important position in enhancing crop yields and soil fertility. Rhizospheric and non-rhizospheric methylotrophs are frequently applied as bioinoculants, and the use of these bacteria for alternatives to chemical fertilizers is growing (Yadav and Yadav, 2018 b).

All the microorganisms under study showed activity in producing IAA, HCN, and ammonia despite the differences in values. In this respect, Indole acetic acid (IAA) promotes cell elongation by increasing the synthesis of components of the cell wall, for example, cellulose and pectin (Rayle & Cleland, 1992). Meantime, the hormone auxin in plants is known to stimulate cell elongation through increased cell wall extensibility (Majda & Robert, 2018). Moreover, IAA stimulates the expression of genes involved in the development of lateral roots, hence IAA encourages lateral root formation (Du & Scheres, 2018). On the other hand, a wide range of microbes creates hydrogen cyanide (HCN), a volatile secondary metabolite that is believed to be important for the biocontrol

of plant diseases (Gupta & Sinha, 2020). HCN is a cytochrome c oxidase inhibitor, that resulted to disrupts cellular respiration, leading to the depletion of ATP and the accumulation of reactive oxygen species (ROS). ROS have the potential to cause damage to cell components, leading to cell death (Cooper & Brown, 2008). Hence, the production of HCN by biocontrol agents is thought to be a key factor in their ability to suppress plant diseases. HCN can act directly on pathogens, inhibiting their growth and development. In addition, HCN can also induce systemic acquired resistance (SAR) in plants. SAR can help to protect the plant from subsequent infections (Díaz-Rueda et al*.,* 2023). However, by producing ammonia, PGPB collects and supplies nitrogen to their host plants, as well as elongate its roots and shoots and increase biomass (Marques et al., 2010).

In the present research, the antibiotic susceptibility test showed that isolates were resistant to different antibiotics as ampicillin and colistin. Meantime, the rhizosphere is home to a wide variety of microorganisms. Certain microorganisms in the soil naturally create antibiotics that are fatal to soil populations of sensitive methylotrophic or Rhizobium bacteria. Hence, innate resistance to antibiotics is a favorable characteristic for methylotrophic bacteria and Rhizobium populations. It improves the odds of survival, growth, and reproduction in an antibiotically stressful environment (Nahar et al., 2017).

In the present work, using the genus *Methylobacterium* and genus Rhizobium as biocontrol agents for controlling root rot disease of faba bean plants (cv. G 843) caused by *R. solani*, also as a biofertilizer showed a respectable result in addition to the possibility of using them for reducing nitrogen fertilization to about one-third rate of the recommended dose.

Under greenhouse and field conditions, results indicated that treating seeds with *M. nodulans* in combination with a mix of rhizobium strains plus one-third of the nitrogen fertilization rate enhanced resistance against pre- and post-emergence damping-off and increased survived plants, in addition to the fact that treating seeds with Rizolex-T greatly reduced the disease incidence. Also, the treatment with a mix of rhizobium strains plus one-third of the nitrogen fertilization rate followed by foliar treatment with *M. mesophillicum* decreased the disease incidence significantly in comparison with the control. Different microorganism treatments under investigation resulted in a significant increase in nodules number and nodules dry weight of faba bean plants in comparison to untreated control grown in infested soil with *R. solani*. There was a noticeable rise in the seeds produced per feddan in the field with the tested microorganism's treatments or Rizolex-T over the control due to the increase of shoots and roots dry weight generated by the treatments.

Meanwhile, *Methylobacterium* spp. are significant endophytes that are thought to be responsible for improved plant height, leaf area, and seed germination as a result of the production of physiologically active metabolites like auxins and cytokinins (Rossetto et al*.,* 2011). Furthermore, *Methylobacterium* spp. encourages plant growth as a bio-promoter by modulating the quantity of ethylene, a key signaling chemical in plants that influences defense system function, cell development, and aging (Hoppe et al., 2011). The application of pink-pigmented facultative methylotrophs (PPFMs) as a foliar spray significantly improved plant height, dry weight, leaf area, number, and dry weight of boll resulting in a rise in seed cotton yield over control (Madhiayan et al., 2006).

However, it has been reported that many rhizobial strains possess biocontrol capabilities. Consequently, potential control can be achieved by using these strains against soilborne pathogens. The rhizobia's biocontrol mechanism involves competition with nutrients (Arora et al., 2001), the synthesis of antibiotics (Bardin et al., 2004; Deshwal et al. 2003a), the secretion of enzymes that degrade the cell wall (Ozkoc & Deliveli, 2001), and the formation of siderophores (Carson et al., 2000; Deshwal et al., 2003b). It has been observed that several strains include *R. Leguminosarum* bv. *trifolii, R. leguminosarum* bv. *viciae*, *R. meliloti, R. trifolii,* and *Bradyrhizobium japonicum* secrete enzymes that break down cell walls and antibiotics that can suppress plant pathogens (Bardin et al., 2004; Siddiqui et al., 2000).

In our study, under artificially infested soil in the greenhouse and natural infection in the field, all treatments significantly enhanced the NPK quantity of faba bean plants in comparison with the control treatment with full nitrogen fertilization. However, individuals in the genus *Methylobacterium* fix nitrogen, solubilize phosphorus, and deliver essential nutrients to plants. Because *Methylobacterium* species are capable of dissolving inorganic phosphates,

phosphate metabolism is encouraged in both microbes and plants (Rodríguez et al., 2006). Additionally, the genus *Methylobacterium* can create low-molecular-mass siderophores that have a high affinity for iron solubilization. As a result, solubilizing iron helps plants absorb it and grow. Radha et al., (2009) reported that treated soybean seeds with *Bradyrhizobium* and PPFM increased nitrogen and potassium uptake in the vegetative plant parts, nodulation, and yields in comparison with control and chemical fertilizer. Furthermore, both nodulating and non-nodulating Methylobacterium spp. with high nitrogenase activity were isolated from legumes (Raja et al., 2006).

On the other hand, all treatments increased the activity of the enzymes peroxidase (PO) and polyphenol oxidase (PPO) which in turn induced an expression of pathogenesis-related (PR) proteins. Once more, the maximum enzymatic activity was obtained by combining bio treatments with the one-third N fertilization, and this resulted in the inhibition of Rhizoctonia root rot. According to Jha et al., (2015), *Methylobacterium* is an inducer of systemic resistance through the synthesis of proteins, such as phenylalanine ammonia-lyase, peroxidase, chitinase, β -1,3, glucanase, and phenolic compounds. Peroxidase is well recognized for being essential to host plant defenses against pathogens (Van Loon et al., 2006). Peroxidases also play a role in the synthesis of phytoalexins and the creation of reactive oxygen species (ROS), which have antimicrobial effects (Almagro et al., 2009).

Meanwhile, ortho-diphenolic compounds can be oxidized to o-quinones (antimicrobial chemicals) by polyphenol oxidases. Quinones are potent inhibitors of the SH family of enzymes that may suppress pathogens (Constabel & Barbehenn, 2008). Polyphenol oxidases can function in four ways: (1) by quinones toxicity; (2) by decreasing bioavailability and alkylation of cellular proteins of the pathogen; (3) by physically forming barriers through crosslinking quinones with protein in the pathogen; and (4) by creating reactive oxygen species (Yoruk & Marshall, 2003). On the other hand, faba bean plants' total phenolic compound content was much higher in comparison with untreated control plants. In this case, Nicholson & Hammerschmidt (1992) proposed that phenolic compounds have a function in disease resistance. They suggested that plants related to phenols have two stages to their defense mechanism. The first is thought to entail the quick build-up of phenols at the site of infection, which inhibits (or even stops) the pathogen's growth. The second stage might include the activation of particular defenses such as the creation of phytoalexins or other compounds associated with stress.

The use of a mixture of biological control agents is expected to achieve better protection against diseases because each biological agent may have a unique mode of action to combat the pathogen. The majority of biological control strategies for soilborne plant pathogens depend on a single biocontrol agent to inhibit the pathogen (Larkin et al., 1998). This strategy needs to be changed since specific biocontrol agents may not perform consistently against all crop diseases under different soil conditions. A broader variety of properties can be found in a mixture of biocontrol agents that inhibit one or more diseases and have these traits manifested under a variety of pathological conditions. Numerous studies demonstrate that combining different biocontrol agents can increase effectiveness and reduce variability in efficacy (Guetsky et al., 2002; Roberts et al., 2005; Pertot et al., 2017).

Conclusion

Eventually, it may be stated that the use of *Methylobacterium* and *rhizobium* in combination with the one-third amount of N recommended fertilization dose could be utilized to replace chemical fertilizers (full nitrogen dose) as an environmentally friendly and cost-efficient method to enhance plant growth. Likewise, methylotrophic bacteria provide an alternate means for biocontrol and plant growth enhancement by producing phytohormones, fixing nitrogen, and solubilizing phosphate. Also effective as a biocontrol agent against Rhizoctonia damping off and root rot disease.

References

Ahlawat, U., Rekha,Priyanka, Bardwaj, S. Wati, L. (2018) Methylotrophic bacteria and their role in sustainable agriculture system. *Chemical Science Review and Letters*, **7**(25), 1-11.

Ali, A. A., Hafez, W. A. (2018). Phosphate solubilizing bacteria (*Rhizobium* and *Azotobacter chroococcum*) and their environmental effects with compost on growth promotion of wheat under saline soil conditions. *N. Egypt. J. Microbiol*., **51**,138-158.

Ali, A. A., Orf, H. O. (2022). Screening and increase of

exopolysaccharide production by rhizobial strains, stress tolerances and its efficiency with. *Egyptian Journal of Agricultural Research,* **100**(1), 11-21.

Almagro, L., Gómez Ros, L. V., Belchi-Navarro, S., Bru, R., Ros Barceló, A., Pedreño, M. A. (2009) Class III peroxidases in plant defence reactions. *Journal of experimental botany*, **60**, 377-390. Doi: org/10.1093/jxb/ern277

Arora, N.K., Kang, S.C., Maheshwari, D.K. (2001) Isolation of siderophore-producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. *Curr Sci*., **81**,673–677.

Atwa, M.A.(2016) Induction of resistance against damping off and root rot diseases in faba bean. *Arab Univ. J. Agric. Sci., Ain Shams*, **24**,555–578. Doi: 10.21608/AJS.2016.14425

Bakker, A.W. Schippers, B. (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* sp. mediated plant growth-stimulation. *Soil Boil. Biochem.,* **4**, 451-457. https://doi.org/10.1016/0038-0717(87)90037-X

Bardin, S.D., Huang, H.C., Pinto, J., Amundsen, E.J. Erickson, R.S. (2004) Biological control of Pythium damping-off of pea and sugar beet by *Rhizobium leguminosarum* bv. *viceae*. *Can J. Bot*., **82**,291–296. https://doi.org/10.1139/b04-003

Bauer, A., Kirby, W. , Sherris, J. Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. **45,** 493-496 . https://doi.org/10.1093/ajcp/45.4_ts.493

Berg, G. (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology*, **84**, 11–18. https://doi.org/10.1007/s00253-009-2092-7

Boeswinkel, H.J. (1976) Storage of fungal cultures in water. *Trans. Br. Mycol. Soc.*, **66**, 183-185. https://doi.org/10.1016/S0007-1536(76)80119-2

Cappuccino, J.C., Sherman, N. (1992) Microbiology: a laboratory manual, 3rd ed. Benjamin/Cummings, New York, pp. 125–179.

Carson, K.C., Meyer, J.M. Dilworth, M.J. (2000) Hydroxamate siderophore of root nodule bacteria. *Soil Biol. Biochem*., **32**,11–21. https://doi.org/10.1016/S0038-0717(99)00107-8

Chakraborty, M.R., Chatterjee, N.C. (2007) Interaction of *Trichoderma harzianum* with *Fusarium solani* during its pathogenesis and the associated resistance of the host. *Asian J. Exp. Sci.,* **21**, 351-355.

Constabel, C.P., Barbehenn, R.V. (2008) Defensive roles of polyphenol oxidase in plants. In: Schaller, A. (Ed.), Induced Plant Resistance to Herbivory, Springer, New York, 253-269.

Cooper, C.E. , Brown, G.C. (2008) The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *J. Bioenerg Biomembr*, **40**, 533–539. Doi:10.1007/s10863-008-9166-6

Cottenie, A., Verloo, M., Kiekns, L. (1982) Chemical Analysis of Plants and Soils. Laboratory of analytical and agrochemistry. State University, Ghent Belgium. 63p. https://lib.ugent.be/catalog/rug01:000239299

Deshwal, V.K., Pandey, P., Kang, S.C., Maheshwari, D.K. (2003a) Rhizobia as a biological control agent against soil borne plant pathogenic fungi. *Indian journal of experimental biology*, **41**, 1160-1164.

Deshwal V.K., Dubey, R.C., Maheshwari, D.K. (2003b) Isolation of plant growth-promoting strains of *Bradyrhizobium (Arachis)* sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal rot of peanut. *Curr. Sci*., **84**,443–444.

Díaz-Rueda, P., Morales de los Ríos, L., Romero, L. C., García, I. (2023) Old poisons, new signaling molecules: the case of hydrogen cyanide. *Journal of Experimental Botany*, **74**, 6040–6051. DOI: 10.1093/jxb/erad317

Du, Y., Scheres B. (2018). Lateral root formation and the multiple roles of auxin. *Journal of Experimental Botany,* **69,** 155–167. https://doi.org/10.1093/jxb/erx223

EUCAST, European Committee on Antimicrobial Susceptibility Testing. (2021) EUCAST disk diffusion method for Antimicrobial Susceptibility Testing, Version 9.0. 22 pages, www.eucast.org

FAOSTAT. (2024) Food and Agricultural Organization (FAO) of the United Nations, http://fao.org

Garrity, G. (2005). Bergey's Manual of Systematic Bacteriology: Volume 2: The Proteobacteria, Part C: The Alpha-, Beta-, Delta-, and Epsilon Proteobacteria. Springer Science & Business

Media, pp. 567-571.

Garrity, G.M, Bell, J.A, Lilburn, T. (2005) Family IX. *Methylobacteriaceae*.fam. nov. In: Garrity, G.M., Brenner, D.J., Krieg, N.R., Staley, J.T. (eds) Bergey's Manual of Systematic Bacteriology: Volume 2: The Proteobacteria, Part C: The Alpha-, Beta-, Delta-, and Epsilon Proteobacteria. Springer Science & Business Media, pp. 567-571.

Gaskill, J.O. (1968) Breeding for Rhizoctonia resistance in sugar beet. *J. Am. Soc. Sugar Beet Technol*., **15**, 105-119.

Glick, B.R. (2012) Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindawi Publishing Corporation, *Scientifica*, **2012**,963401, 15p.http://dx.doi.org/10.6064/2012/963401

Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. L. L., Krishnamurthy, L. (2015). Plant growth promoting rhizobia: challenges and opportunities. *Biotech*., **5**, 355–377. Doi: 10.1007/s13205-014-0241-x

Guetsky, R., Shtienberg, D., Elad, Y., Fischer, E., Dinoor, A. (2002) Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. *Phytopathology*, **92**, 976–985. Doi: 10.1094/PHYTO.2002.92.9.976.

Gupta, D., Sinha, S. N. (2020) Production of hydrogen cyanide (HCN) by purple non-sulfur bacterium isolated from the rice field of West Bengal. *IOSR Journal of Pharmacy and Biological Sciences*. **15**, 16-26. DOI: 10.9790/3008-1501031626

Hall, T. (2005) BioEdit. Version 7.2. 5 Biological sequence alignment editors for Win 95/98/NT/2K/XP.

Holland, M. A., Polacco, J. C. (1992) Urease null and hydrogenase – null phenotypes of a phylloplane bacterium reveal altered nickel metabolism in two soybean mutants. *Plant Physiol*., **98,** 942-948. https://doi.org/10.1104/pp.98.3.942

Hoppe, T., Peters, K., Schmidt, F. (2011) *Methylobacterium bullatum* sp. nov., a methylotrophic bacterium isolated from *Funaria hygrometrica*. *Syst. Appl. Microbiol*.,**34**, 482–486 . https://doi.org/10.1016/j.syapm.2010.12.005

Indiragandhi, P., Anandham, R., Kim, K.Y., Yim, W.J.(2008) Induction of systemic resistance by modulating ethylene biosynthesis pathway by ACC deaminase containing *Methylobacterium oryzae* against *Pseudomonas syringae* in tomato. World *J. Microbiol. Biotechnol.,* **24**, 1037-1045 . https://doi.org/10.1007/s11274-007-9572-7

Jackson, M.L. (1973) Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd., New Delhi, 498 p

Jha, C.K., Maheshwari, D.K., Saraf, M. (2015) Emergence of *Methylobacterium* spp. as potential organism in agroecosystems. In: Maheshwari, D. (eds) Bacterial Metabolites in Sustainable Agroecosystem. Sustainable Development and Biodiversity, vol 12. Springer, Cham. https://doi.org/10.1007/978-3-319-24654-3_3

Jorge, G.L., Kisiala, A. Morrison, E., Aoki, M., Nogueira, A.P.O., Emery, R.J.N. (2019) Endosymbiotic *Methylobacterium oryzae* mitigates the impact of limited water availability in lentil (*Lens culinaris* Medik.) by increasing plant cytokinin levels. *Environ. Exp. Bot.* **162**, 525–540. https://doi.org/10.1016/j.envexpbot.2019.03.028

Jukes, T.H.,Cantor, C.R. (1969) Evolution of Protein Molecules. In: Munro, H.N. (Ed.); Mammalian Protein Metabolism, Academic Press, NY, USA, pp. 21-132.

http://dx.doi.org/10.1016/B978-1-4832-3211-9.50009-7

Karkanis, A., Ntatsi, G., Lepse, L., Fernández, J.A., Vågen, I.M., Rewald, B., Alsin, A. I., Kronberga, A., Balliu, A., Olle, M., Bodner, G., Dubova, L., Rosa, E., Savvas, D. (2018) Faba Bean Cultivation – Revealing Novel Managing Practices for More Sustainable and Competitive European Cropping Systems. *Front. Plant Sci*. **9**,1115, 14p. DOI: 10.3389/fpls.2018.01115

Khamna, S, Yokota, A., Peberdy, J. F., Lumyong, S.(2010) Indole3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *EurAsian Journal of Biosciences***, 4**,23-32. DOI:10.5053/ejobios.2010.4.0.4

Lamari, L., Bernier, C.C. (1985) Etiology of seedling blight and root rot of faba bean (*Vicia faba*) in Manitoba. *Canadian Journal of Plant Pathology,* **7**, 139-145. DOI:10.1080/07060668509501490

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, **23**, 2947-2948. DOI: 10.1093/bioinformatics/btm404

Larkin, R.P., Roberts, D.P., Gracia-Garza, J. A. (1998)

Biological control of fungal diseases. In: Fungicidal Activity-Chemical and Biological Approaches to Plant Protection. (Hutson, D. and Miyamoto, J. eds.). Wiley, New York, NY., pp 141–191.

Lassaletta, L., Bille, G., Grizzetti, B., Anglade, J., Gamier J. (2014) 50-year trends in nitrogen use efficiency of world cropping systems: The relationship between yield and nitrogen input to cropland. *Environ. Res Lett*., **9**,1-9. https://doi.org/10.1088/1748-9326/9/10/105011

Lim, C.H., Jackson, M.L. (1982) Dissolution for Total Elemental Analysis. In: Page, A.L. (Ed.) Methods of Soil Analysis, part 2. Chemical and microbiological properties. Agronomy monograph No. 9, 2nd ed., the American Society of Agronomy, Madison. Wisconsin, U.S.A., pp. 1-12. https://doi.org/10.2134/agronmonogr9.2.2ed.c1

Madhiayan, M., Poonguzhali, S., Sundaram, S.P., Sa, T.M. (2006) A new insight to foliar applied methanol influencing phylloplane methylotrophic dynamics and growth promotion of cotton (*Gossypium hirsutum* L.) and sugarcane (*Saccarm officinarium* L.). *Environ. Exp. Bot*., **57**,168-176. https://doi.org/10.1016/j.envexpbot.2005.05.010

Mahanty, T., Bhattacharjee, S., Goswami, M., Bhattacharyya, P., Das, B., Ghosh, A., Tribedi, P. (2017) Biofertilizers: A potential approach for sustainable agriculture development. *Environ. Sci. Pollut. Res*., **24**, 3315–3335. https://doi.org/10.1007/s11356-016-8104-0

Majda, M., Robert, S. (2018) The Role of Auxin in Cell Wall Expansion. *Int. J. Mol. Sci.,* **19**,951. 21p. DOI:10.3390/ijms19040951

Marques, A. P. G. C., Pires, C., Moreira, H., Rangel, A. O. S. S., Castro, P. M. L. (2010) Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biol. Biochem.*, **42**,1229–1235. https://doi.org/10.1016/j.soilbio.2010.04.014

Mazen, M.M., El-Batanony, N.H., Abd El-Monium, M.M., Massoud, O.N. (2008) Cultural filtrate of *Rhizobium* spp. and arbuscular Mycorrhiza are potential biological control agents against root rot fungal diseases of faba bean. *Global J. Biotechnol. Biochem.*, **3**,32–41.

Munir, S., Li, Y., He, P., Huang, M., He, P., He,P., Cui, W., Wu, Y., He, Y. (2020) Core endophyte communities of different citrus varieties from citrus growing regions in China. *Scientific reports*, **10** (1), 1-12. http://dx.doi.org/10.1038/s41598-020-60350-6

Nahar, N., Begum, A., Akhter, H. (2017) Isolation, identification, and molecular characterization of *Rhizobium* species from *Sesbania bispinosa* cultivated in Bangladesh. *African Journal of Agricultural Research*, **12**(22): 1874-1880. https://doi.org/10.5897/AJAR2017.12321

Nicholson, R. L., Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology*, **30**, 369-389 . https://doi.org/10.1146/annurev.py.30.090192.002101

Ogoshi, A. (1987) Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annual Review of Phytopathology,* **25,** 125-143 . https://doi.org/10.1146/annurev.py.25.090187.001013

Omar, S. (2021) The Importance of Faba Bean (*Vicia faba* L.) Diseases in Egypt. In: Awaad, H.; Abu-hashim, M.; Negm, A., Mitigating Environmental Stresses for Agricultural Sustainability in Egypt. Springer International Publishing, pp.371-388. DOI:10.1007/978-3-030-64323-2_13

Omar, S. (1986) Pathological studies on root rot disease of faba bean (*Vicia faba* L.). FABIS Newsletter, Faba Bean Information Service, ICARDA, No. **14**, 34-37.

Ondřej, M., Dostálová, R., Trojan, R. (2008) Evaluation of virulence of *Fusarium solani* isolates on pea. *Plant Protection Science*, 44(1), 9-18.

Ongley, E.D., Xiaolan, Z., Tao, Y. (2010) Current status of agricultural and rural non-point source Pollution assessment in China. *Environ. Pollut*., **158,** 1159–1168. DOI: 10.1016/j.envpol.2009.10.047

Orf, Heba O. M., Wedad, E. E. Eweda, Sawsan, F. Shehata, Abo Taleb, H. H. (2005) Isolation, purification and identification of some microorganisms produce plant growth-promoting substances (methylotrophic bacteria). *Arab Univ. J. Agric. Sci. Ain Shams, Univ.*, 13 (3),717-729.

Orf, Heba O. M., Wedad, E. E. Eweda, Sawsan, F. Shehata, Abo Taleb, H. H. (2014). comparative studies between nitrogen fixing methylotrophic bacteria and rhizobia of some legume plants. *Minufiya J. Agric. Res.* 39(2),775 – 792.

Ozkoc, I., Deliveli, M.H. (2001) In *vitro* inhibition of the mycelial growth of some root rot fungi by *Rhizobium leguminosarum* bv. *phaseoli* isolates. *Turk. J. Biol.,* **25**,435–445 .

https://journals.tubitak.gov.tr/biology

Papavizas, G.C., Davey, C. B. (1962) Isolation and pathogenicity of Rhizoctonia saprophytically existing in soil. *Phytopathology*, **52**, 834- 840.

Pertot, I., Giovannini, O., Benanchi, M., Caffi, T., Rossi, V., Mugnai, L. (2017). Combining biocontrol agents with different mechanisms of action in a strategy to control *Botrytis cinerea* on grapevine. *Crop Protec.*, **97**, 85-93. https://doi.org/10.1016/j.cropro.2017.01.010

Poorniammal, R., Sundaram, S.P., Kumutha, K. (2009). *In vitro* biocontrol activity of *Methylobacterium extorquens* against fungal pathogens. *Inter. J. of Plant Prot.*, **2**, 59-62. DOI:10.13140/2.1.3086.0163

Radha, T. K., Savalgi, V. P., Alagawadi, A. R. (2009) Effect of methylotrophs on growth and yield of soybean (*Glycine max* (L.) Merrill). *Karnataka J. Agric. Sci*., **22**, 118-121.

Raja, P., Uma, S., Sundaram, S.P. (2006) Non-nodulating pink pigmented facultative *Methylobacterium* sp. with a functional *nifH* gene. *World J. Microbiol. Biotechnol*., **22**, 1381-1384 . https://doi.org/10.1007/s11274-006-9199-0

Ramakrishna, W.,Yadav, R., Li, K. (2019). Plant growth promoting bacteria in agriculture: two sides of a coin. *Appl. Soil Ecol.,* **138**, 10–18. DOI: 10.1016/j. apsoil.2019.02.019

Rayle, D.L., Cleland, R.E. (1992) The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiol*., **99**,1271–1274. DOi: 10.1104/pp.99.4.1271

Roberts, D.P., Lohrke, S.M., Meyer, S.L.F., Buyer, J.S., Bowers, J.H., Baker, C.J., Li, W.; de Souza, J.T., Lewis, J.A., Chung, S. (2005) Biocontrol agents applied individually and in combination for suppression of soil-borne diseases of cucumber. *Crop Protec*., **24,** 141-155 . https://doi.org/10.1016/j.cropro.2004.07.004

Rocha, A. M. C. N., Morais, A. M. (2001) Characterization of polyphenol oxidase (PPO) extracted from 'Jonagored' apple. *Food Control*, **12(**2), 85-90. https://doi.org/10.1016/S0956-7135(00)00026-8

Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, Y. (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant Soil*, **287**: 15–21. DOI:10.1007/978-1-4020-5765-6_2

Rossetto, P.B., Dourado, M.N., Quecine, M.C., Andreote, F.D., Araújo, W.L., Azevedo, J.L., Pizzirani-Kleiner, A.A. (2011) Specific plant induced biofilm formation in *Methylobacterium* species. *Braz. J. Microbiol.*, **42**,878–883. DOI: 10.1590/S1517-83822011000300006

Ryan, R. P., Germaine ,K., Franks, A., Ryan, D. J., Dowling, D. N. (2008) Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters*, **278**,1–9 . https://doi.org/10.1111/j.1574-6968.2007.00918.x

Seema, M., Punith, B. D., Devaki, N. S. (2012) A simple and rapid nuclear staining method for *Rhizoctonia solani* Kuhn. *Biotechnic and Histochemistry*, **87**(3): 169-171. DOI:10.3109/10520295.2011.577097

Siddiqui, I.A., Ehteshamul-Haque, S., Zaki, M.J., Abdul, G. (2000) Effect of urea on the efficacy of *Bradyrhizobium* sp. and *Trichoderma harzianum* in the control of root infecting fungi in mungbean and sunflower. *Sarhad Journal of Agriculture*, **16,**403–406.

Silva, F. de A.S.E. , de Azevedo, C.A.V. (2009) Principal Components Analysis in the Software Assistat-Statistical Attendance. In: World Congress on Computers in Agriculture, 7, RenoNV-USA: American Society of Agricultural and Biological Engineers.

Sneh, B., Burpee, L., Ogoshi, A. (1991) Identification of Rhizoctonia species, 133p. American Phytopathological Society Press, Saint Paul, USA.

Snell, F. D., Snell, C. T. (1953) Colorimetric Methods of Analysis. Third edition, Vol. 3. (Organic I), Nostrand-Reinhold CO. Inc., Princeton, New Jersey, USA, 766 p.

Sutha, R., Ramiah, M. Rajappan, K. (1998) Changes in protein and amino acid composition of tomato due to a tospovirus infection. *Indian Phytopath***.,** **51**, 136-139.

Tamura, K., Stecher, G., Kumar, S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution*, **38**, 3022-3027 . https://doi.org/10.1093/molbev/msab120

Thompson, J. D., Higgins, D. G., Gibson, T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**(22), 4673-4680. DOI: 10.1093/nar/22.22.4673

Van Loon, L. C., Rep, M., Pieterse, C. M. (2006) Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol*., **44**, 135-162. https://doi.org/10.1146/annurev.phyto.44.070505.143425

Vincent, J.M. (1970). A Manual for the Practical Study of Root-Nodule Bacteria. In: International Biological Programme Handbook No. 15, Blackwell Scientific Publications, Oxford.

White, T. J., Brans, T., Lee, S., Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A.G.; D. H. Sninsky; and White, T. J. (Eds.), PCR protocols: A Guide to Methods and Applications. Academic Press, London, U.K., pp. 315-322.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, **18**, 6531-6535.

Yadav, A. N. ,Yadav, N. (2018a). Stress-Adaptive Microbes for Plant Growth Promotion and Alleviation of Drought Stress in Plants. *Acta Scientific Agriculture***, 2**(6), 85-88.

Yadav, N., Yadav, A.N. (2018 b). Biodiversity and biotechnological applications of novel plant growth promoting methylotrophs. *J Appl Biotechnol Bioeng*., **5**,342–344 . https://doi.org/10.15406/jabb.2018. 05.00162

Yin, G., Y. Zhang, Pennerman, K. K., Wu, G., Hua ,S. S. T., Yu, J., Jurick, W. M., Guo, A., Bennett, J. W. (2017) Characterization of blue mold *Penicillium* species isolated from stored fruits using multiple highly conserved loci. *Journal of Fungi*, **3**(1), 1-10. https://doi.org/10.3390/jof3010012

Yoruk, R., Marshall, M. R. (2003). Physicochemical properties and function of plant polyphenol oxidase: a review. *Journal of Food Biochemistry*, **27,** 361-422.https://doi.org/10.1111/j.1745-4514.2003.tb00289.x

Zhang, C., Wang, M.Y., Khan, N., Tan, L.L., Yang, S. (2021) Potentials, Utilization, and Bioengineering of Plant Growth-Promoting *Methylobacterium* for Sustainable Agriculture. *Sustainability*, **13**(7), 3941, 12p . https://doi.org/10.3390/su13073941

البكتيريا مثيلية التغذية وبكتيريا العقد الجذرية كسماد حيوي وعامل مكافحة حيوية لعفن الجذور الرايزوكتوني في الفول البلدي

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يهدف هذا البحث إلى دراسة تأثير الجمع بين البكتيريا مثيلية التغذية إما كمعاملة للبذرة (*Methylobacterium nodulans*) أو معاملة بالرش على المجموع الخضري *Methy-lobacterium mesophilicum)* وبكتيريا العقد الجذرية المتخصصة على الفول البلدي *Rhizobium leguminosarum* biovar *viciae* علي حدوث عفن الجذور الرايزوكتونى تحت ظروف الصوبة والحقل

أدت معاملة (الجمع بين عزلات بكتيريا العقد الجذرية + ثلث التسميد النيتروجيني + معاملة البذرة بالبكتريا *M. nodulans*) إلى انخفاض معنوي في النسبة المئوية لموت البادرات قبل الظهور وبعد الظهور فوق سطح التربة كما سببت في زيادة كبيرة في نسبة النباتات الباقية على قيد الحياة. كما كان لها تأثير تحفيزي معنوي على مكونات المحصول والمحصول النهائي، تلاها في ذلك معاملة (الجمع بين عزلات بكتيريا العقد الجذرية + ثلث التسميد النيتروجيني + معاملة رش المجموع الخضري بالبكتريا *M. mesophilicum*) يليها المعاملة بالمبيد رايزولكس- تي . في الوقت نفسه، أسفرت نفس المعاملات عن زيادة في محتويات النيتروجين والفوسفور والبوتاسيوم. كما أظهرت جميع المعاملات زيادة في نشاط انزيمات البيروكسيديز والبولي فينول اوكسيديز بالإضافة الي زيادة المحتويات الفينولية وكانت أعلي زيادة نتيجة معاملة (الجمع بين عزلات بكتيريا العقد الجذرية + ثلث التسميد النيتروجيني + معاملة البذرة بالبكتريا *M. nodulans*). تنتج جميع السلالات المختبرة من بكتيريا العقد الجذرية والبكتيريا مثيلية التغذية اندول حمض الخليك، والهيدروسيانيد، والامونيا كما إنها مقاومة للمضادات الحيوية الأمبيسيلين والكوليستين وحساسة للمضاد الحيوي التتراسيكلين. في النهاية، أشارت النتائج إلى أن الجمع بين بكتيريا العقد الجذرية والبكتيريا مثيلية التغذية بالإضافة إلى ثلث جرعة التسميد النيتروجيني يقلل من حدوث عفن الجذور الرايزوكتونى كما يؤدى إلى زيادة ملحوظة في محصول الفول البلدي.