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The Inhibitory Effects of Cultural Filtrates of Some Wild *Rhizobium* Spp. On Some Faba Bean Root Rot Pathogens and Their Antimicrobial Synergetic Effect When Combined with *Arbusclar mycorrhiza* (Am)

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Abstract: The antimicrobial activity of cultural filtrate of three wild rhizobial isolates isolated from nodules of wild legumes in Egypt and *Rhizobium leguminosarum* ICARDA 441 were evaluated on the three pathogenic fungi *Rhizoctonia solani, Fusarium oxysporum* and *F. solani* of faba bean. Their potential synergetic activity with *Arbuscular mycorrhiza* (AM) fungi in the biocontrol of these pathogens was investigated. In a laboratory and greenhouse study, different concentrations of cultural filtrates of all the rhizobial isolates reduced the growth and development of the tested pathogens. The inhibitory effect of the rhizobia increased with increasing the concentration of their cultural filtrates (100% concentration). Cultural filtrate treatments of R_2 and R_1 showed higher inhibition (76.33, 56.11 and 51.11%) and (65.22, 52.44 and 65.78%) to *R. solani F. solani, F. oxysporum* respectively. Such treatments caused higher reduction in spore germination % of *F. solani*; *F. oxysporum* and sclerotia formation of *R. solani.* All treatments of combined rhizobial filtrates and AM inhibited the pathogencity of all tested fungi which were manifested by the increase in plant vegetative growth parameters compared with infested controls. The most effective treatment against *R. solani,* was R_1 + AM. *F. solani* was the most sensitive fungus, judged by the lowest percentage of damping-off and the highest percentage of healthy survival of plants, followed by *F. oxysporum* and *R. solani.* All the tested treatments resulted in a significant increase in nitrogenase activity, % of AM infection in roots and NPK contents of faba bean shoot dry matter. It was concluded that the wild rhizobial cultural filtrates and/or AM plants had a significant antagonistic effect against soil borne pathogenic fungi and therefore enhance the plant resistance to diseases.

Key words: Biological control · *Rhizobium* · *Arbuscular mycorrhiza* (AM) · root rots fungi

INTRODUCTION

Faba bean (*Vicia faba*) is used as an important human food in developing countries and as an animal feed, mainly for pigs, horses, poultry and pigeons in industrialized countries. Feeding value of faba bean is high and this legume has been considered as a meat extender or substitute due to its high protein content (20-41%) [1]. In the soil ecosystem, pathogenic and non-pathogenic microorganisms are in competition with each other [2]. Every soil has an antagonistic potential against specific pathogens to prevent or reduce the spread of a pathogen, parasite or deleterious agent [3].

Seed rot, seedling damping-off and root rot diseases of faba bean are considered as limiting factors affecting plant growth and yield [4, 5]. Different disease control methods have been adapted to reduce the impact of the disease on the plant mortality including breeding for disease resistance [6], seed dressing fungicide [7] and biological control [8].

However, more attention has been given to Plant Growth-promoting Rhizobacteria (PGPR), as the most important alternative to chemicals, to help eco-friendly biological control of soil-borne pathogens [9].

The potential of plant growth promoting rhizobacteria to protect plant roots from soil-borne pathogens was demonstrated in several research works [10, 11]. They found that inoculation of faba bean with (PGPR) strains significantly reduced root rot disease caused by *F. oxysporum* and also enhanced nodulation

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status in the roots as well as increasing plant growth. Microorganisms that can grow in the rhizosphere are ideal to be used as biocontrol agents since the rhizosphere provides front line defense for roots against attack by pathogens [12].

Obviously, rhizobia are known to increase nodulation and nodule weight in legumes along with increase in host plant growth and development [13]. In addition, the rhizobia were reported as effective biocontrol agents for the inhibition of certain soil-borne plant pathogens [14] due to production of diverse microbial metabolites like siderophore [9], rhizobitoxin, plant growth enhancement through IAA production, increase of phosphorus uptake and other minerals [11]. Additionally production of plant growth regulators such as auxins, cytokinins and gibberallins like substances by rhizobia that stimulate and enhance plant growth, were also shown [15].

A few strains of rhizobia were reported to inhibit sclerotia germination of *Sclerotium rolfsii* and colony growth of *Phytophthora megasperma* [13]. *Rhizobium meliloti* and *Bradyrhizobium japonicum* bacterized seeds are known to reduce *Macrophomina phaseolina* infection [13].

Arbuscular mycorrhizal (AM) fungi are ubiquitous in nature and constitute an integral component of terrestrial ecosystems, forming symbiotic associations with plant root systems of over 80% of all terrestrial plant species, including many agronomically important species. AM fungi are particularly important in organic and/or sustainable farming systems that rely on biological processes rather than agrochemicals to control plant pathogens. Of particular importance is the bioprotection conferred to plants against many soil-borne pathogens such as species of *Aphanomyces, Cylindrocladium, Fusarium, Macrophomina, Phytophthora, Pythium, Rhizoctonia, Sclerotinium, Verticillium* and *Thielaviopsis* and various nematodes by AM fungal colonization of the plant root [16]. AM fungi are known to enhance plant uptake of phosphorus (P) and other mineral nutrients [17]. This enhanced plant development may lead to disease escape or to higher tolerance against soil-borne pathogens [18]. The nutritional superiority of more vigorous AM plants has been proposed to be a mechanism in reduction of root diseases [17].

The present study aims to investigate the antagonistic activity of cultural filtrate of some wild rhizobial isolates against some root rot pathogens of faba bean. (*R. solani, F. oxysporum* and *F. solani*) *in vitro* and their role with AM fungi in the biocontrol of these pathogens under greenhouse conditions.

MATERIALS AND METHODS

Rhizobial strains, *Arbusclar mycorrhiza* (AM), Pathogenic fungi and Faba bean seeds: Three isolates of *Rhizobium* spp. were used in this study, isolated from root nodules of some wild leguminous plant species, grown in different phytogeographical regions of Egypt [19]. The phytogeographical distribution, life forms, occurrence and collection sites (soil type and habitat) of these wild leguminous species were previously described by Tantawy *et al.* [19]. The three rhizobial isolates were symbolized M.L from nodules of wild legume *Medicago laciniata* (L.) Mill *v.bradycantha* Boiss, $L.C_4$ from nodules of wild legume *Lotus corniculatus* L. and $T.S_1$ from nodules of wild legume *Trigonella stellata* Forssk. Details of morphological and physiological characterization of these wild leguminous species are described in Abdel-Wahab *et al.* [20].

The wild rhizobial isolates used were obtained from the culture collection that previously isolated and provided by Dr. El-Batanony (Environmental Studies and Research Institute (ESRI), Sadat Branch, Menoufiya University, Egypt). Strains of *R. leguminosarum* ICARDA 441 were also used in this study and provided by Biofertilizers Production Unit, Soil, Water and Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt. Pure cultures were routinely maintained on Yeast Extract Mannitol (YEMA) agar plates [21] at 4°C and in YEM broth containing 20% (v/v) glycerol at -80°C.

AM fungi obtained by Dr.Massoud, Soil, Water and Environment Research Institute, Agric. Res. Center (ARC). Giza, Egypt. They include the following genera: *Glomus, Gigaspora* and *Acaulospora.* The AM inoculum was prepared according to Massoud [22].

Pathogenic fungi *R. solani, F. solani* and *F. oxysporum* were kindly provided by plant pathology Research Institute, Agric. Res. Center (ARC), Giza, Egypt. Pure cultures were maintained on Potato dextrose agar (PDA) plates at 4°C.

Faba bean (*Vicia faba* L.) seeds variety (Giza 3) were provided by Unit, Field Crops Research Institute, Agric. Res. Center, Giza, Egypt.

Cultural filtrates of rhizobial strains: The three wild rhizobial isolates and *R. leguminosarum* ICARDA 441 strain were grown in yeast extract-mannitol broth medium [21], in 250 mL Erlenmeyer flasks. They were grown in shaking incubator (200 rpm) for five days at 28-30°C. The cells were then harvested by centrifugation

at 6000 rpm for 20 Min. and the supernatants were filter sterilized through 0.45µm bacterial filter. Three replicates of 250 mL Erlenmeyer flasks prepared for each rhizobial strain.

Screening the antimicrobial activity of the rhizobial isolates: The sterilized culture filtrates of the wild rhizobial isolates and *R. leguminosarum* ICARDA 441 strain were tested for its antimicrobial activity against the root rot pathogens (*R. solani, F. solani* and *F. oxysporum*) of faba bean. This test was conducted as described by El-Abyad *et al.* [23] for studying the antifungal assay, the antifungal potency of the rhizobia was examined and the most potent ones were selected.

Effect of the rhizobial cultural filtrates on growth activities of *R. solani, F. solani* and *F. oxysporum:* Radial growth of the pathogenic fungi was assessed on PDA agar medium in triplicate Petri dishes amended with different concentrations of 25, 50, 75 and 100% (v/v) of sterilized cultural filtrates of the rhizobial isolates. The control and treated plates were then examined and the radial growth was determined. The inhibition% in mycelial growth of the three pathogenic fungi was determined according to the following equation:

$$\text{Reduction\%} = \text{Control-treatment/Control} \times 100.$$

Germination of macroconidia of *F. solani* and *F. oxysporium* were studied using microscopic slides, each covered with 0.5µL of spore suspension (10^6/mL) in different concentrations of sterilized cultural filtrates of the rhizobia or YEM broth as a control in Petri dishes that served as moist chambers. The plates were incubated at 25°C for 24 H after that the percent germination were assessed using the methods described by El-Abyad *et al.* [24]. Sclerotia formation of *R. solani* were counted after 10 D of incubation at 25°C on PDA agar plates amended with different concentrations of antagonistic sterilized cultural filtrate of rhizobia.

Effect of the rhizobial cultural filtrate and *Arbusclar mycorrhizal* (AM) on fungal pathogenicity: Pot experiment was conducted in a greenhouse and healthy seeds of faba bean variety (Giza 3) with homogenous size were chosen for the experiment.

Preparation of Arbusclar mycorrhizal (AM) inoculum: Mixed spores of AM fungi from genera: *Glomus, Gigaspora* and *Acaulospora* were prepared after propagation and mixed with sand as a carrier (200 spore g^{-1}) and then added to the soil at the rate of 10 g pot^{-1}.

Preparation of pathogenic fungal inoculum (*R. solani, F. oxysporum* and *F. solani*): The fungi were grown on autoclaved sorghum sand medium for 15 D at 25°C. They were inoculated into the soil before sowing (5 D) at the rate of 3 g kg^{-1} soil [25].

The soil used: was a clay loamy (pH 7.8) and its chemical properties are: organic carbon%: 1.21, total nitrogen: 0.12, EC ds/m: 2.2. Anions and cations (meq L^{-1}): CO_3^-: trace, HCO_3^-: 3.82, Cl^-:5.94, SO_4^{2-}: 12.26, Mg^{2+}: 3.88, Na^+: 12.0, K^+: 0.84 and Ca^{2+}: 5.9. Soil physical and chemical properties were analyzed as described by Jackson [26].

Ten treatments in pots were conducted for each pathogen of the faba bean root rot fungi (*R. solani, F. oxysporum* and *F. solani*). The treatments were R_1: Culture filtrate of (M.L), R_2: Culture filtrate of ($L.C_4$), R_3: Culture filtrate of ($T.S_1$), R_4: Culture filtrate of (*R. leguminosarum* ICARDA 441 strain), AM: Mixed spores of *mycorrhizal* genera as a single treatment. The other four treatments consisted of AM and an individual cultural filtrate of the selected wild rhizobial isolates (M.L, $L.C_4$ and $T.S_1$); and *R. leguminosarum* ICARDA 441 strain as follow: R_1 + AM, R_2 + AM, R_3 + AM and R_4 + AM. *Vicia faba* seeds were soaked in these cultural filtrates overnight prior sowing. AM fungal inocula were mixed with soil. An infested control treatment was inoculated with only the pathogenic fungi.

The experiment was conducted in plastic pots (300 mm diameter x 250 mm). Each contains 10Kg of soil. The pots were sterilized with 5% formalin solution and left to dry before use. The pots were watered before planting to enhance the fungal growth. Five seeds were sown in each pot, 5mm below the soil surface and five pots were used for each treatment. The pots were kept in the greenhouse (22±3°C), watered and fertilized weekly with Hoagland solution [27]. The cultivation period was extended up to 60 D.

Chemical and microbiological analysis of plants: Sixty day old plants were harvested to determine the shoot height (cm), nodules number and shoots dry weight (g $plant^{-1}$) [28]. The percentage of AM fungi infection in plant root tissues were also determined [29]. Nitrogenase activity was measured as acetylene reduction activity (ARA) by GC analysis using a 5880 HP chromatograph (Hewlett Packard Inc Palo Alto, CA, USA) with an

ionization flame detector at 135°C according to Somasegaran and Hoben, [28]. Total nitrogen, phosphorous and potassium% (NPK) in the shoot samples were determined according to Jackson [26]. The data were subjected to an ANOVA protected Least Significant Difference (LSD) test [30].

RESULTS

Effect of the rhizobial cultural filtrates on fungal growth: The antifungal potency of the three wild rhizobial isolates R_1, R_2 and R_3 displayed high reduction % on the radial growth of *R. solani F. solani,F. oxysporum*. Results in Table (1) showed the effect of different concentrations of cultural filtrates of the tested wild rhizobial isolates as well as *R. leguminosarum* ICARDA 441strain on the radial growth of *R. solani F. solani, F. oxysporum*. It is obvious that, all the rhizobial treatments showed a remarkable reduction on radial growth of the fungi being tested compared with the control. Significant variations among the effect of rhizobial treatments on fungal growth were noticed. Treatments R_2 and R_1 showed the highest effect (76.33, 56.11 and 51.11%) and (65.22, 52.44 and 65.78% reduction), on growth of *R. solani, F. solani, F. oxysporum*, respectively at 100% concentration. Table 2 showed that all the rhizobial filtrate treatments, significantly inhibited spores germination of *F. solani, F. oxysporum* and sclerotia formation of *R. solani* compared with the control. The inhibitory effect increased with increasing the concentration of culture filtrates. Treatments R_1 and R_2 were the most effective ones. They recorded the highest reduction% in spore germination at 100% concentration (84.69 and 93.54% reduction of *F. solani;* 89.70 and 90.58% reduction of *F. oxysporum*, respectively*)*. Concerning sclerotial formation of *R. solani* treatments R_1 and R_2 were the most effective ones. They recorded the highest reduction in sclerotial formeation 100 and 93.33%, respectively, at 100% concentration.

Table 1: Effect of different concentrations of cultural filtrates of wild rhizobial isolates and *Rhizobium leguminosarum* ICARDA 441 on radial growth of *Rhizoctonia solani, Fusarium solani* and *Fusarium oxysporum*

		Rhizoctonia solani		*F. solani*		*F.Oxysporum*	
Treatments		----------		----------		----------	
Conc. (v/v)		R.g (mm)	Reduction (%)	R.g (mm)	Reduction (%)	R.g (mm)	Reduction (%)
R_1	25	5.48	39.11	6.35	29.44	4.83	46.33
	50	4.68	48.00	5.75	36.11	4.43	50.78
	75	4.10	54.44	5.15	42.78	3.53	60.78
	100	3.13	65.22	4.28	52.44	3.08	65.78
R_2	25	4.55	49.44	6.03	33.00	6.05	32.78
	50	4.00	55.56	5.50	38.89	5.68	36.89
	75	3.40	62.22	4.78	46.89	5.08	43.56
	100	2.13	76.33	3.95	56.11	4.40	51.11
R_3	25	6.75	25.00	6.98	22.44	8.45	6.11
	50	6.30	30.00	6.63	26.33	8.28	8.00
	75	5.68	36.89	6.35	29.44	7.78	13.56
	100	4.45	50.65	5.60	37.78	7.40	17.78
R_4	25	9.00	0.00	8.10	10.00	8.65	3.89
	50	9.00	0.00	7.45	17.22	8.58	4.67
	75	8.10	10.0	7.08	21.33	8.28	8.00
	100	7.40	17.78	6.28	30.22	8.05	10.56
Control		9.0	0.0	9.0	0.0	9.0	0.0
$L.S.D_{0.05}$			*R.solani*		*F.solani*		*F.oxysporum*
Treatments			0.1175		0.1093		0.1116
Concentrations			0.1051		0.0977		0.0998
Treatments x concentrations			0.2351		0.2185		0.2232

Treatments (Rhizobial isolate and strain): R_1: M.L, R_2: $L.C_4$, R_3: $T.S_1$, R_4: *Rhizobium leguminosarum ICARDA 441*. R. g: Radial growth (mm). Conc.: Concentrations of cultural filtrates of rhizobial isolates

Table 2: Effect of cultural filtrates of different concentrations of wild rhizobial isolates and *Rhizobium leguminosarum* ICARDA 441 on spore germination of *Fusarium solan, Fusarium oxysporum* and sclerotia formation of *Rhizoctonia solan*

Treatment		*R. solani*		*F. solani*		*F. oxysporum*	
Conc. (v/v)		Slerotia formation	Reduction (%)	Spore germination	Reduction (%)	Spore germination	Reduction (%)
R_1	25	4.30	91.31	53.50	39.34	15.20	83.35
	50	1.80	96.36	28.20	68.03	12.80	85.98
	75	0.00	100.00	23.90	72.90	11.30	87.62
	100	0.00	100.00	13.50	84.69	9.40	89.70
R_2	25	11.50	76.77	38.50	56.35	24.40	73.27
	50	8.80	82.22	21.77	75.32	17.10	81.27
	75	6.50	86.87	12.30	86.05	15.10	83.46
	100	3.30	93.33	5.70	93.54	8.60	90.58
R_3	25	17.00	65.66	33.80	61.68	31.00	66.05
	50	15.30	69.09	18.70	78.80	30.40	66.70
	75	14.00	71.72	17.80	79.82	23.90	73.82
	100	13.50	72.73	12.90	85.37	21.30	76.67
R_4	25	22.30	54.95	59.90	32.09	81.40	10.84
	50	18.00	63.64	30.00	65.99	64.20	29.68
	75	17.50	64.65	28.00	68.25	39.70	56.52
	100	17.00	65.66	25.80	70.75	24.70	72.95
Control		49.50	0.0	88.20	0.0	91.30	0.0

$L.S.D_{0.05}$	*R.solani*	*F. solani*	*F.oxysporum*
Treatment	2.8798	3.7534	5.8569
Concentration	2.0363	2.6541	2.7273
Treatment x concentration	5.7452	7.4880	7.6945

Rhizobial isolate and strain: R_1: M.L, R_2: $L.C_4$, R_3: $T.S_1$, R_4: *Rhizobium leguminosarum* ICARDA 441. Conc.: concentrations of cultural filtrates of rhizobial isolates

Table 3: Effect of interaction of wild *Rhizobium* isolates and *Rhizobium leguminosarum* ICARDA 441 and *Rhizobium Leguminosarum Arbusclar mycorrhizas* on *Vicia faba* root rot diseases after 60 D of sowing under green house conditions

	Rhizoctonia solani			*Fusarium solani*			*Fusarium oxysporum*		
		Survival (%)			Survival (%)			Survival (%)	
Treatment	Damping off (%)	Infected	Healthy	Damping off (%)	Infected	Healthy	Damping off (%)	Infected	Healthy
R1	25^{b}	0^{b}	75^{bcd}	10^{bc}	5^{ab}	85^{ab}	20^{bc}	10^{ab}	70^{bc}
R2	25^{b}	5^{ab}	70^{bcd}	15^{ab}	10^{ab}	75^{bc}	25^{bc}	5^{ab}	70^{bc}
R3	30^{b}	5^{ab}	65^{cd}	10^{bc}	10^{ab}	80^{abc}	20^{bc}	15^{ab}	65^{bc}
R4	30^{b}	10^{ab}	60^{d}	15^{ab}	15^{a}	70^{bc}	25^{bc}	15^{ab}	60^{cd}
AM	30^{b}	5^{ab}	65^{cd}	15^{ab}	10^{ab}	75^{bc}	30^{ab}	10^{ab}	60^{cd}
R1 + AM	15^{cb}	0^{b}	85^{ab}	10^{bc}	0^{b}	90^{ab}	15^{c}	5^{ab}	80^{abc}
R2 + AM	20^{b}	0^{b}	80^{bc}	5^{bc}	5^{ab}	90^{ab}	15^{c}	0^{b}	85^{ab}
R3 + AM	25^{b}	5^{ab}	70^{bcd}	10^{bc}	5^{ab}	85^{ab}	25^{bc}	5^{ab}	70^{bc}
R4 + AM	20^{b}	5^{ab}	75^{bcd}	15^{ab}	5^{ab}	80^{abc}	25^{bc}	10^{ab}	65^{bc}
Control	50^{a}	15^{a}	35^{e}	25^{a}	15^{a}	60^{c}	40^{a}	20^{a}	40^{d}
$L.S.D._{0.05}$	16.612	11.746	19.072	14.603	13.717	21.25	13.717	16.422	20.802

Treatments (Rhizobial isolate and strain): R_1: M.L, R_2: $L.C_4$, R_3: $T.S_1$, R_4: *Rhizobium leguminosarum* ICARDA 441; AM: *Arbusclar mycorrhiza*. Means with the same letter are not significantly different

Effect of the rhizobial cultural filtrates and *Arbusclar mycorrhiza* (AM) on fungal pathogenicity: The treatments of interaction between rhizobia and AM on faba bean plants infested with root rot disease under greenhouse conditions is listed in Table (3).

Combined rhizobial filtrates and AM fungi caused a significant reduction in damping-off and root rot disease compared with control. Moreover, the most effective treatment on *R. solani,* was R_1 + AM, nevertheless, the lowest effective treatments were R_3, R_4 and AM, other treatments ranked in between. However, the treatments R_1, R_3, R_1+AM, R_2 +AM and R_3+ AM significantly affected *F. solani* pathogenicity. All treatments were significantly effective in increasing faba bean emergence except treatment AM in *F. oxysporum* compared with control. On the other hand, *F. solani* was the most sensitive fungus judged by the lowest percentage of damping-off and the highest percentage of healthy survival plants, followed by *F. oxysporum* and *R. solani* respectively.

Table 4 showed the vegetative growth parameters [shoot height (cm), nodule numbers and shoot dry

Table 4: Shoots height (cm), nodules number and shoots dry weigh (gm) of *Vicia faba* plants treated with cultural filtrates of wild rhizobial isolates, *Rhizobium leguminosarum* ICARDA 441, inoculated with *Arbusclar mycorrhiza* and infected with some root rot fungi after 60 d of sowing

	Rhizoctonia solani			*Fusarium solani*			*Fusarium oxysporum*		
Treatments	Sh.h. Pl^{-1} (cm)	Nod. No.pl^{-}	Sh.D.wt. (gm pl^{-1})	Sh.h. Pl^{-1} (cm)	Nod. No.pl^{-1}	Sh.D.wt. (gm pl^{-1})	Sh.h. Pl^{-1} (cm)	Nod. No. pl^{-1}	Sh.D.wt. (gm pl^{-1})
R_1	20.0^{e}	65^{bc}	2.93^{bc}	26.7^{d}	115^{d}	3.19^{d}	22.7^{d}	95^{e}	3.80^{de}
R_2	20.7^{b}	80^{a}	3.11^{bc}	28.7^{c}	139^{b}	4.42^{b}	28.5^{b}	120^{bc}	3.92^{d}
R_3	18.0^{e}	60^{cd}	2.88^{c}	25.7^{e}	97^{e}	3.19^{d}	20.9^{f}	91^{e}	3.76^{de}
R_4	17.3^{f}	61^{cd}	2.52^{d}	25.0^{e}	100^{e}	3.18^{d}	20.7^{f}	70^{e}	3.74^{e}
AM	15.0^{g}	55^{d}	2.15^{e}	22.7^{f}	88^{f}	2.88^{e}	17.3^{g}	50^{f}	2.98^{f}
R_1 + AM	20.7^{b}	71^{b}	3.17^{b}	31.7^{b}	130^{c}	4.06^{c}	24.2^{c}	126^{ab}	4.69^{b}
R_2 + AM	21.8^{a}	83^{a}	3.99^{a}	35.3^{a}	150^{a}	5.05^{a}	30.9^{a}	134^{a}	4.99^{a}
R_3 + AM	18.9^{d}	62^{c}	3.06^{bc}	27.7^{c}	125^{c}	4.03^{c}	22.5^{d}	116^{cd}	4.31^{c}
R_4 + AM	19.3^{d}	60^{cd}	2.84^{c}	27.3^{d}	115^{d}	4.02^{c}	21.9^{e}	110^{d}	4.19^{c}
Control	13.0^{h}	15^{f}	0.072^{f}	16.0^{h}	15^{h}	2.14^{f}	15.7^{h}	17^{g}	1.68^{h}
LSD 0.05	0.5283	6.1053	0.117	0.9652	7.5209	0.133	0.4801	8.9018	0.1521

Treatments (Rhizobial isolate and strain): R_1: M.L, R_2: L.C_4, R_3: T.S_1, R_4: *Rhizobium leguminosarum* ICARDA 441; AM *Arbusclar mycorrhiza.* Sh.h: Shoot height. Nod. No: Nodules number. Sh.D.Wt: Shoot dry weight. Pl. plant. Means with the same letter are not significantly different

Table 5: Nitrogenase activity (µ Mole C_2H_4 h^{-1} gm^{-1} D.Nod.) and *Arbusclar mycorrhiza* infection % of *Vicia faba* plants treated with cultural filtrates of wild rhizobial isolates and *Rhizobium leguminosarum* ICARDA 44, inoculated with *Aarbusclar mycorrhiza* and infected with root rot fungi after 60 D of sowing

	Rhizoctonia solani		*Fusarium solani*		*Fusarium oxysporum*	
Treatments	Nase activity (µ Mole C_2H_4 h^{-1} gm^{-1} D.Nod.)	% of AM infection	Nase activity (µ Mole C_2H_4 h^{-1} gm^{-1} D.Nod.)	% of AM infection	Nase activity (µ Mole C_2H_4 h^{-1} gm^{-1} D.Nod.)	% of AM infection
R_1	12.472^{c}	60^{bc}	20.182^{e}	60^{c}	22.794^{c}	60^{cd}
R_2	13.712^{c}	65^{ab}	22.061^{d}	80^{b}	24.182^{b}	65^{bc}
R_3	11.556^{f}	55^{cd}	18.459^{f}	50^{cd}	17.015^{f}	55^{de}
R_4	9.884^{g}	50^{d}	18.009^{g}	55^{c}	10.953^{g}	50^{e}
AM	8.117^{h}	40e	8.202^{h}	40^{de}	7.636^{h}	40^{f}
R_1 + AM	15.182^{b}	65^{ab}	23.726^{b}	90^{ab}	24.182^{b}	70^{b}
R_2 + AM	17.061^{a}	70^{a}	27.13^{a}	95^{a}	27.041^{a}	85^{a}
R_3 + AM	12.159^{e}	60^{bc}	22.645^{c}	85^{ab}	22.097^{d}	65^{bc}
R_4 + AM	12.019^{e}	60^{bc}	22.815^{c}	85^{ab}	21.991^{e}	60^{cd}
Control	0.65^{d}	15^{g}	0.960^{j}	25^{f}	1.10^{i}	20^{h}
L.S.D. 0.05	0.1533	7.6583	0.1766	12.506	0.0747	6.754

Treatments: (Rhizobial isolate and strain): R_1: M.L, R_2: L.C_4, R_3: T.S_1, R_4: *Rhizobium leguminosarum* ICARDA 441, AM: *Arbusclar mycorrhiza.* Nase: nitrogenase. D. Nod.: Dry nodule. Means with the same letter are not significantly different.

Table 6: NPK % of *Vicia faba* plants treated with cultural filtrates of wild rhizobial isolates, *Rhizobium leguminosarum* ICARDA 44, inoculated with *Arbusclar mycorrhiza* and infected with root rot fungi after 60 D of sowing

	NPK shoots (%)								
	Rhizoctonia solani			*Fusarium solani*			*Fusarium oxysporum*		
Treatments	N	P	K	N	P	K	N	P	K
R_1	0.61^{d}	0.3^{c}	0.201^{bc}	0.88^{de}	0.510^{b}	0.28^{e}	0. 85^{c}	0.411^{e}	0.26^{cd}
R_2	0.66^{c}	0.33^{b}	0.210^{b}	0.98^{c}	0.551^{a}	0.30^{d}	0.9^{b}	0.458^{d}	0.28^{bc}
R_3	0.59^{de}	0.29^{cd}	0.199^{bc}	0.81^{e}	0.509^{b}	0.25^{g}	0.76^{e}	0.410^{e}	0.25^{d}
R_4	0.56^{e}	0.28^{d}	0.165^{e}	0.85^{e}	0.47^{c}	0.26^{f}	0.80^{de}	0.40^{e}	0.25d
AM	0.51^{f}	0.28^{d}	0.173^{de}	0.61^{f}	0.25^{d}	0.20^{h}	0.58^{f}	018^{f}	0.19^{e}
R_1 + AM	0.72^{ab}	0.32^{b}	0.215^{ab}	1.09^{b}	0.55^{a}	0.33^{b}	0.90^{b}	0.517^{b}	0.327^{a}
R_2 + AM	0.75^{a}	0.35^{a}	0.23^{a}	1.2^{a}	0.58^{a}	0.35^{a}	1.07^{a}	0.552^{a}	0.341^{a}
R_3 + AM	0.70^{bc}	0.3^{c}	0.2^{bc}	0.95^{cd}	0.50^{bc}	0.30^{d}	0.84^{cd}	0.499^{c}	0.298^{b}
R_4 + AM	0.69^{bc}	0.29^{cd}	0.185^{cd}	1.00^{c}	0.51^{b}	0.31^{c}	0.82^{cd}	0.496^{c}	0.292^{b}
Control	0.43^{g}	0.035^{f}	0.11^{f}	0.43^{g}	0.100^{f}	0.15^{i}	0.40^{h}	0.134^{h}	0.14^{f}
L.S.D. 0.05	0.0481	0.0168	0.0163	0.0755	0.0305	0.0099	0.0422	0.0144	0.0235

Treatments: (Rhizobial isolate and strain): R_1: M.L, R_2: L.C_4, R_3: T.S_1, R_4: *Rhizobium leguminosarum* ICARDA 441

AM: *Arbusclar mycorrhiza*, NPK: nitrogen, phosphorous and potassium. N: Nitrogen, P: Phosphorous, K: Potassium

Means with the same letter are not significantly different

weight] of plants infested with *R. solani, F. solani* and *F. oxysporum*. The data indicate that all the treatments significantly inhibited all the tested pathogens manifested by increasing in the vegetative growth parameters as compared with the control treatment. The inhibition rate displayed differences according to different fungi. The highest values were recorded in treatment R_2, (shoot height: 28.7 and 28.5 cm) with *F. solani* and *F. oxysporum*, respectively. Consequently, the most effective single treatments were: R_1, R_2 and interacted ones were: R_1+AM and R_2+AM. They resulted the highest dry matter (G) / plant (2.93, 3.11, 3.17 and 3.99), (3.19, 4.42, 4.06 and5.05) and (3.8 0, 3.92, 4.69 and 4.99) for *R. solani, F. solani* and *F. oxysporum,* respectively. Considering nodules number, the same trend as dry matter was obtained. Single R_1, R_2 and combined R_1+AM and R_2+AM treatments gave the highest nodules number (65, 80, 71 and 83), (115, 139, 130 and 150) and (95, 120, 126 and 134) with *R. solani, F. solani* and *F. oxysporum,* respectively. Moreover, results in Table 4 showed that, mycorrhizal treatment (AM), when applied singly, had a little effect. Where as it give the best effect against the tested pathogens when combined with different rhizobial treatments especially against *F. solani.*

Data in Table (5) showed the nitrogenase (Nase) activity (μmole C_2H_4 h^{-1} g^{-1} dry nodule) as well as AM fungal infection% in roots of faba bean plants, infested with the pathogens in the present of the tested rhizobia and AM. It is obvious that, all the treatments resulted in high significant increase in Nase activity comparing with the control. Moreover, rhizobial treatments R_1 and R_2 as well as combined treatments R_1 + AM and R_2 + AM showed heighest values (12.472, 13.712, 15.182 and 17.061), (20.182, 22.061, 23.726 and 27.13) and (22.794, 24.182, 24.182 and 27.041) with *R. solani, F. solani* and *F. oxysporum*, respectively. Table (5) also revealed that AM fungal infection % increased up to the range of 60-95% with the pathogens.

The percentage of NPK analysis as affected by the treatments in accordance with the three pathogens tested in Table 6 cleared that all the treatments showed significant increase in NPK percentage. However, rhizobial treatments R_1 and R_2 as well as combined treatments R_1 + AM and R_2 + AM are the most effective treatments especially with *F. solani* (0.88, 0.98, 1.09 and 1.20), (0.510, 0.551, 0.55 and 0.58) and (0.28, 0.30, 0.33 and 0.35) NPK percentage, respectively.

Generally it was noticed that all the used treatments significantly increased the value of all parameters tested in this study. Moreover, *F. solani* was obviously the most sensitive fungus followed by *F. oxysporum* and *R. solani* in particular to the rhizobial treatments R_1 and R_2 as well as combined treatments R_1 + AM and R_2 + AM.

DISCUSSION

The large use of chemicals in order to control plant diseases has disturbed the delicate ecological harmony of the soil, leading to groundwater contamination, development of resistant races of pathogens and health risks to humans [9]. The present work was conducted under laboratory and greenhouse conditions and revealed that the cultural filtrates of three wild rhizobial isolates (M.L, L.C_4 and T.S_1) and *R. leguminosarum* ICARDA 441 significantly reduced growth and development of *F. solani, F. oxysporum* spore germination and *R. solani* sclerotia formation; the causal fungi of root rot diseases of faba bean plants. This result agreed with different researcher [31], who found that most of *R. leguminosarum biovar phaseoli* isolates inhibited the fungal development (*F. oxysporum, Pythium ultimum* and *R. solani*).

Moreover increased concentration of rhizobial cultural filtrates and /or combined rhizobia with AM fungi gave a remarkable reduction in percentage of seedlings and root rot diseases. These findings are in agreement with those founded by others [11], they showed that *R. leguminosarum* and *B. japonicum* as a PGPR controlled faba bean root disease caused by *F.oxysporum* and promoted plant growth root nodulation, N and P uptake of faba bean shoots. Also it is proved that some *R. leguminosarum bv. viceae* strains, in addition to their use as biofertilizer, have the potential to suppress *Pythium* damping-off and increase the emergence of field pea and sugar beet compared with the untreated control [32]. Many workers also observed that inoculation with bacterial mixtures of nitrogen fixing and phosphate-solubilizing bacteria provided more balanced nutrition for the plants [33]. Hence multiple strains inocula seem to have great potential in plant growth promotion and biological control.

Consequently, the treatments of the cultural filtrates of the wild rhizobial isolates and *R. leguminosarum* ICARDA 441; or combined rhizobia with AM fungi enhanced the vegetative growth and nutrient uptake of the faba bean through the reduction of root rot diseases severity caused by *R. solani, F. solani* and *F. oxysporum.* These results seem to be parallel to those obtained by many workers who found that inoculation of seedlings of *Trifolium subterraneum* L. With *Rhizobium trifolii* reduced the severity of *Phytophthora clandestina* root disease by 14-58% with corresponding increases in dry matter production of 20-73%. They also found that 29 *Rhizobium* isolates caused significant reductions in the hyphal growth of the three *P. clandestina* isolates examined [34]. In addition, AM fungi had a pronounced role in controlling plant pathogens as revealed by many investigators [35]. They showed that the addition of *mycorrhiza* improved the resistance of *Gladiolus grandiflorus* to *F. oxysporum* f. sp. *Gladioli* root rot disease. Furthermore, the effect of mycorrhizal fungi on plant growth and fruit production of tomato inoculated with *F. oxysporum* f. sp. *radicis-lycopersici* under greenhouse conditions were studied and results showed that mycorrhizal inoculation significantly, increased plant growth and fruit production of tomato [36].

One of the most important findings in this paper is that the cultural filtrates of some wild rhizobial isolates, especially L.C_4(R_2) and M.L (R_1) either singly or combined with AM had antifungal potency higher than the strain *R. leguminosarum* ICARDA 441. This concept agreed with other researchers who proved that culture filtrate of *R. leguminosarum* and heat-killed bacterial cells protected lentil plants against infection with the pathogen *F. oxysporum* MR 84 to a high degree [37].

The mechanism of PGPR and/or AM fungi in controlling root diseases and enhancement of plant growth has not been investigated in the present work it will be carried out in the future. Nevertheless, the mechanism that could be accounted for disease control ability of PGPR include competition of infection site, production of (or) defense mechanism, improvement of host nutrition siderophore production [9], production of phytohormons [38] or through the induction of systemic host resistance or immunity [39].

The role of *Rhizobium* in biological control may be due to influence of antifungal compounds of rhizobia on the fungal colonies [15]. Rhizobia are also reported to produce toxic metabolites, enzymes, or volatile compounds [37] which have inhibitory effect on soil borne plant pathogens, like siderophore [9], rhizobitoxin, or plant growth enhancement through IAA production.

Enhancement of phosphorus uptake and other minerals and production of plant growth regulators such as auxins, cytokinins and gibberallins like substances were also suggested [13, 15]. However, there were several hypotheses about the mechanisms of the increased resistance in mycorrhizal plants: improvement of plant nutrient status, changed roots morphology and structure, changed in the rhizospheric microflora, improved the competition ability of some rhizospheric microflora and induced systematic resistance in plant [40].

In conclusion, *Rhizobium* bacteria seem to protect the plant, with which they make symbiotic contact, against the root rot pathogenic fungi particularly

Fusarium and *Rhizoctonia,* by suppressing their pathogenic effects. The protection effects of the rhizobial isolates vary according to the strain. Moreover, AM plants antagonize the soil borne pathogenic fungi or suppress the growth of such pathogens and increase plant resistance to diseases.

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