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Cultural Filtrate of *Rhizobium* spp. and Arbuscular Mycorrhiza are Potential Biological Control Agents Against Root Rot Fungal Diseases of Faba Bean

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Abstract: A field experiment was conducted in 2005/2006 and 2006/2007 to evaluate the potential antagonistic activity of the cultural filtrates of the three wild rhizobial isolates M. L (R1), L. C_4 (R2), T. S_1 (R3), *R. leguminosarum* ICARDA 441 (R4) strain and arbuscular mycorrhiza (AM) fungi in the biocontrol of damping-off and root rot diseases of faba bean plants, when applied in individual or combined treatments, under naturally infested soil with pathogenic fungi: *Rhizoctonia solani, Fusarium spp.* and *F. solani*. The bioassays of the rhizobia were tested. All treatments reduced the damping-off and infected plants as compared with untreated control. The combined treatment R2 + AM was the most effective treatment; it gave the lowest percentage of damping-off (6.67 and 8.89) and the highest percentage of healthy plants (86.58 and 83.33) in the two seasons, respectively. Beside the effect on disease control, seeds treatment with culture filtrates of the tested rhizobia or AM fungi in individual or combined treatments improved plant growth as indicated by the increased plant shoot height, root nodulation by native rhizobia, shoot dry weight and seeds yield of faba bean plants in the two trials, especially combined treatment R2 + AM. On the other hand the used rhizobia especially, isolate (L. C_4), proved to be IAA, exopolysaccharides and chitinase producers. The soaking of faba bean seeds in cultural filtrates of *Rhizobium* spp. individually or in combination with AM fungi acts as a bioprotection agents against faba bean plant root rot diseases, in addition to increasing plant growth and yield.

Key words: Bioprotection · *Rhizobium* cultural filtrate · Arbuscular mycorrhiza · Soil borne diseases · Faba bean

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important legume crops in Egypt. It is grown mostly to fulfill food and feed requirements for human and animal consumption. Seeds of faba bean are rich in protein 26:28% and some other compounds [1]. Soil-borne fungal diseases are among the most important factors, limiting the yield of crop legumes in many countries worldwide. Root rot, caused by *Aphanomyces euteiches*, *Rhizoctonia solani*, *Fusarium spp., Sclerotium rolfsii* are the most destructive soil-borne diseases of pea, chickpea, lentil, faba bean and lupine [2-4]. Regarding to environmental and health concerns about extended use of chemicals; there is considerable interest in finding alternative control approaches for use in biological control strategies for crop diseases [5].

The beneficial effect of *Rhizobium* and *Bradyrhizobium* in legumes in terms of biological nitrogen fixation has been a main focus in the recent past [6]. Many species of rhizobia promote plant growth and also inhibit the growth of certain pathogenic fungi. Rhizobia are reported to inhibit significantly the growth of pathogenic fungi, *Macrophomina phaseolina* (Tassi) Gold, *Rhizoctonia spp, Fusarium* sp. and *Pythium* spp. in both leguminous and non-leguminous plants [7, 8]. Arbuscular mycorrhiza (AM) fungi are recognized as high potential agents in plant protection [9]. In several cases direct biocontrol potential has been demonstrated, especially for plant diseases caused by *Phytophtora, Rhizoctonia* and *Fusarium* pathogens [10].

In natural environment, most cases of naturally occurring biological control result from mixtures of antagonists, rather that from high populations of a single

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antagonist. Most methods for biocontrol of plant diseases have used single biocontrol agents as antagonists to a single pathogen [5]. This may partially account for the reported inconsistent performance by biocontrol preparations, because single biocontrol agents are not likely to be active in all soil environments in which they are applied, or against all pathogens that attack the host plant. Consequently, application of a mixture of introduced biocontrol agents would more closely imitate the natural situation and might broaden the spectrum of biocontrol activity, enhance the efficacy and reliability of control [11] and allow the combination of various mechanisms of biocontrol without the need for genetic engineering [12].

The biocontrol agents have different mechanisms or combinations of mechanisms which may be involved in the suppression of different plant disease; for example, inhibition of the pathogen by antimicrobial substances (antibiosis) [13]; or production of diverse microbial metabolites like siderophore, rhizobitoxin [6]; competition for nutrients supplied by seeds and roots and colonization sites; induction of plant resistant mechanisms; inactivation of pathogen germination factors present in seed and root exudates and degradation of pathogenicity factors of the pathogen such as toxins; parasitism that may involve production of extracellular cell wall-degrading enzymes, for example, chitinase that can lyses pathogen cell walls [13], or plant growth enhancement through IAA production [6].

Previously, in our paper [14] the inhibitory effect of cultural filtrate of some wild rhizobial isolates (M.L, $L.C_4$, $T.S_1$ and of *R. leguminosarum* ICARDA 441 strain) against some fungi causing root rot disease of faba bean. (*R. solani, Fusarium spp.* and *F. solani*) *in vitro* and their antimicrobial synergetic effect when combined with Arbuscular mycorrhiza (AM) fungi, were investigated. So, the objective of the current study was to evaluate the bioactiviy of the isolates of *Rhizobium* spp. and strain to determine the probable mechanisms of the bioprotection. The effect of cultural filtrates of the wild rhizobial isolates and strain on control of damping off, root rot diseases and to evaluate their effect on growth and seeds yield of *Vicia faba* infested naturally with *R. solani, Fusarium spp.* and *F. solani* and their role when combined with AM fungi.

MATERIALS AND METHODS

Rhizobial isolates and strains: The three isolates of *Rhizobium* spp. M.L (R1), $L.C_4$ (R2) and $T.S_1$ (R3) used in this study 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). Their hosts of wild legumes, sites of collection and habitats were recorded in [15]. Strains of *R. leguminosarum* ICARDA 441 were also used in this study, 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 [16] at 4°C and in YEM broth containing 20% (v/v) glycerol at-80°C.

Arbuscular Mycorrhizal fungi (AM): AM fungi were provided by Dr. Massoud, Soil, Water and Environment Research Institute, Agric. Res. Center (ARC), Giza, Egypt. They include the following genera: *Glomus, Gigaspora* and *Acaulospora* [17].

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

Cultural filtrates of rhizobia: Rhizobial cultural filtrates were prepared as described in [14].

Arbuscular mycorrhizal (AM) fungi inoculum: The AM fungi inoculum prepared as modified Dr. Massoud, Soil, Water and Environment Research Institute, Agric. Res. Center (ARC) Giza, Egypt was applied as follow: Mixed spores of AM fungi genera were prepared after propagation and mixed with sterilized peat as a carrier (200 spore/g) and then applied as seed coating for sterilized faba bean seeds (previously soaked overnight in rhizobial cultural filtrates) for each mycorrhizal treatment. To aid adhesion, the inocula are often mixed with a sticker, such as Arabic gum and uniformly coating the seeds, then air dried for 2h before planting.

***In vitro* tests:** Mechanisms of the wild rhizobial isolates (M.L, $L.C_4$ and $T.S_1$) and *R. leguminosarum* ICARDA 441 strain were studied to determine the probable mechanisms of antimicrobial activities of such rhizobial isolates. Siderophore production was tested according to [6], their ability to produce hydrocyanic acid (HCN) was checked as described by Bakker and Schippers [18] and chitinase production were checked according to Chernin *et al.* [19]. Moreover, production of phytohormones like Indole Acetic Acid (IAA) as well as exopolysaccharides was determined according to [20] and [21], respectively.

Field trial: Field experiment was conducted in 2005/2006 and 2006/2007 growing seasons at the station of Agric.

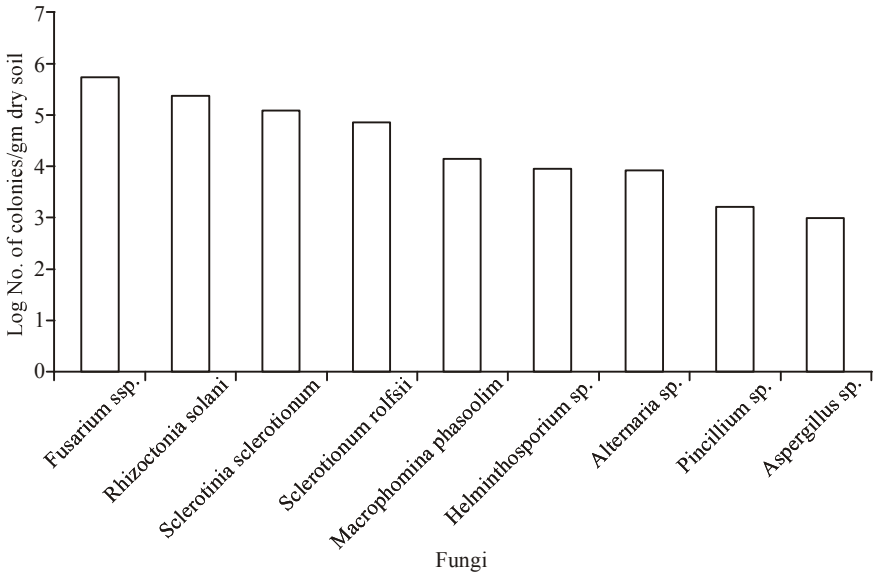

Fig. 1: Population of soil-borne fungi in naturally infested soil before planting of faba bean (colony/gm dry soil)

Res. Center (ARC) at Zarzora-Beheira, Egypt, in a field naturally infested with *Rhizoctonia* and *Fusarium* spp (Fig. 1). The field soil was clay loamy soil in texture having the following characteristics: pH, 7.57; EC 0.57 dSm^{-1}; soluble cations Ca^{+2}, Mg^{+2}, Na^{+} and K^{+} (meq/L), 3.2, 0.7, 1.3 and 1.0, respectively; soluble anions Co_3^{-}, HCo_3, Cl^{-} and So_4^{-2} (meq/L), trace, 1.4, 1.68 and 2.52, respectively; organic matter (%) 0.63; total N (%) 0.021; total P(%) 0.018; total K (%) 0.015.

Ten treatments were used; in treatments R_1, R_2, R_3 and R_4, the faba bean seeds were soaked overnight in the cultural filtrates of the wild rhizobial isolates and the strain. In a single treatment AM; seeds were coated with mixed spores of mycorrhizal genera. In the combined treatments R_1+AM, R_2+AM, R_3+AM and R_4+AM, the faba bean seeds were soaked overnight in the cultural filtrates of the wild rhizobial isolates and coated with mixed spores of mycorrhizal genera before sowing, then air dried for 2h before planting. Treatments were arranged in a complete randomized block design with three replicates. Field plots with standard size 3 x 3 m^2 for every treatment were sown with seeds of faba bean (Giza 3). The control treatment was sown with seeds without any treatment. All treatments received the recommended dose of super phosphate (P_2O_5) at the rate of 50 Kg/fed (1/3 dose), potassium sulphate (K_2So_4) at the rate of 50 Kg/fed and urea nitrogen fertilizer at the rate of 50Kg/fed (1/2 dose) were used.

The plants examined periodically and damping-off was recorded 30d after sowing. Survival plants were recorded 60d after sowing. Plants shoot height (cm), root nodules (number/plant) and shoot dry weigh (g $plant^{-1}$) were recorded after 45 and 75 days of planting while seeds yield were also recorded but at harvest. The infection percentage of AM fungi in plant root tissues was determined according to [22]. The nitrogenase activity in plant root tissues 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 135ºC according to [23]. The percentage of total nitrogen, phosphorous and potassium in the shoot samples were determined according to [24]. The data were analyzed for statistical significance using analysis of variance (ANOVA) test according to [25].

RESULTS

***In vitro* tests:** Results in Table 1 revealed that siderophore and HCN were not detected in all the tested rhizobia. IAA, exopolysaccharides and chitinase were produced in all the tested rhizobia but with different degrees; *Rhizobium* isolate $L.C_4$ was the higher producer than the other isolates.

Field trial: Data presented in Table 2 showed that in both seasons (2005/2006-2006/2007) all treatments significantly reduced damping-off and infected plants and increased healthy survival plants compared with untreated control. Application of wild rhizobial strains filtrates or AM fungi as individual treatments significantly reduced the damping-off and significantly increased the healthy survival plants. R_2 and AM were the most effective treatments; they recorded the highest

Table 1: Qualitative assessment of siderophore, HCN, IAA, exopolysaccarides and chitinase production by wild *Rhizobium* isolates and *R. leguminosarum* ICARDA 441

Isolates	Sidrophore	HCN	IAA	Exopolysaccaride	Chitinase
M.L	-	-	+	++	+
$L.C_4$	-	-	+++	++++	++++
$T.S_1$	-	-	+	++	++
ICARDA 441	-	-	++	+++	+++

HCN: Hydrocyanic acidIAA: Indole acetic acid, (++++): Heavy (+++): Good (++): Moderate (+): Small (-): Negative

Table 2: Effect of cultural filtrates of wild *Rhizobia* and Arbuscular Mycorrhizal fungi (AM) on damping-off and root rot disease of faba bean plants during 2005/2006 and 2006/2007 growing seasons under field conditions

				Survival					
	Damping-off (%)*			Infected			Healthy		
Treatments	2005/2006	2006/2007	Mean	2005/2006	2006/2007	Mean	2005/2006	2006/2007	Mean
R_1	15.45	16.67	16.06	16.66	8.89	12.78	67.89	74.74	71.32
R_2	12.20	13.34	12.77	13.32	12.22	12.77	75.48	74.74	55.11
R_3	16.66	17.78	17.22	14.42	13.33	13.88	68.92	68.89	68.89
R_4	18.89[b]	18.89	18.89	15.55	14.33	14.94	65.56	67.78	66.67
AM	14.46	14.44	14.45	12.21	11.11	11.66	73.33	74.45	73.89
R_1+AM	8.89	10.00	9.45	7.78	8.89	8.34	83.33	81.11	82.22
R_2+AM	6.67	8.89	7.78	6.75	7.78	7.27	86.58	83.33	84.84
R_3+AM	13.33	12.22	12.78	13.34	7.78	10.56	73.33	80.00	76.67
R_4+AM	10.02	15.56	12.79	13.34	8.89	11.12	76.64	75.56	76.10
control	22.19	28.89	25.54	19.99	22.22	21.11	57.82	54.44	56.13
LSD 0.005	5.8907	5.440		5.132	3.671		7.6975	6.501	

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, *Damping-off included pre-and post-emergence-off

percentage of healthy plants, followed by R_1, R_3 and R_4, respectively. Furthermore combined application of rhizobial filtrates and AM fungi were more effective compared with individual treatments and untreated control; especially combined treatment R_2 + AM and R1+AM which were superior, indicated by the lowest damping off Percentage (7.78 and 9.45%) and the highest percentage of healthy survival plants (84.84% and 82.22%). in the two seasons, respectively, compared with the untreated control.

Table 3 showed that all the treatments performed similarly in both trials (2005 2006 and 2006/2007). All the growth parameters tested (Plants shoot height (cm), root nodules (number/plant) and shoot dry weigh (g $plant^{-1}$) were improved by the individual treatments (R_1, R_2, R_3, R_4 a nd AM). Moreover, the treatments R_2 gave the highest growth parameters. However, combined treatments of rhizobia and AM fungi gave significant growth parameters higher than the individual treatments compared with untreated control as Table 3 showed. At the age of 45 d and 75d in both seasons, the combined treatment R_2 + AM exhibited significant increase in all growth parameters tested. It gave significant shoot height (22.6 and 23.33cm) at 45d during 2005/2006 and 2006/2007, whereas, at 75d it gave (93.6 and 97.67 cm) during the two seasons, respectively.

Also, the results in Table 3 cleared that, the nodules formed by native rhizobia had the same manner as shoot height. Combined treatment R_2 + AM recorded significantly increase in nodules number (110 and 120.33) at 45d and (135 and 138) at 75d during the two seasons, respectively). Regarding the shoot dry weight (g/plant), the combined treatment R_2 + AM exhibited significant increase in both seasons. It recorded (60.29 and 57.76 g/plant) after 45d, while at 75d; it gave (120.25 and 119.33 g/plant) during the two seasons, respectively.

The results in Table 4 showed the effect of interaction between AM fungi and rhizobial filtrates on Nitrogenase (Nase) activity and percentage of AM infection during the two seasons of planting. The combined treatment

Table 3: Effect of cultural filtrates of wild *Rhizobia* and Arbuscular Mycorrhizal fungi (AM) on growth parameter of faba bean after 45 and 75 days of planting, during 2005/2006 and 2006/2007 growing seasons under field conditions

	45 days of planting						75 days of planting					
	2005/2006			2006/2007			2005/2006			2006/2007		
Treatments	Sh.h. Pl^{-1} (cm)	Nod. $No.pl^{-}$	Sh.D.wt. pl^{-1} (gm)	Sh.h. Pl^{-1} (cm)	Sh.h. $No.pl^{-}$	Sh.D.wt. pl^{-1} (gm)	Sh.h. Pl^{-1} (cm)	Nod. $No.pl^{-}$	Sh.D.wt. pl^{-1} (gm)	Sh.h. Pl^{-1} (cm)	Sh.h. $No.pl^{-}$	Sh.h. pl^{-1} (gm)
R_1	19.0	94	44.82	19.67	96.33	45.67	86.2	110	103.09	88.33	114.67	104.33
R_2	20.6	106	46.18	21.67	110.00	47.33	92.2	120	117.59	91.00	119.00	113.33
R_3	18.0	81	41.50	18.33	85.00	42.33	86.0	102	85.43	88.67	105.00	91.00
R_4	17.5	76	40.80	17.33	77.00	41.33	87.0	96	86.20	89.00	98.67	90.33
AM	18.4	68	40.00	22.00	66.67	41.33	83.4	78	84.04	86.33	80.00	90.00
R_1+AM	21.8	108	51.87	22.67	115.33	53.67	90.6	115	105.57	94.67	117.67	109.00
R_2+AM	22.6	110	60.29	23.33	120.33	57.67	93.6	135	120.25	97.67	138.00	119.33
R_3+AM	19.4	78	50.49	20.67	84.33	52.67	86.4	88	86.32	89.67	91.33	97.00
R_4+AM	19.0	78	50.74	19.33	87.00	50.67	84.0	93	87.32	88.00	90.33	94.00
control	17.0	60	39.93	17.33	63.33	41.00	80.6	70	81.56	83.67	72.00	89.00
LSD 0.05	0.5545	2.2851	0.3214	1.139	3.460	1.876	2.561	5.194	1.7706	2.157	3.297	3.202

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

Table 4: Effect of cultural filtrates of wild Rhizobia and Arbuscular Mycorrhizal fungi (AM) on AM infection % and nitrogenase activity of faba bean after 45 and 75 days of planting during 2005/2006 and 2006/2007 growing seasons under field conditions

	45 days of planting				75 days of planting			
	2005/2006		2006/2007		2005/2006		2006/2007	
Treatments	Nase activity (µmole/C_2H_4/h/ Nod.D.Wt.)	%of AM infection	Nase activity (µmole/C_2H_4/h/ Nod.D.Wt.)	%of AM infection	Nase activity (µmole/C_2H_4/h/ Nod.D.Wt.)	%of AM infection	Nase activity (µmole/C_2H_4/h/ Nod.D.Wt.)	%of AM infection
R_1	8.402	80	8.70	79.00	66.600	90	66.50	90.00
R_2	15.211	80	16.53	77.67	75.500	100	76.90	98.00
R_3	7.273	75	7.70	75.00	53.500	90	53.17	88.67
R_4	5.979	70	6.10	70.00	51.600	80	51.93	81.00
AM	7.486	85	11.13	85.33	50.560	90	50.75	92.00
R_1+AM	14.084	90	8.03	89.67	88.586	85	89.70	90.00
R_2+AM	30.680	100	30.63	100.00	92.600	100	92.33	100.00
R_3+AM	8.000	90	8.70	89.33	87.250	80	87.47	81.00
R_4+AM	8.400	70	7.83	70.00	85.770	80	86.70	80.00
Control	6.553	30	6.41	36.00	43.254	60	46.20	56.67
LSD 0.05 (%)	0.5838	9.3287	3.499	5.679	0.8551	8.0789	2.708	7.508

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

R_2 + AM gave significantly increased Nase activity and AM infection %. It recorded 30.68 and 30.63 (µmole/C_2H_4/h/ Nod.D.Wt.) Nase activity at 45d of planting, while at 75d of planting it gave 92.6 and 92.33 (µmole/C_2H_4/h/Nod.D.Wt.) Nase activity during the two years, respectively. In addition, it gave 100 % AM infection at both the trials (2005/2006 and 2006/2007). Results obtained in Table 5 indicated that the NPK% in faba bean dry shoots increased in all treatments compared with untreated control and their values were nearly the same in both trials. However, combined treatment R_2 + AM exhibited the highest NPK% values; it recorded (1.0 N%, 0.415 P% and 0.31 K %) and (1.03 N%, 0.47 P% and 0.34 K%) at 45d of planting, during the two seasons, respectively. While at 75d of planting, it gave (1.53 N%, 1.199 P% and 1.786 K%) and (1.60 N%, 1.21 P% and 1.76 K%) during the two years, respectively.

Table 5: Effect of cultural filtrates of wild Rhizobia and Arbuscular mycorrhizal fungi (AM) on N, P and K percentage of faba bean dry shoots after 45and 75 days of planting during 2005/2006 and 2006/2007 growing seasons under field conditions

	45 days of planting						75 days of planting					
	2005/2006			2006/2007			2005/2006			2006/2007		
Treatments	N%	P%	K%	N%	P%	K%	N%	P%	K%	N%	P%	K%
R1	0.801	0.330	0.201	0.79	0.34	0.21	1.360	0.536	1.096	1.32	0.54	1.07
R2	0.990	0.350	0.255	0.95	0.37	0.26	1.400	0.650	1.581	1.41	0.67	1.58
R3	0.800	0.215	0.200	0.80	0.24	0.21	1.300	0.361	1.025	1.31	0.39	1.21
R4	0.800	0.231	0.206	0.80	0.31	0.21	1.125	0.400	1.089	1.16	0.42	1.15
AM	0.785	0.303	0.185	0.77	0.42	0.18	0.900	0.800	0.850	0.90	0.80	0.88
R1+AM	0.890	0.352	0.232	0.88	0.35	0.24	1.400	1.000	1.149	1.40	1.08	1.15
R2+AM	1.000	0.415	0.310	1.03	0.47	0.34	1.530	1.199	1.786	1.60	1.21	1.76
R3+AM	0.851	0.263	0.216	0.84	0.44	0.22	1.310	0.930	1.053	1.35	0.96	1.10
R4+AM	0.870	0.313	0.220	0.88	0.33	0.23	1.350	0.980	1.100	1.37	1.06	1.18
Control	0.456	0.190	0.183	0.48	0.20	0.18	0.587	0.231	1.035	0.59	0.26	1.05
LSD 0.05 (%)	0.0933	0.0216	0.0168	0.054	N. S	N. S	0.1468	0.1092	0.1529	0.094	0.108	0.172

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, N: Nitrogen, P: Phosphorous, K: Potassium

Table 6: Effect of cultural filtrates of wild Rhizobia and Arbuscular Mycorrhizal fungi (AM) on seed yield (ton/fed) of faba bean during 2005/2006 and 2006/2007 growing seasons under field conditions

	Seasons of planting					
	2005/2006			2006/2007		
Treatments	Wt. (gm) of 100 seed	Seed yield (ton/fed)	Increase of seed yield (%)	Wt. (gm) of 100 seed	Seed yield (ton/fed)	Increase of seed yield (%)
R1	61.67	2.49	39.88	60.33	2.64	47.49
R2	64.00	2.90	62.92	62.67	3.12	75.28
R3	60.67	2.24	25.84	59.33	2.33	30.17
R4	60.00	2.32	30.34	58.33	2.38	32.96
AM	59.00	2.08	16.85	57.67	2.10	17.32
R1+AM	69.33	2.91	63.48	69.00	3.13	74.86
R2+AM	72.00	3.39	90.45	70.67	3.43	91.62
R3+AM	62.67	2.42	35.96	64.33	2.70	50.84
R4+AM	61.67	2.38	33.71	62.33	2.37	32.40
Control	55.67	1.78	0.0	52.67	1.79	0.00
LSD 0.05 (%)	2.197	0.626		1.275	0.316	

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, Wt.: weight, fed: feddan

Data in Table 6 showed that in both trials there is a significant increase in seeds yield (ton/fed) of faba bean plants treated with individual treatments of rhizobial filtrates as well as in combined treatments with AM fungi compared with untreated control. However, Table 6 showed that combined treatment R_2 + AM gave the highest values of % increase in seeds yield in the two season of planting (90.45 and 91.62 %) compared with untreated control.

In general the results obtained proved that, the combination of rhizobial filtrates and AM fungi gave more efficient effect on disease incidence of root rot pathogens as well as the growth of faba bean plants.

DISCUSSION

Sustainable farming systems strive to minimize the use of synthetic pesticides and to optimize the use of alternative management strategies to control soil-borne pathogens [26]. Bacteria in plant root zone are a key agent of change in soil agroecosystems. Interactions between plant root systems and rhizobacteria have a profound effect on crop health, yield and soil quality. Root zone bacteria are able to generate a wide array of secondary metabolites which can have a positive influence on plant growth; enhancing the availability of minerals and nutrients, improving nitrogen fixation ability, decreasing susceptibility to frost damage, improving plant health through the biocontrol of phytopathogens, inducing systemic plant disease resistance and facilitating plant establishment, growth and development [27].

The results obtained *in vitro* revealed that none of the rhizobial isolates produce siderophore or HCN and this results agreed with many investigators [28], who found that in rhizobia, the ability to synthesize siderophore is restricted to a limited range of strains, rather than a wide distribution. Moreover they reported a very low incidence of cyanogens in rhizobia..However, they proved that the production of IAA is reported to be more common in rhizobia and this foundation was coinciding with the data obtained in the current study. Also the obtained results reported that the rhizobial isolates produce exopolysaccharide and this result was in agreement with many investigator, they proved that an Extra Cellular Polysaccharide (EPS) was produced by a *Rhizobium* sp. isolated from the root nodules of *Vigna mungo* (L.) [29]. Furthermore, the results obtained showed that the rhizobial isolates produce chitinase, this result were in harmony with data obtained by many workers [19], they reported that many species of bacteria, *Streptomycetes*, *Actinomycetes,* fungi and plants produce chitinolytic enzymes.

Data obtained from the field trial showed that there were heavy attacks on the untreated control plants by the end of the experiment. Treatment of faba bean seeds with individual rhizobial cultural filtrates or AM fungi generally reduced the percentage of infected plants. However, combined treatments of rhizobial cultural filtrates and AM fungi reduce the percentage of infected plants and root rot disease incidence more than the single treatments in comparison with untreated control,, especially the combined treatment R_2 + AM. These results were in harmony with many authors [30], who found that field experiments showed that with only eight isolates of 21 *Rhizobium* isolates significantly reduced wilt incidence of *Fusarium oxysporum* f.sp. *ciceris* (Foc) race 0, the causal agent of *Fusarium* wilt of chickpea.. Furthermore, [31] proved that seed treatment with R. *leguminosarum* bv. *viceae* was effective in controlling damping-off of pea in the field naturally infested with *Pythium* spp. On the other hand obtained results were coinciding with many researchers [32-34]. They found that application of AM fungi alone or combined with rhizobia reduced disease incidence of some soil-born disease. Moreover, [35] found 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.

Although the basic mechanisms behind such protection are not clearly defined; however there are many explanation of such mechanisms, one of them may be the possibility of inducing systemic resistance by the metabolic products found in the cultural filtrates of the tested rhizobia e.g. exopolysaccharaides. Another explanation may be the possibility of producing chitinolytic enzymes which was also detected in the tested rhizobial isolates. These findings were in agreement with many of the previous workers. They demonstrated that living and heat-killed cells of *Rhizobium etli* G12 induced in potato roots systemic resistance against *G. pallida* infection [29, 36, 37]. The results of their studies suggested that heat-stable surface structures of *R. etli* G12 which consist mainly of exopolysaccharides (EPS) and Lipopolysaccharides (LPS) and may be the inducing factors; have been proposed to be involved in the induction of induced systemic resistance. Essalmani and Lahlou [35] proved that the culture filtrate and heat-killed bacterial cells of *R. leguminosarum* contain signals able to induce plant resistance in lentil plants. Furthermore, it is well known, however, that chitin is a major structural component of most fungal cell walls and that many species of bacteria, S*treptomycetes*, *Actinomycetes,* fungi and plants produce chitinolytic enzymes [19].

On the other hand, our finding of protection may be in agreement with [38]. They found that AM fungi can effectively reduced root rot diseases caused by a number of soil-borne pathogens, such as *Fusarium* spp. *Aphanomyces euteiches* and *Phytophthora* spp. Such a kind of bioprotection by AM fungi could protect plants systemically and had been called induced systemic resistance (ISR) [38]. Researchers covering the mechanism of ISR by AM fungi had focused on nutritional effects; sink competition with infection sites, morphological changes in roots and root tissues, changes in chemical constituents in plant tissue, reduction of abiotic stress and microbial changes in the mycorrhizosphere [38].

Besides the effect on disease control, seed treatments with culture filtrates of the tested rhizobia or AM in individual or combined treatments also improved plant growth in the current study, as shown by significant increase in plant shoot height, nodules formed by native rhizobia, shoot dry weight and seeds yield of faba bean plant in the two field trials. Huang and Erickson [31] showed that seeds treatment with *R. leguminosarum* reduced significantly of damping off and promoted growth of pea and lentil plants grown in naturally infested with *Pythium* spp. The increase in growth parameter may be due not only to the reduction in disease incidence but also to the IAA which is found in culture filtrates of tested rhizobia in our investigation. These results were coincide with many workers [39], they showed that *R. leguminosarum* and *B. japonicum* as a plant growth promoting rhizobacteria controlled faba bean root disease caused by *F. oxysporum* and promoted plant growth, root nodulation, N and P uptake of faba bean shoots. In addition, [34] indicated that treatment of chickpea plants with mycorrhizal increased seed germination, plant height, number of pods, seed weigh and biomass production in soil infested with *R. solani*, *Macrophomina phaseolina* and *F. oxysporum*. Furthermore, rhizobial Extracellular Polysaccharide (EPS) as detected in present study, may improve nutrient acquisition and providing protection from environmental stresses and host defenses [40].

Application of culture filtrates of tested rhizobia or AM fungi as individual application under field condition increased the percentage of AM infection compared with untreated control. At the same time application of rhizobial cultural filtrates and AM fungi as a combined treatment resulted in additional increase in percentage of AM infection especially treatment R_2+AM, it recorded 100% AM fungi infection in both seasons as well as high reduction in disease incidence. This result explained according to [41] findings. They showed that how fungal root pathogens and the AM fungi, although colonizing the same host tissues, usually develop in different root cortical cells, indicating some sort of competition for space. If AM fungus colonizes the root cortical cells then this would limit the colonization of the pathogenic fungi to areas of the root which had not been colonized, thus affording protection. Obtained data may agree with those reported by [42], they found that *Phytophthora* dose not penetrate AM fungi-containing cells and its development is also reduced in adjacent uncolonized regions. In addition, mycorrhizal fungi improved plant nutrition indicating by the highest increase in NPK content in plants dry matter as proved in data obtained, this finding was in harmony with many investigators [32, 41, 43]. They found that AM fungi symbiosis results in more vigorous plants which may be more resistant to or tolerant of pathogen attack due to increased nutrient uptake by the mycorrhizal plants.

In conclusion, our findings indicate that soaking faba bean seeds in rhizobial cultural filtrates, individually or in combination with AM fungi acts as bioprotection agents against root rot diseases of faba bean plants, in addition to increasing plants growth and yield by the effect of the metabolic products found in the rhizobial cultural filtrates e.g. exopolysaccharaides, chitinolytic enzymes and IAA, as well as by the effect of AM fungal infection of the plants.

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