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Research Article

Effect of Fungicides on the Growth and Survival of Different Symbiotic N_2-Fixing Rhizobium Strains

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ABSTRACT

Rhizobial populations introduced into legume crop fields as biofertilizer become non-target microbiomes for the fungicides applied in the field. An *in vitro* experiment was carried out to evaluate the response of four *Rhizobium leguminosarum* strains originated from different soil types to twenty-five fungicides using four different techniques (paper disk, hole, streak, and microfermentor techniques). Fungicides were applied at five concentrations (0.0, 0.1, 1.0, 10, and 100 mg/l). The results from this study provide critical baseline information for further examinations to optimize the growth of these symbiotic N_2-fixing bacteria, the microfermentor technique was carried out as following the range of concentrations was tested in 5 ml yeast extract mannitol (YEM) broth medium inoculated with 125 µl bacterial suspension of cell density (106 CFU/ml) and the growth was measured spectrophotometrically at 550 nm after 48 h incubation in rotary shaker (150 rev./min.) at 28°C. The results were presented as relative growth rate of control tubes. Results showed that the sensitivity of investigated *Rhizobium* strains to the fungicides were in agreement accordance with those found earlier. Comparing the results obtained from agar and liquid cultures, it was found that the effect of fungicides on the growth of the tested *Rhizobium* strains at each concentration were similar in both cultures. The degree of growth inhibition zone had the same tendency to the investigated concentrations of each fungicide similar to the degree of turbidity obtained by spectrophotometry. Strain of Lóbab Z (isolated in Hungary) however, proved to have the high tolerance to most of tested fungicides followed by Bükköny 75/4 (Hungarian Strain), HB-3841 (isolated in Libya) and E1012 strain (U.K. origin) was the most sensitive strain. When the fungicides were compared, Zineb-80, Baytan, Agrocit, and Maneb-80 were the least toxic fungicides, but Orthocide was the most toxic one, followed by Dithane DG, PangenR, and Captan. Cober, Ortho-phaltan, Plantvax, Mycozol, Cobox, Thiram, Terreneb, Benlate, Brassicol, Dithane M-45, Ipam, Dithane FL, were moderately affecting the survival and growth of the *rhizobial* strains. MIC was also calculated. Finally, the degree of fungicide toxicity is associated with the *Rhizobium* strain. More investigation can be done with different *rhizobial* species.

Keywords: Fungicides; *Rhizobium*; Relative growth; Inhibition; Minimum Inhibitory Concentration (MIC)

INTRODUCTION

Globally, legume-fixed nitrogen (N_2) is obviously great. Due to the increasing in global population, a strong need for food requires. N limits plant growth in many agroecosystems. Biological N_2-fixation makes an important contribution to soil N and improves plant productivity. The legume plants which are cultivated include major food, forage and pasture plants, as well as some that produce timber and other products. Agriculture today faces challenges such as soil fertility loss, climate change and increased attacks of pathogens and pests. The production of sufficient quantities in a sustainable and healthy farming system is based on environmentally friendly approaches such as the use of biofertilizers, biopesticides and the return of crop residues. The multiplicity of beneficial effects of soil micro biomes, especially the Plant Growth Promotion Rhizomicrobiomes (PGPR), highlights the need to further strengthen the research

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and its use in modern agriculture. Faba bean (Viciafaba L.) is one of the cool season leguminous crops. Broad bean contains high protein (min. 24%) and appreciable amount of minerals and vitamins, and thus it is part of the various popular dishes in the globe like common bean. Due to the higher protein contains, legumes attain the significance of a great food value for human diet particularly in the country of low income and for vegetarians. Faba bean is one of the most effective N_2-fixing plants by forming symbiotic association with *R. leguminosarum* and increases the N content in the soil. Due to this, it is commonly integrated within crop rotation of traditional mixed low-input agricultural system in the highlands of the country.

The rhizosphere of legume plant possesses various microbiomes such as bacteria, actinobacteria, fungi and algae which affect the physical, chemical and biological properties of soil [1]. Among the soil bacteria, there are a group of bacteria that are able to form root-nodules of leguminous plant roots and this group is named *Rhizobia*. They are aerobic, Gram-negative, which belongs to the class of *Alphaproteobacteria, Rhizobiales* order [2,3]. *Rhizobia* are considered as PGPR comes in symbiosis with legumes. *Rhizobial* species belonging to several genera such as: *Rhizobium, Mesorhizobium, Bradyrhizobium, Ensifer, Allorhizobium, Azorhizobium, Neorhizobium* and *Pararhizobium* and can exist as free-living soil saprophytes or as N_2-fixing endosymbionts of legume host plants roots that forming the root-nodules of host legumes or in close association with the plant roots [1]. The symbiotic relationship results in huge quantities of N_2-fixation throughout the world and any adverse effect on *Rhizobia* results in reduced rates of biological N_2-fixation. Many factors influence the growth of N_2-fixing rhizobia. The effects of temperature, light, moisture, soil pH, salinity, antibiotics and nutrition on *rhizobia* are well documented [4]. In addition, fungicides may also influence the growth of *rhizobia* [4,5].

Fungicide used as seed treatment to improve early plant emergence and to control the early attack by the pests.

Some reports showed little damage, which may reflect the considerable variation within and in between various groups of *Rhizobium* in their sensitivity to fungicides [4-8]. Sometimes these agrochemicals negatively affect the growth and multiplication of fungi and bacteria and consequently cause the disturbance of the natural soil microbial balance. Fungicides may harmfully affect the non-target soil microflora. In modern agriculture, fungicides applied as seed dressings protect germinating seeds and young seedlings against fungal pathogens. Fungicides used to increase crop yields by eliminating the harmful effects of non-target microbiomes. The interaction between these organisms and the fungicides can be considered as general and the often harmful interactions of *Rhizobium* and fungicides living in symbiosis with leguminous plants should be taken into consideration. The sensitivity of *rhizobia* to a number of fungicides is known [9-12]. In contrast, some fungicides have less pronounced toxicity, and *rhizobia* are able to tolerate the effects of fungicides [13-15].

Environmental factors, methods of investigation used, soil types and other factors also influence the toxicity of fungicides. Diatloff [16] studied the effects of soils and their compatibility with fungicides, raised the interaction between environmental factors and fungicides. Heinonen-Tanski and Turrki [15] studied ten fungicides against R. *leguminosarum* strains *in vitro*. According to Nirmal et al. [17], fungicides used for seed dressing significantly reduce the population of *Rhizobium* strains. The effects of fungicides on the growth of *Rhizobium* strains under agar conditions [18] or in liquid cultures [4-19] were studied. It was found that There is a drastic mortality of the two strains of *R. leguminosarum*bv. phaseoli on seeds treated with Benlate and Ridomil [20]. The effect of the fungicides on the isolates of *Rhizobium* was variable, depending on the fungicide and isolate; also it was found that Addition of Mancozeb and also Carbendazim were found to decrease growth for *Rhizobium* isolates at concentration recommended for field application [21]. It has been reported that the systemic fungicide Benomyl increased the relative abundance of nodules formed by the inoculated strain [22]. Our investigations focused on the selection of *R. leguminosarum* strains with the best symbiotic N_2-fixing ability and fungicide-tolerant *rhizobial* strains.

Successful *Rhizobium* infection is considered to be important for agricultural crop production by increasing the level of atmospheric N_2-fixation and by selecting strains tolerant to fungicides for the particular soil type.

MATERIALS AND METHODS

Rhizobium strains (Table 1) were grown and maintained on yeast extract mannitol agar medium (YEMA). A suspension of 106 cell forming unit/ml of inoculum (in physiological saline of 0.85% NaCl) of *Rhizobium* strains was used in the following in vitro experimental work and the measurement was completed at the logarithmic stage of reproduction.

Fungicides: The effects of 25 fungicides belonging to 20 different active ingredients mentioned in Table 2 were studied in liquid and agar cultures. The sterile aqueous solution of each of each fungicidal concentrations 0 (control); 0.1; 1.0; 10.0; and final concentrations of 100 mg active ingredient/l were used. Culture medium used in this investigations were YEMA and in broth (YEMB) without agar. Maintenance of *rhizobial*strains was carried out on YEMA + 3 g/l CaCO3. Incubation temperature was at 28°C.

Table 1: *Rhizobium leguminosarum* strains

Strain	Host plant	Origin
HB-3841	Viciafaba	Libya
Lóbab Z	Viciafaba	Hungary
Bükköny 75/4	Viciafaba	Hungary
E1012	Viciafaba	England

Table 2: *In vitro* fungicides used

Fungicides	Active ingredient	Active ingredient	Chemical formula of active ingredient
Actidion	Cycloheximide	20%	Cycloheximide
Agrocit	Benomyl	50%	1-butylcarbamoyl-benzimidazol-2-methylcarbamate
Baytan F	Triadimenol + Fuberidazol	15% + 2%	1-(4-chloro-phenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanol-2-(2-furyl)-benimidazol
Benlate	Benomyl	50%	1-butylcarbamoyl-benzimidazol-2-methylcarbamate
Buvicide K	Captan	50%	N-trichloro-methylthio-tetrahydro-phthalimide
Captan 50 W	Captan	50%	N-trichloro-methylthio-tetrahydro-phthalimide
Terreneb	Chloroneb	50%	1,4-dichloro-2,5-dimetiloxibenzen
Cobox 50 WP	Copper oxychloride	50%	Copper oxychloride
Dithane FL	Mancozeb	455 g/l	(Manganese+zinc) ethylene bis-dithiocarbamate
*Dithane*DG	Mancozeb	75%	(Manganese+zinc) ethylene bis-dithiocarbamate
Dithane M-45	Mancozeb	80%	(Manganese+zinc) ethylene bis-dithiocarbamate
Corber	Fenpropimorph	750 g/l	4-/3-(4-(1,1-dimethyl-ethyl)-2-methyl/propyl-2,6-cis-dimethylmorpholine
Kolfugó 25 WP	Carbendazim	25%	2-(methoxymethyl-carbanyl-amino)-benzimidazole
Maneb-80	Maneb	80%	Manganese-ethylene-bis-dithiocarbamate
Ipam	Metam	40%	Methyldithiocarbamate
Mikál 75 WP	Efozite-Al+Folpet	50%+25%	Aluminum tris (0-ethyl hydrogen phosphonate)+N-(trichloromethyl-thio) phthalimide
Mycozol	Micozol	40%	2-(thiazol-4-yl) benzimidazole
*Orthocide*50 WP	Captan	50%	N-trichloro-methylthio-tetrahydro-phthalimide
*Ortho-phaltan*FL	Folpet	450 g/l	N-(trichloromethyl-thio) phthalimide
*Plantvax*75 WP	Oxycarboxin	75%	5,6-dihydro-2-N-phenyl-1,4-oxathiin-3-carboxamide 4,4-dioxide
Panogen(R)	Hg content	1.5/2.5	(Equivalents with mercury) methoxyethyl mercury acetate
Brassicol	PCNB	50%	1,2,3,4,5-penta-chloro nitrobenzene
Ronilan FL	Vinclozolin	500 g/l	3-(3,5-dichlorophenyl)-5-methylvinyl-1,3-oxazolidine-2,4-dione
Thiram	Thiuram	80%	Bis-(dimethyl-thiocarbamate) disulfide
Zineb-80	Zineb	80%	Zinc ethylene bis-dithiocarbamate

Interactivity and tolerance of four selected of Viciafabarhizobial strains against 25 selected fungicides was assessed to the extent of inhibition zone formation through agar plate diffusion by different techniques. Methods used: Each fungicide is used to determine its effect on the growth of *rhizobial* strains.

The different methods were used for all concentrations of all fungicides.

Disc method: 8 mm sterile paper disc impregnated with the above fungicidal concentrations in a petri dish. The diameter of the inhibition zone was determined in triplicate after 48 hours incubation at 28°C.

Hole technique: Known concentrations of each fungicide similar to the above method was placed in wells cut into agar.

The band method according to Stapharest and Strigjdöm [11].

Liquid Culture: 125 µl of the applied fungicidal concentrations were added to 5 ml of liquid YMA medium. Optical density was determined at 550 nm from a shaken culture (150 rpm) after 48 hours incubation with a DR-2000 spectrophotometer. The measured data were statistically evaluated [4].

Statistical analysis: The experimental was used completely randomized design with three replications. The averages and standard deviation calculated and the significant differences were calculated at the 5% of probability level.

RESULTS AND DISCUSSION

Fungicides markedly affect the population of saprophytic soil microbiomes as equally as the pathogens they are designed to control. The degree of inhibition and the duration vary with the chemical, the soil and the environmental conditions [23]. Fungicides differ in their effects on the growth and survival of *Rhizobium* and *Bradyrhizobium*strains depending on the strain and the concentration of the fungicide [24].

There are studies showing the incompatibility of some fungicides with *R. leguminosarumbiovarphaseoli* affecting its survival on seeds, and subsequently nodulation and N_2-fixation [25,26]. Protectant fungicides are commonly used to aid in the establishment of legume crops and pastures by protecting the seedlings from soil-borne fungal pathogens. In general, *rhizobia* are more susceptible to fungicides *in vitro* than when growing in situ [27].

Comparing the results obtained in liquid cultures, the effect of fungicides on the growth of the four *Rhizobium* strains showed a similar tendency at all concentrations. Each concentration of fungicide gave similar results in both inhibition zones and multiplication. Based on the results, growth inhibition was measured for *Rhizobium* strains as fungicide concentrations were increased.

Data from different methods were comparable (data not shown for similarity). The effects of fungicides on the four *Rhizobium* strains tested in liquid cultures are shown in Table 3. It was found that the English E1012 strain was the most sensitive, while the Hungarian strain Lóbab Z proved to be one of the most tolerant, followed by Bükköny 75/4 and HB-3841 in sensitivity.

Based on the interaction between fungicides and *Rhizobium* strains, Zineb-80, Baytan F, Agrocit, Maneb-80 appear to be less toxic fungicides to the investigated four strains, while Orthocide 50 WP was most toxic followed by Dithane DG, Panogen and Captan 50 W. Cober, Orthophaltan, Plantvax, Mycozol, Cobox, Thiram, Terreneb, Benlate, Brassical, Dithane M-45, Ipam, Dithane FL moderately influenced the growth of the studied Rhizobium strains.

The four *R. leguminosarum* strains studied here responded differently to different fungicidal concentrations. The approximate Minimum Inhibitory Concentration (MIC) was determined in each case, taking into account the growth response of each strain (Table 4). For example, it was found that the MIC of Actidion, Agrocit, Baytan F and Zineb-80 was>100 mg/l for all four *rhizobial* strains, while MIC of Dithane FL was >10.

Table 3: Effect of fungicides on the growth of *Rhizobium leguminosarum* strains (Relative growth%)

Fungicides	*Rhizobium* strains			
	HB-3841	**Lóbab Z**	**Bükköny 75/4**	**E1012**
	Average ± SD	Average ± SD	Average ± SD	Average ± SD
Actidion	79.1 ± 9.0	79.0 ± 9.1	77.9 ± 10.1	80.0 ± 11.8
Agrocit	69.4 ± 14.7	75.1 ± 14.2	76.3 ± 16.0	64.5 ± 16.8
Baytan F	83.9 ± 7.6	86.3 ± 9.0	85.1 ± 8.1	65.8 ± 17.4
Benlate	74.9 ± 14.3	55.3 ± 13.8	65.8 ± 13.3	62.1 ± 16.4
Buvicide K	85.6 ± 9.7	82.2 ± 14.4	82.8 ± 17.1	44.2 ± 17.1
Captan 50 W	45.5 ± 36.8	59.5 ± 27.1	55.8 ± 25.0	39.0 ± 27.6
Terreneb	62.4 ± 21,1	65.5 ± 16.3	70.7 ± 15.2	52.3 ± 17.3

Cobox 50 WP	58.7 ± 23.8	64.9 ± 15.3	68.2 ± 27.0	50.9 ± 29.4
Dithane FL	71.2 ± 11.9	71.4 ± 14.5	72.6 ± 16.0	62.8 ± 23.9
Dithane DG	40.8 ± 32.7	40.9 ± 25.2	38.9 ± 25.2	53.3 ± 36.4
Dithane M-45	57.1 ± 22.1	68.9 ± 15.8	73.1 ± 16.5	62.4 ± 15.0
Cober	48.9 ± 14.4	57.7 ± 15.1	59.4 ± 15.0	38.4 ± 17.6
Kolfugó 25 WP	75.7 ± 14.7	89.8± 9.7	76.9 ± 13.0	74.9 ± 13.1
Maneb-80	64.3 ± 23.7	74.1 ± 19.2	79.6± 14.7	68.9± 22.0
Ipam	66.3 ± 10.8	60.7 ± 15.4	78.1 ± 12,6	66.3± 15.7
Mikál 75 WP	76.2 ± 16.3	80.1 ± 9.0	77.1 ± 8.7	64.3 ± 15.6
Mycozol	59.7 ± 16.1	70.6 ± 14.2	65.5 ± 15.5	46.1 ± 18.4
Orthocide 50 WP	27.5 ± 28.0	55.5 ± 34.6	48.0 ± 16.9	32.8 ± 34.6
Ortho-phaltan FL	35.1 ± 35.4	77.1 ± 9.8	44.0 ± 16.2	48.5 ± 39.7
Plantvax 75 WP	42.0 ± 18.9	56.4 ± 17.3	63.2 ± 17.6	48.7 ± 20.5
Panogen(R)	35.5 ± 16.3	57.9 ± 14.4	47.3 ± 12.1	36.9 ± 14.9
Brassicol	59.0 ± 23.4	71.6 ± 17.3	67.4 ± 18.5	63.4 ± 21.0
Ronilan FL	75.1 ± 16.7	82.6 ± 12.0	76.9 ± 15.1	70.6 ± 18.2
Thiram	54.0 ± 18.2	70.5 ± 20,0	67.6 ± 17.6	57.4 ± 15.7
Zineb-80	80.8 ± 13.2	81.8 ± 13.1	78.7 ± 13.2	86.1 ± 11.3

Table 4: The minimum inhibitory concentration of fungicides the growth of *Rhizobium* strains

Fungicides	**MIC (mg/l)**			
	***Rhizobium* strains**			
	HB-3841	**Lóbab Z**	**Bükköny 75/4**	**E1012**
Actidion	>100	>100	>100	>100
Agrocit	>100	>100	>100	100
Baytan F	>100	>100	>100	100
Benlate	>10	10	>10	>1
Buvicide K	>100	>100	>10	>1
Captan 50 W	>1	10	>10	>1
Terreneb	10	10	>10	>1
Cobox 50 WP	>1	>10	>10	>1

Dithane FL	>10	>10	>10	>10
Dithane DG	>1	>0.1	>0.1	>1
Dithane M-45	>1	>10	>10	>1
Cober	>0.1	>1	>1	>0.1
Kolfugó 25 WP	>10	>100	>10	>10
Maneb-80	>10	>100	>100	>100
Ipam	>10	>1	>100	>10
Mikál 75 WP	>10	>100	>100	>1
Mycozol	>1	>10	>1	>1
Orthocide 50 WP	>1	>1	>0,1	>0.1
Ortho-phaltan FL	>1	>100	>0.1	>1
Plantvax 75 WP	>0.1	>1	>1	>0.1
Panogen(R)	>0.1	>1	>0.1	>0.1
Brassicol	>1	>10	>10	>1
Ronilan FL	>10	>100	>10	>10
Thiram	>1	>1	>10	>1
Zineb-80	>100	>100	>100	>100

Table 5 summarizes the effects of fungicides in general. It was found that Zineb-80 (81.9% ±2.7), Baytan F (80.3% ± 8.4), Actidion (79.0% ± 0.8), Kolfugó 25 WP (79.3% ± 6.1), Ronilan FL (76.3% ± 4.3), Mikál 75 WP (74.4% ± 6.0), Buvicide K (73.7% ± 17.1), Maneb-80 (71.7% ± 5.7), Agrocit (71.3% ± 4.7). Meanwhile, the most effective inhibitors of fungicides were Orthocide 50 WP (42.0% ± 11.3), and Dithane DG (43.5% ± 5.7), Panogen(R) (44.4% ± 9.0), Cober (51.1% ± 8.4), and Ortho-phaltan FL (51.2% ± 15.7). The fungicide viability of the studied four *R. leguminosarum* strains was reduced in most cases, but *Rhizobium* strains were able to survive at medium to high concentrations of the fungicides tested, except for a highly toxic fungicide such as Orthocide 50 WP. A remarkable result is the similarity of the data obtained by different methods.

Among non mercurous fungicides, Dithane Z-78 was observed to be the most toxic showing safe limit between 0 and 50 ppm while Thiram, Captan and Dithane M-45, the safest fungicide, showed the safe limit as high as to 5000 ppm depending upon the type species of Rhizobium. Some of the non-mercurous fungicides like Thiram, exerted stimulatory effect on rhizobialgrowth at their lower concentration [28].

The results provided a partial explanation of the compatibility of *Rhizobium* strains and fungicides under laboratory conditions. Because fungicides are often non-specific in their action, it is necessary to be aware of the effects of these chemicals on the roots, and some care should be taken when inoculating chemically treated seeds. Shahid and Khan [29] studied the effect of Hexaconazole fungicide and established that tolerant *Bradyrhizobiumjaponicum* mitigate toxicity and enhance green gram production under Hexaconazole stress. Our results can be supported by the earlier work of several researchers. At low concentrations (0.03 ppm), Afifi et al. [7] found Captan and Thiram toxic to some but not all *Rhizobium* strains, although at concentrations of 300-3000 ppm, most strains inhibited reproduction. We found that Captan was highly inhibited at 10 mg/l, while Thiram moderately inhibited *Rhizobium* at 100 mg/l. Aynalem and Assefa [30] showed that in YEMA medium almost all *Rhizobium* isolates were affected at lower (100 mg/l) concentration of Mancozeb.

Gillberg [31] mentioned that *R. leguminosarum* is sensitive to higher doses of Captan and already inhibits reproduction by 50 ppm, as confirmed [6] and our findings too, as well as the fact that Kecskés and Vincent [23] found that copper oxychloride is less toxic to *R. leguminosarum* than Cersan. The data reported by Faiza et al. [19] on the inhibitory effects of fungicides are still valid as we have similar results.

There was no significant difference in the proliferation of the tested strains when treated with PCNB, and is consistent with

Pillai and Nair]32], who found no difference in effect on the growth of the two PCNB-treated *Rhizobium* strains.

Table 5: The average inhibiting effect of fungicides on *Rhizobium* strains (Relative growth%)

Fungicides	Average inhibiting effect (% ± SD)
Actidion	79.0 ± 0.8
Agrocit	71.3 ± 4.7
Baytan F	80.3 ± 8.4
Benlate	64.5 ± 7.1
Buvicide K	73.7 ± 17.1
Captan 50 W	50.0 ± 8.1
Terreneb	62.7 ± 6.7
Cobox 50 WP	60.7 ± 6.6
Dithane FL	69.5 ± 3.9
Dithane DG	43.5 ± 5.7
Dithane M-45	65.5 ± 6.0
Cober	51.1 ± 8.4
Kolfugó 25 WP	79.3 ± 6.1
Maneb-80	71.7 ± 5.7
Ipam	67.9 ± 6.3
Mikál 75 WP	74.4 ± 6.0
Mycozol	60.5 ± 9.2
Orthocide 50 WP	42.0 ± 11.3
Ortho-phaltan FL	51.2 ± 15.7
Plantvax 75 WP	52.6 ± 8.0
Panogen(R)	44.4 ± 9.0
Brassicol	65.4 ± 4.7
Ronilan FL	76.3 ± 4.3
Thiram	62.4 ± 6.9
Zineb-80	81.9 ± 2.7

High concentrations of PCNB were toxic to the two strains of *rhizobia*. Fisher and Cliffon [33] found that Benomyl 200 ppm had little effect on the various *R. leguminosarum* strains grown on agar medium. According to Hamed and Salem [34], Dithane has an effect on the proliferation of *R. leguminosarum*.

Staphorst and Stijdön [11] investigated the effects of 13 fungicides on *Rhizobiumin vitro* and the result showed that Thiram, Mancozeb and Maneb 80 were the most toxic. Backman [35] showed that *Rhizobium* survival in relation to fungicides depends on the species and the doses used.

Mohamad and Al-Naser [36] showed that Mancozeb fungicide greatly inhibited the growth of all tested *rhizobial* strains. Strzelec and Martyniuk [37] mentioned that a reduction in the growth of three strains of *R. leguminosarum*and four strains of R. meliloti with R. meliloti surviving well on fungicide (*Thiram*) treated seeds.

Thiophanate-methyl at 50-500 μg/l stimulated *Rhizobium* growth in YEM broth indicating that the fungicide does not have any bactericidal activity [38] whereas Mancozeb decreased the growth of two *Rhizobium* strains by 50% [39]. It was found [40] that *in vivo* experiment, Benlate reduced drastically the occurrence of inoculated strains in nodules, while the fungicide treatment and inoculation applied in the seed furrow did not affect the survival of the inoculated strain.

The results obtained by various experimental methods prove that the survival of the strains is dependent on genetic (tolerance/resistance or sensitivity) or ecophysiological adaptation. The experimental results obtained can be used in field cultivation of legume plants with respect to the interaction between fungicides and *R. leguminosarum.*

CONCLUSION

This study was designed to evaluate the effects of twenty-five fungicides using four different techniques on the growth rate of four various *rhizobial* strains. It has shown that some of investigated fungicides had no negative effect on the growth of N_2-fixing bacterial population *in vitro.*

From this study, it can be concluded that the results obtained under *in vitro* experiment show the sensitivity of the studied *Rhizobium* strains was as described in the literature. Comparing the results obtained with the various methods, it was found that the fungicides had similar effects on the investigated four *Rhizobium* strains.

It is concluded that *Rhizobium* strains vary in their sensitivity to the fungicides. The degree of growth inhibition at each concentration of fungicides tested using different techniques show the same trend, similar to that of the spectrophotometer. The Lóbab Z strain (isolated from Hungarian soil) proved to be broad-tolerant (the most tolerant) to most of the fungicides investigated, in contrast to the Bükköny 75/4 (Hungarian) and HB 3841 (Libyan) strains. Strain E1012 (of English origin) was the sensitive strain. On the other hand, fungicides react differently with the strains depending on their toxicity and concentration.

It was found that Zineb-80, Baytan, Agrocit and Maneb-80 were the least toxic fungicides, compared with Orthocide 80 WP being the most toxic, followed by Dithane DG, Panogen and Captan. Cober, Orthophaltan FL, Plantvax 75 WP, Mycozol, Cobox, Thiram, Terreneb, Benlate, Brassicol, Dithane M-45, Ipam, Dithane FL moderately influenced strain survival and reproduction.

Depending on the high tolerance of Lóbab Z strain to the tested 25 fungicides, this strain can be used an attractive choice to cultivate the faba bean seeds in fungicide polluted soils and to concurrently enhance faba bean production under stressed environment. More investigations should be carried out on different genera and species of symbiotic N_2-fixing bacteria. Further studies are needed to study the genes involved in fungicide tolerance and the relationship between these genes and the symbiotic genes. These strains can be assessed for their symbiotic efficiency and suitability as inoculant for cultivated Viciafaba.

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