SIDEROPHORES MEDIATED INTERACTIONS IN RELATION TO GROWTH OF *FUSARIUM OXYSPORUM* F.SP. *GLYCINE*, *BRADYRHIZOBIUM JAPONICUM* AND SOYBEAN PERFORMANCE UNDER IRON LIMITED CONDITIONS

ABSTRACT

Different concentrations (50,100,150 or 200 µL) of extracted siderophores produced by *Pseudomonas fluorescens* B, were added into the culture media of *Bradyrhizobium japonicum* ARC 501 or *Fusarium oxysporum* f.sp. *glycine*. The synthetic chelator ethylene diamine di-o-hydroxy phenyl acetic acid (EDDHA) with the high capacity to bind Fe, was also supplemented into concentrations of 200, 400, 600, 800, 1000, 1500, 2000 or 2500 mg/L of M9 medium. The treated media were used to grow *F.oxysporum* f.sp *glycine*,*F.solani* or *Rhizoctonia solani*. Data showed that *B.japonicum* ARC501 efficiently utilized the increased concentrations of *Ps. fluorescens* B siderophore for growth, but *F.oxysporum* f.sp. *glycine* gave concomittantly reduced biomass. The latter fungus along with *F. solani* and *Rh. solani*, also gave similar responses with increased concentrations of EDDHA. Iron starved *B.japonicum* ARC501 cells applied for soybean seedlings grown in test tube - sand culture supplemented with *Ps. fluorescens* B siderophore, showed gradual proliferation and induced levels of soybean root hair curling, to nearly similar those obtained from Fe-EDDHA treatment. Acid washed sand amended with Fe as Fe-EDDHA or Fe $(OH)_3$, was used to grow soybean inoculated with *B.japonicum* ARC 501, or in conjugation with either of 2 strains of *Ps. fluorescens* (B or 1) varied in their siderophore mediated antagonism to the root nodule bacterium. Developed plants were kept under net-house conditions and harvested after 45 days, to record shoot and root dry weights, nodulation and N as well as Fe uptake under different conditions. The effects of inoculation treatments were retested in sandy soil naturally limited in Fe content.Soybean grown in sand amended with Fe $(OH)_3$ and dually inoculated with *B.japonicum* ARC501 plus *Ps. fluorescens* B(the non-antagonist), gave positive responses, which were insignificantly different to those obtained from Fe-EDDHA treatment. However, the levels of enhancement were significant, as compared with the single inoculation with *B.japonicum* ARC501, or when conjugated with *Ps. fluorescens* 1(the antagonist). These findings were also reported in results obtained from plants grown in sandy soil naturally limited in Fe-content.

Keywords:Siderophore, *Fusarium oxysporum*, *Bradyrhizobium japonicum*, *Pseudomonas fluorescens*, Fe $(OH)_3$, Fe-EDDHA, Soybean Performance.

INTRODUCTION

The number of iron chelating siderophores produced by microorganisms were listed by **Ratledge and Dover (2000)** to exceed 500.Fluorescent psudomonads are amongst the most effective siderophores producing bacteria **(Cox,1980; Bezbaruah et al. 1996 ;Terano et al. 2002)**.This pronounced cabability has reflected in potential interactions with highly Fe demanding N2-fixing associations and phytopathogenic fungi.The role of siderophores produced by many *Psudomonas* has been clearly demonstrated in the control of *Pythium* and *Fusarium* species,either by

M. I. Mostafa; Sohair A. Nasr; Enas A. Hassan and M.El-Sawy
Microbiol. Dept., Fac. Agric., Ain-Shams Univ., Shoubra El-Kheima, Cairo, Egypt.

comparing the effects of the purified pyverdin siderophore with synthetic iron chelators or through the use of pyoverdin minus mutants**(Loper and Buyer, 1991; Duijff et al. 1993)**.The study of **Raaijmakers et al.(1995)** demonostrated that the sole mechanism in suppression of Fusarium wilt of radish by *Ps.putida* strain WSC358 was the production of pseudobactin 358.The study of the antifungal mechanism with *Ps.fluorescens*GL20-S101(sid),a mutant defective in siderophores synthesis was responsible for the fungal inhibition by siderophore-mediated Fe (III) competition under iron –deficient conditions **(Hoseong et al. 1999)**.

Jadhav et al.(1994) found that addition of 10uM of Fe plus 10uM of either of the 2 chelator (siderophore of cowpea rhizobium or desferrioxamine B) gave significant increases in growth and chlorophyll content of peanut compared with the control.Individual inoculation of soybean with *Pseudomonas* JLOZ-3 or *B.japonicum* SB-12 also improved emergence,seedling stand and increased dry matter accumulation in shoots and roots, nodulation and nutrient uptake over uninoculated control plants.However, the highest records were obtained from mixed inoculation **(Zaidi 2003).**

The experimental work reported in this study was planned to verify a) the level of interaction between extracted siderophore and growth of phtytopathogenic fungi and *B.japonicum* in culture media,b)the effect of siderophore on early stages of nodule initiation in soybean and c) the role of siderophore mediated antagonistic effect iduced by *Pseudomonas* strains against *B.japonicum* in relation to soybean performance in sand culture and sandy soil naturally limited in Fe content.

MATERIALS AND METHODS

Growth media

Acid-washed sand as well as sandy soil were used for experimental purposes. The sandy soil was collected from Al-Nubaria, Al-Bahaira governorate. The physico-chemical characterstics of that soil are given in **Table (1)**.

Seeds

Soybean (*Glycine max* cv. Crawford) was used as a host plant for experimental studies. Seeds of this host plant were kindly obtained from Field Crops Research Institute, Department of Leguminous Crops, Agricultural Research Centre (ARC), Giza, Egypt

Microorganisms

A range of microbial strains belonging to fluorescent Pseudomonads (*Ps. fluorescens* B and *Ps. fluorescens***1**), root nodule bacteria (*B. japonicum* ARC 501) and phytopathogenic fungi (*Fusarium solani, F. oxysporum* f.sp. *glycine and Rhizoctonia solani*) were used in this study. Those strains were kindly supplied by different research institutes as shown in **Table (2)**.

Table(1): Physico – chemical characteristis of the soil sample taken from Al-Nubaria to be used in this investigation.

Physical analysis

Soil particles size distribution (Mechanical analysis)					Soil texture
$CaCO_3$ %	Gravels > 2 mm %	Sand %	Silt %	Clay %	
7.8	7.4	86.5	8.6	4.9	Sandy soil

Chemical analysis

pH	SP	ECe	TDS	Water soluble cations and anions (in saturated water extract)							
(1 : 2.5)	(%)	(ds / m)	(mg / L)	Ca (mg / L)	Mg (mg / L)	K (mg / L)	Na (mg / L)	CO_3 (mg / L)	HCO_3 (mg / L)	Cl (mg / L)	SO_4 (ppm)
8.65	24.9	1.65	2976	5.58	2.79	0.19	36.27	0.75	1.5	36.27	6.31

Available nutrients (ppm)							Organic matter (%)
P	K	Ca	Mg	Fe	Mn	Zn	
Olsen	In Amm .acetata extract			In *DTPA extract			
3.8	12.7	235.0	16.4	1.1	2.3	0.7	0.057

*DTPA: Ethylene triamine pentaacetic acid

Table (2): Microbial isolates or strains used in this study and their sources.

Fluorescent Pseudomonads	Source	Root nodule bacteria	Source	Phytopathogenic fungi	Source
Ps. fluorescens B *Ps. fluorescens* 1	Cairo MIRCEN, Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt	*B. japonicum* ARC 501	Culture collection of Biofertilizers Production Unit, Agric. Research, Center, Giza, Egypt.	*F. solani* *F. oxysporum* f.sp. *glycine* *Rhizoctonia solani*	Plant Pathology Dept., National Research Center, Dokki, Giza, Egypt Plant Pathology Dept., Faculty of Agriculture, Ain-Shams Univ., Cairo, Egypt

Culture supplement

EDDHA(Ethylenediamine di o-hydroxy phenyl acetic acid) was used as a chelating agent with a high affinity for Fe binding in culture media.

Iron sources

Fe-EDDHA (ferric ethylenediamine di o-hydroxy phenyl acetic acid) with the trade name sequestrene solurapide Fe 100 SG contained 6% Fe, and $Fe(OH)_3$ (ferric hydroxide) contained 52.14% Fe were used as sources of iron in pot experiments.

MINERAL NUTRIENTS

The N free nutrient solution of **Somasegran and Hoben (1985)** was used to feed the leguminous host grown in sand culture. Potassium nitrate (13.86 % N) was used a mineral fertilizer for sandy soil.

Experimental techniques

Utilization of introduced siderophore by *B. japonicum* ARC 501 and *F. oxysporum* f. sp. *glycine*

Siderophore extraction

For this purpose, cells of *Pseudomonas fluorescens* B grown in 1000 ml of modified M9 liquid medium **(Schwyn and Neilands, 1987)** for 2 days at 28–30°C, were harvested by centrifugation (4000 rpm / 30 min). Siderophore was obtained by filteration of the supernatant through a 0.45um membrane filter to ensure complete removal of cells and was then acidified to pH 3 with concentrated HCl. The supernatant was extracted three times with equal volumes of ethyl acetate. The organic solvent was evaporated and the concentrated extracts were dissolved in 3 ml of a 0.01 M phosphate buffer (pH 7) as described by **Guan *et al.* (2000).**

Effect on microbial growth under limited iron condition

Two hundreds and fifty ml Erlenmeyer flasks containing 100 ml of sterilized (121°C / 15 min) Fe-deficient M9 or defined medium **(Wiebe,2002),** was supplemented with 0.1 μM Fe III and either of 0.0, 50, 100, 150 or 200 μl of *Ps. fluorescens* B siderophore extract. The mixture was allowed to stand for at least 24 h at 7 °C prior to

the assay to permit the slow chelation of all adventitious iron. Media with different treatments, were inoculated with 1 ml of *B. japonicum* ARC 501 (contained about 10^5 cell) or an agar disk of *Fusarium oxysporum* f.sp. *glycine* . Inoculated media were incubated at 28–30 °C. Samples of growing bacterial culture was taken every 6 h and growth density was plotted against time and the logarithmic linear phase of the growth curve was used to estimate the following parameters: specific growth rate (μ) – doubling time (td) – multiplication rate (MR) - number of generations (N). The dry weight (g/100 ml medium) of fungal biomass was also recorded at the end of the experimental period (6 days).

Utilization of ethylene diamine di-o-hydroxy phenyl acetic acid (EDDHA) as a synthetic iron chelator by phytopathogenic fungi

100 ml Erlenmeyer flasks containing 25 ml of sterilized (121 °C / 15 min) Fe-deficient modified M9 medium was supplemented with increased concentrations (0.0, 200, 400, 600, 800, 1000, 1500, 2000, 2500 mg / L) of EDDHA (sterilized by filtration). Mixtures were then allowed to stand for at least 24 h at 7 °C prior to the assay to permit the slow chelation of all adventitious iron. Media with different treatments were inoculated with agar disks (7 mm) of fungal inoculum (contained actively growing mycelia of 7 days old cultures) of *Fusarium oxysporum* f. sp. *glycine*, *Fusarium solani* or *Rhizoctonia solani*. Inoculated media were incubated at 28 – 30 °C for 7 days. After incubation period, fungal growth was determined as dry weight (g/L) and culture filtrates of each treatment were used to determine the amount of produced siderophore as μM DFOM (**Alexander and Zuberer ,1991**).

Interaction between *B. japonicum* and soybean roots in sand culture supplemented with *Ps. fluorescens* B siderophore or Fe- EDDHA

Soybean seeds selected for uniformity in size and viability (95%) were surface sterilized with ethanol (95%) and 0.1% $HgCl_2$ for 5 min, followed by several washing changes of sterilized distilled water. Seeds were then aseptically sown at a rate of one seed /test tube (4 x 25 cm) containing 100 g sterilized (121 °C / 2 h) acid washed sand supplemented with 2 mg N as KNO_3 and 25 ml of N and Fe free nutrient solution (Somasegran and Hoben, 1985). The tubes were kept in growth chamber and germinated seeds were left to grow for 10 days where developed seedlings were inoculated with 1 ml of *B. japonicum* ARC 501 active culture (containing about 5.9 x 10^6 CFU). Tubes were then divided into 2 groups, where Fe (III) - siderophore of *Ps. fluorescens* B complex or Fe-EDDHA were applied at a rate of 0.1 μM Fe / tube. Cell density of *B. japonicum* ARC 501 in the whole sand – culture was determined immediatley after inoculation (zero time) along with root hair curling percentages after 2, 5 and 8 days from inoculation. The density of *B. japonicum* ARC 501 cells was determined by decimal plate count technique using yeast extract mannitol agar medium supplemented with 10 ml of 0.25% congo red and percentages of root hair curling were estimated following root staining with 0.01% methylene blue for 15 min and light microscopic examination.

Performance of soybean as affected by coinoculation with *Brady. japonicum* and *Pseudomonas fluorescens*
In sand culture

Acid washed sand packed in pots with 2 kg capacity was amended with 20mg N/kg sand as KNO_3 . Sand was either unamended or amended with poorly soluble iron

[0.2 or 0.5 m mol Fe as Fe $(OH)_3$] or available iron (0.5 m mol Fe as Fe- EDDHA). *B. japonicum* ARC 501 inoculation was common for all Fe treatments,but *Ps. fluorescens* B or 1 inoculation represented an additively application for Fe $(OH)_3$ treatments in particular. Inoculation was carried out with planting by pippetting 10 ml of the bacterial culture (contained about 10^8 CFU / ml) / pot of either of both organisms according to the treatment, around the seeds.All treatments were fed twice weekly with Fe free nutrient solution (**Somasegran and Hoben 1985**)

In sandy soil

Sandy soil limited in iron content was packed in pots with 2kg capacity, suplemented with 20 mg N / kg as KNO_3, and the inoculation treatments comprising *B. japonicum* ARC 501 alone or conjugated with either *Ps. fluorescens* B or 1 were only applied as shown above. . Pots were kept in net house and watered with tap water when necessary to keep moisture content at 60% W.H.C.

For both experiments,five replicates were made for each treatment. The plants were harvested after 45 days from sowing and the number nodules, dry weight of nodules (mg / plant), as well as , dry weight of shoots and roots (g / plant), nitrogen (mg / plant) and iron (ppm) uptake in shoot were determined according to **Jackson(1967)**. Total chlorophyll content (mg /g) was only determined in leaves samples of plants grown in sandy soil according to the method described by **Arnon (1949).**

RESULTS

Utilization of introduced siderophore by *B. japonicumn* and *F. oxysporum* f. sp. *glycine*

The growth kinetics of *B. japonicum* ARC 501 calculated from log phase of the growth curve in modified M9 liquid medium supplemented with increased amounts of siderophore extracted from *Ps. fluorescens* B culture are given in Table (3). Generally calculations of specific growth rates (μ) multiplication rates (MR) and doubling times (td) of *B. japonicum* ARC 501 showed positive correlation with increased concentration of introduced siderophores being 0.11, 0.16 and 6.3 with supplementation of 200 μl of extracted siderophores, respectively. Data in Fig. (1) present the positive correlation coefficient between the optical density of *B. japonicum* ARC 501 and amount of introduced siderophores. On the other hand, *F. oxysporum* was not able to utilize the bacterial siderophores for growth under iron limited condition as fungal dry weight showed a gradual decrease with increased concentrations of incorporated *Ps. fluorescens* B siderophore being 2.91, 2.43, 2.01, 1.72 and 1.51 g100 ml^{-1} medium with 0.0, 50, 100, 150, and 200 μl 100 ml^{-1} medium of introduced siderophores respectively (Fig. 2).

Table (3): Growth kinetics of *Bradyrhizobium japonicum* ARC 501 grown with increased concentrations of siderophore extracted from *Ps. fluorescens* B.

Amount of extracted siderophore		Growth parameters			
		Specific growth rate (μ)	Doubling time (td)	Multiplication rate (MR)	Number of generation (N)
(- Fe)	0.0 µl	0.061	11.36	0.080	6.69
0.1 µM + Fe (III)	0.0 µl	0.065	10.66	0.090	7.13
	50 µl	0.068	10.19	0.100	7.46
	100 µl	0.088	7.88	0.127	7.36
	150 µl	0.091	7.61	0.130	6.83
	200 µl	0.110	6.30	0.160	7.30

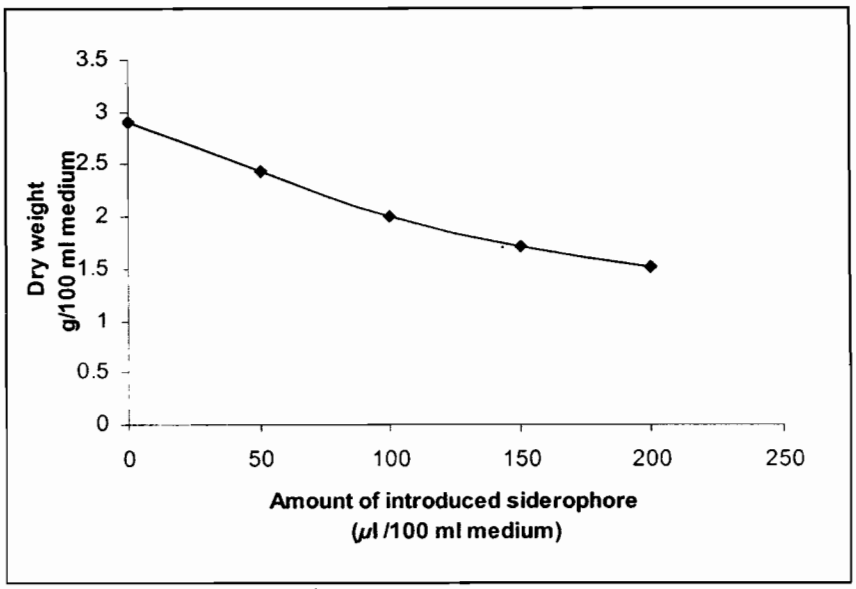


Fig.(1):Growth of Fusarium oxysporum f.sp. glycine as influenced by introduced siderophore extracted from Ps. fluorescens B.

Effect of EDDHA as a synthetic iron chelator on growth and siderophores production by phytopathogenic fungi

The increased concentrations of EDDHA with Fe binding affinity similar to bacterial siderophore also resulted in gradual decrease or even complete elimination of fungal growth with the highest concentration of EDDHA. While *Rh. solani* growth was completely depressed with incorporation of 1500 mg EDDHA / L medium into culture medium, *F. solani* and *F. oxysporum* f. sp. glycine gave the same response with 2000 mg EDDHA / L medium. Siderophore production, on the other hand, showed a gradual increase with increased concentrations of added EDDHA up to 1500 mg / L medium of *F. solanii* and *F. oxysporum* f.sp. *glycine*. However,*F. oxysporum* f. sp. *glycine* gave a relatively greater amount of dry mass and siderophores, than the other 2 tested fungi (Fig. 2).

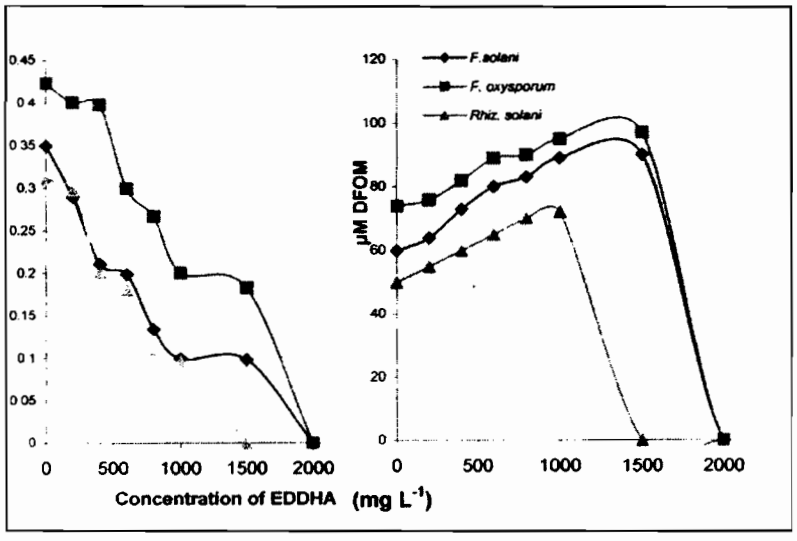


Fig.(2): Effect of increased concentrations of EDDHA on growth and siderophore production by 3 strains of phytopathogenic fungi on modified M9 liquid medium.

Effect of introduced siderophore and Fe-EDDHA on cell density of *B. japonicum* and its ability to induce root hair curling in soybean

Data in Table (4) clearly demonstrate that *B. japonicum* ARC 501 effectively utilized the introduced siderophore of *Ps. fluorescens* B as shown by gradual increase in cell density to nearly similar levels of those obtained from available iron (Fe-EDDHA) treatment. The recorded figures were 7.5, 20 and 38 against 9, 28 and 41 CFU/ g sand, for introduced siderophore and Fe-EDDHA, respectively. No changes in root hair morphology were observed in seedling roots examined after 2 days from inoculation. However, 3.7 and 8.5 % of root hairs of soybean seedling supplemented with *Ps. fluorescens* B siderophore, showed curling phenomenon after 5 and 8 days from inoculation, respectively. The corresponding records of Fe-EDDHA treatment were 4.4 and 9.7 % at the same above-mentioned respective order. The ability of the bacterium to induce root hair curling under the two treatments is shown in Figure (4).

Performance of soybean as affected by coinoculation with *B. japonicum* and *Ps. fluorescens*.

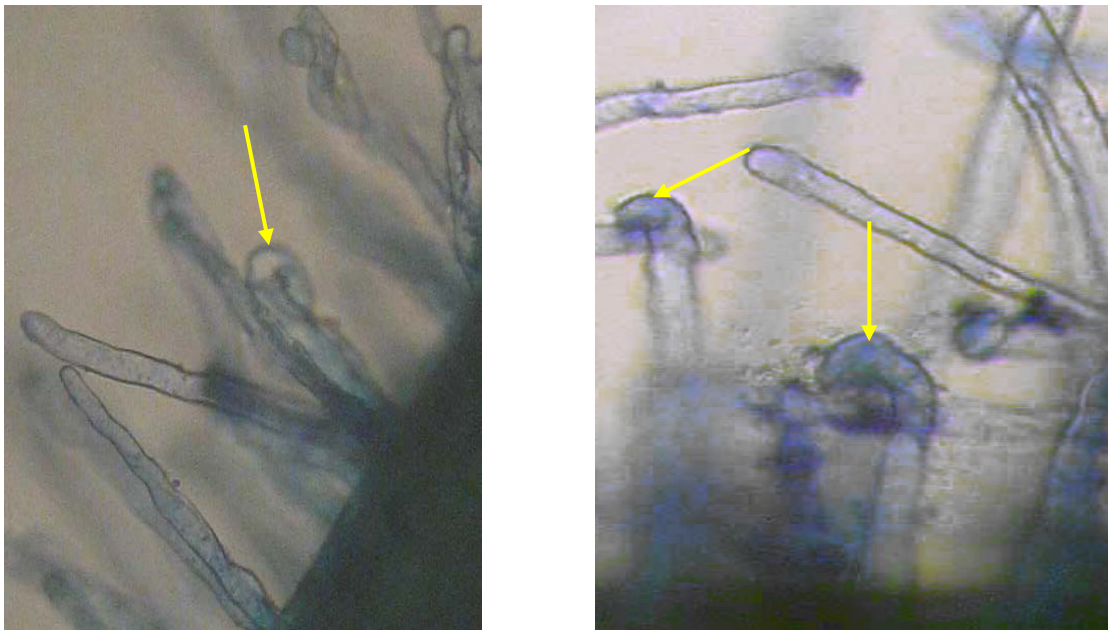
The finding that *B. japonicum* ARC 501 showed varied response to 2 effective siderophore producers *Ps. fluorescens* strains in the antagonism test, i.e. being tolerant to *Ps. fluorescens* B and suscetiple to *Ps. fluorescens* 1 under Fe limited condition was used to study their interactions in relation to nodulation, soybean growth and N as well as Fe uptake in sand culture supplemented with $Fe(OH)_3$ and sandy soil naturally limited in available iron

Sand culture experiment

Significantly, higher values of dry weights, nodulation, N and Fe uptake were reported for soybean plants inoculated with *B. japonicum*

Table (4): Effect of introduced siderophore and Fe-EDDHA on cell density of *B. japonicum* ARC 501 and its ability to induce root hair curling in soybean.

Time in days	Treatment			
	Introduced siderophore		Fe-EDDHA	
	B. japonicum ARC 501 CFU x 10^4/g sand	Root hair curling (%)	*B. japonicum* ARC 501 CFU x 10^4/ g sand	Root hair curling (%)
0	4.3	0.0	4.3	0.0
2	7.5	0.0	9.0	0.0
5	20.0	3.7	28.0	4.4
8	38.0	8.5	41.0	9.7



A **B**

Fig. (3): Root hair curling as induced by *B. japonicum* ARC 501 grown with introduced *Ps. fluorescens* B siderophore (A) and Fe-EDDHA (B).

ARC 501 and supplemented with 0.5 m mol Fe as Fe-EDDHA compared with those obtained from plants grown without Fe. Similar effects were obtained when *B. japonicum* ARC 501 was conjugated with the non-antagonistic *Ps. fluorescens* B in the presence of 0.2 m mol Fe as $Fe(OH)_3$. In this respect, no significant differences were generally observed between the effect of sand amendement with 0.2 or 0.5 $Fe(OH)_3$. When soybean plants were inoculated with *B. japonicum* ARC 501 conjugated with its antagonist, i.e. *Ps. fluorescens* 1, insignificant growth responses were recorded, as compared with that of singly inoculated plants grown with either 0.2 or 0.5 m mol Fe as $Fe(OH)_3$. This finding was also true for the records of number of nodules but nodule dry weight, N and Fe uptake (Table 5) showed significant increases due to that treatment. However, the non- antagonestic *Ps. fluorescens* B gave significantly higher records of all tested parameters than those obtained from the antagonestic *Ps. fluorescens* 1 in dual inoculation treatment.

Table (5): Effect of siderophore producing *Ps. fluorescens* strains 1 and B on growth, nodulation and N & Fe up take of soybean (*Glycine* max cv. crowford) inoculated with *Bradyrhizobium japonicum* ARC 501 and grown in the presence of Fe – EDDHA or 2 concentrations of iron as Fe $(OH)_3$.

Inoculation treatment	Fe source		Total dry weight (g / plant)	No. of nodules (/ plant)	Dry wt. of nodules (mg / plant)	Nitrogen up take (mg/shoot)	Fe uptake (ppm)
B.japonicum ARC 501	- Fe		1.30c	7 e	60 f	6.82 f	68 g
	Fe-EDDHA* (0.5m mol Fe)		2.90a	23 ab	120 b	50.60 b	200 b
B. japonicum ARC 501	$Fe(OH)_3$	0.2 m mol	1.90b	12 d	90 e	20.25 a	125 f
		0.5 m mol	2.04b	16 c	95 d	23.05 d	135 a
B. japonicum ARC 501 +*Ps. fluorescens* 1		0.2 m mol	2.00b	13 cd	102 c	24.16 d	160 d
		0.5 m mol	2.10b	14 cd	102 c	27.03 c	168 c
B. japonicum ARC 501 + *Ps. fluorescens* B		0.2 m mol	3.00a	22 b	122 ab	61.25 a	200 b
		0.5 m mol	3.00a	26 a	125 a	61.00 a	218 a

Means in a column not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

* Fe-EDDHA : Ferric ethylenediamine – di (0-hydroxy phenyl acetic acid)

Sandy soil experiment

The effects of *Ps. fluorescens* B co-inoculated with *B. japonicum* ARC 501 in sandy soil was significantly suprior to the single treatment of *B. japonicum* ARC 501, in enhancing chlorophyll content and total dry weight being 19.1 mg/g leaves and 3.71 g / plant against 7.9 mg / g leaves and 2 g/ plant, respectively (Table 6).

Table (6) Effect of siderophore producing *Ps. fluorescens* strains 1 and B on growth, nodulation and N & Fe uptake of soybean (*Glycine max* cv. crawford) inoculated with *Bradyrhizobium japonicum* ARC 501 grown in sandy soil.

Parameter	Treatment		
	B. japonicum ARC 501	*B. japonicum* + *Ps. fluorescens* 1	*B.japonicum* + *Ps. fluorescens* B
Total chlorophyll content (mg /g leaves)	7.9 c	9.70 b	19.1 0a
Shoot dry weight (g / plant)	1.6 b	1.80 b	3.01 a
Root dry weight (g / plant)	0.4 b	0.49 ab	0.70 a
Total dry weight (g / plant)	2.0 b	2.29 b	3.71 a
No. of nodules (/ plant)	23.0 c	29.00 b	38.00 a
Nodules dry weight (mg / plant)	100.0 c	119.00 b	150.00 a
Total nitrogen uptake (mg/shoot)	28.8 c	34.20 b	71.63 a
Fe uptake (ppm)	165.0 c	174.00 b	213.0 a

Means in a raw not followed by the same letters are significantly different by Duncan's LSD test (≥ P 0.05)

Variable increases were also reported for the above-mentioned treatment, in the number and dry weight of nodules as well as N and Fe uptake of dually inoculated plants compared with those only inoculated with *B. japonicum* ARC 501 being 65, 50, 149 and 29% respectively. In fact, soybean plants grown under the latter treatment showed iron deficiency symptoms (Figure 5 A). On the other hand, insignificant differences were observed between the combined effect of *B. japonicum* ARC 501plus *Ps. fluorescens* 1 regarding plant dry matter. However, the effect of that combination on chlorophyll content, N and Fe uptake were remarkable, when compared to those of *B. japonicum* ARC 501 applied alone. However, the records of nodulation parameters were also significantly higher for the dual inoculation treatment compared to single treatment. In conclusion, the superiority of *Ps. fluorescens* B to *Ps. fluorescens* 1 in dual inoculation treatments was evident in 9 out of the 10 recorded parameters.

(A)

(B)

Fig. (4): Growth and nodulation of soybean plants in sandy soil limited in available iron content

A. Inoculated with *B. japonicum* ARC 501 (notice Fe deficiency symptoms on leaves)

B. Inoculated with *B. japonicum* ARC 501 + *Ps. fluorescens* B

DISCUSSION

Siderophores mediated interactions are known to affect microbial activities in plant rhizosphere. In this study, the role of siderophore extracted from the effective producer *Ps. fluorescens* B was evaluated in relation to iron acquistion capacity of microbial strains representing two major groups affecting soybean growth, i.e. root nodule bacteria and phytopathogenic fungi. Incorporation of ethylendiamine di o-hydroxy phenyl acetic acid (EDDHA) into the culture medium reduced fungal growth to a level comparable to added concentration. This finding is in harmony with that reported by **Vandenbergh *et al.* (1983)**. The bioassay using an EDDHA chelator to compete with the siderophore – producing organisms was first introduced by **Miles and Kjimji (1975)**. The test indicates the efficiency of iron-binding siderophores as compared with that of the chemical chelator EDDHA. *F.oxysporum* f.sp. *glycine* was also unable to utilize *Ps. fluorescens* B siderophore and gave decreased amounts of dry mass comparable to increased concentration of introduced siderophore. This finding may indicate that the fungal siderophore had a lower affinity for Fe than the introduced *Pseudomonas* siderophore. **Bezbaruah *et al.*, (1996)**, similarly reported that purified siderophores from *Pseudomonas* strains RRLJ 30 and RRLj 17 inhibited the growth of *Fomes lamoenses,* a common pathogen on tea. **Manwar *et al.* (2001 & 2004)** also reported that siderophores extracted from a culture supernatent of *Pseudomonas* SCI, inhibited the growth of *Aspergillus flavus, F. oxysporum* and *Sclerotium rolfsii.* On the other hand, *B. japonicum* ARC 501 grown with *Pseudomonas fluorescens* B siderophore gave positive growth responses which were correlated with the concentration of introduced siderophore. *B. japonicum* USDA 110 and 61A152 were also found to utilize the hydroxamate type siderophores ferrichrome and rhodotorulate, as well as the pyoverdin – type siderophore pseudobactin St 3 in addition to ferric citrate, to overcome iron starvation **(Plessner *et al.*, 1993)**. Interestingly, **Jadhav and Desai (1994)** showed that a siderophore non – producing mutant, which was unable to grow on a medium containing synthetic iron chelators unless and until iron was added exogenously in the medium, could use siderophore of the wild type for iron uptake. Stimulation of bacterial growth with exogenous siderophores under iron – limited and low nutrient conditions was also reported by **Guan *et al.* (2000).**

In sand culture supplemented with either of 2 concentrations of $Fe(OH)_3$, the *Ps. fluorescens* B conjugated with *B. japonicum* ARC 501 showed significant increases in growth, nodulation and N as well as Fe uptake compared to inoculation with the *B.japonium* ARC501 +*Ps. fluorescens* 1, and both were superior to *B. japonicum* ARC 501 inoculation alone. These positive effec could be explained by the ability of *B. japonicum* ARC 501 to utilize *Ps. fluorescens* B siderophore under Fe limited condition as shown in cultured soybean seedlings. This ability was examplified by gradual increase in cell density and induction of root hair curling to nearly similar levels of those obtained from available iron (Fe-EDDHA). The finding that the antagonitic strain *Ps. fluorescens* 1, didn't show negative effect on *B. japonicum* ARC 501 is in agreement with that reported by **Kosslak and Bohlool (1985)** who attempted to modify nodulation competition between strain 110 and 123 by inoculation with actinomycete displaying *in vitro* inhibition against strain 110, but reported negative results. The importance of siderophore produced by microorganisms to supply Fe to plants has been suggested by many workers. For example, the catecholate siderophore agrobactin stimulates Fe uptake by pea and bean plants **(Becker *et al.*, 1985)**. **Jurkevitch *et al.*, (1988)** also reported that bacterial siderophores may help to overcome lime-induced chlorosis in groundnut (*Arachis hypogaea*) grown in calcerous soils. This effect was

also reported in this study for soybean plants grown on sandy soil limited in Fe content (Fig. 5 B). **Sakthivel *et al.* (1986)** also showed that co-inoculation of green gram with *Pseudomonas* strains MRS 13, MRS 16 and *Bradyrhizobium* sp. (vigna) strain S24, showed a significant increase in nodule weight, plant dry weight and total plant N as compared to inoculation with *Bradyrhizobium* strain S24 alone. **Sharma and Johri (2003)** used the siderophore producing *Pseudomonas* GRP 3 to inoculate mung bean in the presence of Fe-citrate, Fe-EDTA and $Fe(OH)_3$ in different concentrations with Hoagland's solution. After 45 days, the GRP 3 bacterized plants showed a reduction of chlorotic symptoms and enhanced chlorophyll level. In 10 μM Fe – citrate along with GRP 3 treatment, total chlorophyll contents increased significantly by 39% compared to the control. There was also a significant increase in total and physiologically available iron. A closely similar pattern was generally observed in chlorophyll content in Fe-EDTA and $Fe(OH)_3$ treated plants.

CONCLUSON

The variability of sideraphores utilizing ability among root nodule bacteria and phytopathogenic fungi seemed to be strongly related to enahcned nodulation from one side and suppression of phytophthogenic fungi from the other side. Therefore, *Ps. Fluorescens* producing siderophores represent an effective tool to improved nodulation growth and N and Fe uptake of soybean under iron limited condition.

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التداخلات المتعلقة بالسيدروفورس وعلاقتها بنمو
Bradyrhizobium japonicum , Fusarium oxysporum f.sp. glycine
وفول الصويا تحت ظروف نقص الحديد

مجدى اسماعيل مصطفى ، سهير أحمدابراهيم نصر

ايناس عبد التواب حسن ، محمد الصاوى مبارك

قسم الميكروبيولوجيا – كلية الزراعة – جامعة عين شمس – القاهرة – مصر

تم فى هذه الدراسة إضافة تركيزات متزايدة (٥٠ ، ١٠٠ ، ١٥٠ ، ٢٠٠ ميكرولتر) من السيدروفور المستخلص من السلالة *Pseudomonads fluorescens* لبيئات نمو *F. oxysporum f.sp. glycine* , *B. japonicum ARC 501* ومن ناحية أخرى أضيفت المادة المخلبية الإصطناعية ايثيلين داى امين – داى – اورثو – هيدروكسى فينيل حمض الخليك (EDDHA) المعروفة بقدرتها العالية على ربط الحديد ، بتركيزات ٢٠٠ ، ٤٠٠ ، ٦٠٠، ٨٠٠ ، ١٠٠٠ ، ١٥٠٠ ، ٢٠٠٠ ، ٢٥٠٠ مجم / لتر من بيئة M9 التى استخدمت لتنمية النوع الفطرى السابق بالإضافة للنوعين *Fusarium solani* , *Rhizoctonia solani* . وقد أظهرت النتائج أنه فى حين كانت *B. japonicum* ARC 501 ذات كفاءة فى تمثيل التركيزات المتزايدة من السيدروفور المستخلص من B *Ps fluorescens* قلت كمية نمو الفطر *F. oxysporum* f.sp. *glycine* بزيادة هذه التركيزات وكان هذا أيضاً هو نمط استجابة الفطريات الثلاثة للتركيزات المتزايدة من مادة EDDHA وعند تلقيح بادرات فول الصويا النامية فى الرمل بأنابيب اختبار بالسلالة *B. japonicum* ARC 501 والسابق تجويعها من عنصر الحديد مع إضافة السيدروفور المستخلص زادت أعداد الخلايا مع حدوث انحناءات للشعيرات الجذرية بمستويات تشابه تلك المتحصل من التلقيح مع إضافة Fe- EDDHA بدلاً من السيدروفور المستخلص. ثم أجريت تجربة استخدم فيها رمل مغسول بالحامض ومسمد بالحديد على صورة Fe- EDDHA , $Fe(OH)_3$ منفردة أو لتنمية فول الصويا مع تلقيحه بالسلالة *B. japonicum* ARC 501

مختلطة مع السلالتين *Ps. fluorescens* B أو *Ps. fluorescens* 1 المتباينتين فى تضادهما لسلالة البكتريا العقدية ونميت النباتات تحت ظروف الصوبة السلكية لمدة ٤٥ يوم حيث جمعت النباتات لتسجيل الوزن الجاف للمجموع الجذرى و الخضرى ودرجة التعقيد وكمية النتروجين والحديد الممتص بالمجموع الخضرى للنباتات النامية تحت المعاملات المختلفة ثم أعيد اختبار معاملات التلقيح مرة أخرى فى تربة رملية تتسم بقلة محتواها من الحديد بصورة طبيعية وقد أظهرت النتائج أن نباتات فول الصويا النامية فى الرمل المغسول والمسمد بـ $Fe(OH)_3$ والملقحة بالسلالتين *Ps. fluorescens* B , *B. japonicum* ARC 501 (الغير مضادة) قد أظهرت استجابة إيجابية لا تختلف معنوياً عن تلك المتحصل عليها عند التلقيح بسلالة البكتريا العقدية منفردة فى وجود Fe-EDDHA . وعلى أى حال كانت مستويات التحسن معنوية إذا ما قورنت بمعاملة التلقيح الفردىبسلالة البكتريا العقدية منفردة أو مختلطة بالسلالة *Ps. fluorescens* 1 (المضادة) وتم التحصل على نفس النتائج بالنسبة للنباتات النامية فى التربة الرملية ذات المحتوى القليل من الحديد طبيعياً.