Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2012, 3 (3):1661-1666
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

The Effect of *Thiobacillus* and *Pseudomonas fluorescent* Inoculation on Maize Growth and Fe Uptake

***Zarrin Taj Alipour[1] and Adeleh Sobhanipour[2]**

[1]*Department of Soil Science, Damghan Branch, Islamic Azad University, Damghan, Iran*
[2]*Department of Plant Protection, Damghan Branch, Islamic Azad University, Damghan, Iran*

ABSTRACT

Although Fe is easily found in earth crust and soil, it is not available to plants in most soils especially in calcareous soils of dry warm climates with high pH. So this study was conducted in 2010 to evaluate the effect of Thiobacillus and Pseudomonas fluorescent inoculation on maize growth and Fe uptake in a calcareous soil. The experimental design was factorial in the form of a completely randomized, with four replications. Treatments of the experiment were sulfur (0 and 100 kg/ha), P. fluorescent (with and without inoculation) and Thiobacillus (with and without inoculation). Five maize seeds of S.C. 704 cultivar were planted in each pot containing 7 kg soil, after germination two of them were thinned to reach the density of three plants/pot. During the 90 days of growth period, soil moisture was held at 70% of field capacity. Before the final harvest, leaves chlorophyll content were measured. Plant's fresh and dry weight, plant height and Fe uptake were also measured after harvest. Results indicated that Thiobacillus had no significant effect on any of the measured traits; sulfur and P. fluorescent significantly affected the measured traits ($P \leq 0.05$). Maize Fe uptake was also affected by P. fluorescent inoculation.

Keywords: organic farming, plant nutrition, siderophores, sulfur.

INTRODUCTION

Although large amount of Fe exists in earth crust and soil, Fe is usually a limiting nutrient to plants especially in calcareous soils of dry warm climates with high pH. Some plants faces Fe deficiency and chlorosis in soils with carbonate calcium content of more that 5%; this chlorosis is called lime induced chlorosis as it is caused by high lime concentration [1-3]. However, there are cultivars that can absorb soil Fe more efficiently than the others and grow better in calcareous soils [2, 4].

Some microorganisms such as *Thiobacillus* and *Pseudomonas* improve plant Fe absorption. The mechanisms used by the two microorganisms are totally different.

Pseudomonas bacteria produce siderophores under Fe deficiency conditions. Siderophores are organic molecules which bound Fe^{+3} and act like chelates; transporting Fe into plant roots. Siderophor production is a very important mechanism against low available Fe conditions [5-7]. There are also documents proving the effects of siderophores on plant resistance to pathogens [8-9].

Different researchers have studied the effects of *Pseudomonas* on crops growth and yield. Waller and Cook (1982) reported that inoculating wheat seeds with *Pseudomonas fluorescent* increased wheat yield by 147% in sterile soil and by 27% in unsterile soils [10]. In another experiment, application of some *P. fluorescent* strains increased wheat yield by 17% [11].

Thiobacillus bacteria are chemolithotrophs and secure their energy by sulfur oxidation [12]. This feature of *Thiobacillus* bacteria is also effective on plants Fe uptake. When there is sufficient population of *Thiobacillus* bacteria in soil, they start sulfur oxidation which results in the reduction of soil pH; increasing the availability of nutrients to plants roots [13].

Finally, this experiment was conducted to improve maize growth, yield and nutrient uptake by the application of *Thiobacillus*, *Pseudomonas* and sulfur, in a high pH, calcareous soil.

MATERIALS AND METHODS

This experiment was conducted in factorial in the form of a completely randomized design with four replications. Treatments include: *Pseudomonas* (with and without inoculation), *Thiobacillus* (with and without inoculation) and sulfur (0 and 1000 kg/ha).

Preparation of *Thiobacillus* inoculant. To prepare the *Thiobacillus* inoculant, 250 ml of the inoculant including 10^7 *Thiobacillus* bacteria/ml was received from Karaj Biology Laboratory; the volume was then increased to 8 L. To do this, the Postgate culture medium was used, which is a suitable medium for *Thiobacillus*.

Preparation of *Pseudomonas* inoculant. To prepare the *Pseudomonas* inoculant, "M" type sampling was conducted on maize farms to collect *P. fluorescent*. Samples were put in plastic bags and sent to the laboratory.

In laboratory, 1kg of each sample was put in test tube containing 9 ml distilled water and shook with a shaker. Different concentrations were prepared from each tube, in serial form, and 0.1 ml of each concentration was poured in King B medium. Petri dishes were kept in 25-28^{o}C for 48 h. After colonies formation, petri dishes with the best concentration were selected and fluorescent colonies were determined using 366 nm UV ray, and were purified on a NA medium by the method of streaking. To make sure the isolates were gram negative, the test of sensitivity to 3% KOH was carried out. Finally, colonies were put in sterile distilled water and kept in 4^{o}C, for long time preservation.

Siderophor production assay. The Waller and Cook (1982) method was used to evaluate the production of siderophores [10]. Bacterial isolates were cultured on KB culture medium containing 0, 25, 50, 100 and 1000 µmol iron (III) chloride and kept in 25^{o}C for 48 h. Then, the fungus *Geotrichum candidum* spore suspension, which was obtained by its 48 h culture on PDA medium with sterile distilled water, was sprayed on the bacteria inside the petri dishes. Absence of fungus growth around the bacteria is the indicator of the siderophor production. This experiment had three replications for each concentration.

Preparation of *Pseudomonas* strain inoculant for soil treatment experiments. First, all *Pseudomonas* strains were cultured on PDA medium and kept in 26-28^{o}C for 24 h. Then, the petri dishes surface was washed by 10 ml sterile distilled water and strains suspension were evaluated by spectrophotometer for their optical absorption. 48 h after maize seeds were planted in pots, the suspension was used to inoculate seeds.

After preparing the microorganisms inoculants, in May 2010, samples were taken from a calcareous soil (20%) in depth of 0-30 cm. soil samples were dried in open air and passed through 4 mm sieve. 10 kg of this soil was used to fill each pot. Maize seeds were first sterile by 2% Tween (Sigma) solution for 10 minutes and finally, washed with distilled water for 10 minutes. After planting seeds in pots, 1000 kg S/ha and 100 cc *Pseudomonas* and *Thiobacillus* (58 cfu/g) suspension was added to each pot. The properties of the soil and the irrigation water are listed in Tables 1 and 2, respectively.

Table 1. Properties of the soil applying the treatments

Zn (mg/kg)	Fe (mg/kg)	Gypsum (%)	Lime (%)	O.C (%)	pH	EC (ds/m)	Texture
1.8	2.1	5	20.7	0.07	8.1	1.7	Loamy silt

Table 2. Properties of the irrigation water

Na (Meq/l)	Cl (Meq/l)	Bicarbonate (Meq/l)	Carbonate (Meq/l)	Mg (Meq/l)	Ca (Meq/l)	TDS (mg/l)	pH	EC (ds/m)
0.8	0.9	1.4	0	1.5	3	0.6	7.9	0.54

All the end of maize growth period, in Sep. 2010, sampling was conducted. Before harvest, leaves chlorophyll content was determined by SPAD chlorophyll meter. Then, maize plants were removed from soil (both root and shoot), to measure the other parameters.

Soil, leaf and water analysis were conducted according to the suggested methods by Iranian Soil and Water Research Institute. In the samples, soil pH was measured by pH meter, organic carbon by Walky-Blacky method, available K by ammonium acetate extraction, TNV based on calcium carbonate, and the micronutrients in leaves by digestion according to the method of dry burning with 2 normal chloridric acid by the means of atomic absorption instrument. In the samples of irrigation water, pH, EC, bicarbonate, chlorine, sulfate, calcium, magnesium, sodium, potassium and SAR were measured.

Finally, data were analyzed using SAS (1988) [14] and means were compared by Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Analysis of the variances indicated that *Thiobacillus* had no significant effect on plant dry weight, but *Pseudomonas* and sulfur significantly affected the traits at $P \leq 0.01$ and $P \leq 0.05$, respectively (Table 3). Mean comparison also showed that the highest yield was achieved when *Pseudomonas* was applied; the lowest yield was related to the control. *Thiobacillus* in this experiment had no effect on maize growth. This can be attributed to the low population of *Thiobacillus* bacteria in soil or to the lack of colonization in the rhizosphere [15].

Application of sulfur significantly affected dry weight at $P \leq 0.05$. Sulfur is an acidifier which reduces the pH of calcareous and alkaline soils and facilitates the absorption of some nutrients. Sulfur also improves the condition of sodium soil and controls some plant pathogens.

Kaplan and Erman (1998) conducted greenhouse and filed experiments and found that sulfur application increased sorghum yield and Fe, Zn, P and Mn uptake [16]. Singh et al. (1991) reported that application of 12.5, 25 and 50 mg S/kg increased lentil grain yield by 7.4, 16.8 and 11.7%, respectively [17]. Klopper et al. (1980) concluded that inoculating radish with *Pseudomonas* significantly increased yield [18]. Growth promoting bacteria improve plant growth and yield by the production and exudation of different growth promoting substances such as phytohormones and vitamins. Duffy et al. (1996) proved that inoculating wheat seeds with special strains of *Pseudomonas fluorescent* increased grain yield by 17% [11].

Analysis of the variances represented that *Pseudomonas* significantly affected plant height; the effect of *Thiobacillus* and sulfur was not significant (Table 3). Attoe and Olson (1996) attributed the low improvement of wheat yield in sulfur treatment to insufficient time for sulfur oxidation which results in low sulfur oxidation [19]. Results of this experiment showed that the lowest plant height was achieved in the treatment with *Pseudomonas* and without *Thiobacillus* and sulfur.

Co-application of sulfur and *Pseudomonas fluorescent* strains significantly increased Fe uptake. Mean comparison revealed that leaves Fe content was significantly higher in plants inoculated with *Pseudomonas* and received sulfur, than in the control. Sulfur increased Fe content by 25% and *Pseudomonas* increased it by 23%, compared with the control. Kaplan and Erman (1998) in their greenhouse and field experiments observed that application of sulfur increased sorghum yield and Fe, Zn, P and Mn uptake [16]. *Pseudomonas* bacteria produce siderophores which results in the improvement of plant Fe uptake. An increased Fe uptake promotes chlorophyll synthesis, and as the results of our experiment showed, *Pseudomonas* inoculation increased chlorophyll production.

Results indicated that application of *Thiobacillus* had no effect on chlorophyll production. The non-significant effect of *Thiobacillus* on sulfur oxidation and chlorophyll production can be attributed to low population of the bacteria, incompatibility of the bacteria with the soil and climatic condition, low colonization rate and short period of the experiment [15].

Table 3. Analysis of the variances for the measured traits

SOV	df	Mean Squares (MS)				
		Dry weight	Plant height	Fe	Zn	Chlorophyll
Sulfur (S)	1	*	ns	*	*	*
Thiobacillus (T)	1	ns	ns	ns	ns	ns
Pseudomonas (P)	1	**	*	*	ns	*
T×S	1	*	ns	ns	ns	ns
P×S	1	*	ns	ns	ns	ns
T×P	1	ns	*	*	ns	ns
T×P×S	2	ns	ns	ns	ns	ns
Error	-	147	86	36.29	1.06	5.6
CV (%)	-	9.4	10.46	7.64	7.9	3.6

*ns, nonsignificant; *, significant at $P\leq0.05$; **, significant at $P\leq0.01$*

Zn uptake was significantly affected only by sulfur; the effect of *Thiobacillus* and *Pseudomonas* was non-significant (Table 3). Sulfur can be oxidized in soil and produce sulfuric acid which results in the reduction of soil pH; lower pH releases the fixed nutrients such as Zn and makes the available to plant roots.

Pseudomonas improves plant height and shoot growth by producing plant growth promoting substances such as auxin and cytokinin. The bacterium also produces rhizobiotoxin which reduces ethylene content in plant. This condition promotes the development of plant root system so plant can use higher soil volume as the source of water and nutrients; increasing water and nutrients absorption efficiency.

Table 4. The effect of *Thiobacillus* (T), *Pseudomonas* (P) and sulfur (S) on the measured traits

Treatments	Dry weight (g/pot)	Plant height (cm)	Fe (mg/kg)	Zn (mg/kg)	Chlorophyll
S0	59.66a	83.00a	89.33b	25.08b	60.62b
S1	69.08a	91.50a	112.66a	29.10a	70.45a
T0	62.70a	84.33a	97.16a	26.60a	65.20a
T1	66.50a	90.25a	104.83a	27.75a	67.00a
P1	79.25A	95.50a	110.16a	27.32a	70.79a
P0	49.50b	79.08b	91.02b	27.02a	61.29b
T0S0P1	94.00a	108.00a	127.30a	30.30a	75.000a
T1S1P1	77.30b	106.60a	120.00b	29.80a	71.667bc
T1S1P0	76.60b	90.00b	114.00c	28.20b	69.500bc
T1S0P1	76.60b	88.30b	107.60d	27.90b	68.333bc
T0S1P1	69.00b	80.00cb	101.60e	25.80c	67.000c
T0S1P0	53.30c	79.60cb	85.60f	25.30c	66.833c
T1S0P0	35.60d	79.00cb	81.30f	25.00c	55.167d
T0S0P0	32.30d	66.60c	70.30g	24.90c	54.833d

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$

CONCLUSION

Overall, results of this experiment proved the significant effect of *Pseudomonas* on all the measured traits except for Zn uptake. Sulfur had also a significant effect on all the measured traits except for plant height. On the contrary, *Thiobacillus* application had no effect on any of the measured traits. The effects of two-fold and three-fold interactions were nonsignificant in most cases.

Acknowledgement
This paper is derived from a research project funded by Islamic Azad University, Damghan branch, Iran.

REFERENCES

[1] L Bavaresco, E Giachino and S Pezzutto, *J Plant Nutr*, **2003**, 7, 1451-1465.
[2] N Jelali, I Ben Salah, W M'sehli, S Donnini, G Zocchi and M Gharsalli, *Scientia Horticulturae*, **2011**, 1298, 548-553.
[3] R Ksouri, M Gharsalli and M Lachaal, *J Plant Physiol*, **2005**, 162, 335-341.
[4] RJ Goos and BE Johnson, *Agron J*, **2000**, 92, 1135-1139.
[5] H Marschner and V Rmheld, *Plant and Soil*, **1994**, 165, 375-388.
[6] A Sharma and BN Johri, *Microbiological Research*, **2003**, 158, 77-81.
[7] A Sharma, BN Johri, AK Sharma and BR Glick, *Soil Biol Biochem*, **2003**, 35, 887-894.
[8] M Shoda, *Journal of Bioscience and Bioengineering*, **2000**. 69, 515-521.
[9] LS Thomashow and DM Weller, *Plant and Soil*, **1990**, 129, 93-99.
[10] DM Waller and RJ Cook, *Phytopathology*, **1982**, 73, 463-469.
[11] BK Duffy, A Simon and DM Weller, *Phytopathology*, **1996**, 86, 188-194.
[12] SL Tisdale, WL Nelson and JD Beaton, Soil Fertility and Fertilizers, 4th ed, Mc Millan Publishing Company, New York, **1984**.

[13] K Killham, Soil Ecology, Cambridge University Press, UK, **1994**.
[14] SAS Institute Inc, SAS/STAT User's Guide, Version 6, 4th ed. Statistical Analysis Institute Inc, Cary North Carolina, **1988**.
[15] SD Sharma and VP Bhutani, *Horticultural-Journal*, **1998**, 11, 1-8.
[16] M Kaplan and S Erman, *J Plant Nutr*, **1998**, 21 (8), 1655-1665.
[17] V Singh, AK Parashar and VS Mehta, *J Ind Soc Soil Sci*, **1991**, 39, 727-729.
[18] JW Klopper, MN Schroth and TD Miller, *Phythology*, **1980**, 70, 1078-1082.
[19] OJ Attoe and RA Olson, *Soil Sci*, **1996**, 101, 317-324.