JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT
ISSN: 2339-076X, Volume 3, Number 2 (January 2016): 507-516
DOI:10.15243/jdmlm.2016.032.507

Research Article

Effects of *Pseudomonas fluorescens* and sulfur on nutrients uptake, growth and yield of groundnut in an alkaline soil

H. Pratiwi[1,2*], N.Aini[3], R. Soelistyono[3]

[1] Postgraduate Programme, Faculty of Agriculture, Brawijaya University, Jl. Veteran, Malang 65145, Indonesia.
[2] Indonesian Legumes and Tuber Crops Research Institute, Indonesian Agency for Agricultural Research and Development, Jl. Raya Kendalpayak Km 8 PO BOX 66, Malang 65101 Indonesia
[3] Department of Agronomy, Faculty of Agriculture, Brawijaya University

*corresponding author: herdina_p@mail.com

Abstract: The aim of this research was to study the influence of *P. fluorescens* and sulfur on nutrients uptake, growth and yield of groundnut in alkaline soil. The experiment was conducted in the greenhouse in Malang from January 2015 to Mei 2015. The experiment was laid out in factorial randomized block design consisted of two treatment factors and three replications. The first factor were concentration of *P. fluorescens* (0 cfu/mL; 10^7 cfu/mL; and 10^9 cfu/mL). The second factor were elemental sulfur doses (0 g/kg soil; 1 g/kg soil; 2 /kg soil; and 3 g/kg soil). Soil used was collected from Lamongan East Java, Indonesia. Soil previously was given 40 g $Ca(OH)_2$ /kg soil to achieve pH >8. There was no interaction between *P. fluorescens* and sulfur on all of parameters observed. *P. fluorescens* concentration of 10^9 cfu/mL independently significantly increased availabe Fe in soil as 34.75% compared with the control and could maintain the populations of *P. fluorescens* better than the concentrations of 0 and 10^7 cfu/mL. Sulfur significantly correlated with decrease in soil pH expressed by equation y = -0,226x + 8.218. Sulfur independently significantly increased N, P, S, Ca and Mn uptake by plants. Sulfur dose of 3 g/kg of soil provided leaves and stems growth better, increased 80.74% of pod yield and 34.09% of harvest index compared to control.

Keywods : *alkaline soil, PGPR, Pseudomonas fluorescens, sulfur*

Introduction

Groundnut is the fourth leading food crop after rice, maize and soybean in Indonesia. National yield is only about 1.2 t/ha with harvested area is steadily declining from year to year (Central Bureau of Statistics Indonesia, 2014), whereas the potential yield of groundnut is above 2 t/ha (Indonesian Legumes and Tuber Crops Institute, 2012). Alkaline soil is one cause of the low yields of groundnut (Taufik, 2001). Alkaline soil with pH >7 is found in calcareous soil that is derived from parent material of calcium carbonate ($CaCO_3$) (Singer and Munns, 2002). The obtacles of peanuts cultivation on alkaline soil are the high pH, low organic matter and lack of essential nutrients N, P, S and Fe. The shift in land use and decreasing arable land may extent groundnutcultivation to marginal lands with higher pH. Symptoms of nutrients deficiency on groundnut plants in alkaline soil is chlorosis starting from the youngest leaves to the whole leaves that cause decreasing on pod yield (40–60)% (Harsono et al., 1998).

The main causes of chlorosis allegedly due to the high pH and deficiency of Fe and S (Taufik and Sudaryono, 1998; Taufik, 2001). Element Fe has been known to play an important role in the chlorophyll synthesis and photosynthesis activity of the plant (Briat et al., 2014) while S is the main component of amino acids cysteine and methionine which play a role in the process of photosynthesis (Brady, 1984). The element Fe and S in plant form a close bond Fe-S that acts as a cofactor protein constituent. On the soil with iron and S deficiency, the application of one of them or both will increase Fe and S uptake by plants (Muneer et al., 2014). Chlorosis causes a decrease

in weight and leaf area and changes leaf structure both at the epidermal and mesophyll (Fernandes et al., 2008). The disorders eventually led to lowering plant growth and yield.

Underway efforts to improve the plant growth and yield in alkaline soil is lowering soil pH and augment Fe to the ground by $FeSO_4$, but $FeSO_4$ is inefficient because Fe^{2+} is tend to settle (bound to organic compounds in the soil), while ion SO_4^{2-} easily leached by water. Decreasing soil pH can also be done with aplication of elemental sulfur which eventually oxidized to sulfate in the soil (Scherer, 2001). Decreasing in soil pH is intended to increase the availability of nutrients Fe, S and other essential nutrients (Taufiq et al., 2001; Motior et al., 2011; Soaud et al., 2011a,b). Elemental sulfur is more effective because slow release, but aplication with excessive doses can suppress the biological activity of the soil (Gupta et al., 1988) and increase soil salinity (Orman and Kaplan, 2011).

Element Fe is one of abundant element in the earth layers, but often can not absorbed by plants because of adsorbtion by colloidal mineral soil (Ammari and Mengel, 2006). Therefore, another attempt to increase the availability of Fe in soil is using synthetic chelating agent such as Fe-EDDHA (iron ethylene diamine dio-hydroxy phenyl acetate) that can change the Fe^{3+} to Fe^{2+} that absorbed by plants (Schenkeveld et al., 2008). However, Fe-EDDHA is unstable at pH above 6.5. Fe ions easily exchanged by other cations such as Ca^{2+}, Zn^{2+} and Cu^{2+} then undergo deposition so that need repeated application every year (Pestana et al., 2003). More stable Fe chelating compounds is produced by a microorganism namely "siderophore". Increasing soil microorganisms producing siderophore can be done with organic fertilizer or manure (Taufiq et al., 2007), however in addition to limited availability, fertilizer manure do not necessarily contain Fe chelating microbes in large numbers. The alternative that can be selected is using specific microbial inoculum that can chelate Fe in soil through siderophore compound i.e *Pseudomonas fluorescens* (Tate, 2000; Sharma and Johri, 2003).

As plant growth promoting rhizobacteria (PGPR), *P. fluorescens* contribute plant growth by producing growth hormones such as auxin and gibberellin (Ryu et al., 2005), dissolve nutrients and increase nutrients uptake in plants (Mullen, 1998; Rodriguez and Fraga, 1999; Karthikeyan et al., 2010), and suppress plant diseases (Leeman et al., 1995; Dwivehdi and Johri, 2003; Saleh-Lakha and Glick, 2007; Alemu and Alemu, 2013; Lutenberg et al., 2013). It also produces a citrate compound which can lower the pH of the growth environment (Hoberg et al., 2005). Previous studies showed that *P. fluorescens* increase the growth and yield of groundnut (Dey et al., 2004), wheat (Egamberdieva, 2010) and rice (Meera and Balabaskar, 2012) in alkaline soil. The combination of *P. fluorescens* and sulfur have also been attempted to increase maize yields (Alipour et al., 2012) and rapa (Eslamyan et al., 2013). But it has not yet known the role of *P. fluorescens* in replacing sulfur in alkaline soil. Increasing population of *P. fluorescens* in the soil by applying high concentrations of *P. fluorescens* in alkaline soil are expected to increase the availability of Fe and also can lower the soil pH thereby assist nutrient availability of other nutrients including S. Thus *P. fluorescens* can ultimately reduce or even replace elemental sulfur to enhance nutrient uptake, growth and yield of groundnut in alkaline soil. This research was aimed to study the effect of the *P. fluorescens* and sulfur on enhancement of nutrient uptake, growth and yield of ground nut Alfisol alkaline soil.

Materials and Methods

The greenhouse experiment was conducted in Indonesian Legumes and Tuber Crops Research Institute (ILETRI) Malang on January 2015 until May 2015. The research was laid on factorial randomized block design. The study consisted of two factors and three replications. The first factor were *P. fluorescens* concentration consisted 0 cfu/mL, 10^7 cfu/mL, and 10^9 cfu/mL The second factor were sulfur doses consisted 0 g S/kg soil, 1 g S/kg soil, 2 g S/kg soil, and 3 g S/kg soil. *P. fluorescens* formulation obtained from Department of Plant Pests and Diseases Brawijaya University collection with concentrations 10^7 cfu/mL and 10^9 cfu/mL. Elemental sulfur with S content 99% was used for this experiment. Groundnut variety used was Local Tuban (Spanish type) produced by Seed Resources Management Unit of ILETRI. Neutral soil was collected from Brondong, Lamongan, East Java.

Total of 7.5 kg of air-dry soil was put into a plastic pots. To obtain high soil pH (> 7.5), the soil was given extra calcium hydroxide ($Ca(OH_2)$) with dose of 40 g/kg soil and then incubated for 15 days before the application of sulfur. Soil chemical properties after liming are presented in Table 1. The sulfur powder was mixed into the soil at each dose according to the treatment and then incubated for 30 days before planting. During incubation, the soil maintained at field capacity. Plant watering used distilled water (pH

= 6, DHL = 0 dS/m). Applications of *P. fluorescens* were conducted twice that were after planting the seeds and at 15 das (days after sowing) at the afternoon. Application was done by pouring *P. fluorescens* solution in the soil around the seed and the rhizosfer. The first volume application for each treatment was 100 mL/pot and the second was 50 mL/pot.

Table 1. Soil properties after liming

Soil properties	Content
pH H_2O	9.5
Organic-C	0.94 %
N	0.11 %
P_2O_5	14.7 mg/kg
SO_4^{2-}	2.36 mg/kg
Fe	28.54 mg/kg
Total Fe	0.14 %
Zn	5.20 mg/kg
K	0.36 cmol/kg
Na	0.33 cmol/kg
Ca	37.33 cmol/kg
Mg	11.23 cmol/kg
CEC	23.97 cmol/kg

The research collected data of soil nutrient level soil and nutrient uptake at 60 das. Soil pH was measured using distilled water (ratio 1: 5) (Indonesian Soil Research Institute, 2005). Total N was measured using Kjedahl method. Ca was measured with NH_4OAc pH 7.0 extractor. Available P was measured using Bray I method. SO_4^{2-} was measured using turbidimetry method. Available Fe was measured using DTPA extractor.Total Fe was measured using HNO_3-$HClO_4$ extractor. Measured of plant nutrients uptake included the absorption of N, P, S, Ca, Fe and Mn. All of elements were measured based on procedure of Indonesian Soil Research Institute (2005). The population density of *P. fluorescens* was measured at 45 and 97 days after sowing (DAS). It was measured by taking 10 g soil in the rhizosfer and analyzed using dilution method (Alexander, 1998).

Colonies of *P. fluorescens* were counted under ultra violet (UV) light. ata of plant growth were collected at 60 DAS included plant height, number of leaves, leaf area, biomass of leaves and stems. Plant height was measured from stem butt until growing tip at main stem. One of tetrafoliate was measured as one leaf. Leaf area was measured using combination of punch and gravimetric methode (Sitompul and Guritno, 1995). Harvest was done at 97 das based on mature standart (Boote, 1982). Yield componenst were measured on filled pod weight, harvest index, total number and weight of pods, total number and weight of kernels per plant. The pod weight was on 14% moisture content.

Results

Soil properties

The results showed that there was no interaction between *P. fluorescens* concentration and sulfur doses on the soil pH, the content of N, available P, Ca, SO_4^{2-} and Fe in the soil. *P. fluorescens* concentration independently significantly affected the available Fe and total Fe, however did not significantly affect soil pH and the content of N, available P, Ca and SO_4 in the soil. Sulfur independently significantly affected soil pH and soil SO_4^{2-} and did not significantly affect the soil content of N, available P, Ca, SO_4^{2-}, available Fe, and total Fe (Table 2). The average available Fe and total Fe in soil differ in the treatment of *P. fluorescens*.

The higher of the bacteria concentration the higher available Fe and total Fe in the soil. *P. fluorescens* concentration of 10^9 cfu/mL resulted in the highest increase of available Fe in soil by 34.75% compared to control. Available Fe between the concentrations of 10^7 and 10^9 cfu/mL were significantly different, however when measured in total Fe were not significantly different. Sulfur significantly increased SO_4^{2-} in the soil and decreased soil pH. The higher of sulfur dose the higher of SO_4^{2-} content and the lower of soil pH. Sulfur dose of 1, 2, and 3 g/kg soil increased SO_4 in the soil respectively at 45, 75, 102 times compared with the control, and conservely decreased soil pH respectively for 3.64%, 5.82%, and 8.48% compared to control.

Nutrients uptake

There was no interaction between *P. fluorescens* and sulfur on the uptake of N, P, S, Ca, Fe and Mn by plants. *P. fluorescens* independently did not increase the uptake of N, P, S, Ca, Fe and Mn while the sulfur significantly increased the N, P, S, Ca, and Mn uptake by plant. The highest increase was achieved by dose of 3 g S/kg soil repectively increased N, P, S, Ca and Mn by 21.26 %, 10.99%, 30.91%, 84.62%, and 163.83% compared to control (Table 3).

Table 2. Soil pH and nutrients content on the *P. fluorescens* and sulfur treatments.

Treatments	pH	N (%)	Available P (mg/kg)	Ca (cmol/kg)	SO_4^{2-} (mg/kg)	Available Fe (mg/kg)	Total Fe (%)
Pf Concentration							
0 cfu/mL	7.95	0.13	19.10	47.39	389.88	5.64 c	5.81 b
10^7 cfu/mL	7.88	0.14	19.16	48.65	362.77	6.40 b	6.45 a
10^9 cfu/mL	7.81	0.13	17.85	50.66	347.72	7.60 a	6.34 a
LSD 5%	ns	ns	ns	ns	ns	0.44	0.32
S Dose							
0 g/kg soil	8.25 a	0.14	17.04	48.57	6.60 d	6.38	6.07
1 g/kg soil	7.95 b	0.13	16.90	48.08	294.67 c	6.34	6.24
2 g/kg soil	7.77 c	0.13	20.61	49.57	491.78 b	6.74	6.06
3 g/kg soil	7.55 d	0.13	20.28	49.39	674.11 a	6.73	6.42
LSD 5%	0.18	ns	ns	ns	75.65	ns	ns

Notes: Pf = *P. fluorescens*, S = Sulfur, cfu = colony forming unit, LSD = Least Significant Different, ns = not significant, the numbers followed by different letters are significantly different at LSD 5%

Table 3. Nutrients uptake by groundnut plants on *P. fluorescens* and sulfur treatments

Treatments	N	P	S	Ca	Fe	Mn
	……(mg/plant) ……					
Pf Concentration						
0 cfu/mL	70.59	6.95	4.85	56.25	2.12	0.85
10^7 cfu/mL	56.06	4.99	5.12	44.34	1.75	0.70
10^9 cfu/mL	54.80	5.28	5.22	42.47	1.32	0.80
LSD 5%	ns	ns	ns	ns	ns	ns
S Dose						
0 g/kg soil	39.15 c	3.65 c	2.09 c	36.29 b	1.21	0.47c
1 g/kg soil	64.46 ab	6.28 ab	4.28 b	44.85 b	1.99	0.76b
2 g/kg soil	55.09 bc	5.36 b	5.35 b	42.61 b	1.47	0.68bc
3 g/kg soil	83.23 a	7.66 a	8.55 a	67.00 a	2.24	1.24a
LSD 5%	1.29	0.39	0.48	1.31	ns	0.12

Notes:Pf=*P. fluorescens*, S=Sulfur, cfu=*colony forming unit*, LSD=Least Significant Different, ns = non significant, the numbers followed by different letters are significantly different at LSD 5%. Data used for analysis were transformed by $\sqrt{(x+0.5)}$

Population density of P. fluorescens in the rhizosfer

There was no interaction between *P. fluorescens* with sulfur dose on population of *P. fluorescens* in the rhizosphere at 45 and 97 DAS. *P. fluorescens* populations independently influenced by the concentration of *P. fluorescens* at 97 das and were not affected by sulfur dose neither at 45 nor 97 DAS (Table 4).

Population of *P. fluorescens* in the rhizosfer was not different between treatments at age 45 DAS with an average of 21.58 x 10^3 cfu/g soil, however different at 97 DAS. Population *P. fluorescens* on the concentration of 10^9 cfu/mL were significantly higher than treatment with concentrations of 0 and 10^7 cfu/mL with increase by 256.18% compared to control.

Plant growth

There was no interaction between *P. fluorescens* and sulfur on the parameters of plant height, number of leaf, leaf area and plant dry matter at all observations ages. *P. fluorescens* independently also had no significant effect on all plant growth parameters. Sulfur doses significantly increased plant height, number of leaves, leaf area, dry matter of leaves and stems compared to control (Table 5). The dominant plant growth response to sulfur treatment was at 60 das when the stage of the plant was pod development and seed fulfillment. Sulfur dose of 3 g kg^{-1} soil resulted in the highest of number of leaves, leaf area, leaf and stem dry matter with increase respectively by 32.55%, 84.66%, 129.32%, 143.96%, and 87.72% compared to control.

Table 4. Population density of *P. fluorescens* in rhizosfer on *P. fluorescens* dan sulfur treatment.

Treatments	*P. fluorescens* Population (cfu/g soil)	
	45 DAS	97 DAS
Pf Concentration		
0 cfu/mL	25.92 x 10^3	8.33 x 10^3 b
10^7 cfu/mL	19.00 x 10^3	17.25 x 10^3 b
10^9 cfu/mL	19.83 x 10^3	29.67 x 10^3 a
LSD 5%	ns	0.32
S Dose		
0 g/kg soil	18.00 x 10^3	9.67 x 10^3
1 g/kg soil	17.67 x 10^3	13.44 x 10^3
2 g/kg soil	21.00 x 10^3	19.44 x 10^3
3 g/kg soil	29.67 x 10^3	31.11 x 10
LSD 5%	ns	ns

Notes: Pf = *P. fluorescens*, S=Sulfur, cfu = colony forming unit, DAS = days after sowing, LSD = Least Significant Different, ns = not significant, the numbers followed by different letters are significantly different at LSD 5%. Data used for analysis were transformed by ^{10}Log x.

Table 5. Plant Growth at 60 days after planting on *P. fluorescens* dan sulfur treatments.

Treatments	Plant height (cm)	Leaf number/ plant	Leaf area (cm^2/plant)	Leaves dry mass (g/plant)	Stem dry mass (g/plant)
Pf Concentration					
0 cfu/mL	16.06	16.29	293.77	1.80	0.90
10^7 cfu/mL	16.23	12.88	243.56	1.41	0.67
10^9 cfu/mL	15.79	13.33	242.07	1.39	0.73
LSD 5%	ns	ns	ns	ns	ns
S Dose					
0 g/kg soil	14.50 b	10.89 b	167.05 c	0.91 c	0.57 b
1 g/kg soil	15.28 b	13.89 b	248.64 b	1.61 b	0.76 b
2 g/kg soil	15.11 b	11.78 b	240.44 b	1.40 b	0.66 b
3 g/kg soil	19.22 a	20.11 a	383.08 a	2.22 a	1.07 a
LSD 5%	0.36	0.61	2.81	0.46	0.10

Notes: Pf = *P. fluorescens*, S = Sulfur, cfu = colony forming unit, LSD = Least Significant Different, ns = non significant, the numbers followed by different letters are significantly different at LSD 5%. Data used for analysis were transformed by $\sqrt{(x+0.5)}$.

Yield and yield components of peanut

There was no interaction between *P. fluorescens* and sulfur in filled pod yield, harvest index and yield components. Sulfur significantly improved filled dry pods per plant compared without sulfur while *P. fluorescens* had no significant effect (Table 6).

Sulfur dose of 1, 2, 3 g/kg soil improved pod yield with increase respectively were 41.88%, 47.40%, and 80.74% compared to control. Sulfur significantly increased harvest index with the highest increase in sulfur dose of 3 g/kg soil that was 34.09% compared to control. There was no interaction between *P. fluorescens* concentration with sulfur dose on the total number and dry weight of pods, total number and dry weight of kernels per plant (Table 6). *P. fluorescens* independently did not significantly affect all components of the yield. Sulfur significantly increased the total of pods number, pod weight, kernels number and kernel weight per plant. The largest increase was resulted by sulfur at dose of 3 g/kg soil with increase respectively by 71.60 % for pods number, 79.43 % for pod weight, 75.47 % for kernels number, and 81.67% for kernel weight compared to control.

Table 6. Yield, harvest index and yield components per groundnut plant on *P. fluorescens* dan sulfur treatments.

Treatments	Filled pods (g)	Harvest index	Total pod number	Total pod weight (g)	Total kernel number	Total kernel weight (g)
Pf Concentration						
0 cfu/mL	4.52	0.51	8.67	4.61	11.85	4.61
10^7 cfu/mL	4.43	0.52	8.32	4.50	12.38	4.51
10^9 cfu/mL	4.21	0.54	8.85	4.36	11.83	4.27
LSD 5%	ns	ns	ns	ns	ns	ns
S Dose						
0 g/kg soil	3.08 c	0.44 c	5.74 b	3.16 c	8.44 c	3.11 c
1 g/kg soil	4.37 b	0.52 b	9.31 a	4.49 b	12.00 b	4.50 b
2 g/kg soil	4.54 b	0.55 ab	9.54 a	4.65 b	12.81 ab	4.59 b
3 g/kg soil	5.56 a	0.59 a	9.85 a	5.67 a	14.81 a	5.65 a
LSD 5%	0.94	0.07	1.54	0.95	2.28	0.92

Notes: Pf = *P. fluorescens*, S = Sulfur, cfu = colony forming unit, LSD = Least Significant Different, ns = not significant, the numbers followed by different letters are significantly different at LSD 5%.

Discussion

P. fluorescens and sulfur treatment affeted the chemical properties of the soil separately. It was shown from the absence of interaction between *P. fluorescens* and sulfur in soil chemical properties. It seemed that *P. fluorescens* used in this study have a role only in chelating Fe. The evident was showed from its effect on soil chemical properties only increased available Fe and total Fe in soil. Provision of *P. fluorescens* to the soil did not increase the levels of soil N, P, and S, and did not lower the pH and Ca levels in the soil.

Sulfur affected the soil chemical properties after oxidized to H_2SO_4 by oxidizing bacteria in an aerobic condition. Provision of sulfur significantly increased availability SO_4 in soil up to 100 times higher than control. Increase of SO_4 in soil and significantly negatively correlated with soil pH. Provision of sulfur up to 3 g/kg soil lowered the soil pH from 9.5 to 7.6. However, it was not able to increase the content of N, available P, available Fe in soil.

Stress of high pH caused bacteria could not grow well. In this study, the bacterial population could be seen on population of 10^3 cfu/g soil. At the appropriate conditions (soil pH near neutral 7.5) populations of *P. fluorescens* in the soil usually reach 10^6 to 10^7 cfu/g soil. Low populations of *P. fluorescens* due to poor soil organic C and N that were needed as a starter for early bacterial development. Previous research showed that population of *P. fluorescens* on groundnut rhizosfer grown in soil with pH 7.9, organic-C content 0.52% and Fe content 5–7 mg/kg ranges between 10^5 to 10^6 cfu/g soil (Dey et. al., 2004). *P. fluorescens* has the optimal pH for growth closer to 7-8 and the growth is inhibited at pH less than 5 (Fernandes-Calvino and the Baath, 2010; Fernandes-Calvino et al., 2011). According to Kamble et al. (2014), growth of bacteria at high pH increase with the increasing of C, N and P, while in this study, the levels of C and N in the soil is very low even though P is high. In addition, the success of *P. fluorescens* to form colonies in the rhizosfer is also influenced by exudates released by plants such as amino acids, flavonoids and sugars while the exudatesis influenced by the availability of plant species and environmental conditions (Botelho and Mendonça-Hagler, 2006). The limitation of organik-C and N also decreased the secretion of organic compounds by plant roots. The lack of food stuffs at high pH conditions resulted in competition between *P. fluorescens* so that why the amount of *P. fluorescens* at 45 das were lower than the population at 97 das. On the other hand the addition of sulfur to soil could not increase the soil N therefore the bacteria remain low.

The Interaction between *P. fluorescens* and sulfur did not occur may also caused by the sufficient level of available Fe content in soil. Available Fe content was said deficient in the range below 2.5 mg/kg and enough if the range of 4.5 mg/kg (Indonesian Soil Research Institute, 2005). Fe and S in plants form a bond Fe-S cluster which is a cofactor of protein that has an important function in the process of photosynthesis, respiration, N and S metabolism, plant hormones and the synthesis of coenzyme (Balk and Pilon, 2011). Supply one or both of these elements increase the uptake of Fe and S as well as increasing the total chlorophyll of plants.

Muneer et al. (2014) reported that aplication of Fe together with S increase levels of S and Fe and chlorophyll content of plant leaves compared to single aplication. In addition, Lehtoranta et al. (2015) reported that SO_4^{2-} in soil can reduce Fe and other elements to make more available. It explains why in this study there was no interaction between *P. fluorescens*. Soil analysis showed that available Fe content in the soil is sufficient although it has been limed in to pH 9.5, therefore provision of *P. fluorescens* and sulfur did not increase levels of Fe and S in the plant compared to the control.

Previous studies showed that interactions between *P. fluorescens* and sulfur that applied together occured in soil pH below 8.5 with available Fe content deficient until marginal. Alipour et al. (2012) reported that interaction between *P. fluorescens* and sulfur increase dry weight, plant height, levels of Fe, Zn and chlorophyll of maize grown in calcareous soil with pH 8.1 and available Fe content 2.1 mg/kg. At the same soil conditions, the interaction between *P. fluorescens* and sulfur improve the content of Fe and Zn, and oil content of rapa (*Brassica napus)*, but the interaction is not able to improve the plant growth and seed yield (Eslamyan et al., 2013). It reveals that the interaction between *P. fluorescens* with *sulfur* is influenced by soil pH and crop types.

The effect of P. fluorescens on nutrients uptake, growth and yield of peanut

Generally *P. fluorescens* has no effect on the growth and yield of peanut. *P. fluorescens* only affected the available Fe and total Fe content in the soil. *P. fluorescens* has extensive habitat on earth that can be found in colonies soil, rhizosphere, water, and plant surfaces (Nickel et al., 2014). Non significant effect of *P. fluorescens* maybe caused by natural population in the soil used for the research. It was shown from *P. fluorescens* population that calculated at 45 DAS and 97 DAS in the control (without *P. fluorescens* application). *P. fluorescens* have been seen in treatment concentration 0 cfu/mL. It contained *P. fluorescens* with the same population density at 45 DAS and slightly lower at the 97 DAS. Previous research showed that there are many different strains of *P. fluorescens* in the same rhizosphere. Dey et al. (2014) invented four different strains of *P. fluorescens* on groundnut rhizosphere. On soil with pH 7.9, the four strains increase the levels of N, the number and weight of roots, growth and yield of groundnut compared to control but with different capabilities. It reinforces the suspicion that there was natural strains in the rhizosphere of groundnut plants in this study. It seemed that applied *P. fluorescens* had chelating ability of Fe better than naturally one. Because of the ability, apllied strain eventually could sustain their life therefore had population higher than natural populations at the end of the study.

Increase of *P. fluorescens* concentrations on soil significantly improve the availability of Fe in the soil by 13.48 and 34.75% compared to control. Since the available Fe content in soil of control was sufficient, therefore addition of *p. fluorescens* did not increase the growth and yield of peanut. This is possible because available Fe was excess while plant demand is not much as can be seen from the Fe uptake by plants that was not differ between the treatment of *P. fluorescens*. Increasing concentrations of *P. fluorescens* also were unable to lower the pH of the soil therefore the availability of nutrients Fe, N, P, S in the soil and plant absorption was also not significantly different.

The effect of sulfur on nutrients uptake, growth and yield of peanut

Sulfur is an element of secondary nutrient needed by plants as a constituent amino acids. Applications sulfur in soil with low levels of S is beneficial to plants especially for legumes which is producing the protein (Brady, 1984). Soil analysis showed that S content in the soil was extremely low therefore sulfur applications significantly increase nutrient uptake, stems and leaves growth, and groundnut yield. Sulfur doses were positively and significantly correlated with SO_4^{2-} levels in the soil and negatively correlated with soil pH. The higher the sulfur dose the higher SO_4^{2-} levels in soil and conversely the lower the soil pH. The relationship between sulfur dose and SO_4^{2-} concentration in the soil is expressed by the equation $y = 219{,}9x + 36.84$ ($R^2 = 0.987$). The relationship between the dose of sulfur and soil pH is expressed in the equation $y = -0{,}226x + 8.218$ ($R^2 = 0.989$) (Figure 1). Element N and S are important constituent of plant chlorophyll and amino acids formation. S is one of the constituent parts of the protein molecule chromoproteid on chlorophyll (Marschner, 1995). N and S have close relation on the plant. Improvement of S in the soil will increase the utilization of N soil by plants (Jamal et al., 2010; Soaud et. al., 2011b), conversely, application N in soil with S deficiency will increase the S uptake by plants (Rahman et al., 2011). Although in this study sulfur did not increase the content of N in the soil at 60 das, it effected on N uptake by plant.

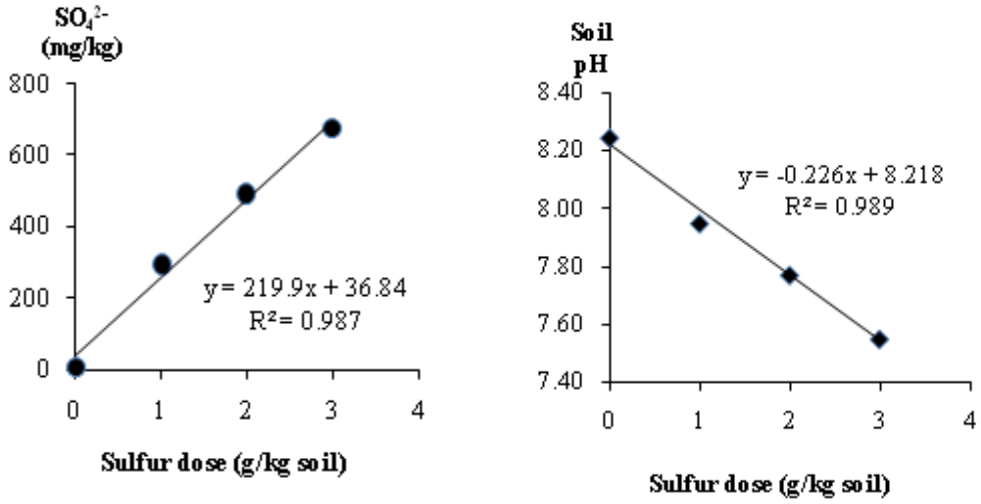

Figure 1. Relationship between sulfur doses with SO_4^{2-} level in soil (left) and soil pH (right)

Application of sulfur significantly increased SO_4^{2-} level of sulfur in the soil 50, 80 and 100 times compared with control, however the application did not increase the levels of nutrients N, available P, Ca and Fe in the soil. Decreasing in soil pH with the highest sulfur dose (3 g/kg soil) reached soil pH 7.55, while it is known that almost all of the nutrients available on the neutral pH 6.5-7.5 (Marcsner, 1995). In this study, decrease in soil pH has not yet reached the optimal soil pH. Kaya et al. (2009) reported that application of elemental S up to 1200 kg/ha on soils with pH 8.1 increased the levels of N, Mg, Ca, Fe in the soil and increased the levels of N, P and Ca in *Phaseolus vulgaris* plant. In this study, application of S did not increase the nutrient content in soil, but significantly increased uptake of N, P, S, Ca, Mn by plant. The highest uptake occurred in sulfur dose of 3 g kg^{-1} soil therefore increased the groundnut yield.

Sulfur significantly increase filled pod yield and harvest index that was supported by the components total number and weight of pods, total number and total seed weight per plant. N elements that play a role in vegetative growth was shown on the leaf number and leaf area, dry matter of leaves and stems, that also supported by the high uptake of S. P uptake effected on generative growth, while the increase in Ca played a role in the development of pods and seed filling. Aplication of sulfur with dose of 3 g/kg soil on high pH soil (9.5) significantly increased the soil pod yield up to 80.74% compared with control and the yield continued to rise. Figure 2 shows the relationship between the sulfur dose up to 3 g/kg soil was linear with the equation y=0,761x + 3,242 (R^2 = 0.931).

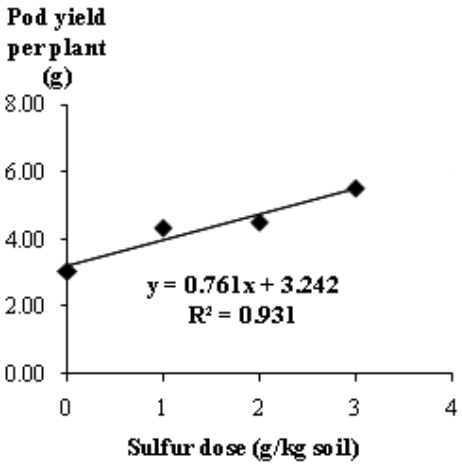

Figure 2. Relationship between sulfur doses with filled pod yield per groundnut plant

Conclusion

Application of *P. fluorescens* could not replace the role of elemental sulfur in alkaline soil with pH 9.5, especially with sufficient available Fe level. Domination of sulfur on growth and yield of groundnut was caused by S deficiency in the soil. *P. fluorescens* concentration of 10^9 cfu/mL independently resulted in increase of available Fe by 34.75% compared the control and could maintain the populations of *P. fluorescens* better than the concentrations of 0 and 10^7 cfu/mL. Sulfur doses significantly negatively correlated with soil pH expressed by equation y= -0,226x +

8.218. Sulfur significantly increased uptake of N, P, S, Ca and Mn by plants, promoted growth, yield, harvest index and yield components compared with control. Sulfur dose of 3 g/kg soil resulted in the growth of leaves and stems better, increasing pod yield by 80.74% and harvest index by 34.09% compared to control. Low populations of *P. fluorescens* in the rhizosphere showed that it is necessary to maintenance *P. fluorescens* in order to thrive in soil by the addition of organic matter as early growth starter.

Acknowledgement

The first author thanks Indonesian Agency for Agricultural Research and Development for the research funding.

References

Alemu, F. and Alemu, T. 2013. Antifungal activity of secondary metabolites of *Pseudomonas fluorescens* isolates as a biocontrol agent of chocolate spot disease (*Botrytis fabae*) of faba bean in Ethiopia. *African Journal of Microbiology Research* 7 (47): 5364–5373.

Alexander, D.B. 1998. Bacteria and Archaea. In: Sylvia, D.M., Fuhrmann, J.J., Hartel P.G. and Zuberer. D.A.(eds), *Principles and Application of Soil Microbiology*. Prentince Hall, New Jersey, pp 44─71

Alipour, Z.T. and Sobhanipour, A. 2012. The effect of *Thiobacillus* and *Pseudomonas fluorescent* inoculation on maize growth, and Fe uptake. *Annals of Biological Research* 3 (3): 1661–1666.

Ammari, T. and Mengel, K. 2006. Total soluble Fe in soil solutions of chemically different soils. *Geoderma* 136: 876–885.

Balk, J and Pilon, M. 2011. Ancient and essential: the assembly of iron–sulfur clusters in plants. *Trends Plant Science* 16(4):218–226

Boote, K.J. 1982. Growth stages of groundnut (*Arachis hypogaea* L.). *Peanut Science*. 9: 35–40.

Botelho, G. R. and Mendonça-Hagler, L.C. 2006. Fluorescent *Pseudomonads* associated with the rhizosphere crops-an overview. *Brazilian Journal of Microbiology* 37:401–416.

Brady, N.C. 1984. The Natures and Properties of Soils.9th ed. Macmillan Pub.Co., New York. pp. 189–383.

Briat, J.F., Dubos, C. and Gaymard, F. 2014. Iron nutrition, biomass production, and plant product quality. *Trends in Plant Science* 20 (1): 33-40.

Central Bureau of Statistics Indonesia. 2014. Harvested Area, Productivity and Production of Peanut in Indonesia. http //: www.bps.go.id.

Dey, R., Pal, K.K., Bhatt, D.M. and Chauhan., S.M. 2004. Growth promotion and yield enhancement of groundnut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiological Research* 159: 371—394.

Dwivedi, D. and Johri, B.N. 2003. Antifungals from fluorescent *Pseudomonads*: biosynthesis and regulation. *Current Science* 85 (12): 1693–1703.

Egamberdieva, D. 2010. Growth response of wheat cultivars to bacterial inoculation in calcareous soil. *Plant, Soil and Environment 56* (12): 570–573.

Eslamyan, L., Alipour, Z.T., Beidokhty, S. R. and Sobhanipour, A. 2013. *Pseudomonas fluorescens* and sulfur application affect rapeseed growth and nutrient uptake in calcareous soil. *International Journal of Agriculture and Crop Sciences* 5 (1): 39–43.

Fernandez, V., Eichert, T., Del Rio, V., Lopez-Casado. G., Heredia-guerrero, J.A., Abadia, A., Heredia, A. and Abadia, J.2008. Leaf structural changes associated with iron deficiency chlorosis in field-grown pear and peach: physiological implications. *Plant and Soil* 311: 161–172.

Fernandez-Calvino, D. and Bååth, E. 2010. Growth response of the bacterial community to pH in soils difering in pH. *FEMS Microbiology Ecology* 73: 149–156

Fernández-Calviño, D., Rousk, J., Brookes, P. C. and Bååth, E. 2011. Bacterial pH-optima for growth track soil pH, but are higher than expected at low pH. *Soil Biology and Biochemistry* 43:1569–1575.

Gupta, V.V.S.R, Lawrence, J. R. and Germida, J. J. 1988. Impact of elemental sulfur fertilization on agricultural Soils: I. Effects on microbial biomass and enzyme activities. *Canadian Journal of Soil Science* 68: 63–473.

Harsono, A., Anwari, M., Krisdiana, R., Antarlina, S.S., Supriyatin dan Sunardi. 1998. ILETRI Annual Report 1997/1998. ILETRI, Malang. pp. 40–64.

Hoberg, E., Marschner, P. and Lieberei, R. 2005. Organic acid exudation and pH changes by *Gordonia* sp. and *Pseudomonas fluorescens* grown with P adsorbed to goethite. *Microbiological Research* 160: 177–187

Indonesian Legumes and Tuber Crops Institute. 2012. Description of Legumes and Tuber Varieties. ILETRI, Malang. pp. 77-110. (in Indonesian)

Indonesian Soil Research Institute, 2005. Technical Guidelines for Chemical Analysis of Soil, Plant, Water and Fertilizer. Bogor. pp. 119-121. (in Indonesian)

Jamal, A., Yong-Sun, M., Abdin, M. Z. 2010. Sulphur-a general overview and interaction with nitrogen. *Australian Journal of Crop Science* 4 (7):523–529.

Kamble, P. N., Gaikwad, V. B., Kuchekar, S. R., Bååth, E. 2014. Microbial growth, biomass, community structure and nutrient limitation in high pH and salinity soils from Pravaranagar (India). *European Journal of Soil Biology* 65:87–95.

Karthikeyan, B., Joe, M.M., Jaleel, C.A. and Deiveekasundaram, M. 2010. Effect of root inoculation with plant growth promoting rhizobacteria (PGPR) on plant growth, alkaloid content and nutrient control of *Catharanthus roseus* (l.) G. Don. *Natura Croatica* 19 (1): 205–212.

Kaya , M., Küçükyumuk, Z. and Erdal, I. 2009. Effects of elemental sulfur and sulfur-containing waste on nutrient concentrations and growth of bean and maize plants grown on a calcareous soil. *African Journal of Biotechnology* 8 (18):4481–4489.

Leeman, M., Van Pelt, J.A., Den Ouden, F.M., Heinsbroek, M., Bakaer, P.A.H.M. and Schipper, B. 1995. Induction of systemic resistance against Fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens. Phythopathology* 85 (9): 1021-1027.

Lehtoranta, J., Ekholm, P., Tallberg, S. P., Uusitalo, R. 2015. Labile organic carbon regulates phosphorus release from erodedsoil transported into anaerobic coastal systems. *AMBIO* 44 (2):S263–S273.

Lutenberg, B.J.J, Malfanova, N., Kamilova, F. and Berg, G. 2013. Microbial control of plant root disease. In: de Bruijn, F.J. (ed), *Molecular Microbial Ecology of Rhizosphere*. vol. 1–2. John Wiley & Sons, USA., pp 575–585.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. Academic Press, London. pp. 269–523.

Meera, T., and Balabaskar, P. 2912. Isolation and cauterization of *Pseudomonas fluorescens* from rice fields. International *Journal of Food, Agriculture & Veterinary Sciences*. 2 (1): 113-120.

Motior M.R., Abdou, A.S., Al Darwish, F.H., El-Tarabily, K.A., Awad, M.A., Golam, F. and Sofian-Azirun, M. 2011. Influence of elemental sulfur on nutrient uptake, yield and quality of cucumber grown in sandy calcareous soil. *Australian Journal of Crop Science* 5 (12): 1610–1615.

Mullen, M.D. 1998. Transformation of other elements. In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G. and Zuberer, D.A. (eds), *Principles and Application of Soil Microbiology*. Prentince Hall, New Jersey, pp 369–386.

Muneer, S., Sang-Hyun, P., Bok-Rye, L., Qian, Z., Kil-Yong, K. and Tae-Hwan, K. 2014. Involvement of sulphur nutrition in modulating iron deficiency responses in photosynthetic organelles of oilseed rape (*Brassica napus* L.). *Photosynthesis Research* 119:319–329.

Nickel, P. I., Martínez-García, E. and de Lorenzo, V. 2014. Biotechnological domestication of pseudomonads using synthetic biology. *Nature Reviews Microbiology* 12: 368–379.

Orman, S. and Kaplan, M. 2011. Effects of elemental sulphur and farmyard manure on pH and salinity of calcareous sandy loam soil and some nutrient elements in tomato plant. *Journal of Agricultural Science and Technology* 5 (1): 20–26.

Pestana, M., de Varennes, A. and Faria, E.A. 2003. Diagnosis and correction of iron chlorosis in fruit trees: a review. *Journal of Food, Agriculture and Environment* 1 (1): 46–51.

Rahman, M. M. , Soaud, A. A., Al Darwish, F. H. and Sofian-Azirun, M. 2011 Responses of sulfur, nitrogen and irrigation water on *Zea mays* growth and nutrients uptake. *Australian Journal of Crop Science* 5(3):350–360.

Rodríguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advance* 17: 319–339.

Ryu, C., Hu, C., Locy, R.D. and Kloepper, J.W. 2005. Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana. Plant and Soil* 268: 285–292.

Saleh-Lakha, S. and Glick, B. R. 2007. Plant growth-promoting bacteria. In Van Elsas, J.G., Jansson J.K. and Trevors, J.T. (eds), *Modern Soil Microbiology*. Second Ed. CRC Press, USA, pp 503–520.

Schenkeveld, W.D.C., Dijcker, R., Reichwein, A.M., Temminghoff, E.J.M. and van Riemsdjik, W.H. 2008. The effect of soil-applied FeEDDHA treatments in preventing iron chlorosis in soybean as a function of the o,o-FeEDDHA content. *Plant and Soil* 303 :161–176.

Scherer. H.W. 2001. Sulphur in crop production. *European Journal of Agronomy* 14: 81–111.

Sharma, A. and Johri, B.N. 2003. Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiological Research* 158: 243–248.

Singer, M.J. and Munns, D.N.. 2002. Soils: An Introduction. Fifth edition. Prentince Hall, New Jersey. pp. 233–259.

Sitompul, B.C. and B. Guritno. 1995. Analysis of Plant Growth. Gadjah Mada University Press, Yogyakarta. pp. 81-124. (in Indonesian)

Soaud, A.A., Al Darwish, F.H., Saleh, M.E., El-Tarabily, K.A. and Rahman, M.M.. 2011a. Effects of elemental sulfur, phosphorus, micronutrients and *Paracoccus versutus* on nutrient availability of calcareous soils. *Australian Journal of Crop Science* 5 (5): 554–561.

Soaud, A.A., Al Darwish, F.H., Saleh, M.E., El-Tarabily, K.A. and Rahman, M.M.. 2011b. Effect of elemental sulfur application on ammonia volatilization from surface applied urea fertilizer to calcareous sandy soils. *Australian Journal of Crop Science* 5 (5): 571–579.

Tate, R.L. 2000. Soil Microbiology. Second edition. John Wiley 7 Sons, Inc. New York. pp. 189–214.

Taufik, A. dan Sudaryono. 1998. Sulfur (S) fertilization and organic matter on the groundnut in the Mediterranean (Alfisol) reacts bases. Crops Agricultural Research 17 (1): 76–82. (in Indonesian)

Taufiq, A. 2001. Nutrient evaluation of alfisol and the productivity increment for groundnut. *Agricultural Sciences* 8 (1): 16–25. (in Indonesian)

Taufiq, A., Radjagukguk, B., Syukur, A. 2001. Chlorotic symptom in groundnut (Arachis hypogaea) grown on a calcareous soil. *Agrosains* 14 (3): 297–312. (in Indonesian)

Taufiq, A., Rahmianna, A.A., Hardaningsih S. and Rozi, F. 2007. Increasing groundnut yield on dryland Alfisols in Indonesia. *SAT e-journal* 5 (1): 1–5