

# The Study on the Fixed Nitrogen and Nitrogenase Activity in the Day-Round of *Azotobacter* and *Azospirillum* Grown with Maize in KamphaengSaen Soil Series

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## Abstract

The aim of this study was to determine not only the activity of the nitrogenase enzyme, the fixed nitrogen in the day-round of Suwan 5 maize when inoculation with *Azotobacter chroococcum* and *Azospirillum lipoferum* but also the correlation between the nitrogenase activity and total fixed nitrogen. The statistical arrangement was  $3 \times 4$  factorial in completely randomized design with 4 replications. The first factor was inoculation (*Azotobacter chroococcum*, *Azospirillum lipoferum* and non-inoculation (control)). The second factor was the observation time of nitrogenase activity (6:00, 12:00, 18:00, 24:00 hr.). The data were collected at 4 and 6 weeks. The results showed that the different time in a day-round did not affect the nitrogenase activity. Inoculation of azotobacter gave the highest total soil nitrogen at 4 and 6 week. (729.77 and 750.47 mg/ 10kg-soil (while, the highest total plant nitrogen was found as inoculated with azospirillum (159.11 and 840.35 mg/plant of 4 and 6weeks). However, the total fixed nitrogen of azotobacter and azospirillum treatments were in the same level. Azotobacter treatment gave higher nitrogenase activity in soil than that of azospirillum treatment, while that in plant it was lower. The total nitrogenase activities of both inoculums were not different during the time of this study. The bulk (over all) correlation of total fixed nitrogen and total nitrogenase activity at week 4 and 6 were linear equations as  $y = 0.0224x + 18.045$ ,  $R^2 = 0.8687$  and  $y = 0.0117x + 14.57$ ,  $R^2 = 0.7604$  respectively.

**Keywords:** *Azotobacter* sp., *Azospirillum* sp., nitrogenase activity, nitrogen fixation

## 1. Introduction

Nitrogen is a nutrient which plants need it in a great amount. It plays the most important role for the growth of plants and it is an important complement of enzymes catalyzing and controlling reactions in plants to be proceeded normally. Plant available nitrogen found in soil is derived from fertilization, rain water, and lightning. Most of nitrogen, however, is found in a form of nitrogen gas ( $N_2$ ) which approximately amounts to 78% in the atmosphere. As plants cannot use this form of nitrogen directly, some microbes can change the nitrogen gas into ammonia. Most free living microbes in soil which can fix nitrogen and whose activities in enhancing the growth of plants are bacteria namely *Azotobacter* sp. and *Azospirillum* sp. These two bacteria can fix nitrogen and engage good activity with tropical plant like maize. The majority of azotobacter is in root zone soil rather than inside the root, while azospirillum primarily grows in cell cortex of root rather than in soil. Azospirillum acquires carbohydrate directly from sieve tube as a resource of carbon which promotes its growth (Olivera et al., 2004). Azospirillum can be used to promote the growth of sprouts under normal and arid conditions (Alejandra Pereyra et al., 2009). Azospirillum also provides more flexibility to cell wall which enhances the growth (Pereyra, 2010) and increases products of wheat in waterless plot of land (Martin & Maria, 2009). Furthermore, azospirillum had the highest efficiency in nitrogen fixation at the root of sweet corn and it would reach the highest point of nitrogen fixation in the week 4 amounting to  $0.20 \text{ mgNhr}^{-1}\text{m}^{-2}$  (Toopakuntho, 2010). Azospirillum can also create auxin, a substance promoting growth of maize, of 53.57 mg/ml (Phookkasem, 2011). The amount of nitrogen fixation may vary based on types of microbe and conditions of soil. Several methods can be adopted for measurement of nitrogen fixation such as

measuring the growth of bacteria in culture media without nitrogen to see the ability of nitrogen fixation (Tate, 2000), N-analysis due to Kjeldahl method by measuring the difference of total amount of nitrogen before and after the growth (Burns & Hardy, 1975), following up nitrogen by  $^{15}\text{N}$ , analyzing nitrogen with the acetylene reduction assay which is indirectly measuring nitrogen fixation by gauging nitrogenase activity (Bergersen, 1970), and using gas chromatography which is the fastest method at present. These methods have different restrictions. The measurement of nitrogen fixation by acetylene reduction assay would inform amounts of nitrogenase activity and fixed nitrogen. Therefore, we aimed to study the fixed nitrogen and nitrogenase activity in the day-round of azotobacter and azospirillum with Suwan 5 maize in KamphaengSaen soil series and to make comparison on the correlation between nitrogenase activity and amount of fixed nitrogen in order to estimate the actual amount of fixed nitrogen in growing maize for more convenience and speed.

## 2. Materials and Method

### 2.1 Experimental Design

Statistical planning was  $3 \times 4$  factorial in completely randomized design with 4 replications. It comprises two factors; 1) inoculation (non-inoculation, *Azotobacter chroococcum* and *Azospirillum lipoferum*) and 2) the observation time of nitrogenase activity (6:00, 12:00, 18:00 and 24:00 hr.).

### 2.2 Inoculum Powder

They are *Azotobacter chroococcum* strain 20AS3 and *Azospirillum lipoferum* strain LB<sub>10</sub> (Phoockasem, 2011) in peat which are produced in soil microbiology laboratory, Department of Soil Science, Faculty of Agriculture at KamphaengSaen, Kasetsart University (KamphaengSaen Campus). The viable cell of *Azotobacter chroococcum* inoculum was  $60.03 \times 10^7$  cell/g, *Azospirillum lipoferum* inoculum was  $35.89 \times 10^7$  cell/g, approximately.

### 2.3 Preparation of Soil

The researcher collected samples of soil which are KamphaengSaen soil series (pH 7.2, electrical conductivity 0.04 dS/m, organic matter 0.74%, total N 0.04%, available phosphorus ( $\text{P}_2\text{O}_5$ ) 37.56 mg/kg, exchangeable potassium ( $\text{K}_2\text{O}$ ) 72.67 mg/kg) at the depth of 15 centimeter from its surface collected in random all over the entire experimental plot. The soil was solarized for 3 days and allocated into pots; each pot contains 10 kilograms of soil. The chemical fertilizers are added, they are triple superphosphates 15 mg  $\text{P}_2\text{O}_5$ /kg soil and potassium chloride 15 mg  $\text{K}_2\text{O}$ /kg soil equally to all pots.

### 2.4 Planting

1) The Suwan 5 maize seeds were surface sterilized in 5% sodium hypochlorite for 10 minutes and rinsed with distilled water. The cleaned and dried seed then were inoculated with related inoculum by using gum acasia 40% in distilled water as sticker. The 100 g of inoculum powder was mixed with 75 ml of gum acasia, then, the 500 g of seeds was subsequently mixed with them (Kaewmueng, 2013).

2) Drop 4 seeds of maize inoculated with each type of powder per pot at a depth of 2-3 centimeters. At the age of two weeks, plants were thinned to obtain one plant per pot and weeds must be eradicated until the harvest date.

### 2.5 Data Collection

1) Soil and Plant nitrogen. The amount of total nitrogen in soil and maize at week 4 and 6 were measured by the method as described by Attanand and Chancharoensuk (1999).

Total fixed N;  $N_f$  is calculated by the following equation.

$$N_f = (N_{sa} + N_p) - N_{sb} \quad (1)$$

As  $N_f$  = Total fixed nitrogen (mg/po)  
 $N_{sa}$  = Total soil nitrogen after planting (mg/10 kg-soil or pot)  
 $N_p$  = Total plant nitrogen after planting (mg/plant or pot)  
 $N_{sb}$  = Total soil nitrogen before planting (mg/ 10kg-soil or pot)

2) (The soil and maize root nitrogenase activity at week 4 and 6 were determined (Hardy et al., 1973).

The nitrogenase activity is calculated by the following equation (Toopakuntho, 2010).

$$\text{Nitrogenase activity in soil} = \frac{n\text{C}_2\text{H}_4 \times \text{MWN}_2 \times \text{ms}}{c} \quad (2)$$

$$\text{Nitrogenase activity in root} = \frac{n\text{C}_2\text{H}_4 \times \text{MWN}_2 \times N_p}{c} \quad (3)$$

As  $nC_2H_4$  = mole of ethylene per gram soil or gram dry plant/hour  
 MWN<sub>2</sub> = molecular weight of nitrogen gas (28 grams per mole of nitrogen)  
 ms = mass of ripened soil (100 grams)  
 Np = mass of ripened root (gram per plant)  
 c = the constant number for changing the amount of ethylene to the fixed nitrogen (3 moles of ethylene per mole of nitrogen)

Total nitrogenase activity (TNA) is calculated by the following equation.

$$TNA = SNA + RNA \quad (4)$$

As TNA = Total Nitrogenase Activity (mgNpot<sup>-1</sup>day<sup>-1</sup>)

SNA = Soil Nitrogenase Activity (mgNpot<sup>-1</sup>day<sup>-1</sup>)

RNA = Root Nitrogenase Activity (mgNpot<sup>-1</sup>day<sup>-1</sup>)

### 2.6 Statistical Analysis

The collected data were statistically analyzed using SPSS-program for Windows, version 21, and the treatment means were compared using Duncan's Multiple Range Test (DMRT) method.

## 3. Results

### 3.1 Total Nitrogen Found in Soil and Suwan 5 Maize

The time of collecting samples did not affect amount of total nitrogen found in soil and Suwan 5 maize both in week 4 and 6 (table 1 and 2). However, the different inoculations of microbe significantly affected average total nitrogen. The azotobacter and azospirillum treatment had total nitrogen higher than that of non-inoculation on average. The azotobacter treatment had the highest total nitrogen in week 4 and 6 with the average of 729.77 and 750.47 mg/10 kg-soil. The azospirillum treatment carried produced less nitrogen in soil on average amounting to 610.73 and 631.43 mg/10kg-soil. The non-inoculation hold the least nitrogen with average amounts of 502.04 and 507.21 mg/10 kg-soil.

The total nitrogen in maize provided different results. That is to say, the azospirillum treatment provided the highest total nitrogen in maize on average in week 4 amounting to 159.11 mg/plant. In week 6, as the maize rapidly grew up, the total nitrogen in plant increased as well with an average of 840.35 mg/plant. The azotobacter treatment resulted in less total nitrogen of maize with average of 132.20 and 713.91 mg/plant in week 4 and 6, respectively, while those of the non-inoculation treatment carried the least nitrogen in maize with average amounts of 60.79 and 553.63 mg/plant, respectively (table 2).

Table 1. The total soil nitrogen (mg/10 kg-soil) grown with Suwan 5 maize at week 4 and 6

Times (T)	Week 4				Week 6			
	Inoculation (I)			Average	Inoculation (I)			Average
	Non inoculation	Azotobacter	Azospirillum		Non inoculation	Azotobacter	Azospirillum	
6.00 am.	434.76	724.59	621.08	593.48	517.57	745.30	621.08	627.98
12.00 pm.	538.27	745.30	641.78	641.78	455.46	766.00	683.19	634.88
6.00 pm.	538.27	724.59	579.67	614.18	496.86	766.00	600.38	621.08
12.00 am	496.86	724.59	600.38	607.28	558.97	724.59	621.08	634.88
Average	502.04c	729.77a	610.73b		507.21c	750.47a	631.43b	
F-test								
I		**				**		
T		ns				ns		
I×T		ns				ns		
%CV		18.41				18.74		

Remarks: ns has no statistical difference at the confidence level of 95%.

\*\* Statistical difference at the confidence level of 95%.

The same alphabets which are in the same row bear no statistical difference at the confidence level of 95% based on DMRT.

Table 2. The total plant nitrogen (mg/plant) grown with Suwan 5 maize at week 4 and 6

Times (T)	Week 4				Week 6			
	Inoculation (I)			Average	Inoculation (I)			Average
	Non inoculation	Azotobacter	Azospirillum		Non inoculation	Azotobacter	Azospirillum	
6.00 am.	49.09	103.24	140.82	97.72	455.12	682.12	824.01	653.75
12.00 pm.	53.25	140.91	171.06	121.74	492.77	756.20	772.89	673.95
6.00 pm.	62.63	133.13	149.99	115.25	541.67	717.43	875.63	711.58
12.00 am	78.16	151.51	174.58	134.75	724.94	699.91	888.85	771.23
Average	60.79c	132.20b	159.11a		553.63c	713.91b	840.35a	
F-test								
I		**				**		
T		ns				ns		
I×T		ns				ns		
%CV		20.41				22.46		

Remarks: ns has no statistical difference at the confidence level of 95%.

\*\* Statistical difference at the confidence level of 95%.

The same alphabets which are in the same row bear no statistical difference at the confidence level of 95% based on DMRT.

### 3.2 Total Fixed Nitrogen ( $N_f$ ) of Suwan 5 Maize

The time of collecting samples did not affect total fixed nitrogen in week 4 and 6 and did not cause statistical difference. Nevertheless the inoculation significantly influenced the amount of  $N_f$ . The  $N_f$  of azotobacter and azospirillum treatment bore no statistical difference. Their  $N_f$ , nonetheless, were higher than that of non-inoculation. In week 4, azotobacter and azospirillum treatment generated average  $N_f$  of 365.10 and 272.97 mg/pot respectively. Moreover,  $N_f$  increased in week 6 amounted to 967.52 and 974.91 mg/pot respectively. Meanwhile, the non-inoculation held average  $N_f$  of 81.78 and 563.97 mg/pot respectively (table 3).

Table 3. The total fixed nitrogen ( $N_f$ ) (mg/pot) grown with Suwan 5 maize at week 4 and 6

Times (T)	Week 4				Week 6			
	Inoculation (I)			Average	Inoculation (I)			Average
	Non inoculation	Azotobacter	Azospirillum		Non inoculation	Azotobacter	Azospirillum	
6.00 am.	48.32	330.97	265.03	214.78	475.82	930.55	948.23	784.87
12.00 pm.	94.65	389.33	315.97	266.66	451.36	1025.33	959.22	811.97
6.00 pm.	88.36	360.85	232.79	227.34	541.67	986.57	979.14	835.79
12.00 am	95.77	379.23	278.09	251.03	787.05	927.64	1013.06	909.25
Average	81.78c	365.10a	272.97a		563.97b	967.52a	974.91a	
F-test								
I		**				**		
T		ns				ns		
I×T		ns				ns		
%CV		20.45				27.46		

Remarks: ns has no statistical difference at the confidence level of 95%.

\*\* Statistical difference at the confidence level of 95%.

The same alphabets which are in the same row bear no statistical difference at the confidence level of 95% based on DMRT.

### 3.3 Soil Nitrogenase Activity (SNA) and Root Nitrogenase Activity (RNA) of Suwan 5 Maize

According to the study of SNA of Suwan 5 maize, it was found that the time of collecting samples did not cause the statistical difference of SNA both in week 4 and 6. Nevertheless, the inoculation brought the significantly

statistical difference to SNA on average. The azotobacter and azospirillum treatment generated higher SNA on average compared with that of non-inoculation. In week 4, the azotobacter and azospirillum treatment carried average SNA in similar level, but their amounts were higher than that of non-inoculation. The average SNA amounted to 17.52, 16.90, and 14.38 mgN/10 kg-soil/day respectively. In week 6, the inoculation held statistical significance. The highest average SNA was derived from azotobacter treatment, followed by azospirillum treatment, and the smallest amount of SNA resulted from non-inoculation (18.38, 17.62, and 15.61 mgN 10kg soil<sup>-1</sup>day<sup>-1</sup>) (table 4).

Average RNA in week 4 and 6 was presented in table 5. The inoculation caused statistically significant difference of average RNA in week 4. The azospirillum treatment exerted the highest influence to average RNA, followed by azotobacter treatment, and the non-inoculation gave the lowest RNA (8.57, 7.89, and 4.98 mgN plant<sup>-1</sup>day<sup>-1</sup>). In week 6, averages of RNA were closed to those in week 4 amounting to 8.71, 7.85, and 4.90 mgN plant<sup>-1</sup>day<sup>-1</sup> respectively.

Table 4. The nitrogenase activity in soil (mgN 10kg soil<sup>-1</sup>day<sup>-1</sup>) of Suwan 5 maize at week 4 and 6

Times (T)	Week 4				Week 6			
	Inoculation (I)			Average	Inoculation (I)			Average
	Non inoculation	Azotobacter	Azospirillum		Non inoculation	Azotobacter	Azospirillum	
6.00 am.	14.92	17.54	17.22	16.56	15.79	19.73	17.83	17.79
12.00 pm.	14.05	17.56	16.63	16.08	16.61	18.32	17.66	17.53
6.00 pm.	14.11	17.35	16.55	16.01	14.80	17.94	17.56	16.77
12.00 am	14.42	17.62	17.19	16.41	15.25	17.53	17.42	16.73
Average	14.38b	17.52a	16.90a		15.61c	18.38a	17.62b	
F-test								
I		**				**		
T		ns				ns		
I×T		ns				ns		
%CV		10.78				8.77		

Remarks: ns has no statistical difference at the confidence level of 95%.

\*\* Statistical difference at the confidence level of 95%.

The same alphabets which are in the same row bear no statistical difference at the confidence level of 95% based on DMRT.

Table 5. The root nitrogenase activity (mgN/plant/day) of Suwan 5 maize at week 4 and 6.

Times (T)	Week 4				Week 6			
	Inoculation (I)			Average	Inoculation (I)			Average
	Non inoculation	Azotobacter	Azospirillum		Non inoculation	Azotobacter	Azospirillum	
6.00 am.	4.34	7.07	8.59	6.67	4.33	7.72	9.09	7.05
12.00 pm.	4.78	8.43	9.09	7.43	5.17	8.12	8.58	7.29
6.00 pm.	5.44	8.06	8.67	7.39	4.91	7.79	8.80	7.17
12.00 am	5.38	7.98	7.94	7.10	5.21	7.79	8.38	7.13
Average	4.98c	7.89b	8.57a		4.90c	7.85b	8.71a	
F-test								
I		**				**		
T		ns				ns		
I×T		ns				ns		
%CV		24.99				25.25		

Remarks: ns has no statistical difference at the confidence level of 95%.

\*\* Statistical difference at the confidence level of 95%.

The same alphabets which are in the same row bear no statistical difference at the confidence level of 95% based on DMRT.

### 3.4 Total Nitrogenase Activity (TNA) of Suwan 5 Maize

According to the study, the time of collecting samples did not cause statistical difference to TNA both in week 4 and 6 (table 6). Nevertheless, the inoculations provided statistical difference to TNA in week 4 and 6. The azotobacter and azospirillum treatment carried higher TNA compared with that of non-inoculation. The averages of TNA amounted to 25.40 and 25.47 mgN pot<sup>-1</sup>day<sup>-1</sup> respectively. However, the non-inoculation resulted in the lowest TNA amounting to 19.36 mgN pot<sup>-1</sup>day<sup>-1</sup>. The averages of TNA rose in week 6 of azotobacter and azospirillum treatment were in the similar level being 26.23 and 26.33 mgN pot<sup>-1</sup>day<sup>-1</sup>, while that of non-inoculation was the lowest at 20.52 mgN pot<sup>-1</sup>day<sup>-1</sup>.

Table 6. The total nitrogenase activity (TNA) (mgN pot<sup>-1</sup>day<sup>-1</sup>) grown with Suwan 5 maize at week 4 and 6

Times (T)	Week 4				Week 6			
	Inoculation (I)			Average	Inoculation (I)			Average
	Non inoculation	Azotobacter	Azospirillum		Non inoculation	Azotobacter	Azospirillum	
6.00 am.	19.26	24.61	25.81	23.23	20.12	27.44	26.93	24.83
12.00 pm.	18.83	26.00	25.72	23.52	21.78	26.44	26.24	24.82
6.00 pm.	19.56	25.41	25.22	23.40	19.70	25.73	26.36	23.93
12.00 am	19.80	25.60	25.13	23.51	20.46	25.32	25.80	23.86
Average	19.36b	25.40a	25.47a		20.52b	26.23a	26.33a	
F-test								
I		**				**		
T		ns				ns		
I×T		ns				ns		
%CV		13.81				12.31		

Remarks: ns has no statistical difference at the confidence level of 95%.

\*\* Statistical difference at the confidence level of 95%.

The same alphabets which are in the same row bear no statistical difference at the confidence level of 95% based on DMRT.

## 4. Discussions

### 4.1 The Effect of Day-Round Time of Observation on the Fixed Nitrogen and Nitrogenase Activity

From the study of correlation of TNA and nitrogen fixation in the day-round of azotobacter and azospirillum in week 4 and 6, it was found that the time of collecting samples did not affect nitrogenase activity and total nitrogen in soil and maize because nitrogen fixation can occur well in case of low concentration of oxygen.

Due to their heterotrophic habit, organic carbon is the important source for their growth and nitrogen fixation. Both bacteria derived carbon and energy sources mainly from root exudate and root debris during plant growth therefore, kinds and quantity of organic substance in root exudate control the growth and activity of bacteria (Liste & Alexander, 2000; Suwanpinij & Preecha, 2011). Nitrogen fixation activity uses some amount of this organic carbon. The soil organic carbon by level and form, thus, will be the important role to regulate the nitrogenase activity. In this case, during the day time, the organic C from root exudate may not different but may be enough for stimulating growth and activity of bacteria.

Both species of bacteria are hetero-aerobes. They consume oxygen as electron acceptor for their growth. But, the nitrogen fixation must be in reducing condition. Azotobacter maintain the reduced condition for nitrogen fixation by some mechanisms such as respiratory protection, conformation protection, slime production and autoprotection (Bais et al., 2006) while that of azospirillum fix nitrogen in microaerophily condition that oxygen will not damage the nitrogenase enzyme. Nitrogen fixation and growth of azotobacter proliferate in rhizosphere and rhizoplane. However, some quantity of them can enter the plant root epidermis and show nitrogenase activity in aerenchyma and intercellular region of plant root (Giller, 2001; Tate, 2000). Azospirillum carry higher nitrogen fixation in plant root than that in rhizosphere due to the microaerophily condition in this region. (Osmar et al., 2004).

#### 4.2 The Effect of *Azotobacter Chroococcum* and *Azospirillum Lipoferum* towards TNA and Total Nitrogen in Soil and Maize

*Azotobacter chroococcum* and *Azospirillum lipoferum* affected TNA and total nitrogen in soil and maize. The *Azotobacter chroococcum* generated the highest average SNA and total nitrogen in soil (18.38 mgN/10 kg-soil/day and 750.47 mgN/10 kg-soil respectively). The *Azospirillum lipoferum* provided the highest average RNA and total nitrogen in maize (8.71 mgN plant<sup>-1</sup> day<sup>-1</sup> and 840.35 mg/plant respectively). It can be seen that the *Azotobacter chroococcum* and *Azospirillum lipoferum* created total nitrogen in soil and maize higher than that of non-inoculation; this was because azotobacter and azospirillum are free living nitrogen fixer. Because of the rise in nitrogen fixation some part of fixed nitrogen release to soil and some part transfer to upper ground stem. Then, total nitrogen in soil and maize increased (Shabaev, 1991). The azotobacter and azospirillum also can create substance fostering the growth of plant, so the plant acquired benefits from such substance. In accordance with the study of Farah et al. (2008), it was found that 47 out of 72 azotobacter species can produce IAA which supported 83.3%, growth of plant. Therefore, the plant had nitrogen fixation and increasingly grew. Meanwhile, azospirillum helped synthesize auxin. Phookkasem (2011) found that isolates of azospirillum can produce the maximum auxin of 53.75 µg/ml and support the growth of maize more than that of non-inoculation. These plant growth promoting substances stimulate the uptake nutrient and release higher amount of root exudate of maize, then bacteria can get more carbon and energy sources leading to fixing more nitrogen as well.

Most of azotobacter influenced in root zone instead of being inside the root (Olivera et al., 2004). However, azospirillum is highly correlated to the root system. It can enter root, consume surrounding organic substance, fix N<sub>2</sub> and release into the surrounding soil (Steenhoudt & Vanderleyden, 2000). From the study of Tejera (2005), it was found that azotobacter whose species are selected from soil (60%) and root (40%) revealed no close correlation, particularly in the area of fibrous root. No azotobacter was found inside the root of maize in the studying zone because most of azotobacter live freely and prevail in the soil at the appropriate temperature of 30 °C. Kongsorn (2010) found that inoculation of azotobacter and azospirillum maximized the efficiency of nitrogen fixation, but the nitrogen fixation in the root was lower than that in the soil. Adding azotobacter maximized nitrogen fixation in soil in week 4 and 8 equivalent to 3.30 and 3.35 mgN hr<sup>-1</sup> m<sup>-2</sup> and inoculating azospirillum created nitrogen fixation in the root with a maximum of 0.20 mgN hr<sup>-1</sup> m<sup>-2</sup>. As most of free living microbe lived in the soil, nitrogen in the root was higher, which was in line with Toopakuntho (2010) who found that azospirillum could cause the maximum nitrogen fixation in the maize root of 0.046 mgN hr<sup>-1</sup> m<sup>-2</sup>. Such result aligned with the finding of this research, that is, *Azotobacter chroococcum* resulted in the highest nitrogenase activity and total nitrogen in soil on average. Meanwhile, *Azospirillum lipoferum* generated the highest total nitrogenase activity in root and nitrogen in maize on average. Nevertheless, there were some nitrogenase activity and nitrogen in non-inoculation. Therefore, it showed that there were some microbes in soil which could fix nitrogen, but the amount of microbes may be not much. The amount of fixed nitrogen was relatively low and not adequate to the demand of maize.

#### 4.3 The Correlation of Total Fixed Nitrogen (N<sub>f</sub>) and TNA of Suwan 5 Maize

Figure 1 demonstrated the correlation of TNA and N<sub>f</sub> in week 4 and 6. The tendency of correlation was positive. It was found that the relation between TNA and N<sub>f</sub> assumed linear equation  $y = 0.0224x + 18.045$ ,  $R^2 = 0.8687$  (left) and  $y = 0.0117x + 14.57$ ,  $R^2 = 0.7604$  (right) for week 4 and 6 respectively, demonstrating that TNA and N<sub>f</sub> had relatively high correlation. The amount of N<sub>f</sub> changed accordingly to the amount of TNA in which the equation was consistent with Phookkasem (2011). Toopakuntho (2010) studied the total amount of nitrogen and found that the total amount of nitrogen moved in the same direction.

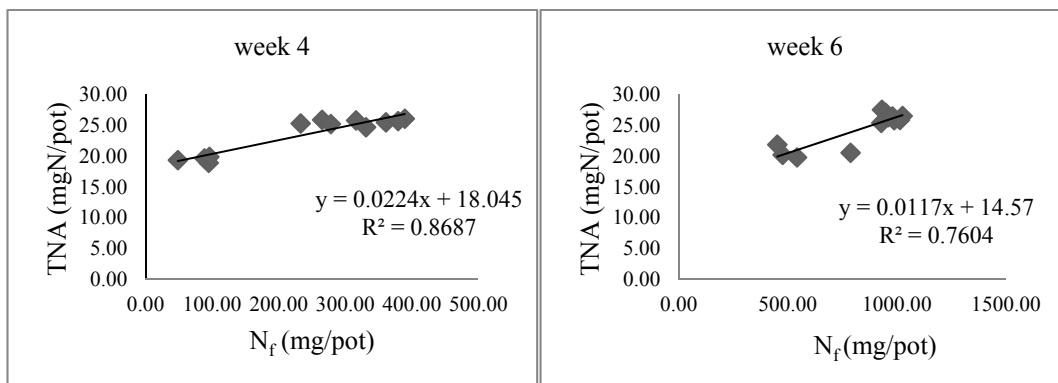


Figure 1. The correlation between total fixed N ( $N_f$ ) and total nitrogenase activity (TNA) at week 4 (left) and 6 (right)

In accordance with the correlation between SNA and TNA of *Azotobacter chroococcum* presented in figure 2, it was found that TNA ( $\text{mgNpot}^{-1}\text{day}^{-1}$ ) and SNA ( $\text{mgNpot}^{-1}\text{day}^{-1}$ ) carried linear correlation which  $y = 0.8153x - 3.102$ ,  $R^2 = 0.7805$  (left). The correlation between RNA ( $\text{mgNpot}^{-1}\text{day}^{-1}$ ) and TNA ( $\text{mgNpot}^{-1}\text{day}^{-1}$ ) of *Azotobacter chroococcum* was  $y = 0.1847x + 3.102$ ,  $R^2 = 0.1543$  (right). In comparing the correlation between TNA and SNA of *Azotobacter chroococcum*, their correlation was higher than that of TNA and RNA. This aligned with the experimental result. That is, *Azotobacter chroococcum* put the strongest effect to nitrogenase activity in soil on average and this was higher than that in maize root because most of azotobacter lives freely in soil (Tejara, 2005)

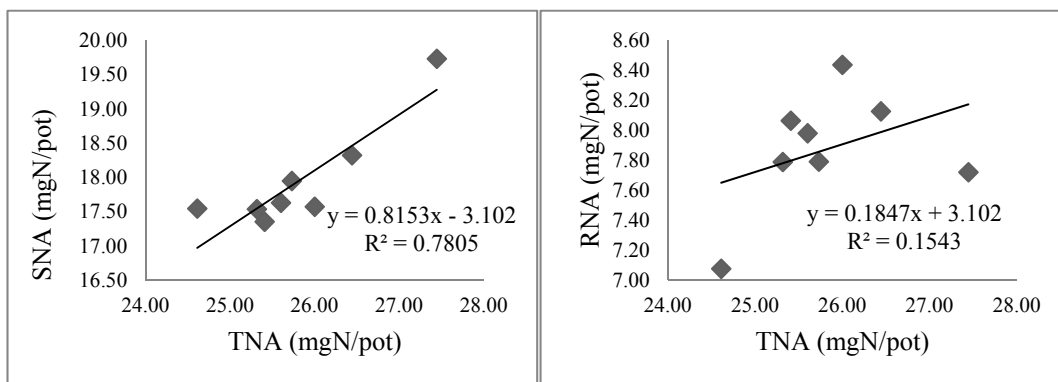


Figure 2. The correlation between total nitrogenase activity (TNA) and soil nitrogenase activity (SNA) (left) and the correlation between total nitrogenase activity (TNA) and root nitrogenase activity (RNA) (right) of *Azotobacter chroococcum*

The correlation between SNA and TNA of *Azospirillum lipoferum* presented in figure 3 demonstrated that TNA ( $\text{mgNpot}^{-1}\text{day}^{-1}$ ) and SNA ( $\text{mgNpot}^{-1}\text{day}^{-1}$ ) carried linear correlation which  $y = 0.6015x + 1.6812$ ,  $R^2 = 0.5973$  (left), while that RNA was  $y = 0.3985x - 1.6812$ ,  $R^2 = 0.3944$  (right). In comparing the correlation between TNA and SNA of *Azospirillum lipoferum*, it revealed that they had correlation which was higher than that of the correlation between TNA and RNA. This differed from Tejara (2005) who found that azospirillum had high correlation in the root area and root surface of sugar cane. No other microbes were found at the surface and inside the root of sugar cane in Spain. In this regard, this may be due to environment and kinds of plant which affect the amount of inocula powder and nitrogenase activity of azospirillum. In the first phase of maize's growth, *Azospirillum lipoferum* increased rapidly in week 2-4 and decreased quickly in a period that the maize fully grew in week 6-8, leading to the decline of azospirillum to move into the root (Toopakuntho, 2010). Consequently, the correlation between RNA and TNA was lower than that of SNA and TNA.



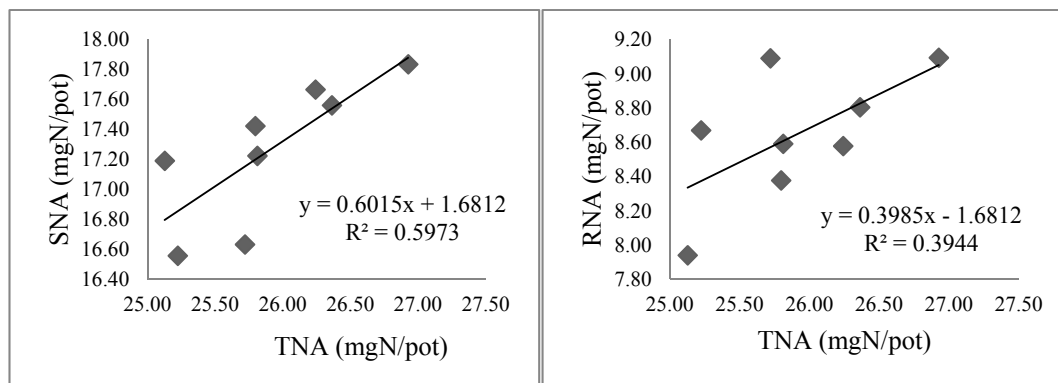


Figure 3. The correlation between total nitrogenase activity (TNA) and soil nitrogenase activity (SNA) (left) and the correlation between total nitrogenase activity (TNA) and root nitrogenase activity (RNA) (right) of *Azospirillum lipoferum*

When comparing the correlation between TNA and SNA of *Azotobacter chroococcum* and *Azospirillum lipoferum* (figure 2, 3 left), it could be seen that the correlations in of *Azotobacter chroococcum* were higher than that of *Azospirillum lipoferum*. In considering the correlation between TNA and RNA (right) of two inoculum powders (figure 2, 3 right), the correlation in *Azospirillum lipoferum* was higher than that of *Azotobacter chroococcum*. This had the highest alignment with nitrogen and nitrogenase activity of azotobacter in soil on average. The *Azospirillum lipoferum* generated the highest nitrogen in maize and nitrogenase activity in maize root on average.

## 5. Conclusions

From the results on correlations between TNA and total fixed nitrogen in the day-round of *Azotobacter chroococcum* and *Azospirillum lipoferum*, it was found that the time in the day-round of collecting samples had no effects on total nitrogenase activity in soil and total nitrogen in soil and maize. *Azotobacter chroococcum* and *Azospirillum lipoferum* caused the statistical difference in total nitrogenase activity and nitrogen in soil and maize and their amounts were higher than those of non-inoculation. The correlation between the total fixed N ( $N_f$ ) and total nitrogenase activity (TNA) was linear equation  $y = 0.0224x + 18.045$  and  $y = 0.0117x + 14.57$  for week 4 and 6 respectively.

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