



# CONTRIBUTION OF NITROGEN FROM BIOFERTILIZER INOCULUM TO YOUNG OIL PALM UNDER FIELD CONDITION

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# Introduction

- Improper and excessive use of synthetic fertilizer has creates damaging impact to global environments such as water pollution from algal bloom which detrimental to aquaculture industry and water quality (Shumway, 1990; Tanga et al., 2003), greenhouse emission in excess creates imbalance atmospheric gaseous (Zou et al., 2005; Kim and Dale, 2008), groundwater pollution negatively affect water resource for human consumption (Johnson et al., 1991) and soil acidification making soil less fertile for agricultural activity (Campbell et al., 1995; Barak et al., 1997).
- The impact is cascading, difficult to control and manage, and it cost billions to rehabilitate.
- Oil palm is an economic crop, highly traded in the international market is one of the agricultural sector that generates large income to the countries like Malaysia and Indonesia, major oil palm producers. An estimated 74 per cent of global palm oil usage is for food products and 24 per cent is for industrial purposes (USDA 2010).
- Since the 1990s, around 43 per cent area occupied by oil palm cultivation has expanded worldwide driven mainly by demand from India, China and the European Union (RSPO 2011).
- However, in parallel, the sector also contributes to imbalance ecosystem through plantation activities, one of the major activities is manuring. Active promotion to enhance the oil palm production for palm oil, making the oil palm as nutrient-demanding crop. Therefore extensive fertilizer manuring programme has been employed, leading to harmful effect to agro-ecosystem.









# Introduction, contd.

- Application of biofertilizer containing plant growth-promoting rhizobacteria for agricultural production has seen one of the potential solutions to mitigate the harmful effects from synthetic fertilizer (Mohamed and Babiker, 2012; Khan et al., 2012).
- The biofertilizer could reduce synthetic fertilizer dependency or may be an alternative. Plant growth-promoting rhizobacteria (PGPR) belong to several genera namely *Azospirillum*, *Bacillus*, *Pseudomonas* and several others (Esquivel-Cote et al., 2010; Mia et al., 2010; Prasanna et al., 2011; Aziz et al., 2012).
- Plant growth promotion by PGPR involved several direct and indirect mechanisms such as biological nitrogen fixation, phosphate solubilization, phytohormone production and antagonism against plant diseases (Mia et al., 2010; Aziz et al., 2012; Sayyed et al., 2012; Zakry et al., 2012).
- However, the use of biofertilizer for commercial agricultural production especially in oil palm is still in doubt, questionable and inconsistent because of its effectiveness and cost-effective in delivering nutrient to the crop as compared to synthetic or inorganic fertilizer application and also lack of evidence from field experimentation (El-Sirafya et al., 2006).





Study based on previous experiments on oil palm seedlings



**Arrangements of pots in the FELDA field nursery station,  
Bukit Mendi, Pahang**

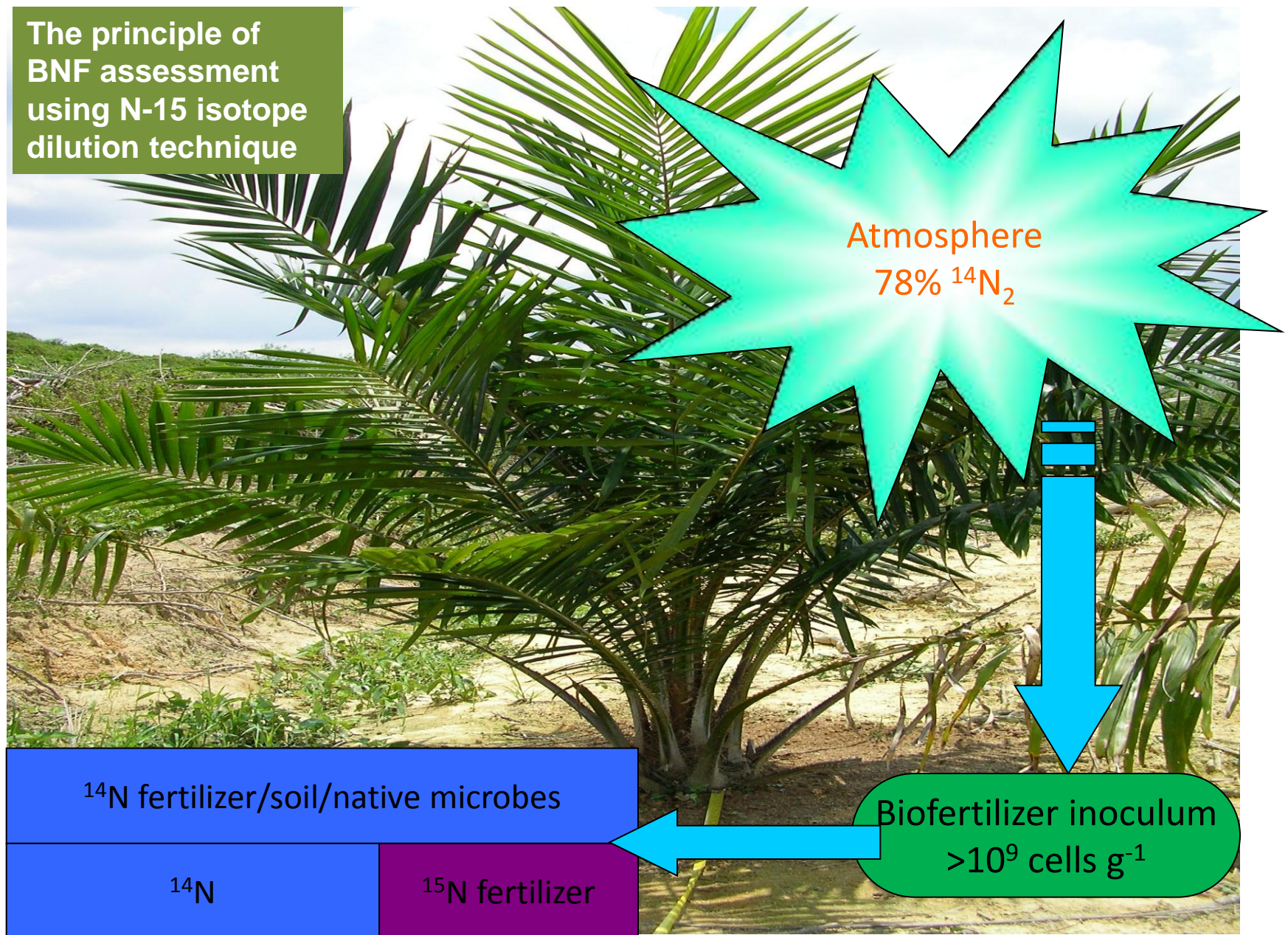


# Objectives

- To evaluate contribution of N from biofertilizer containing diazotrophic plant growth promoting rhizobacteria in comparison with conventional chemical fertilizer in field grown young oil palms using  $^{15}\text{N}$  isotope dilution method.



The principle of  
BNF assessment  
using N-15 isotope  
dilution technique



(from Zakry Fitri Abdul Aziz et al. 2005; 2006)



# Methodology

- The experiment was conducted in a field plot at Tangkah Estate, Sime Darby Plantation Berhad (formerly Golden Hope Plantation Berhad), Tangkak, Johor, in southern Peninsular Malaysia (2° 21' N, 102° 40' E) and soil chemical data as presented in Table 1. Fourteen-month-old GH500 cloned oil palms were allowed to establish for 5 months after transplantation in the field.
- The upkeep and maintenance of the trial plots included a normal estate fertilizer application schedule of inorganic straight fertilizers, comprising N as ammonium sulfate, P as Christmas Island Rock Phosphate, K as muriate of potash, Mg as kieserite and B as borate (Goh and Härdter, 2003).
- *Bacillus sphaericus* UPMB-10, isolated in Malaysia from oil palm roots (Amir et al., 2003), was subcultured on tryptic soy agar (TSA) (Merck KGaA) to produce a pure mother culture for inoculum production (as described in Zakry et al., 2012). The minimum population of strain UPMB-10 was  $\geq 10^9$  cfu g<sup>-1</sup> during field inoculation.

**Table 1. Chemical properties of the soil<sup>2</sup> (Ultisol) from the oil palm experimental field in Tangkak, Johor**

pH (KCl) (1:2.5)	mg kg <sup>-1</sup>				
	Available				
	Total N	P <sup>1</sup>	K	Ca	Mg
4.7	16.0	22.3	19.0	55.0	15.0

<sup>1</sup>Extracted with an aqueous solution of 0.05 M HCl and 0.0125 M H<sub>2</sub>SO<sub>4</sub>

<sup>2</sup>Bungor sandy clay loam soil, with 1.2% total C content

From Zakry et al. (2012)





Packages of UPMB-10 biofertilizer

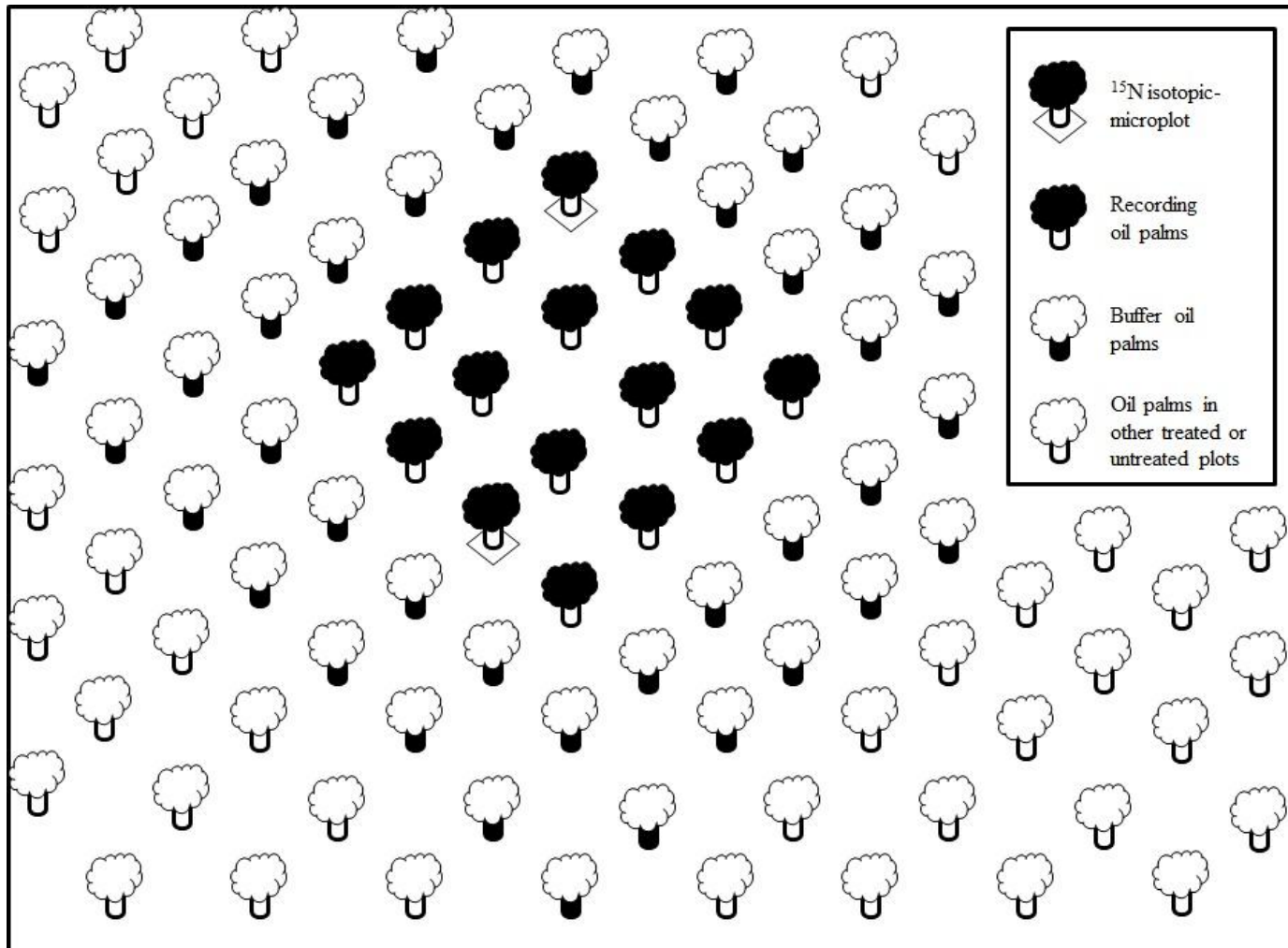
# Methodology

- In the field, the plants were laid down in randomized complete block design with 4 treatments and 4 replicates (Figure 1). The (Uninoculated–Ni+<sup>15</sup>Ni) and (Uninoculated+100%Ni+<sup>15</sup>Ni) treatments served as negative and positive controls, respectively, and also as a benchmark for deficient N (negative control) and optimum N (positive control).
- The (Inoculated+67%Ni+<sup>15</sup>Ni) treatment involved inoculation with *B. sphaericus* strain UPMB-10 inoculum.
- The (Uninoculated+67%Ni+<sup>15</sup>Ni) control treatment had similar Ni (67%) to the inoculated treatment.
- All uninoculated treatments were provided with killed inoculum (gamma-irradiated at 50 kGy) per palm. ‘100% Ni’ and ‘67% Ni’ refer to the full recommended inorganic N fertilizer regime and 67% of the full N fertilizer regime, respectively (as described in Zakry et al., 2012).



# Methodology

- Recordings were made from 16 palms for each of the 16 plots (4 treatments by 4 replicates). Palms in the two outermost rows served as a buffer. The  $^{15}\text{N}$ -labeled fertilizer used was  $(^{15}\text{NH}_4)_2\text{SO}_4$  (ammonium sulfate) with 10.13 atom %  $^{15}\text{N}$  excess (at. %  $^{15}\text{N}$ ) serving as a tracer.
- The field experiment was initiated by the application of  $^{15}\text{N}$ -labeled fertilizer 5 months after transplanting. Within the 16 recording palms, 2 palms (micro-plot) received labeled  $^{15}\text{N}$ , with 10.13 at.%  $^{15}\text{N}$  ammonium sulfate at a rate of  $1 \text{ g N m}^{-2}$ . The  $^{15}\text{N}$ -labeled fertilizer was uniformly applied in liquid form using 2 L distilled water per isotopic plot of  $1 \text{ m}^2$  size.
- The plots were then covered with black polythene sheets evenly to reduce  $^{15}\text{N}$ -labeled fertilizer loss. A week later, the black polythene sheets at the  $^{15}\text{N}$  isotopic microplots were removed after the inner surface of each sheet was rinsed with water prior to inoculum application. The black polythene sheets were used once only for all inoculated and uninoculated  $^{15}\text{N}$  isotopic microplots. Inoculum for the first inoculation was then applied followed by the second inoculation four months later.
- The (Inoculated+67%Ni+ $^{15}\text{Ni}$ ) treatment was carried out at a rate of 2 kg inoculum (containing more than  $10^9 \text{ cfu g}^{-1}$  *B. sphaericus* UPMB-10) by raking the surface of soil to a depth of approximately 5 cm within an area of  $1 \text{ m}^2$ , and at a rate equivalent to  $296 \text{ kg ha}^{-1}$ .



**Figure 1:** A plot with 16 recording oil palms (including 2 randomly selected palms receiving  $^{15}\text{N}$  labelled-labeled fertilizer) and two outermost rows serving as a buffer. The buffer oil palms help to prevent cross- contamination between plots. They were treated the same as the recording palms in the  $^{15}\text{N}$  isotopic- microplot. Recording palms were also used to conduct vegetative growth measurements (Zakry et al., 2012).





















# Methodology

- Harvesting was carried out 240 days (8 months) after the  $^{15}\text{N}$ -labeled fertilizer application. Four palms from each treatment were harvested destructively, and separated into leaflets, rachis, stem and roots.
- The major roots were extracted with a backhoe tractor, and the remaining roots were excavated by shoveling and sieving the soil within the area occupied by the harvested palm.
- Fresh weights and weights of oven-dried ( $70^{\circ}\text{C}$  for 72 h) sub-samples were recorded.
- Samples were ground to pass through 0.5 mm sieves and analyzed for total N by the semi-micro Kjeldahl method (Bremner, 1996) and atom % $^{15}\text{N}$  excess using the NOI-6PC emission spectrometer at Malaysian Nuclear Agency, Bangi, Selangor, Malaysia.
- The  $^{15}\text{N}$  abundance found in palm tissue was corrected for the atom % $^{15}\text{N}$  excess present in the atmosphere (0.3663 at.% $^{15}\text{N}$ ) (Warembourg, 1993).

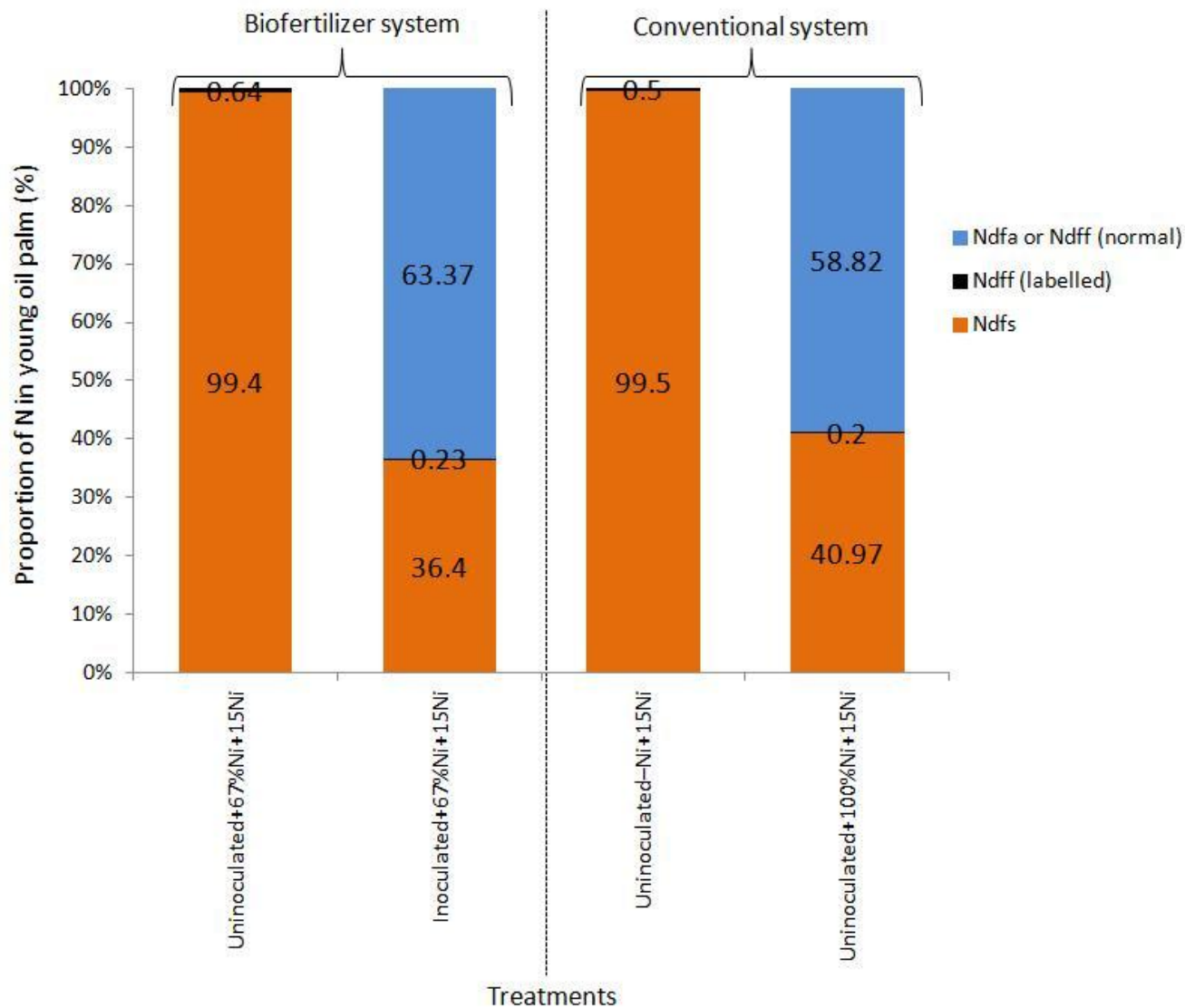


# Methodology

- N<sub>2</sub> fixation in the whole palm was calculated from weighted atom excess (WAE) in the inoculated palm (Inoculated+67%Ni+<sup>15</sup>Ni) and uninoculated palm (Uninoculated+67% Ni+<sup>15</sup>Ni), using the following formula (Zakry et al., 2012):
- where AE, TN, Lf, Rc, St and Rt are atom % <sup>15</sup>N excess, total N, leaflets, rachis, stems and roots, respectively.
- 
- The % of N derived from atmospheric N (%Ndfa) was then calculated as follows:
- 
- To determine the proportions of N from the unlabeled fertilizer (% Ndff (normal fertilizer)), labelled fertilizer (% Ndff (<sup>15</sup>N fertilizer)) and from the soil (% Ndfs), the following formula was used:
- $$\% \text{ Ndff (}^{15}\text{N fertilizer)} = \frac{\text{atom \%}^{15}\text{N excess in plant tissue}}{\text{atom \%}^{15}\text{N excess in labeled fertilizer}} \times 100$$
- (e.g. 10.13 atom %<sup>15</sup>N excess in ammonium sulfate)
- 
- $$\% \text{ Ndfs} = 100 - \% \text{ Ndff (}^{15}\text{N fertilizer)}$$
- 
- $$\% \text{ Ndff (normal fertilizer)} = 100 - \% \text{ Ndff (}^{15}\text{N fertilizer)} - \% \text{ Ndfs}$$
- 
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# Results

- At 240 of plant growth, the inoculated young oil palm had accumulated 0.23% N from the  $^{15}\text{N}$  labeled fertilizer (% Ndff ( $^{15}\text{N}$  fertilizer)) and 36.40% N from the soil (% Ndfs), besides the 63.37% from  $\text{N}_2$  in the atmosphere (% Ndfa) (Figure 1).
- The uninoculated young oil palm at normal fertilization rate accumulated 0.20% Ndff ( $^{15}\text{N}$  fertilizer), 40.97% Ndfs and 58.82% Ndff (normal fertilizer).
- On average, the inoculated young oil palms had slightly higher of N from atmosphere than the fully fertilized young oil palms that received fertilizer-N at 58.82% (Figure 2).
- The results indicated that young oil palm that received biofertilizer containing *B. sphaericus* UPMB-10 had accumulated N at 63.37%, comparable with normal fertilizer at 58.82% (uninoculated+N) and this may be even better as presented in previous study, the biofertilizer containing *B. sphaericus* UPMB-10 also improved total N and dry matter accumulations as presented in Figure 3 and 4 (Zakry et al., 2012).

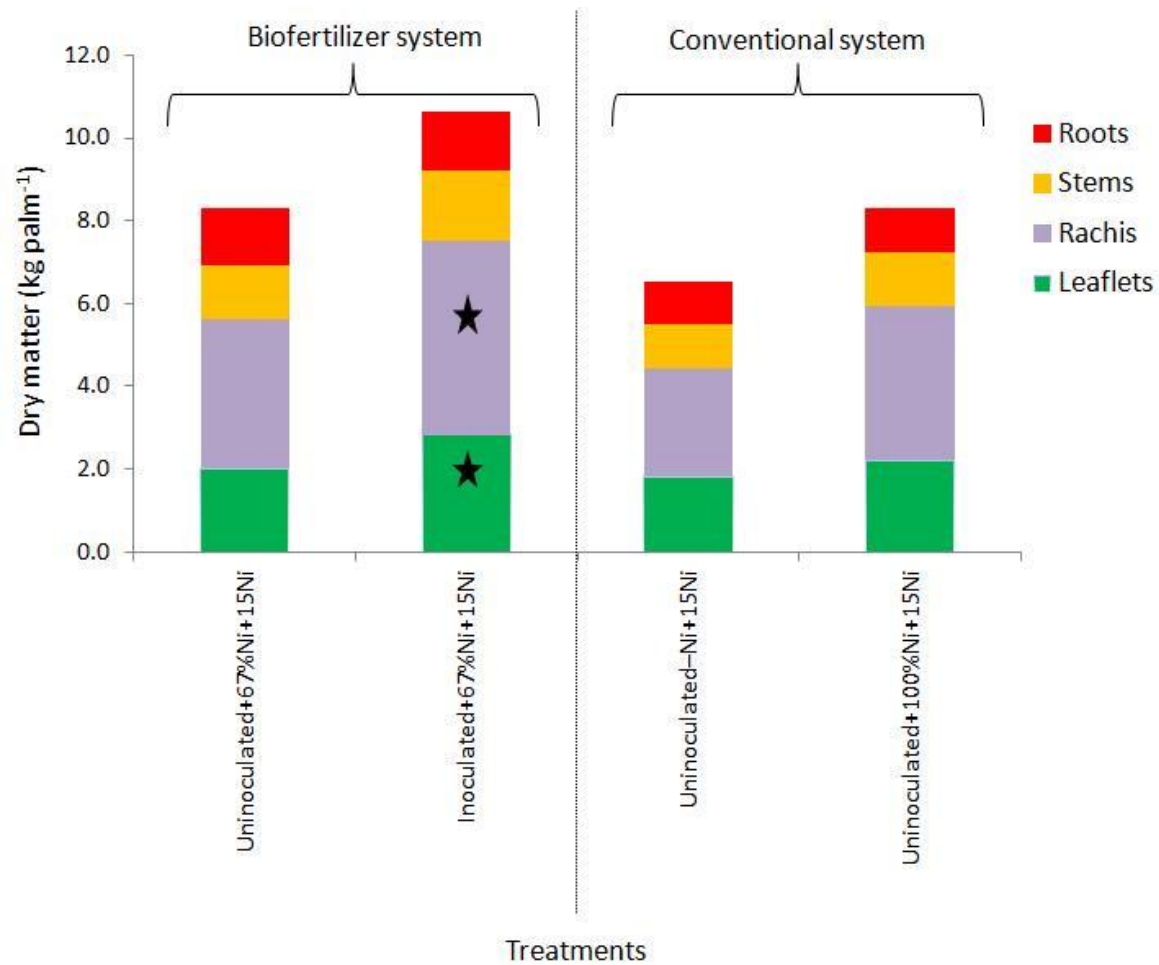


Proportion of N sources in oil palm biomass receiving biofertilizer and conventional fertilizer package

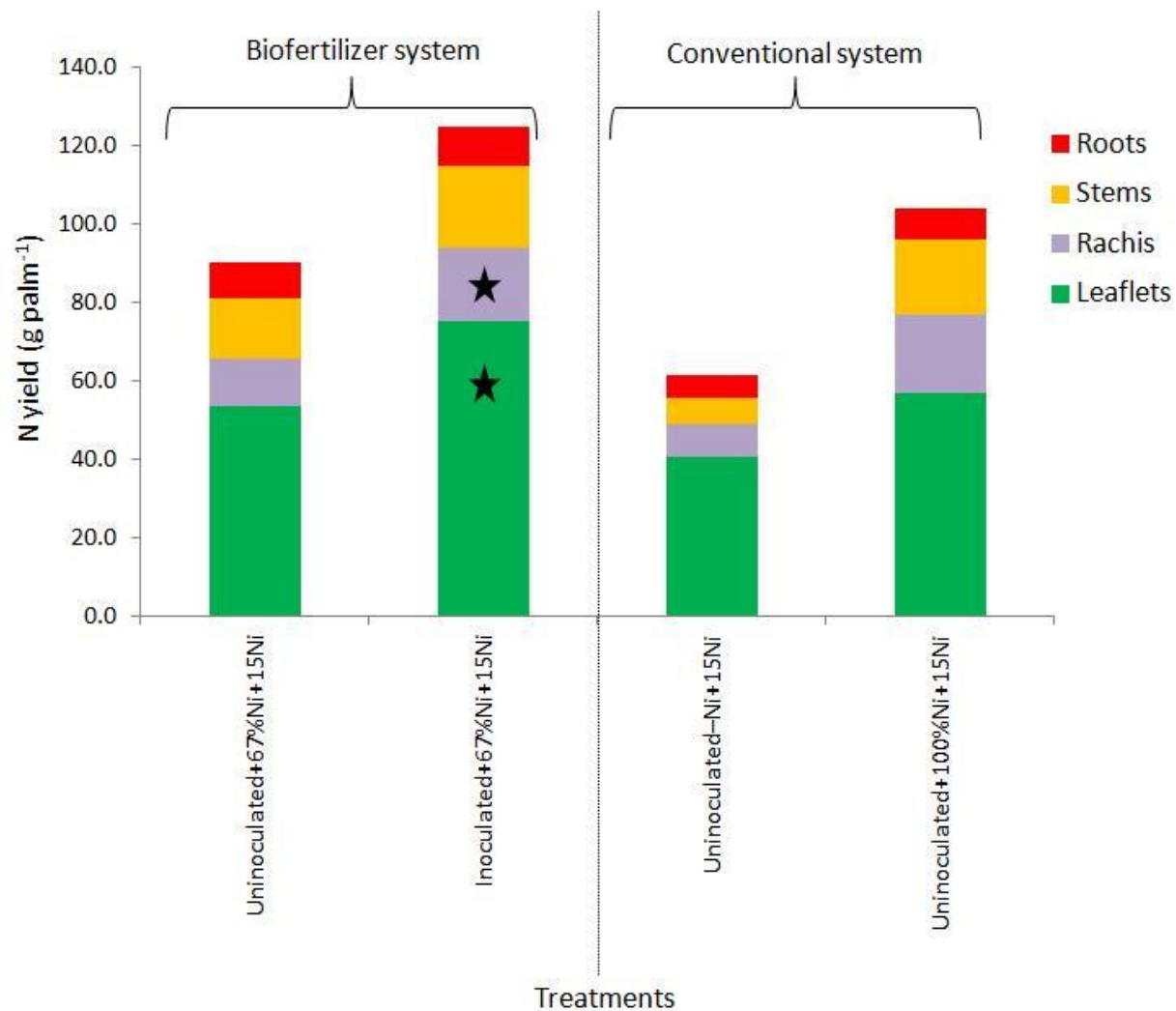


# Results

- Dry matter yield and its distribution in immature young oil palm as presented in Figure 3 indicated that rachis and leaflets dry matter increased significantly in inoculated young oil palm over uninoculated young oil palm.
- Stems and roots dry matter accumulation of inoculated young oil palm similar with fully fertilized young oil palm and this phenomenon may be even better.
- A phenomenal increment as indicated in dry matter accumulation was also similar and consistent with total N yield of young immature oil palm.
- Rachis and leaflets N yield of young immature oil palm increased significantly in inoculated young immature oil palm over uninoculated young immature oil palm.
- Leaflets, rachis, stems and roots dry matter of inoculated young oil palm similar with fully fertilized young immature oil palm and may be even better.



Dry matter yield of oil palm parts receiving biofertilizer and conventional fertilizer package



N yield of oil palm parts receiving biofertilizer and conventional fertilizer package



# Discussion

- This phenomenon indicates an increased amount of N uptake by oil palm especially to rachis and leaflets part which the most nutrient demanding part in oil palm, leaflets, rachis which need for photosynthetic activity.
- Photosynthetic rate positively correlated with total leaf N content and subsequently contributing to the development of vegetative growth (Cassman et al., 1995). In addition, Shaobing et al. (1991) reported that leaf photosynthetic rate in sorghum significantly correlated with biomass and grain production.

# Discussion

- This is great and promising application of biofertilizer in the field or in the commercial practices. In recent years, more biofertilizer application in the field has been conducted to demonstrate its effectiveness to promote nutrient uptake and growth.
- Ahmad et al. (2008) demonstrated two-year pot and field trials and revealed that the organic-fertilizer supplemented with  $88 \text{ kg ha}^{-1} \text{ N}$  was equally effective compared to full dose of N-fertilizer ( $175 \text{ kg ha}^{-1}$ ) in improving root weight, fresh biomass, and ear and grain yields of maize. Interestingly, bio-fertilizer supplemented either with 88 or  $132 \text{ kg N ha}^{-1}$  significantly increased the growth and yield of maize over full dose of N-fertilizer and exhibited superiority over organic-fertilizer.
- El-Sirafya et al. (2006) reported mixed findings. They found a slight additional increase in grain and straw yields when a biofertilizer was applied along with N fertilizer. A slightly higher grain and straw yield was measured with the polymer-coated urea treatment than with the ammonium nitrate treatment. However, the biofertilizer materials were not as effective as N fertilizers in producing grain ( $4.02\text{--}4.09 \text{ Mg ha}^{-1}$ ) or straw ( $7.71\text{--}8.11 \text{ Mg ha}^{-1}$ ) for either year, although the Nitrobien + Phosphorien combination increased these parameters over the N-fertilizer control.

# Discussion

- The advantage of the fixed  $N_2$  over fertilizer-N may be related to the characteristics of N supply by the two modes of application.
- *B. sphaericus* UPMB-10 fixes  $N_2$  directly from the atmosphere within the plant itself, so 'uptake' is almost complete (100%) with no losses to the environment, the N accumulation rate can be solely depend on the effectiveness of bacterial strain to fix  $N_2$ .
- However, much of the fertilizer-N applied may be lost (volatilized, denitrified and leached) (Bijay-Singh et al., 1995) or simply remain in the soil unabsorbed. For example, applying urea will generally only give less 50% recovery by the plants (Halvorson et al., 2002).

# Conclusion

- Biofertilizer use can complement use on conventional chemical fertilizers in oil palm plantation.
- The use of biofertilizer and implementing biofertilizer system in agricultural production can potentially be exploited towards environmentally friendly and sustainable agricultural crop production and environmental health.
- Even though present study successfully demonstrated that biofertilizer containing PGPR *Bacillus sphaericus* strain UPMB-10 can efficiently delivers nutrients, its effect on the whole crop productivity is still unclear.
- Thus in future field trial needs to be conducted to evaluate the effect of biofertilizer inoculation on growth, yield and oil productivity in mature oil palm.



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