Ministry of Education, Culture, Sports, Science and Technology (MEXT)
Forum for Nuclear Cooperation in Asia (FNCA) Biofertilizer Project

FNCA Biofertilizer Newsletter

Issue No. 13
March, 2015

Overview of FNCA Biofertilizer Project 2014

Tadashi Yokoyama, Tokyo University of Agriculture and Technology (TUAT)
Shotaro Ando, Japan International Research Center for Agricultural Sciences (JIRCAS)

In order to promote environmental friendly sustainable agriculture in Asia, FNCA Biofertilizer Project aims to develop biofertilizers with radiation sterilization technology, using benefical microorgamisms, which increase the yields of crops while reducing the environmental burden of excessive use of chemical fertilizers.

At the first phase of this project from 2001 to 2006, FNCA Biofertilize Manual was published, which gives information and experiences of biofertilizer use in Asian countries, including their effectiveness, efficient production processes, storage and application on different crops, as an important outcome.

At the second phase from 2007 to 2011, main objectives were 1) developing multifunctional biofertilizer having different two functions such as plant growth promoting and resistance against plant pathogens, and 2) dissemination of radiation sterilization method of carrier using ^{60}Co to improve quality of carrier for biofertilizers.

From 2012, third phase was started to enhance above 2 objectives and it was also focused on synergy effect between biofertilizer and irradiated oligochitosan as a new theme.

FNCA 2014 Workshop on Bifertilizer Project was held on November 24th - 27th in Kajang, Malaysia with 12 participants from 8 countries.

This is the final fiscal year of the third phase; therefore, progress and research summaries for the past three years were reported by each member country. The participants also discussed actively on the core topics involving research and development of biofertilizers: (1) application of radiation sterilization of carriers for production of better quality commercial biofertilizers, (2) development of multifunctional biofertilizers and strategy for extension to farmers, (3) evaluation for results of experiments on the synergistic effect of biofertilizers and irradiated oligochitosan, and (4) development of the FNCA Guideline Vol.2 Production of biofertilizer carriers using radiation.

Proposals were made during discussion regarding the three year evaluation and project activity plans for the next phase, including 1) accumulation of data on the beneficial effects of irradiation carriers to maintain biofertilizer organisms in comparison to autoclaved carriers, and 2) publication of the benefits of irradiated carriers in comparison with autoclaved carriers. An agreement was reached that member countries would continue to disseminate the use of irradiated carriers to biofertilizer producers, to study combinations of microbes to develop multi-functional biofertilizers, and to evaluate experiments for synergistic effects of biofertilizers and irradiated oligochitosan.

Participants of the Workshop

Panel Discussion of Open Seminar

Development and Application of High Effective Biofertilizers in China

Fan Bingquan, Chinese Academy of Agricultural Sciences (CAAS)

1. Under application of $Ca_3(PO_4)$, inoculation of fungus A734 and A66 as P-solubilizing biofertilizers increased both peanut and sunflower biomass by more than 100%. Under application of rock phosphate, inoculation of P-solubilizing biofertilizer had a biomass increase by more than 80% for peanut and over 70% for sunflower, respectively. (Table1).

2. Inoculation of A44, PG24 and A7 with $Ca_3(PO_4)_2$ increased the peanut by 21.3%, 20.1% and 20.1%, and increased the biomass of sunflower by 22.3%,11.3% and 9.2%, respectively. Increasing rate of peanut inoculated with PG24, A7, A55 and C2 with rock phosphate were 16.2%, 13.3%, 10.8% and 11.3%, and increased the biomass of sunflower by 12.5%, 14.5%, 20.4% and 19.0%, respectively (Table1).

3. A field experiment of sunflower with biofertilizer was conducted in Inner Mongolia, strain F6 as antagonistic biofertilizer got the highest yield, averaged 6010.1 kg/ha and increased by 21.5%; the yield of strain Y16 as biofertilizer reached the better yield, averaged 5858.6 kg/ha, the increase of sunflower was 18.4% (Table 2).

4. The effect of oligochitosan combined with P-solubilizing fungi on garlic growth was studied. Strain A52 with oligochitosan had a higher synergetic effect on garlic biomass, strain A52 plus oligochitosan increased garlic biomass by 24.6% than treatment of only strain A52. Strain A5 got a higher biomass both with and without chitosan (Table 3).

Table 1 Biomass of sunflower and peanut with new P-solubilizing trains with Ca3-P and rock phosphate (g/pot)

Strain	Phosphate added to soil	Peanut fresh weight (g)		Sunflower fresh weight (g)	
		yield	Increase%	yield	Increase %
CK	$Ca_3(PO_4)_2$	5.27	-	4.04	-
A734		11.34	115.2	8.59	112.6
A66		10.74	103.8	11.57	186.4
A44		31.7	21.3	31.5	22.3
PG24		31.4	20.1	28.7	11.3
A7		31.4	20.1	28.2	9.2
CK	rock phosphate	5.05		4.706	
A734		9.94	88.6	10.12	114.9
A66		10.48	98.9	8.11	72.2
A7		33.2	13.3	29.2	14.5
PG24		34.0	16.2	28.7	12.5
A55		32.4	10.8	30.7	20.4
C2		32.6	11.3	30.4	19.0

Table 2. Effect of biofertilizer on sunflower yield with chemical fertilizers (Inner Mongolia)

Treatment	Seed yield		
	kg/plot	kg/ha	Increase %
CK	3.27	4949.5	-
T22	3.53	5353.5	8.2
Y16	3.87	5858.6	18.4 **
F6	3.97	6010.1	21.5 **

Table 3. Garlic fresh biomass influenced by P-solubilizing strains and chitosan (g/pot)

Strain	P-solubilizing strains (PS)	PS strain+chitosan	Biomass Increase of PS+ Chitosan over PS	Increase %
	fresh wt	fresh wt		
without	small	3.89	-	-
A85	3.93	4.45	0.52	13.2
A93	3.48	4.31	0.83	23.9
A5	4.53	5.55	1.02	22.5
C2	4.28	5.11	0.83	19.4
A55	3.53	3.93	0.4	11.3
A52	3.74	4.66	0.92	24.6
A42	4.16	4.56	0.4	9.6

Status and Strategy of Commercial Application of Biofertilizer in Indonesia

Dr. Iswandi Anas, Bogor Agricultural University (IPB), Indonesia

Introduction

Chemical fertilizers had been introduced to Indonesian farmers since the earlier 1960 during the era of green revolution. At that time, most of agriculture soils in Indonesia were still good, agricultural soils were rich in organic matter. Only by adding chemical fertilizers mainly N, P and K, a high yield of several crops was obtained. Therefore, most of the farmers only used chemical fertilizers and they forgot to use organic fertilizers. The reasons using only chemical fertilizers was more handy, less work and need less labour compared to using organic fertilizers. However, in the earlier 1980-ies, some farmers or scientists had reported that there was significat drop in yield of several crops. In order to keep the yield high, the farners mostly use more chemical fertilizers up to unreasonable amount of chemical fertilizer. In reality, the yield did not increase by increasing the rate of chemical fertilizers application, the cost of fertilizers certainly increase, the environment become more degraded. In the earlier 2000, the goverments, scientists and the farmers realized that the use only chemical fertilizers organic fertilizer was not the right choice of using fertilizers for agriculture. The use of organic fertilizers together with biofertilizers in combination with chemical fertilizers or when the soil is already good in properties and the good quality of organic fertilizers are available, the use of organic fertilizers only is possible. At the some time, the use of biofertilier related to nitrogen fixing microbes, phosphate solubilizing microbes, antagonists are also introduced to farmers.

Regulations related to commercialization of organic fertilizer and biofertilizer in Indonesia

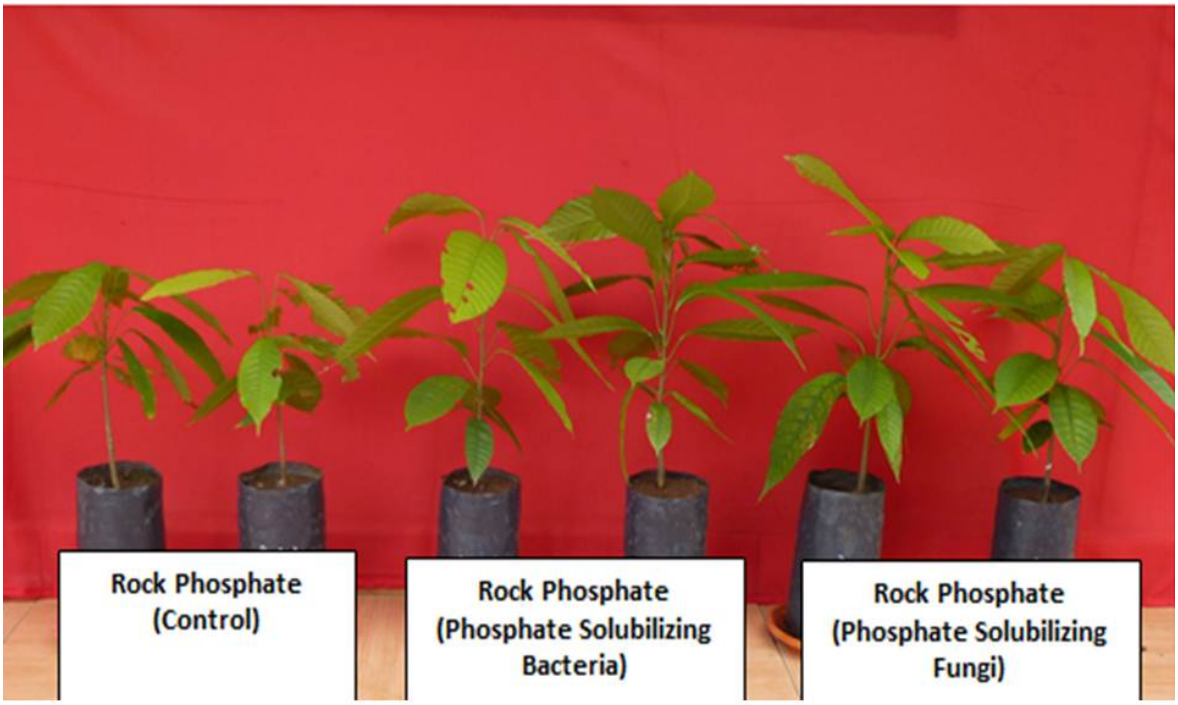
According to Government Regulation No. 8, 2001, about fertilizer for agricultural crops, point 6 mentioned that all fertilizers produced in Indonesia or imported ferilizers, before their distribution in Indonesia, the fertilizres must be registered to obtain Registration Number at Ministry of Agricuture. For this purpose, the Minister of Agriculture had issued Ministry of Agriculture Regulation No. 28/Permentan/SR.130/5/2009 related to the prosedure for registration of organic fertilizer, biofertilizer and soil conditioner. This regulation has been revised by Ministry of Agriculture through Ministry of Agriculture Regulation No. 70/Permentan/SR140/10/2011. Registration of fertilizers is done through Center for Plant Variety Protection and Registration under Directorate General of Sarana and Prasarana Pertanian and the administrative works is done under Center for Plant Variety Protection and Perizinan Pertanian. Since 2005 till 2011, 533 organic fertilizers, 126 biofertilizer and 162 soil conditioners have been registered at Ministry of Agriculture.

Status and strategy of commercial application of biofertilizer

Several universities, National Sciences Agency, some private cmpanies and research institutions do researches and promoting biofertilizer. These institutions developed cost-effective technologiesof inoculants such as nitrogen fixing bacteria, phosphate solubilizing microbes, mycorrhizae and antagonists againt plant pathogens. Since the number of bio-organic fertilizers and biofertilizers becoming more populair nowadays in Indonesia, the Ministry of Agriculture in 2012 took inisiative to evaluate intensively several bio-organic and biofertilizers. The evaluation of these bio-organic and biofertilizers are being done through several stages until now. The evaluation of these bio-organic and biofertilizers are being done for seven main important crops for Indonesia, i.e. rice, corn, soybean, potato, chilli, sugarcane, red-onion. The main purpose of this evaluation is to select several best bio-organic and biofertilizers that can reduce the rate of chemical

fertilizers currently applied by farmers for these crops. As much as 17 bio-organic and biofertilizers are being evaluated by Ministry of Agriculture. The name of bio-organic and biofertilizers are being evaluated are: Agrimeth, Agrifit, Gliocompost, Bio-PF, Agrisoy, Biotrico (produced by Agricultural Research and Development Agency, Ministry of Agriculture), Probio and Super-Biost (Bogor Agricultural University-IPB), Kedelai Plus, Biovam, StartMik, Beyonic (National Sciences Agency), BOC-SRF, Bio-SRF and Biopin (Technology Assessment and Application Agency), and Bio-Up and Bio-Padjar (University Padjadjaran).

Temporary results show that some of these selected bio-organic and biofertilizers have excellent performance in reducing the rate of chemical fertilizers. However, intensive evaluation and multi-location pf these bio-organic and biofertilizers evaluation are really needed before the government recomment these bio-organic and biofertilizres to be used in large area by farmers.



Effect of phosphate solubilizing bacteria and phosphate solubilizing fungi on growth of cacao seedlings fertilized with rock phosphate

Commercialisation of Biofertilizer – From the Laboratory to the Field

Khairuddin Abdul Rahim and Phua Choo Kwai Hoe,
Malaysian Nuclear Agency (Nuclear Malaysia)

The Ministry of Science, Technology and Innovation, Malaysia (MOSTI) announced the year 2014 as "MOSTI Commercialisation Year" to promote commercialisation of research outputs. With the support from MOSTI, Malaysian Nuclear Agency (Nuclear Malaysia) received two ScienceFund grants for biofertilizer projects in 2007 and 2011 for research and development of biofertilizer products. Nuclear Malaysia has developed a series of multifunctional bioorganic fertilizers from these grants, which include two multifunctional bioorganic fertilizers namely, MULTIFUNCTIONAL BIOFERT PG & PA and a pellet bioorganic fertilizer as MF-BIOPELLET, in an effort to reduce dependency on chemical fertilizer for crop production. Acceptance of biofertilizer by agroindustry especially oil palm and rubber plantation has increased over the years. In 2009, Malaysian Agri Hi-Tech Sdn. Bhd. (MYAGRI), the bioproducts company with BioNexus status, utilising *Bacillus megaterium*, a biofertilizer inoculum generated through R&D of Nuclear Malaysia in one of its products, agriCare®ORGANIC-N. In 2011 the company produced about 40t of agriCare®ORGANIC-N. To date, more than 200t has been produced, with worth of RM 630,000 in sales. On 28 August 2013, the Minister of MOSTI launched a liquid biofertilizer (BIOLIQUIFERT) produced by Nuclear Malaysia as one of the products generated through the government ScienceFund grants. This is an effort of government to commercialise R&D products, and this could be capitalised to promote the use of biofertilizer to our agroindustry.

Nuclear Malaysia had demonstrated effectiveness of biofertilizer in paddy rice field trials in 2013 and 2014. This effort is to promote biofertilizer to the farmer through R&D. The trial was carried out at MADA plots, Kedah in 2013. There were four treatments, namely, T1 (Commercial fertilizer), T2 (Liquid biofertilizer), T3 (50% Commercial fertilizer + 50% liquid biofertilizer) and T4 (Control, non-treatment). Liquid biofertilizer contains phosphate solubiliser, potassium solubiliser and plant growth promoter. Two paddy plots, under flooded (wet) and aerobic (dry) conditions, with three rice varieties MR 219 (parent) and mutant varieties MR 219-4, and MR 219-9, were used in these studies. Biofertilizer treatment showed highest yields estimation for MR 219-9, which was at 8.51 t/ha (flooded plot) and 9.55 t/ha (aerobic plot) as compared to other treatments (Figure 2). Yields estimation of controls were 6.78 t/ha (flooded plot) and 4.05 t/ha (aerobic plot). Figures 1 and 3 show high yield estimation for biofertilizer treatments for another two varieties under flooded plot (MR 219-4) and aerobic plot (MR 219). Estimation yields for MR 219-4 was 7.81 t/ha and MR 219 was 8.06 t/ha. Performance of biofertilizer on paddy rice depended on varieties and planting conditions. From these results, combination variety MR 219-9 with biofertilizer, which was adaptable for flooded and aerobic conditions was recommended to the farmer.

Another paddy rice field trial was conducted in farmers' plots in Perlis in 2014 and 2015. Three varieties MR 219 parent), and mutants MR219-4 and MR 219-9 were used in this trial. Four treatments were T1 (Biofertilizer + liquid smoke), T2 (Biofertilizer + liquid smoke + Oligochitosan), T3 (Biofertilizer + oligochitosan) and T4 (Control, famer practice). Biofertilizer, liquid smoke and oligochitosan were produced by Nuclear Malaysia. There were no significant difference ($p \leq 0.05$) among the treatments for MR 219 and MR 219-9 (Figure 5 and 6). Combination treatment T2 showed significant yields as compare to treatment T1 and treatment T3 in variety MR 219-4 (Figure 4). Estimation yields for T1, T2, T3 and T4 were 12, 15, 11 and 17 t/ha, respectively. There was no significant difference between treatment T2 and treatment T4, which was farmer practice. Thus, combination treatment consist biofertilizer, liquid smoke and oligochitosan was suggested to be adopted in the paddy rice field.

In conclusion, R & D of biofertilizer should not end in the market but need to move forward to the farmer.

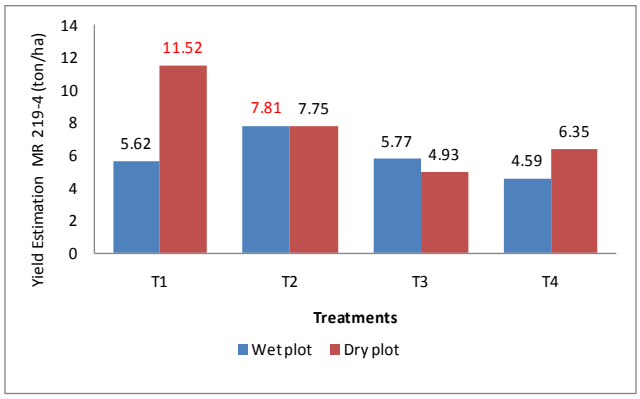


Figure 1: Yield estimation MR 219-4 (t/ha), MADA, Kedah

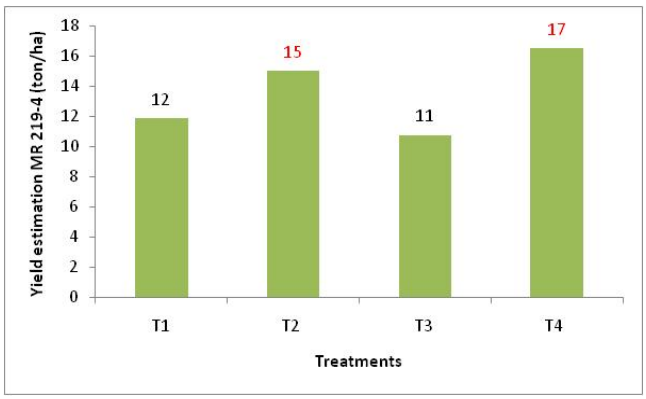


Figure 4: Yield estimation MR219-4 (t/ha), Perlis

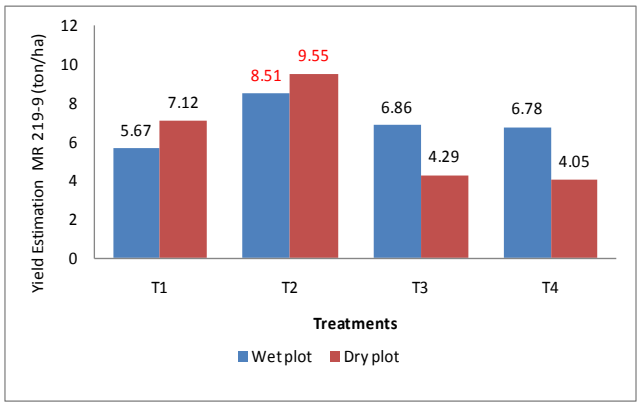


Figure 2: Yield estimation MR 219-9 (t/ha), MADA, Kedah

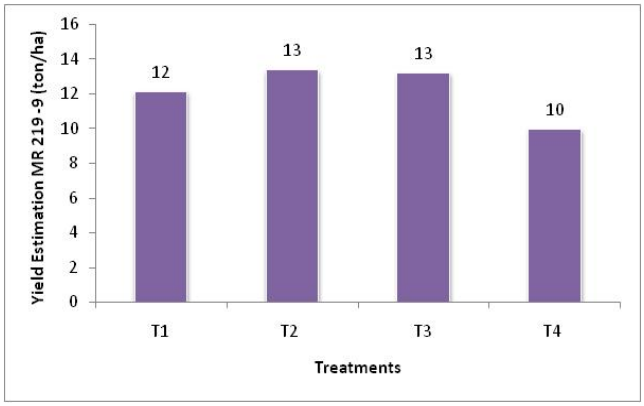


Figure 5: Yield estimation MR219-9 (t/ha), Perlis

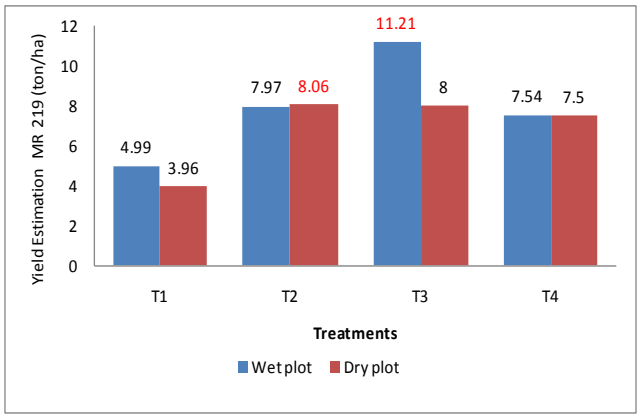


Figure 3: Yield estimation MR219 (t/ha), MADA, Kedah

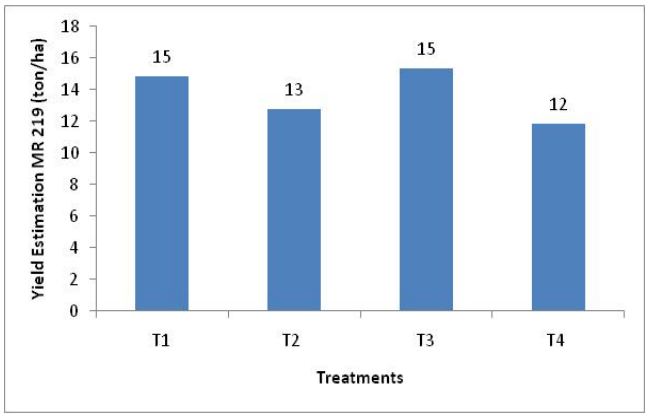


Figure 6: Yield estimation MR219 (t/ha), Perlis

Perlis field trial

FNCA Biofertilizer Research Activity in 2014

Delgermaa Bongosuren, Plant Science Agricultural Research Institute (PSARI)

At present new varieties of wheat is being used by farmers. The need for generating more data on the response of *biofertilizer* to new varieties is very essential to convince more farmers on the *Rhizobacterial biofertilizer* usage.

There were experiments conducted in 2014, three at the experimental field area at PSARTI. The plant materials that were used in the experiments conducted during the period were wheat, barley and tomato.

1) Effect of rhizobacterial biofertilizer inoculation for wheat and barley

We have been studied effect of Rhizobacterial biofertilizer were developed on wheat and barley crops in field experiment. The wheat and barley crops were planted in norm of 3,5-4,0 mln seed/hectare by seeder SZS-2,1. The soil of a skilled site chestnut, sandy with easy mechanical structure.

Experience according to the scheme:

1. Control without fertilizer **- T-1**
2. Liquid biofertilizer 10 l/ha, mixing with seeds **- T-2**
3. Rhizobacterial biofertilizer/dry type/8 kg/ha, mixing with seeds **- T3**

And nutrient content of the soil increased, allowing for sustainable improvement of its fertility.

Higher yield followed by inoculums biofertilizer higher than the control. Use all of biofertilizer on wheat grain yield increased by 16.4-19.8%, and barley grain yield increased by 12.7-17.3 % more than control. Application of Rhizobacterial biofertilizer gave a positive effect and also affected the soil fertility.

The results showed that biofertilizer can increase soil fertility and biomass, as well as crop yield.

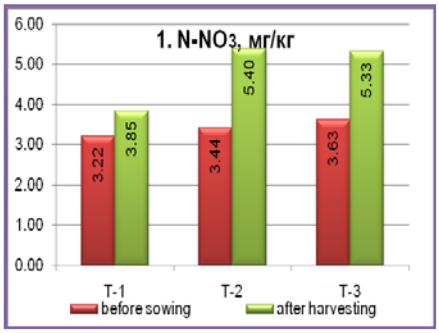
2) Experimental results on synergy between biofertilizer and irradiated oligochitosan

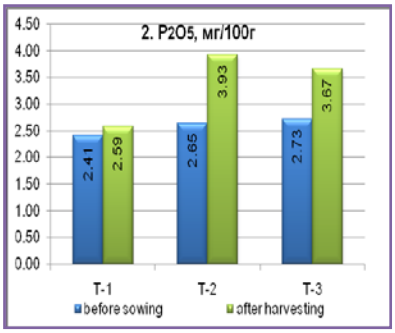
Experimental design was RCBD with 4 replications. Treatments as fallow;

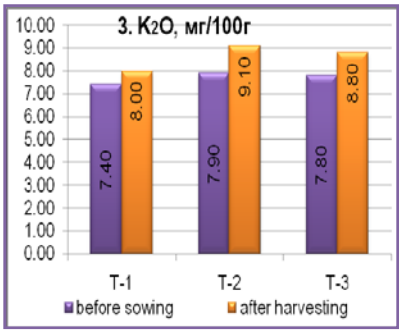
- Oligochitosan 7(every week) **T-1**
- Oligochitosan 14 (every 2 weeks) **T-2**
- Rhizobacterial biofertilizer + Oligo **T-3**
- Rhizobacterial biofertilizer + Oligo 14 **T-4**
- Rhizobacterial biofertilizer **T-5**
- Control **T-6**

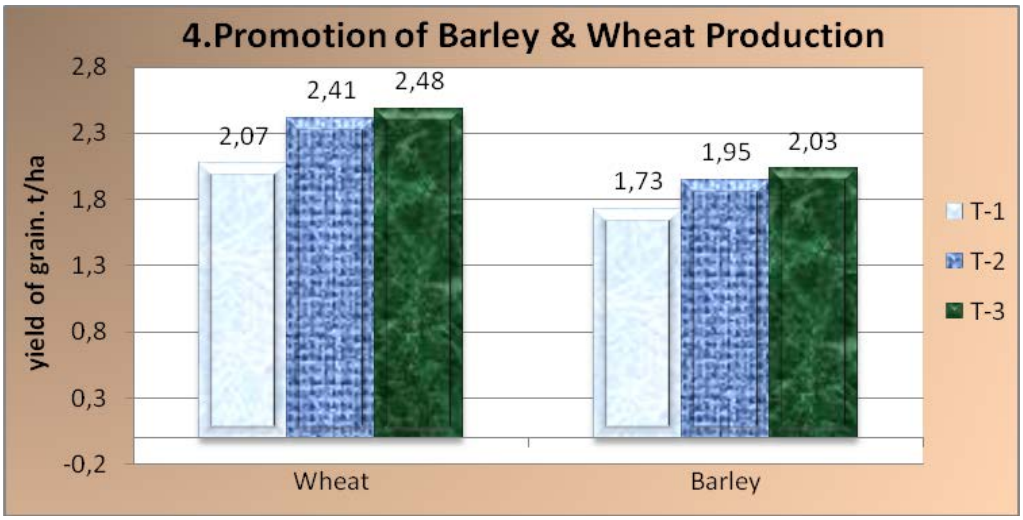
Pot contained 20 kg of sterile soil. Pathogenic fungus is Fusarium sp. For rhizobacterial biofertilizer inoculation treatment seed were coated at sowing. Cultivate pathogenic fungus and mix them into sterile soil were planting the seedlings before one week. The performances of tomatoes seedling were spraying the oligochitosan solution (100ppm) before 1 day transplanting. In conclusion the effect of integrated use of Oligochitosan, with Fusarium on tomato seedling were investigated in this experiment. The performances of tomatoes seedling were spraying the oligochitosan solution (100ppm) every week and every two weeks during the 2 months. Survey tomato biofertilizer effect on the growth of the highest measured at 40th days after planting. However, the highest harvest yield measurement O-14 releases version 1335 g, and higher than 860 g of control

Results of soil agro chemistry









Green house experiment of tomato

1. Tomato harvest

Treatments	Height of plant, cm	Yield of fruit, /by harvesting/		Yield of fruit	
		Number of fruit per plant	Weight of fruit, g	g/ plants	g/fruit
T-1	57.8	24	875	218.8	36.4
T-2	57.5	39	1335	333.8	34.2
T-3	58.8	16	609	152.3	38.1
T-4	57.8	25	785	196.3	31.4
T-5	60.3	29	1065	266.3	36.7
T-6	49.0	23	475	118.8	20.7

Status and Strategy of Commercial Application of Biofertilizer (*Bio N*^{TM}) in the Philippines

Julieta A. Anarna, University of the Philippines Los Baños (UPLB)

Introduction

The National Institutes of Molecular Biology and Biotechnology (BIOTECH) University of the Philippines Los Banos (UPLB) developed cost-effective technologies inoculants such as nitrogen fixing bacteria, mycorrhiza or forms of phosphate solubilizers for the production of goods and services that are cheaper alternatives to conventional products, making use of locally available materials which are safe to the environment.

There are many forms of biofertilizers which are readily available in the market and being produced by private and government sectors. Among these biofertilizers, the most studied and developed biofertilizer is *Azospirillum*, with a registered brand name of *Bio N* which has been shown to increase the income of the farmers by increasing yield of rice and corn by eleven percent (11%) and reduced on its chemical usage.

Status and strategy of commercial application of biofertilizer.

Transfers of technology on use of biofertilizers requires the acceptance of the end users and support of the government. In order to promote biofertilizers we conducted trainings and briefing on Bio N technology meetings with cooperatives and seed growers, field demonstrations (Figure 1), tapping broadcast and distributed flyers/posters, collaboration with seed producers/distributors and presentation and participation in trade show/Agricultural Exhibits/Conventions/Forum (Figure 2).

The establishment of mixing plants is one of the strategies in commercial application of biofertilizer. The mixing plants are considered sales centers and distribution channels to make it available to local consumers at affordable price. Currently eighty three (83) mixing plants were already established throughout the country to make Bio N ^{TM} available for the farmers. (Figure 3)

Figure1. Field demonstration of Bio N ^{TM} for rice, corn and tomato in the Philippines

Figure2. Presentation, distribution of brochure and participation in trade show/Agricultural Exhibits/Conventions/Forum

Figure3. Establishment of Bio N Mixing Plant

Effectiveness of Gamma Radiated Solid Carrier on Survival of Effective Microbe in PGPRs Biofertilizer

Phatchayaphon Meunchang and Achara Nuntagit
Department of Agriculture (DOA), Ministry of Agriculture and Co-operative (MOAC)

Major problem of of Biofertilizer products is the contamination of indigenous microbes in solid carriers. The contaminated strains might inhibit survival of the active biofertilizer strain by produced antibiotic and other prohibits substances. Therefore, free from indigenous microbe of solid carriers was studied to develop high potential of PGPRs biofertilizer product for crops production.

The bacterial strain used in this study was *Azospirillum* sp.TS8-42 which mutated from electron beam 150 Gy at 8 Mev. It resists the highest temperature at 43 ºC, fixed nitrogen 65±15 nmole C_2H_4 $tube^{-1}$ h^{-1}, IAA production for 70±4 mg ml^{-1} and positive sideropore production. The medium for culture the liquid starter was NFb Dobereiner, (1980). The liquid starter was cultured to mid log phase at 48 hours by using starter 9 percent in aerobic condition. The liquid bacterial was injected to the 25 KGy of gamma irradiated bark compost for 150 g per bag for 50 ml or moisture content in carrier between 50-60 percent by weight. The gamma irradiated carrier showed synergic effect on support longer survival of *Azospirillum* sp.TS8-42 used as model of PGPRs microbe in this study (Figure 1), which extended survive of *Azospirillum* sp.TS8-42 from 30 days of non sterile carrier kept in room temperature to 240 days for treatment of gamma irradiated carrier and 360 days for treatment gamma radiated carrier kept at 25 ºC.

Benefit from this project could lead to improve biofertilizer production in Thailand to under control of new biofertilizer act of the kingdom of Thailand.

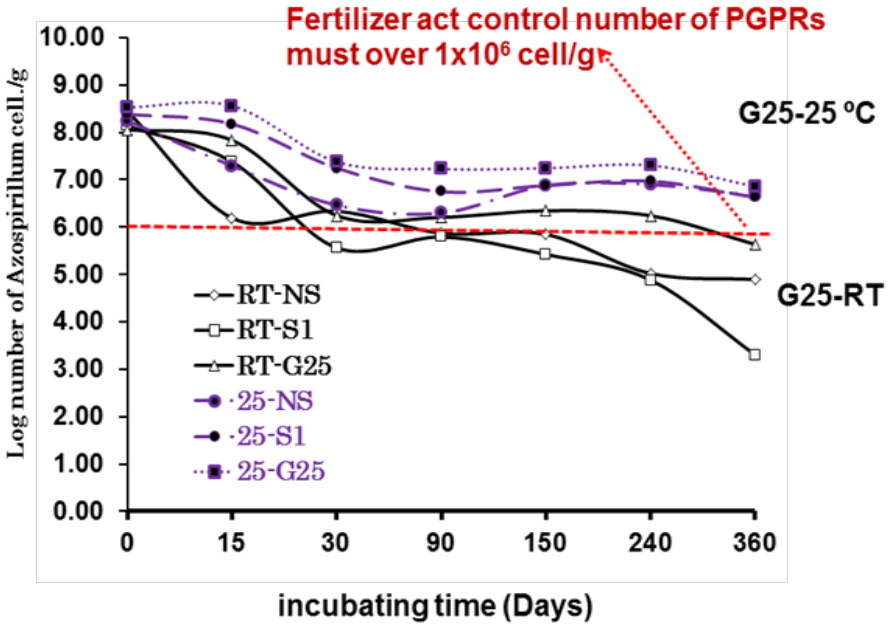


Figure 1 The effect of non sterile (NS), autoclave (S1), gamma sterile (G25) carriers and storage in room temperature (RT) and 25 ºC (25) on survival of *Azospirillum*.

Multifunctional Biofertilizer Research and Development in 2014

Pham Van Toan, Ministry of Agriculture and Rural Development (MARD)

1. Introduction

Continuing the research and development of biofertilizer for sandy soil, in 2014 the biofertilizer project concentrated on testing the effect biofertilizer on growth, yield of peanut cultivated in sandy soil. The improvement of fertility of sandy soil applied with biofertilizer is also evaluated.

2. Material and methods

Biofertilizer for sandy soil contained N-fixer *Bradyrhizobium japonicum*, P-solubilizer *Bacillus megaterium*, Cilicate solubilizer *Paenibacillus castaneae* and polysaccharid producer *Lipomyces starkeyi* with the density of more than 10^8 CFU/gram is used in the study. Trials was conducted in Binhdinh province with following treatments: 1. Base fertlizer (100% NPK: 30.60.90), 2. 100% NPK (30.60.90) + Biofertilizer, 3. 90% NPK (30.60.90) + Biofertilizer, 4. 80% NPK (30.60.90) + Biofertilizer and 5. 70% NPK (30.60.90) + Biofertilizer.

3. Results

As results of effect of multi-functional biofertilizer on growth, yield of peanut in sandy soil of Binhdinh province can be see that biofertilizer saved 10% of required NPK. In this case, the green biomas and grain yield of peanut showed no significant different to control. Reduction of required NPK more than 10% brings lower yield and benefit for farmer. The same tendency can be obtained in the economic effect evaluation (Figure 1, 2). Sandy soil applied multi-functional Biofertilizer had higher moisture and higher population of beneficial microbes than the control without biofertilizer.

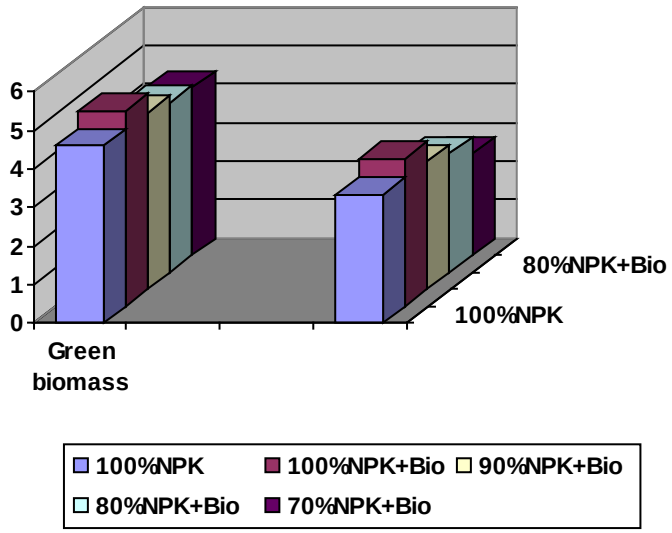


Figure 1. The effects of biofertilizer on the yield of peanut cultivated in sandy soil

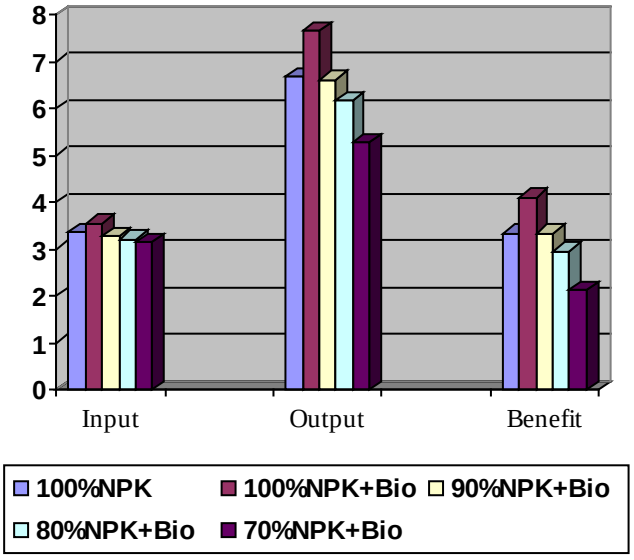


Figure 2. Economical effect of biofertilizer on peanut production in sandy soil (10.000VND)

Figure 3. Field experiment and demonstration on the benefit of biofertilizer