

Increasing Vigor, Nitrogen, and Phosphat Content of Oil Palm in Pre Nursery by Seed Coating and Enrichment *Trichoderma asperellum*, *Enterobacter sacchari*, and Arbuskular Mycorrhiza Fungi (AMF)

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Abstract. Seed coating and enrichment have a central objective to improve seed performance. The aim of this study is to enhance seed vigor, nitrogen and phosphate content in shoot and rhizosphere by seed coating and enrichment in pre nursery. Treatments were controls, negative controls, coated and uncoated seed with enrichment of *E. sacchari*, coated and uncoated seed with enrichment of AMF, coated and uncoated seed with enrichment of *T. asperellum*, coated and uncoated seed with enrichment of consortium *T. asperellum* + *E. sacchari*, coated and uncoated seed with enrichment of consortium AMF + *E. sacchari*, coated and uncoated seed with enrichment of consortium *T. asperellum* + AMF, coated and uncoated seed with enrichment of consortium *T. asperellum* + AMF + *E. sacchari*. Observation were conduct towards seed germination, rate of germination, nitrogen and phosphate content in rhizosphere and shoot. Generally, coating seed with CMC 1,5% was not given significantly differences result for all treatment. Enrichment with consortium microbes promising to increase vigor, and nutrient content of seedling in pre nursery.

Keywords: Seed enhancement, technology, PGPR, phosphate, consortium

1 Introduction

Seed coating is the treatment of coating the seeds with a thin layer without changing the shape of the seeds in order to improve seed appearance, improve storage capacity, reduce the risk of the disease from the environment around the seed, and can be used as a carrier of additives, such as antioxidants, anti-microbes, growth regulators [1]. Coating materials that can be used include arabic gum, sodium alginate, CMC, gypsum, and other non-toxic ingredients. The combination of enrichment and coating of seeds has been widely used, for example in rice, Coating with using 3% alginate + 1% peat + *P. diminuta* A6 was able to increase the percentage of pithy and empty grain weight per panicle and the percentage of pithy and hollow grain per panicle [2]. Enrichment and coating of seeds using talk + CMC 1.5% + 1% glycerol + probiotic bacteria can also be used to maintain the viability of rice seeds infected with *X. oryzae* pv. *oryzae* for 6 weeks in storage [3].

Endophytic microbes have the potential to be added through seed enrichment. The microbes that can be utilized are AMF and *Trichoderma* sp. The use of these two microbes has become a standard operational procedure (SOP) in the preparation of oil palm seedlings, especially if the seeds will be planted on land infected with *Ganoderma boninense*. The dose of microbial use continues to increase along with the stage of seedling development. The dose of AMF was given is 5 g per seed, increasing to 50 g per tree in the field [4]. A hectare land of oil palm has around 136 plants, it needs about 6.800 g/ha. Problems arise because of AMF carried along with the carrier material, zeolite, so that the transport of AMF to the land will high. Application of AMF through enrichment and coating of seeds hoped can be a solution for transporting these microbes to the land effectively and efficiently.

The application of *T. asperellum* in oil palm plants mainly aims to control *Ganoderma boninense*. [5] applied *T. asperellum* by soaking the seed for 1 hour on liquid suspension of *T. asperellum* followed by 1.5% CMC coating, effective to increase the growth of oil palm

seedlings in the pre-nursery and can maintain *T. asperellum* on roots until 12 weeks after sowing. In addition, enrichment techniques with soaking technique is more effective to inserting microbes into seeds because microbes directly contact with seeds. Another microbe that is widely used in seed enrichment is *Enterobacter sacchari*. This bacterium is an endophyte bacteria in sugarcane which has the ability to fixation nitrogen from the air [6].

Enrichment and coating are generally applied to horticulture seeds, while application to oil palm seeds has not been done much because the structure of seed is more susceptible to mechanical damage than other seeds. The enrichment and coating of oil palm seed has been investigated by [5] using *T. asperellum* and [7] using *Burkholderia* sp. use various types of coating materials. The application of consortium AMF, *T. asperellum*, and *E. sacchari* followed by CMC 1.5% coating is expected to increase the vigor of oil palm seedlings at the pre-nursery stage.

2 Method

2.1 Seed

The seed was DxP Tenera aged 21 days from PT Dami Mas Sejahtera, Riau. Length of plumule 1-3 cm and radicle 2-3 cm. Microbes were *Trichoderma asperellum*, AMF genus *Accaulaspora*, and *Enterobacter sacchari* from collection of PT SMART Tbk.

2.2 Increased seed vigor through seed enrichment with microbes and coating

This experiment used a randomized block design with two treatments, i.e. enrichment with microbes and 1.5% CMC coating. The enrichment process was carried out according to the Jawak method (2016). One repetition of the treatment consisted of 30 seed. The volume of distilled water to soak 90 seed was 1,5 L. The dose of AMF used was 5 g of inoculum for one seed (5 g of inoculum containing 132 spores). The *E. sacchari* and *T. asperellum* inoculums used were pellets with a density of 10^9 (*E. sacchari*) and 10^7 cfu /mL (*T. asperellum*). Treatment consisted of controls (without microbes and without coating), negative controls (without microbes and with 1.5% CMC coating), coated and uncoated seed with enrichment *E. sacchari*, coated and uncoated seed with enrichment of AMF, coated and uncoated seed with enrichment of *T. asperellum*, coated and uncoated seed with enrichment of consortium *T. asperellum* + *E. sacchari*, coated and uncoated seed with enrichment of consortium AMF + *E. sacchari*, coated and uncoated seed with enrichment of consortium *T. asperellum* + AMF, coated and uncoated seed with enrichment of consortium *T. asperellum* + AMF + *E. sacchari*. Microbial culture for single microbial enrichment was made by dissolving each microbial inoculum into 1.5 L of distilled water. The microbial consortium culture was made by dissolving two or three types of microbial inoculum according to the treatment into 1.5 L of the same distilled water. After enrichment, seeds were coated with 1.5% CMC using seed coating machine type AGH 2016, with a speed of 25 rpm for \pm 3 minutes. CMC is sprayed manually on seeds. After perfect coating the seeds were dried for \pm 30 minutes, then planted in a 21 x 15 cm polybag containing top soil media. The polybag was then placed on the bed.

2.3 Observation

Plant growth parameters

Seed germination : percentage of seed that grow into normal seedling on 20th and 30th days after planting (DAP). Rate of germination : the number of seeds that have grown normally every day until 40th DAP.

Analysis of levels of nitrogen (N) and phosphate (P) in soil

Analysis of soil N and P was carried out before and after the planting medium was planted with seeds (3 months after sowing (MAS)). Analysis was carried out from three soil samples taken randomly in every replication, then samples of test soil in PT SMART Tbk analytical laboratory. The analysis method for total N is + FIA. The total P analysis method is ICP-OES and available P is Bray.

Analysis of N and P levels in leaf tissue

Analysis of N and P leaf tissue was used to determine nutrient status of nitrogen and phosphorus in plants. The material used is leaves. The leaves were cleaned then dried in an oven at 70°C for 24 hours (Huda 2015). After that, the leaves were crushed using a blender and sifted with a 0.5 mm sieve. The leaves that passed the filter were then tested at the PT SMART Tbk analytical laboratory. The analytical method used is dry ashing + ICP-OES, N (colorimetry).

2.4 Data calculation and statistical analysis

The data obtained from the experiment was calculated with analysis of variance (ANOVA) to determine the statistical significance of measurement parameters among treatments. If the result from ANOVA showed significant differences ($P < 0.05$), Duncan's multiple range test (DMRT) was continually used to compare each treatment. The analysis was done using STAR statistical analysis software.

3 Result

3.1 Seedling Vigor

Treatments were significantly different to seed germination and rate of germination. Enrichment with consortium microbes AMF + *E. sacchari*, *T. asperellum* + AMF, *T. asperellum* + AMF + *E. sacchari* in coated seed showed the highest seed germination percentage. As for uncoated seed, consortium microbes *T. asperellum* + *E. sacchari*, AMF + *E. sacchari*, and *T. asperellum* + AMF + *E. sacchari* showed seed germination percentage above 90% (Table 1). Seed germination was calculated based on normal seedling in 20 and 30 days after planting (DAP). Enrichment with consortium microbes for coated or uncoated seed has highest seed germination percentage. Meanwhile, control, enrichment with *E. sacchari*, AMF, and *T. asperellum* needed more that 30 days to reach normal seedling above 90%.

Table 1. The Effect of Interaction between enrichment and coating on seedling germination

Seed Enrichments	Seed germination (%)	
	Coated CMC 1,5%	Uncoated CMC 1,5%
Control	61,1 dB	73,3 dA
<i>E. sacchari</i>	82,2 cB	88,9 cA
AMF	53,3 eA	45,6 fB
<i>T. asperellum</i>	64,4 dA	60,0 eA
<i>T. asperellum</i> + <i>E. sacchari</i>	87,8 bB	95,6 abA
AMF + <i>E. sacchari</i>	95,6 aA	98,9 aA
<i>T. asperellum</i> + AMF	96,7 aA	92,2 bcA
<i>T. asperellum</i> + AMF + <i>E. sacchari</i>	95,5 aA	95,6 abA

Note : Means in the same row suffixed with different lowercase letters or in the same column with different uppercase letters are different at 5% levels of significance according to DMRT

Seed enrichment with *T. asperellum* + *E. sacchari* showed the highest percentage rate of germination for uncoated seed (Table 2). Generally, coated seeds with CMC 1,5% were not gave significantly different result with control.

Table 2. The Effect of Interaction between Enrichment and Coating on Rate of Germination

Seed Enrichments	Rate of germination (% etmai ^{l-1})	
	Coated CMC 1,5%	Uncoated CMC 1,5%
Control	3,3 abA	2,8 cB
<i>E. sacchari</i>	3,1 bA	3,4 bA
AMF	2,3 cA	1,9 dA
<i>T. asperellum</i>	2,5 cA	2,4 cdA
<i>T. asperellum</i> + <i>E. sacchari</i>	3,6 abB	4,3 aA
AMF + <i>E. sacchari</i>	3,6 abA	3,7 bA
<i>T. asperellum</i> + AMF	3,6 abA	3,5 bA
<i>T. asperellum</i> + AMF + <i>E. sacchari</i>	3,7 aA	3,8 abA

Note : Means in the same row suffixed with different lowercase letters or in the same column with different uppercase letters are different at 5% levels of significance according to DMRT

Generally, enrichment seed with consortium microbes were more vigorous than control. It happened because of consortium microbes can help in vigorizing seedling and more over to reducing production cost by reducing the use of fertilizer in pre nursery. When two or three organisms are inoculated together, they are known to show better performance over single inoculation of microbes with different functional properties as long as they are compatible. Consortium microbes also useful to enrich microbial diversity in the rhizosphere of crop plants and enhance the interaction of microbes [8].

3.2 Nitrogen and Phosphat Content in Plant

Seed coating is application of substance or material to the seed surface, which enhance the seed performance without altering shape on the seed coat which improve germination. Application of microbes in seed coating refers to enhance nutrients in the surrounding of the seed when the seed is planting. Nitrogen content in soil before and after treatment was not significant different. Enrichment with *E. sacchari* could not increase nitrogen content. It happens probably because of the treatments directly insert the microbes into the roots. The seed directly use the nitrogen from fixation [9].

Soil which has highest nitrogen content was uncoating seed with AMF enrichment. Coating treatment gave the significant differences of nitrogen content in soil. The nitrogen soil content of coated seeds with enrichment *E. sacchari*, *Mycorrhiza* sp. + *E. sacchari*, and *T. asperellum* + *Mycorrhiza* sp. were higher than uncoating seed. Otherwise, uncoated seed with enrichment *Mycorrhiza* sp., *T. asperellum* + *E. sacchari*, and *Mycorrhiza* sp. + *T. asperellum* + *E. sacchari* were higher than coated seed (Table 3).

Table 3. The Effect of Treatments on Nitrogen Soil Content

Seed Enrichments	N-soil (%)	
	Coated CMC 1,5%	Uncoated CMC 1,5%
Before Planting	0,12 gA	0,12 eA
Control	0,16cA	0,16aA
<i>E. sacchari</i>	0,14 eB	0,15 bA
AMF	0,18aA	0,16 aB
<i>T. asperellum</i>	0,14 eA	0,14 cA
<i>T. asperellum</i> + <i>E.sacchari</i>	0,15 dA	0,13 dB
AMF + <i>E. sacchari</i>	0,13 fB	0,14 cA
<i>T. asperellum</i> + AMF	0,12 hB	0,16 aA
<i>T. asperellum</i> + AMF + <i>E. sacchari</i>	0,17 bA	0,16 aB

Note : Means in the same row suffixed with different lowercase letters or in the same column with different uppercase letters are different at 5% levels of significance according to DMRT

Total phosphorus in soil was not influenced by the interaction between seed coating and enrichment. Coating treatments decreased total phosphorus in soil. Enrichment treatments were not give significantly differences with control (Table 4).

Table 4. The Effect of Treatments on Total Phosphorus Soil Content

Seed Enrichments	Total Phosphorus (ppm)		
	Coated CMC 1,5%	Uncoated CMC 1,5%	Means
Before Planting	2925	2925	2925 d
Control	4450	4761	4605 ab
<i>E. sacchari</i>	4798	5330	5064 a
AMF	4315	4655	4485 b
<i>T. asperellum</i>	4399	4525	4462 b
<i>T. asperellum</i> + <i>E.sacchari</i>	3884	4508	4196 bc
AMF + <i>E. sacchari</i>	4340	4167	4254 bc
<i>T. asperellum</i> + AMF	3640	4196	3918 c
<i>T. asperellum</i> + AMF + <i>E. sacchari</i>	4149	5126	4638 ab
Means	4100,04 b	4465,93 a	

Note : Means in the same row suffixed with different lowercase letters or in the same column with different uppercase letters are different at 5% levels of significance according to DMRT

Uncoated seed with enrichment AMF + *E. sacchari* has highest available phosphat content in soil. As for uncoated seed, seed with enrichment consortium *T. asperellum* + AMF + *E. sacchari* and AMF + *E. sacchari* were highest among all treatments (Table 5).

Table 5. The Effect of Treatments on Available-P Soil Content

Seed Enrichments	Available-P (ppm)	
	Coated CMC 1,5%	Uncoated CMC 1,5%
Before Planting	8,39 iA	8,39 gA
Control	19,50 hA	24,90 fB
<i>E. sacchari</i>	28,80 dA	25,90 eB
AMF	27,00 eA	26,00 eB
<i>T. asperellum</i>	23,80 fB	31,40 bA
<i>T. asperellum</i> + <i>E.sacchari</i>	22,10 gB	28,60 cA
AMF + <i>E. sacchari</i>	36,10 aA	34,30 aB
<i>T. asperellum</i> + AMF	30,40 cA	27,90 dB
<i>T. asperellum</i> + AMF + <i>E. sacchari</i>	34,10 bA	34,50 aA

Note : Means in the same row suffixed with different lowercase letters or in the same column with different uppercase letters are different at 5% levels of significance according to DMRT

The highest shoot-N content was coated and uncoated seed with enrichment AMF (2,65 %) and 2,77 %), uncoated seed with enrichment *E. sacchari* (2,63%) and coated seed with enrichment *T. asperellum* + AMF + *E. sacchari* (2,72%) (Tabel 6). Generally, enrichment with

AMF increase shoot-N content, but it still need more time to make sure that the effect of treatments. Observation until plant in main nursery was needed. [10] reported that AMF could increase phosphat and nitrogen uptake by plant.

Table 6. The Effect of Treatments on Shoot-N Content

Seed Enrichments	Shoot-N Content (%)	
	Coated CMC 1,5%	Uncoated CMC 1,5%
Control	2,23 bA	2,06 cdA
<i>E. sacchari</i>	2,14 bcB	2,63 aA
AMF	2,65 aA	2,77 aA
<i>T. asperellum</i>	2,21 bA	2,34 bA
<i>T. asperellum</i> + <i>E. sacchari</i>	2,06 bcA	2,11 bcdA
AMF + <i>E. sacchari</i>	1,90 cB	2,19 bcA
<i>T. asperellum</i> + AMF	2,10 bcA	1,90 dA
<i>T. asperellum</i> + AMF + <i>E. sacchari</i>	2,72 aA	2,03 cdB

Note : Means in the same row suffixed with different lowercase letters or in the same column with different uppercase letters are different at 5% levels of significance according to DMRT

The main purpose of AMF in this research was to increase phosphat content in both of soil and shoot, but it didn't show up. It happens probably because of the lack amount of AMF spores that we use (5 g propaguls contained of 132 spora). Research of [11] reported that the number of spores that effectively increase plant growth was 500 spores and it takes more than 15 months to see the effect of inoculation AMF-plant.

Research of [12] reported that first stage of AMF growth in infected plant occurred more than 3 months. On the end of first month after inoculation, there were appresion and intraradical hypae in root cortex tissue. On the end of second month after inoculation, the formation of hypae intraradical tissue was begun. On the end of third month after inoculation, formation of arbuscula was begun. The presence of the spore has been seen before transplanted to main nursery. Therefore, this research need more evaluated and longer time to see the real effect of consortium microbes and seed coating with CMC 1,5%.

4 Conclusion

Seed coating was not affected seedling vigor or increasing nutrient content in shoot and soil. Seed enrichment was promising to enhance seedling vigor and nitrogen and phosphat content in shoot and soil.

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6 Reference

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