

# The Effectiveness Of Pre-Planting Seeds Bio-Matriconditioning And Application Of Fertilization With Leisa Technique On Growth Of Areca Nut Seedling (*Areca Catechu L.*)

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## Abstract.

*Areca nut is widely used as industrial raw material, both for health and cosmetics and as a source of renewable energy. This study aimed to examine the combination of seed biomatriconditioning and LEISA fertilization treatments that were effective in increasing the growth of areca nut seedlings. The research was carried out in the Wua-Wua Kendari Village, from March to July 2021. The study used a split-plot design with a completely randomized design (CRD). The main plot is seed biomatriconditioning treatment which consists of 3 treatments. While the subplots were fertilized with the LEISA technique which consisted of 6 treatments so that 18 treatment combinations were obtained with 3 replications. Observations were made on plant height, number of leaves, stem diameter, number of roots, wet weight and dry weight of shoot. Observational data were analyzed using analysis of variance, followed by the DMRT  $\alpha_{0.05}$  if there was a significant effect. The results showed that seed biomatriconditioning treatment with rhizobacteria integrated with the LEISA technique was able to increase the growth of areca nut seedlings. The integration between LIR biomatriconditioning and the application of organic plus fertilizer + 100% inorganic fertilizer showed a better growth performance of areca nut on plant height, number of leaves, stem diameter, number of roots, wet weight and dry weight of betel nut which were significantly different with control and application 100% inorganic fertilizer, but not significantly different from organic plus fertilizer, organic plus fertilizer + 50% inorganic fertilizer and organic plus fertilizer + 25% inorganic fertilizer. As a conclusion, pre-planting seed treatment with LIR biomatriconditioning is very important to increase areca nut seed germination. To increase the growth of areca nut seedlings, further fertilization needs to be done with organic plus fertilizer or a combination of organic plus fertilizer + 25% inorganic fertilizer.*

**Keywords:** *Areca nut; biomatriconditioning; inorganic fertilizer; LEISA; organic plus; seedling*

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## I. INTRODUCTION

Areca nut is one of the most widely used palm plants as industrial raw materials, both for health and cosmetics. However, the areca nut cultivation system is limited by cultivation techniques, especially seeds that are in the latent phase. Areca nut contains arecoline, an alkaloid that is beneficial for health<sup>1</sup>. Areca nut is also reported to contain high phenolic and flavonoid compounds<sup>2</sup> and also contains cellulose, hemicellulose and lignin<sup>3</sup>. In addition, areca nut can also be used as a source of renewable energy<sup>4</sup>. However, in reality, areca nut has not been widely developed by the community even though this plant provides many benefits and has the opportunity as an export crop. Southeast Sulawesi has a large dry land and very potential for the development of areca nut. The main obstacle to the development of areca nut in Southeast Sulawesi is the use of poor quality seeds that affect plant growth in the field. In addition, other factors that affect plant growth and yield are application of fertilizers for plant nutrient needs. Therefore, in order to overcome these problems, technological inputs are needed that can overcome the problem of quality seeds and fulfill sufficient nutrient needs for plant growth. Technological input that can be done is by using the biomatriconditioning technique of pre-planting seeds. Seed biomatriconditioning was reported to be able to increase plant growth and yield<sup>5,6,7</sup>. In addition to pre-planting seed treatment, additional treatment inputs are also required by integrating the use of organic plus and inorganic fertilizers to meet crop needs through an integrated approach to sustainable agriculture in the form of Low External Input Sustainable Agriculture (LEISA).

The LEISA fertilization technique refers to a form of agriculture that aims to optimize the use of existing local resources<sup>8,9,10</sup>. The LEISA fertilization technique is not intended to maximize production in the short term, but rather to achieve stable and adequate production levels in the long term. The integration of the use of microbes with the LEISA technique is able to support food security and the sustainability of agricultural systems<sup>11</sup>. It was further reported that the use of the LEISA technique was able to improve the physical, chemical and biological properties of the soil, improve crop quality and production<sup>12</sup>. Another study reported that the combination of organic fertilizers with the addition of inorganic fertilizers was able to increase crop yields<sup>13,14,15</sup>. The use of organic plus fertilizers is reported to be able to increase plant growth and yields. This is because organic plus fertilizer contains nutrients needed by plants and also contains biological agents that can increase plant growth. The biological agents contained in organic plus fertilizers play a role in synthesizing growth hormones in the form of IAA, dissolving phosphate and fixing nitrogen and can act as biological control agents<sup>16,17,18,19</sup>.

Meanwhile, the addition of inorganic fertilizers is expected to be able to provide plant nutrients in a fast time according to the dose needed by plants. Thus, this combination is expected to be able to provide a significant effect in increasing the growth of areca nut which in turn has an impact on increasing crop yields. Based on these advantages, the integration of biomatriconditioning and LEISA techniques is also expected to increase the growth of areca nut. This study aims to test the combination of seed biomatriconditioning treatment and LEISA technique fertilization which is effective in increasing the growth of areca nut seedlings. Potato cultivation experiment was carried out using five different doses of chicken manure compost, organic compost, and chemical fertilizers, which contained nitrogen, phosphate, and potassium. Chemical fertilizer was applied at 250 kg/ha, while the compost doses were 10, 12.5, 15, 17.5, and 20 tons/ha. The microbial Minerals decomposed the compost into macro and micronutrients, which improved the soil cation exchange capacity between  $24 \pm 0.8$  -  $27.6 \pm 1.2$  meg/100 g. The results showed improved physical and chemical properties of the soil, increasing the productivity and quality of potatoes produced.

## II. METHODS

The research was carried out in Wua-Wua Kendari Village, from March to July 2021. The study used a split-plot design with the basic design of a completely randomized design (CRD). Main plot is seed biomatriconditioning technique which consists of 3 treatments, namely control, biomatricontinuing L1R and biomatriconditioning LA2E. Meanwhile, the sub-plots were using the LEISA technique which consisted of 6 treatments, namely control (without application of organic plus and inorganic fertilizers) (G0), organic plus fertilizer (G1), 100% inorganic fertilizer (G2), organic plus + inorganic fertilizer 100% (G3), organic plus fertilizer + 50% inorganic fertilizer (G4) and organic plus fertilizer + 25% inorganic fertilizer (G5). Each treatment was repeated 3 times so that there were 54 experimental units in total.

The planting media used in this study were Ultisol soil and rice husk charcoal. The two media were mixed and then filtered through a 0.5 cm diameter sieve. Then the planting medium was put into a polybag measuring 25 cm x 25 cm. The areca nut seeds used in this study had been previously applied with the seed biomatricontioning treatment<sup>20</sup> and have germinated. Seedlings were selected of uniform size with a height of  $\pm 5$  cm. Furthermore, the areca nut seedlings were planted in polybags that had been previously provided as many as 1 seed per polybag according to the treatment. The application of the LEISA technique is carried out 2 weeks after transplanting. The observed variables were plant height, number of leaves, stem diameter, number of roots, wet weight and dry weight of wilted. Observational data were analyzed using analysis of variance. The results of the analysis that showed a significant effect were continued with the Duncan Multiple Range Test (DMRT)  $\alpha=0.05$ .

## III. RESULT AND DISCUSSION

### Seedling height

There was an interaction between the seed biomatriconditioning treatment and the LEISA technique on the height of areca nut seedlings aged 8 WAP. The interaction of the L1R biomatriconditioing treatment with the application of organic plus fertilizers and 100% inorganic fertilizers, showed better plant high

performance compared to other treatments, but was not significantly different from the organic plus fertilizer treatment. Meanwhile, L1R or LA2E biomatriconditioning gave the same effect, but significantly different from the control (Table 1).

**Table 1.** The interaction of seed biomatriconditioning treatment and LEISA technique on the height of areca nut seedlings aged 8 WAP

Biomatriconditioning treatments	LEISA technique					
	G0	G1	G2	G3	G4	G5
Control	13.50 b R	18.67 c PQ	15.33 c QR	19.67 c P	18.17 b Q	17.83 b Q
Biomatric. L1R	17.67 a R	33.50 a PQ	23.00 a R	35.00 a P	32.17 a Q	31.00 a Q
Biomatric. LA2E	18.67 a S	32.67 b P	21.00 b R	32.33 b P	31.17 a Q	31.00 a Q

Note: The numbers followed by the same lowercase letter (a-b) in the same column, and the numbers followed by the same capital letter (P-R) in the same row show no significant difference at the DMRT  $\alpha_{0.05}$

#### Number of leaves

There was an interaction between the seed biomatriconditioning treatment and the LEISA technique on the number of leaves of areca nut seedlings aged 8 WAP. Except for the control (either the biomatriconditioning treatment or the LEISA technique), all treatments showed the same performance in producing leaf count at 8 WAP. The application of organic plus fertilizer, either alone or in combination with inorganic fertilizers, gave the same number of leaves. Only application of 100% inorganic fertilizer gave lower number of leaves, but statistically it was not significantly different from other treatments, except control (Table 2).

**Table 2.** Interaction of seed biomatriconditioning treatment and LEISA technique on the number of leaves of areca nut seedlings aged 8 WAP

Biomatriconditioning treatments	LEISA technique					
	G0	G1	G2	G3	G4	G5
Control	1,00 b P	1,00 b P	1,00 b P	1,00 b P	1,00 b P	1,00 b P
Biomatric. L1R	1,00 b Q	2,00 a P	1,83 a PQ	2,00 a P	2,00 a P	2,00 a P
Biomatric. LA2E	1,67 a Q	2,00 a P	1,83 a PQ	2,00 a P	2,00 a P	2,00 a P

Note: The numbers followed by the same lowercase letter (a-b) in the same column, and the numbers followed by the same capital letter (P-R) in the same row show no significant difference at the DMRT  $\alpha_{0.05}$

#### Stem diameter

There was no interaction between seed biomatriconditioning treatment and LEISA technique on stem diameter of areca nut seedlings. Independently, the biomatriconditioning treatment significantly increased the diameter of the areca nut seedlings compared to the control. Biomatriconditioning L1R was better able to increase stem diameter of areca nut which was significantly different from control and isolate LA2E. Independently, the LEISA technique, which combines the use of organic and inorganic fertilizers, significantly increased the stem diameter of areca nut seeds compared to the control. The application of organic plus fertilizers either independently or in combination with inorganic fertilizers gave significantly different stem diameters with control and application of 100% inorganic fertilizers (Table 3).

#### Number of roots

There was no interaction between the biomatriconditioning treatment and the LEISA technique on the number of roots of areca nut seedlings. Independently, the seed biomatriconditioning treatment significantly increased the number of roots of areca nut seedlings compared to the control. Biomatriconditioning L1R and LA2E produced a higher number of roots and was significantly different from the control. Independently, the LEISA technique significantly increased the number of roots of areca nut seedlings compared to the control. The application of organic plus fertilizer combined with 100% inorganic fertilizer gave a higher number of roots, not significantly different from other combination treatments, but significantly different from the control and application of 100% inorganic fertilizer (Table 3).

**Table 3.** Effect of seed biomatriconditioning treatment and LEISA technique on stem diameter (SD), number of roots (NR), wet weight of shoot (WWS) and dry weight of shoot (DWS)

Biomatriconditioning treatments	Observation variable			
	SD	NR	WWS	DWS
Control	59.27 c	9.67 b	10.17 b	4.14 c
Biomatric-L1R	72.32 a	16.67 a	17.32 a	8.73 a
Biomatric-LA2E	67.67 b	15.72 a	16.16 a	7.33 b
LEISA technique				
Control	49.33 c	7.78 d	9.81 c	3.12 c
Organic plus fertilizer	71.56 a	16.56 ab	16.82 a	8.95 a
100% inorganic fertilizer	57.75 b	10.11 c	11.91 b	4.63 c
Organic plus fertilizer + 100% inorganic fertilizer	76.77 a	17.11 a	17.54 a	8.85 a
Organic plus fertilizer + 50% inorganic fertilizer	70.87 a	16.22 ab	15.53 a	7.60 ab
Organic plus fertilizer + 25% inorganic fertilizer	71.35 a	16.33 ab	15.70 a	7.75 ab

Note: The numbers followed by the same letter in the column for each variable show no significant difference in the DMRT  $\alpha_{0.05}$

### Wet weight and dry weight of shoot

There was no interaction between biomatriconditioning treatment and LEISA technique on shoot wet weight and dry weight of areca nut seedlings. Independently, the biomatriconditioning treatment significantly increased the shoot wet weight and dry weight of areca nut seedlings compared to the control. Biomatriconditioning L1R was better able to increase shoot wet and dry weight of areca nut seedlings, which was significantly different from control and isolate LA2E. Independently, the LEISA technique treatment significantly increased the shoot wet weight and dry weight of areca nut seedlings compared to the control. Application of organic plus fertilizer combined with 100% inorganic fertilizer resulted in better shoot wet weight and dry weight, not significantly different from other combination treatments, but significantly different from control and application of 100% inorganic fertilizer (Table 3). The growth performance of areca nut in the L1R biomatriconditioning treatment and the LEISA technique is shown in Figure 1.



**Fig 1.** Growth performance of areca nut seedlings on L1R biomatriconditioning treatment and LEISA technique

Seed biomatriconditioning treatment which was integrated with biological agents was significantly able to increase the growth of areca nut seedlings. The results of this study are also in line with the results of previous studies which showed that the use of biological agents as seed treatment was able to improve and increase plant growth and yield. Endophytic-rhizobacteria inoculation which was integrated with the matriconditioning technique of husk charcoal powder gave better results than the control. As previously explained, *Bacillus* spp. is a group of PGPR (Plant Growth Promoting Rhizobacteria) that are effective in increasing plant growth and yield. The role of these endophytic-rhizobacteria as PGPR is because these bacteria have the ability to dissolve phosphate, fix nitrogen, and produce growth hormones such as IAA and cytokinins<sup>21</sup>. The results of previous studies that are relevant to this study indicate that the use of biological agents can increase seed germination<sup>22,23,24,25</sup>. In addition to the improvements caused by the use of endophytic-rhizobacteria independently, the application of biomatriconditioning techniques as a medium for inoculation of rhizobacteria in seeds also provides a positive role that cannot be ignored. As previously explained, the seed biomatriconditioning technique is a treatment on seeds (seed conditioning) which aims to accelerate and uniform growth and increase the percentage of sprouts and seedlings appearing. The principle

is to mobilize resources owned by seeds (internal) plus external resources (external) to maximize the improvement of plant growth and yields.

Seed conditioning is a physiological and biochemical improvement associated with the speed and simultaneousness, improvement and increase in germination potential in seeds during delayed germination by low matrix potential media (matricconditioning) or by low osmotic potential media (priming or osmoconditioning). The use of seed matricconditioning techniques has been shown to be effective in increasing seed viability and vigor<sup>26</sup> and protecting planted seeds from seed-borne and soil-borne fungi<sup>27,28,29</sup>. Meanwhile, as previously explained, the seed biomatricconditioning technique through seed conditioning treatment is indeed used to accelerate and uniform growth and increase the percentage of sprouts and seedlings appearing<sup>30</sup>. The principle of seed conditioning is to mobilize the resources owned by the seed (internal) plus external resources to maximize the improvement of plant growth and yield. Seed conditioning is a physiological and biochemical improvement associated with the speed and simultaneous, increased germination potential in seeds during delayed germination by low matrix potential media (matricconditioning) or by low osmotic potential media (osmoconditioning)<sup>31</sup>. Seed matricconditioning technique proved to be effective in increasing seed viability and vigor<sup>26,30</sup>. In addition to promoting early seed growth, this technology has also been shown to be able to protect seeds from seed-borne and soil-borne fungi during an important early stage of growth<sup>32,33</sup>. Seed biomatricconditioning treatment with rhizobacteria integrated with the LEISA technique was also able to increase the growth of areca nut seedlings.

The integration between the biomatricconditioning technique using LIR and the application of organic fertilizer plus + 100% inorganic fertilizer showed a better growth performance of areca nut and significantly different from the control and application of 100% inorganic fertilizer, but not significantly different from the application of organic fertilizer plus, application of organic plus fertilizer. + 50% inorganic fertilizer and application organic plus fertilizer + 25% inorganic fertilizer. This is in line with the results of previous studies that endophytic-rhizobacteria present in the plant rhizosphere have an important role in improving plant growth and yield<sup>6,7</sup>. The increase in the growth of areca nut is also due to the input of inorganic and organic fertilizers in the form of organic plus fertilizers containing biological agents. The use of inorganic and organic fertilizers is reported to be able to improve the physical, chemical and biological properties of the soil<sup>12</sup>. In addition, the use of organic plus fertilizers also plays a role in supplying nitrogen and phosphate needs because the biological agents contained in organic plus fertilizers have the ability to dissolve phosphate, fix nitrogen and produce growth hormones<sup>34,35,36</sup>. It was further reported that biological agents can chelate important elements from the area around plant roots so that they can be utilized by plants<sup>37</sup>. *Bacillus* spp., and *P. fluorescens* are also capable of synthesizing growth hormones, fixing nitrogen or dissolving phosphate<sup>38,39,40,41</sup>. As previously explained, *Bacillus* spp. is a group of PGPR bacteria that is proven to be effective in increasing plant growth and yield<sup>42,43,44</sup>. *Bacillus* spp. reported to be able to synthesize indole acetic acid (IAA)<sup>46</sup>, gibberellins<sup>47</sup> and dissolve phosphate<sup>44,49,50</sup>.

Previous research showed that rhizobacteria isolates from the *Bacillus* sp. able to produce growth hormone IAA<sup>51</sup>. Furthermore, it was reported that rhizobacteria from the *Bacillus* sp. capable of producing the hormone IAA and dissolving phosphate<sup>41</sup>. Besides being able to produce IAA hormone and dissolve phosphate, rhizobacteria from the *Bacillus* sp. also able to fix nitrogen<sup>52,53,54</sup>. The IAA hormone produced by rhizobacteria is thought to be able to stimulate seed germination, resulting in accelerated seed germination. The application of gibberellins can increase the percentage of seed germination by increasing the amino acids contained in the embryo and this is important to maximize hydrolytic enzymes in the endosperm when new seeds germinate<sup>55</sup>. As with rhizobacteria, the use of endophytic bacteria as plant growth promoters is also one of the contributions of biotechnology in increasing plant growth and production. This group of endophytic bacteria from the roots of areca nut is proven to be able to produce the hormone IAA, so the presence of endophytic bacteria in plants can increase the growth of areca nut seedlings<sup>37</sup>. IAA hormone is a type of auxin, involved in physiological processes in plant growth such as cell elongation and division, tissue differentiation and root initiation.<sup>56,57</sup>

#### IV. CONCLUSION

Pre-planting seed treatment with LIR biomatriconditioning is very important to increase areca nut seed germination. To increase the growth of areca nut seedlings, further fertilization needs to be done with organic plus fertilizer or a combination of organic plus fertilizer + 25% inorganic fertilizer.

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