World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:18, No:2, 2024

The Optimal Production of Long-Beans in the Swamp Land by Application of Rhizobium and Rice Husk Ash

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Abstract**—**The swamp land contains high levels of iron and aluminum, as well as a low pH. Calcium and magnesium present in the rice husk ash can mitigate plant poisoning, thereby enhancing plant growth and fertility. Two main factors were considered in the study: The dosage of rice husk, and the rhizobium inoculant dosage, which was varied at 0.0 g/kg seed, 4.0 g/kg seed, 8.0 g/kg seed, and 12.0 g/kg seed. The plants were cultivated under controlled lighting conditions with a photoperiod of 11.45 to 12.15 hours. The combination of rhizobium inoculant and rice husk ash has demonstrated an interacting effect on the production of fresh weight in long bean pods. The mean relative growth rate, net assimilation rate, and pod fresh weight are increased by a combination of husk rice ash and rhizobium inoculant. Rice husk ash enhances nitrogen availability in the soil, even in cases of poor nutritional conditions. Rhizobium plays an active role in nitrogen fixation from the atmosphere, as it enhances both intercellular and symbiotic nitrogen capabilities in long beans. The combination of rice husk ash and rhizobium can effectively contribute to thriving soil conditions.

Keywords**—**Aluminum, calcium, fixation, iron, nitrogen.

I. Introduction

SWAMP and marginal land are spread almost throughout the Riau region, especially in the coastal region. The swamp is characterized by soil with excess iron and aluminum, low pH, and poor fertility. Excessive levels of iron and aluminum can be detrimental to root hairs, potentially leading to plant damage and reduced production. Rice chaff ash consists of the following components: SiO_2 (52%), C (31%), K (0.3%), N (0.18%), Fe (0.08%), and potassium (0.14%). Additionally, it contains trace amounts of other nutrients like Fe_2O_3, K_2O, MgO, CaCo, MnO, and Cu, as well as various organic materials [7], [8], [15]. Rice chaff ash can be a suitable substitute for calcium or magnesium in agricultural applications.

Rice husk ash contains both calcium and magnesium. The presence of calcium and magnesium can mitigate plant poisoning, leading to improved plant growth and fertility.

When rice husk ash is combined with rhizobium, it has been observed to alleviate the issues caused by excessive aluminum and iron in the soil, which can otherwise hinder plant growth and reduce overall production. Rice husk ash is obtained from unshelled paddy rice, separated from the stalk. When this waste is burned, it transforms into ash with high concentrations of calcium and magnesium. Calcium and magnesium are valuable materials for raising soil pH and can effectively counteract the presence of excessive iron and aluminum, especially in in swampy regions and coastal areas.

Hence, rice husk ash proves especially valuable for enhancing soil conditions by increasing its absorbent capacity and reducing the availability of toxic substances such as aluminum and iron. These improved conditions create a more conducive environment for rhizobium to effectively fix nitrogen. Additionally, rice husk ash contributes to the enrichment of soil nutrients. Rice husk ash is derived from the husk of unshelled paddy, separated from the stalk. When this waste is incinerated, it transforms into ash with elevated levels of calcium and magnesium. These two elements are essential for raising soil pH and can effectively mitigate the presence of iron and aluminum, particularly in swampy regions, especially along the coastal areas.

Calcium and magnesium serve as essential materials for elevating soil pH and have the ability to mitigate the presence of iron and aluminum, particularly in swampy regions, especially along coastal areas. Furthermore, calcium and magnesium are crucial macronutrients required for plant growth. The leguminous inoculant is a powdered form of rhizobium bacterial spores [7], [8].

Rhizobium has achieved remarkable ecological and evolutionary success, significantly influencing our biosphere. Despite its complexity, it exhibits a dual lifestyle involving both intracellular infection and a free-living phase in cultivated land. The symbiosis established by Rhizobium has spread to hundreds of bacterial species and is geographically distributed across the globe [2] The dual capacity of intracellular and symbiotic nitrogen fixation in soybean and other legumes is effective in preserving rhizobium in the soil. Even with reduced nitrogen fertilizer usage, up to 50%, legumes continue to grow normally [7], [8], and seed production remains consistent when rhizobium is inoculated into the seeds. This study aims to explore the connection between rice husk ash and rhizobium inoculant concerning the growth and yield of long beans.

II. Materials and Methods

Study Area

This research was conducted from August to December 2019. This experiment was designed using a simple random design with two factors. The first factor involved varying

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doses of rice husk ash: 0.0 g/plot, 0.6 g/plot, 1.2 g/plot, and 1.8 g/plot. The second factor was the amount of rhizobium inoculant per kilogram of seed: 0.0 g rhizobium inoculant/kg seed, 4.0 g rhizobium inoculant/kg seed, 8.0 g rhizobium inoculant/kg seed, and 12 g rhizobium inoculant/kg seed. The plants were maintained under controlled lighting conditions with a photoperiod of + 11.45 to 12.30 hours.

Parameters

Mean Relative Growth Rates

The accumulation of dry weight describes as the increase of cell elongation and cell number in the long beans every day during vegetative growth. The mean relative growth rate (MRGR) can be calculated by sampling plant size for the first time on 7 days after planting (t1) and the second time on 14 days after planting (t2), 21 days after planting for the third time, and 28 days after planting for four times. The equation for calculating the MRGR [12] is as follows:

$$\text{MRGR} = \frac{\ln W2 - \ln W1}{t2 - t1} \quad (1)$$

where W1 and W2 are the biomass dry of long beans at the time measured, beginning (t1) and end (t2) of the sampling until four periods of measurement during vegetative growth, and *ln* is the natural logarithm. Equation (1) is the most common formula used when comparing relative differences between rice husk ash and rhizobium inoculant treatments.

Net Assimilation Rates

The Net Assimilation Rate (NAR) is the weight of total dry weight per unit area of leaf in a certain time.

$$E = \frac{1}{L}\frac{dW}{dt} \quad (2)$$

Leaf area was measured on a sub-sample using a leaf area meter and image analysis software. The leaf area (L) of long beans was measured four times on days 7, 14, 21, and 28 after planting. The W and L means may then be used to accumulate E_M, an estimate of the mean E for each time- interval (t2–t1), usually as proposed by [14] shown in (3):

$$E_M = \frac{(W2) - W1)\ (Log.E\ L2 - LogE\ L1)}{(T2 - T1(L2 - L1)} \quad (3)$$

III. Results and Discussion

NAR is meaning the buildup of the plant's dry weight of every centimeter square of any kind leaf area of any time. The treatment without rice husk ash is different from any other treatment where the lowest treatment is averaged. Rice husk ash's release of the right amount would certainly increase vegetation growth, which, in turn, would prompt generic growth in this issue to increase the NAR.

The growth rate will be comparable to the pattern followed by the net precipitous rate. In addition, the growth rate in plants is likely to indicate a similar pattern. It is just true that the large number of effective leaves carrying out photosynthesis is the dominant factor. In the MRGR count, the leaf area is not considered. If the increase in NAR is at the age of 14-21 days, it will also result in increased growth rates in the MRGR, as shown in the comparison in Fig. 1. The trend of expansion in the NAR directly affects the rate of growth in the MRGR.

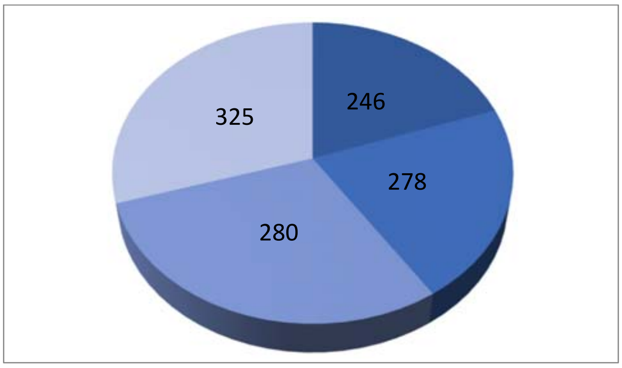

Fig. 1 (a) NAR 7-14 days (246×10^{-4} mg/cm^2/day from treatment of 0 g rhizobium/kg seed, 278×10^{-4} mg/cm^2/day from treatment of 4 g rhizobium/kg seed, 280×10^{-4} mg/cm^2/day from treatment of 8 g/rhizobium/kg seed, and 325×10^{-4} mg/cm^2/day from treatment of 12 g Rhizobium/kg seed)

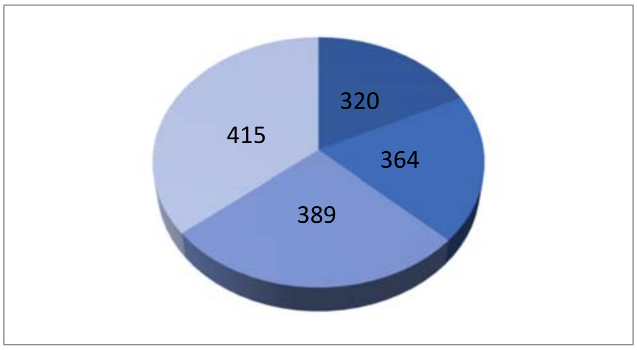

Fig. 1 (b) NAR 14-21 days (320×10^{-4} mg/cm^2/day from treatment of 0 g rhizobium/kg seed, 364×10^{-4} mg/cm^2/day from treatment of 4 g rhizobium/kg seed, 389×10^{-4} mg/cm^2/day from treatment of 8 g rhizobium/kg seed, and 415×10^{-4} mg/cm^2/day from treatment of 12 g rhizobium/kg seed)

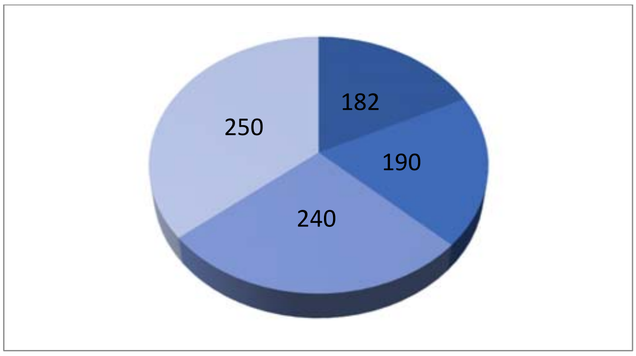

Fig. 1 (c) NAR 21-28 days (1826×10^{-4} mg/cm^2/day from treatment of 0 g rhizobium/kg seed, 190×10^{-4} mg/cm^2/day from treatment of 4 g rhizobium/kg seed, 240×10^{-4} mg/cm^2/day from treatment of 8 g rhizobium/kg seed, and 250×10^{-4} mg/cm^2/day from treatment of 12 g rhizobium/kg seed)

MRGR is employed to compute the rate of plant growth swiftly within the seedbed. It is also useful for making rapid decisions in large-scale studies without incurring significant costs. In this study, the above-mentioned watering pattern is consistently applied.

Additional parameters, such as the quantity of root nodules and the individual plant weights, would also exhibit significant variations under the same treatment with rice husk ash at 1.8 kg/plot.

This treatment exhibits a positive interaction with 12 g/kg rhizobium inoculation in seeds. This observation is depicted in Fig. 3. Long beans are typically consumed when the pods are young and fresh, as they are considered a vegetable. To determine if a treatment has a positive effect, it should noticeably impact plant growth, either positively or negatively, or influence the overall thriving of the plants. The resulting growth will then be seen in the generic growth of the flowers and the seedlings. The combination of 1.8 kg of rice husk ash per plant and 12 g of rhizobium per kilogram of seeds has proven effective in significantly increasing root nodule production, with an average of 51.00 nodules per plant. When using rice husk ash alone, the highest count was 48.83 root nodules per plant. On the other hand, the effect of rhizobium treatment alone yielded an average of 46.91 root nodules per plant, as illustrated in Fig. 2. This means that the rice husk ash is interacting positively with rhizobium, bringing the number of root nodules to 51.00 for each plant. The application of rhizobium in combination with an appropriate dose of rice husk ash led to enhanced vegetative growth in long beans. The vegetative growth increased progressively, starting from 4 g/kg seed of rhizobium and extending to 22 g/kg seed. When there is a high count of root nodules on the plant's root hair, it signifies a greater capacity of the plant to fix nitrogen from the atmosphere in the soil. Following the proper dosages of rice husk ash and rhizobium inoculant treatments, there was an increase in the accumulation of nitrogen molecules in the root system.

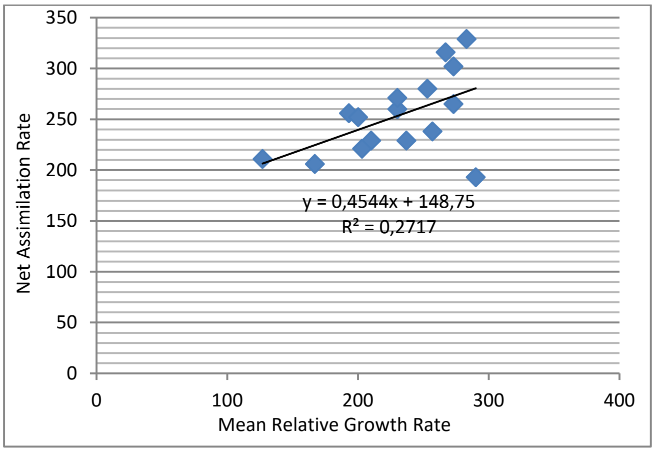

Fig. 2 Relationship between NAR and MRGR disclosed as linear pattern

The connection between NAR and MRGR is closely linked and illustrated as linear in Fig. 2, with the equation represented as $Y = 0.4544x + 148.75$, and an R^2 value of 0.2717. This equation signifies that an increase in NAR corresponds to improvements in MRGR, as indicated in Table I. This is because the accumulation of assimilates in plant tissues is stored. The process of assimilation within plant organs is primarily facilitated by increased photosynthesis activity in the leaves.

The weight of the long bean pods is influenced by the efficacy of the treatment involving the combined increase of rice husk ash and rhizobium. The synergy between these two treatments enhances the plant's capability to yield heavier pods, as illustrated in Fig. 3.

A large leaf area indicates that the leaf is generating more assimilates in the plant's organs. The relationship between MRGR and NAR is highly correlated. This implies that if photosynthetic activities in the leaf are rapid, it leads to an increase in the accumulation of assimilates in plant organs, resulting in a higher MRGR.

TABLE I
MRGR OF LONG BEANS AFTER TREATED WITH RICE HUSK ASH AND RHIZOBIUM INOCULANT (G/DAY)

Rice Husk g/plant	Plant age (day)			
	7-14	14-21	21-28	Average
0 g/kg seed	0.299	0.243	0.224	0.254
4 g/kg seed	0.379	0.268	0.241	0.296
8 g/kg seed	0.413	0.303	0.261	0.326
12 g/kg seed	0.476	0.328	0.283	0.362
Average	0.329a	0.243b	0.231c	

Mean value followed by different alphabet/s within column do not differ significantly over one other at $P < 0.05$ lead by Duncan's Multiple Range Test

A substantial nitrogen supply has a positive impact on plant growth and can fulfill up to 75% of the total nitrogen requirements for plants. Consequently, only 25% of the nitrogen fertilizer addition is necessary. Excessive levels of aluminum and iron in the soil can become toxic, ultimately posing a threat to root hair and leading to plant demise.

This experiment suggests that both treatments have a similar effect on increasing pod weight. Rice husk ash provides a favorable condition for root hair development, while rhizobium, with its persistent root interaction, enhances nitrogen efficiency and productivity.

Long beans, like other legumes, rely on bacteria such as rhizobium to provide them with the necessary nitrogen. This nitrogen helps maintain abundant organic compounds, such as sucrose and glucose, within the root nodules, as depicted in Fig. 4. The interaction between rhizobium and legume plants establishes the legume as the host for these essential nitrogen-fixing bacteria [3], [4], [10], [13].

The rhizospheric site serves as a habitat for a range of organic substrates secreted by plants, including hormones, modulators, and enzymes, which facilitate the production of organic compounds. As the quantity of organic matter increases, so does the population of microorganisms. When a specific protein compound is present, rhizobium can infect the host plant of long beans. This specific protein is secreted by plants and serves as a signal recognized by bacteria [3], [5], [9], [13]. In response, the bacteria release lipooligosaccharide compounds, commonly known as nod factors [1], [6], [11], which trigger a cascade of 147 host cell divisions. Therefore, it is suggested that symbiosis may occur because of the suitability of the respective substrates produced [5], [10], [13].

When compatibility is established, bacteria multiply and create structures to penetrate the root, known as infection threads. These bacteria, in turn, reproduce within a root cell, which is termed a bacteroid. The infected root cell subsequently swells, forming nodules, within which the internal structure, between the host cells and the bacteroid, is enveloped by leg-hemoglobin, giving it a reddish-purple appearance. The mechanism of plant root infections by microbes is that the glucose photosynthesis produced is partially sent to root hair, and then the microbes consume their glucose for their growth together with infection occurring in the root hair. The effectiveness of the root nodule implies that it is a reddish-purple in color.

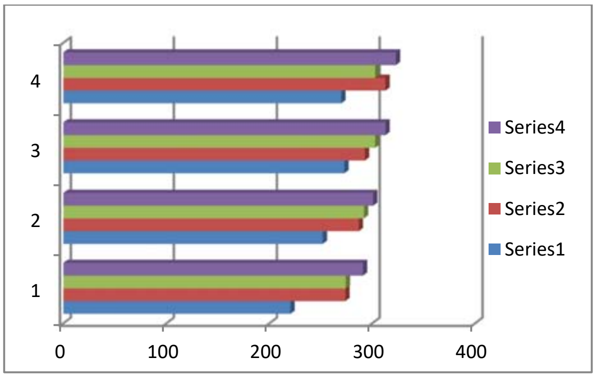

Fig. 3 Mean dry weight of pod on the long beans after treatment with rice husk ash and rhizobium inoculant (g/plant): Series 1 (0 g/plant of rice husk), Series 2 (0.6 g/plant of rice husk ash Series 3 (1.2 g/plant of rice husk ash), Series 4 (1.8 g/plant of rice husk ash), 1 (0 g rhizobium inoculant), 2 (4 g rhizobium inoculant), 3 (8 g rhizobium inoculant), Series 4 (12 g rhizobium inoculant)

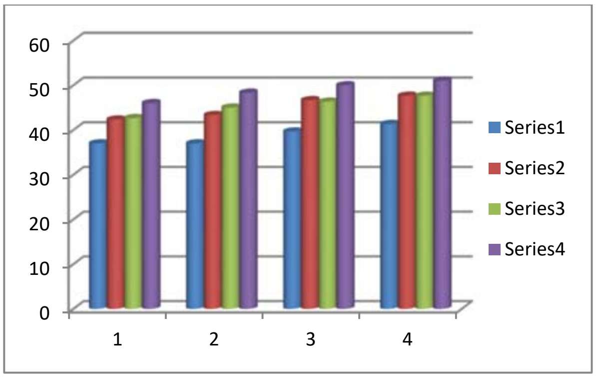

Fig. 4 The number of root nodules of long beans on the treatment of rice husk ash and rhizobium inoculant (piece/plant): Series 1 (0 g/plant of rice husk), Series 2 (0.6 g/plant of rice husk ash Series 3 (1.2 g/plant of rice husk ash), Series 4 (1.8 g/plant of rice husk ash), 1 (0 g rhizobium inoculant), 2 (4 g rhizobium inoculant), 3 (8 g rhizobium inoculant) Series 4 (12 g rhizobium inoculant)

To form reduction power (for instance, NDPH2 and ferredoxin) and ATP to regulate reactions, a bacteroid requires a specific amount of energy. This energy is derived from the host plant's photosynthesis. Subsequently, sucrose, glucose, and organic acids are transported into the nodules, where the oxidation of these substances generates additional energy through oxidative phosphorylation. This respiration process necessitates a certain quantity of oxygen, which is bound by leghemoglobin in the vicinity of the bacteroid. The mechanism is analogous to hemoglobin in the bloodstream of mammals, which serves as an oxygen carrier required for the respiratory process. The enzyme nitrogenase catalyzes the reduction of N2 molecules [6]. Various factors influence nitrogen fixation, including (I) the supply of photosynthesis, (ii) oxygen availability, (iii) temperature, (iv) soil pH, and (v) nitrogen availability. The ultimate outcome of nitrogen fixation by rhizobium in the root hairs is distributed to the long bean plant. Consequently, the long bean plant becomes more efficient in its utilization of nitrogen.

IV. Conclusion

Rice husk ash serves as an excellent organic fertilizer, meeting all the necessary criteria for fertilization. Furthermore, rhizobium consistently engages in a symbiotic relationship with legumes to enhance nitrogen efficiency in long bean plants. The combination of 1.8 kg/plot of rice husk ash and 12 g/kg of rhizobium seed inoculation is the ideal dosage for long beans. Rice husk ash not only provides nutrition for long beans but also maintains a nutritional balance to support the life of rhizobium, addressing any nitrogen deficiencies in long bean plants.

Acknowledgment

We give our thanks to the Rector Islamic University of Riau for the financial support of this research and published paper, and special thanks to the Director of Post Graduate in Agronomy program for their assistance with the administrative affairs.

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