

Effect of Nodule Bacteria from Several Leguminous Weeds on Nodulation and Growth of Peanut Plants Grown Following Paddy Rice

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ABSTRACT

Previous studies showed that microbes from root washing of *Mimosa pudica* from dryland in Pringgabaya significantly increased growth and yield of mungbean plants. This study aimed to examine the effect of root nodule bacteria from several leguminous weeds on nodulation and growth of peanut plants grown following paddy rice. The experiment was conducted at the Experimental Farm of the Faculty of Agriculture, University of Mataram, located in Narmada, from September to November 2024. The experiment was arranged according to Split Plot Design with three blocks and two treatment factors, namely leguminous weed sources of nodule bacteria (L1: pinto peanut; L2: *Mimosa pudica*; L3: *Crotalaria* sp.) as the main plots, and root nodule bacteria (B0: without bacteria, B1: with root nodule bacteria) as the subplots. These root nodule bacterial isolates were applied by soaking the seeds before planting, and peanut plant samples were harvested at the pod formation phase. The results showed that application of root nodule bacteria from several weeds significantly increased shoot fresh weight (FW), total plant FW, nodule FW and root FW of peanut plants, but different leguminous species as sources of nodule bacteria only showed different effects on shoot FW, total plant FW, and nodule FW of peanut plants. There were also significant interaction effects on the first three measurement variables, in which peanut's shoot FW and total plant FW were highest (167.36 and 171.80 g/clump) under the application of *Crotalaria* nodule bacteria, but peanut's nodule FW was highest under the application of pinto peanut nodule bacteria.

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1. INTRODUCTION

Peanuts are a multi-functional commodity, namely they can be consumed directly in the form of boiled or fried seeds, and can be used as raw materials for various types of processed food and vegetable oil industries, as well as stems and leaves for animal feed. Therefore, the development of the peanut-based food and animal feed industry has led to an increase in demand for domestic peanuts. The increasing demand for peanuts is a large market opportunity for the development of peanut production. According to Hutabarat (2003) [1], the demand for food sources of protein will continue to increase along with population growth, urbanization, education, and community income. Likewise, the demand for peanuts as a source of protein and vegetable fat also continues to increase. Although peanuts are one of the food commodities as a source of protein and vegetable oil with high economic value, their popularity is not as high as soybeans [2].

In Indonesia, peanuts are not considered as a leading commodity nationally [3]. The government's attention is not the same as for soybeans which are programmed to achieve self-sufficiency. The program for increasing the production that has been implemented for new food crop commodities is limited to rice, maize, and soybeans. Therefore, the cultivation technology applied by farmers is still traditional and simple, so that the productivity achieved by farmers is still relatively low. In Indonesia, most

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peanuts are only used for household food such as boiled peanuts, crispy peanuts, fried peanuts, cooking spices, and other snacks. In fact, peanuts have the potential to be processed in the food industry into various processed food products such as various cakes, vegetable milk, high protein flour, ice cream, and vegetable oil [4].

However, peanut production in Indonesia is still relatively low. One way to increase production is by planting superior varieties. Superior varieties are one of the main technological components that play an important role in the peanut production increasing program. Compared to new superior varieties of hybrid rice and maize, new varieties of legumes, especially peanuts, are relatively slow to be adopted by the farmers. Available data shows that of the 34 superior peanut varieties that have been released, only a few are popular among farmers [5].

One of the obstacles to the development of peanut production is the need for soil types because peanut plants are less suitable for production on vertisol land. In dry land which is classified as sandy soils, the availability of land for peanut production also competes with maize production, which has a much higher yield compared to peanuts, while for production in the rice fields, the availability of land for peanut production always competes with rice, so peanuts are usually planted in the dry season. Another obstacle to production of peanut plants is the need to form a symbiosis with root nodule bacteria (*Rhizobium* sp.), while on the land that is always planted with paddy rice the availability of *Rhizobium* bacteria in the soil can be very low [6], so that the *Rhizobium* bacteria inoculation process must be carried out as in planting soybeans following paddy rice. This is because more than 60% of the N requirements of peanut plants must be met from the N₂ fixation process through symbiosis with root nodule bacteria [7].

This study aimed to examine the effect of the application of nodule bacteria isolated from several species of leguminous weeds on the growth and formation of root nodules in peanut plants grown following paddy rice.

II. MATERIALS AND METHODS

This study followed an experimental method by conducting a field experiment on a dried paddy rice land located in the experimental farm belong to the Faculty of Agriculture, University of Mataram, which is located in Nyurlembang Village, Narmada (West Lombok Regency). The experiment was conducted from September to November 2024. The materials used in this experiment included the root nodules of several species of leguminous weeds, i.e. pinto peanut (*Arachis pintoi*), *Mimosa pudica*, and *Crotalaria* sp., peanut seeds of Bison variety, Phonska (NPKS 15-15-15-9) fertilizer, bamboo stakes, raffia rope, and insecticides. The isolation, purification, and preparation of the bacterial isolate suspensions of those root nodule bacteria were done following the procedures described in previous studies [8,9].

Experimental Design

The field experiment was arranged according to Split Plot design, testing two treatment factors, namely the leguminous weed species as the source of root nodule bacterial isolates (L1: pinto peanut; L2: *M. pudica*; L3: *Crotalaria* sp.) as the main plots, and the bacteria types (B0: without bacteria; B1: root nodule bacteria) as the subplots. Thus, there were six treatment combinations, each of which was made in three replications (blocks). The B0 treatment (without bacteria) in this experiment was the autoclave sterilized bacteria suspension of each legume source.

Implementation of the Experiment

Land preparation and plotting.-- After clearing the land from grass and previous paddy rice plant residues, the drained rice land was prepared by plowing once and harrowing once with a hand tractor under dry soil condition. Planting beds were then made with a length of 2.0 m and a width of 0.8 m and a height of about 20 cm for each treatment. Furrows of 40 cm width and 20 cm depth were made between the planting beds, and a 50 cm wide ditch was made between the blocks.

Planting, treatment and fertilization.-- The process of isolating root nodule bacteria of various types of leguminous weeds and preparing the suspension of the bacterial isolates were carried out in the Microbiology Lab of the Faculty of Agriculture, University of Mataram. The application of the bacterial suspension was carried out by soaking the peanut seeds in the microbial suspension of each treatment for six hours, before seeding on the planting beds. The peanut seeds of the Bison variety that had been soaked in the bacterial suspension were then seeded on the planting beds by dibbling 3-4 seeds per planting hole, with a planting distance of 20 cm in rows and 25 cm between rows. At 10 days after seeding (DAS), thinning was carried out by allowing only 2 plants to grow per planting hole, then fertilization was carried out by dibbling the Phonska fertilizer at a dose of 200 kg/ha (1 g/clump), then watering was carried out by flowing water through the ditch between the planting beds. Crop maintenance included weeding, which was carried out at 14, 28 and 42 DAS, and pest control using a systemic insecticide (Regent 50 SC). Sample plant harvesting was carried out at the end of flowering phase, namely at 42 DAS for growth and nodulation measurements.

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Measurement Variables and Data Analysis

The measurement variables were shoot and root fresh weight (FW), total plant FW, and nodule FW. Data were analyzed with analysis of variance (ANOVA) and Tukey's HSD at 5% significance level using the CoStat for Windows ver. 6.303. Regression analysis between observation variables was done using MS Excel for Windows.

III. RESULTS AND DISCUSSION

Based on the summary of the ANOVA results in Table 1, it can be seen that the leguminous weed species as the source of root nodule bacterial isolates and the type of bacteria applied showed a significant effect on all observation variables. Likewise, their interaction effects were also significant, except for the root fresh weight.

Table 1. Summary of ANOVA results of the effects of the treatment factors and their interactions on all measurement variables

Treatments	Shoot fresh weight (g/clump)	Nodule fresh weight (g/clump)	Total fresh weight (g/clump)	Root fresh weight (g/clump)
B0: Without bacteria	107.86 b	1.12 b	109.86 b	2.00 b
B1: With bacteria	140.15 a	1.49 a	143.04 a	2.89 a
Tukey's HSD	10.17	0.12	10.65	0.77
L1: <i>A. pintoi</i>	113.77 b	1.49 a	116.07 b	2.30 a
L2: <i>M. pudica</i>	120.57 b	1.19 b	122.91 b	2.34 a
L3: <i>Crotalaria</i> sp.	137.68 a	1.25 b	140.37 a	2.69 a
Tukey's HSD	11.29	0.19	11.81	1.09
B*L interaction effect	*	**	*	ns

Remarks: ns = non-significant; *, ** = significant at p-value <0.05 and 0.01, respectively

Table 1 shows that compared to without the application of root nodule bacteria, the application of these microbes resulted in higher shoot fresh weight, nodule fresh weight, total plant fresh weight, and root fresh weight. This indicates the positive contribution of the bacterial isolates to the plant growth process, especially to the fresh weight of root nodules, which could also indicate the potential contribution of N₂ fixing activity of the inoculated nodule bacteria to better meet N requirement of the inoculated peanut compared with the uninoculated peanut plants. By meeting the N needs of peanut plants, the formation of chlorophyll, photosynthetic rates and growth of peanut plants are more stimulated because more than 60% of the N needs of peanut plants are obtained from the N₂ fixation process [7]. In addition, nodule fresh weight of peanut also differ significantly between species of leguminous weeds that were the sources of the root nodule bacterial isolates, with the highest average nodule FW (1.49 g/clump) was on peanut plants treated with root nodule bacterial isolate from root nodules of pinto peanut (*A. pintoi*). Unfortunately, this trend is not consistent with shoot and total fresh weight of the peanut plants, in which the highest averages were in peanut plants treated with root nodule bacteria isolated from root nodules of *Crotalaria* sp. (Table 1).

However, there was a significant interaction effect of the treatment factors on peanut nodule FW, indicating different response of peanut plants to different leguminous species as sources of the root nodule bacterial isolates. As can be seen from Figure 1, the highest average of peanut nodule FW (1.83 g/clump) was resulted from the treatment using root nodule bacteria isolated from pinto peanut (*A. pintoi*), with an increase in nodule FW by 59.1%, compared with only 28.4% and 9.7% in peanut plants treated with root nodule bacteria isolated from nodules of *Crotalaria* sp. and *M. pudica*, respectively. According to results reported by Khetmalas and Bal (2005) [10], peanut and *A. pintoi* share compatible microsymbionts.

Although all treatments resulted in an increase in peanut shoot FW (Figure 2) and total plant FW (Figure 3), the patterns of the interaction effect of the treatment factors on peanut nodule FW are not similar to those on shoot FW (Figure 2) and total plant FW (Figure 3), in which the highest mean values are on peanut plants treated with root nodule bacteria isolated from root nodules of *Crotalaria* sp. This could mean that the nodulation of peanut root was not 100% effective. This requires further investigations. In addition, correlation analysis also confirm that nodule FW was not significantly correlated with shoot FW or total plant FW, with a correlation coefficient of +0.389 with shoot FW and +0.396 with total plant FW (p-value > 0.05).

Nevertheless, correlation and regression analysis show that peanut nodule FW had a significantly positive correlation with root FW, with a regression equation $Y = 0.7799 + 1.2714 X$ ($R^2 = 25.96\%$, p-value = 0.0308), in which Y = root FW and X = nodule FW (Figure 4). Root FW of peanut showed a stronger correlation with shoot FW, with a regression equation $Y = 65.744 + 23.845 X$ ($R^2 = 51.49\%$, p-value = 0.0008), in which Y = shoot FW and X = root FW (Figure 5). This could mean that better nodulation improved growth of peanut roots for improved absorption of water and nutrients to improve peanut growth (shoot FW and total

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plant FW) as the final growth effect of the treatments. Table 1 also confirms that root FW was also significantly higher on peanut plants treated with root nodule bacteria isolated from those leguminous weed species.

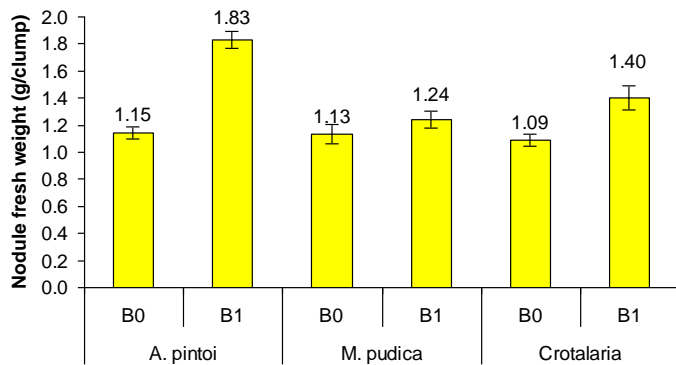


Figure 1. Average (Mean ± SE) peanut nodule fresh weight (g/clump) as affected by the interaction effect of the treatment factors

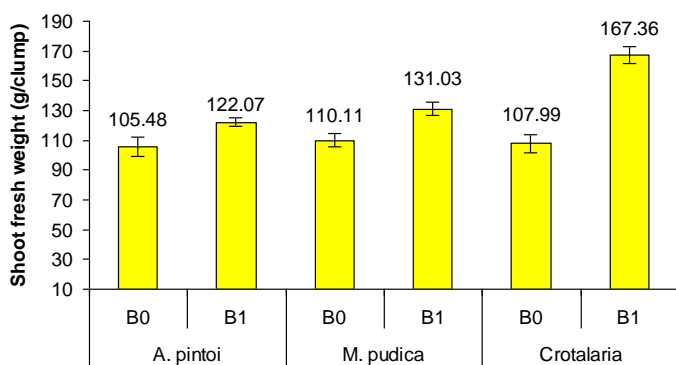


Figure 2. Average (Mean ± SE) peanut shoot fresh weight (g/clump) as affected by the interaction effect of the treatment factors

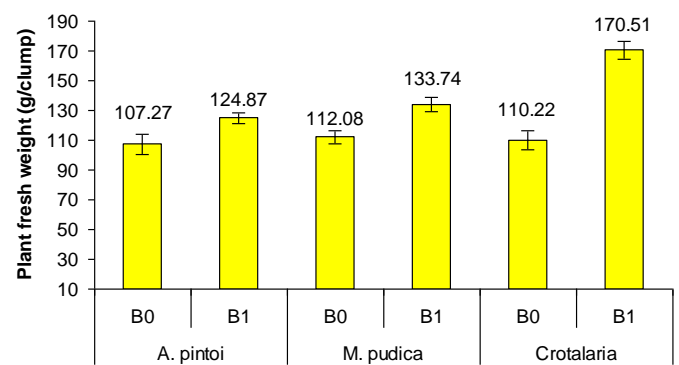


Figure 3. Average (Mean ± SE) peanut total fresh weight (g/clump) as affected by the interaction effect of the treatment factors

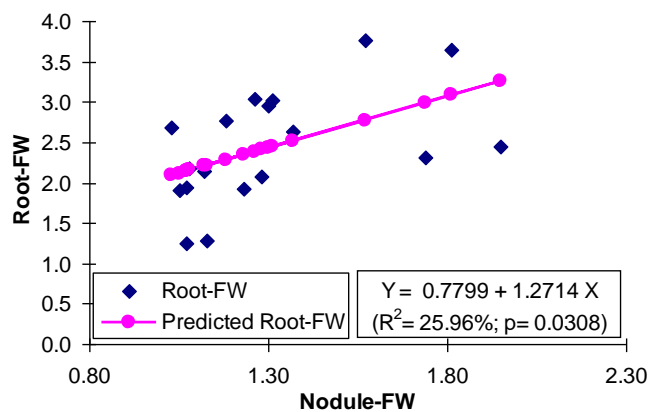


Figure 4. Regression between nodule FW (g/clump) and root FW (g/clump) of peanut plants

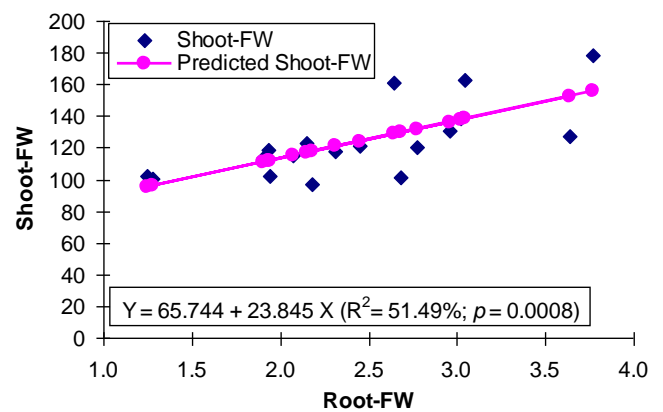


Figure 5. Regression between root FW (g/clump) and shoot FW (g/clump) of peanut plants

In this study, a consortium of root nodule bacteria isolated from root nodules of each leguminous weed species was used. To find out the specific species of the root nodule bacteria resulting growth promoting effects, further investigations are needed in addition to the need for identification of individual species of the root nodule bacteria. The current findings show that different sources of root nodule bacteria had significantly different effects on nodulation of peanut plants, indicating that one or more species of the bacterial consortium had produced the significant effect. Thus, further investigations are also needed to find out whether the significant effect can be produced by a single species or a consortium of more than one species. To find out these in more precisely, then experiment under sterilized growing media is also needed because in this study, the experiment was done in the field.

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IV. CONCLUSION

It can be concluded that application of leguminous weed nodule bacterial isolates significantly increased shoot FW, total plant FW, nodule FW, and root FW of peanut plants, while the difference in leguminous weed species as sources of the nodule bacterial isolates showed different main effects and the interaction effects only on the first three measurement variable. Based on the patterns of the interaction effect, the improvement of nodule FW of peanut plants was highest under application of root nodule bacteria isolated from *A. pintoii*, while the highest improvement of shoot FW and total plant FW of peanut was highest under application of root nodule bacteria isolated from *Crotalaria* sp. The regression analysis indicated that improvement of shoot FW due to application of root nodule bacteria isolated from leguminous weeds occurred indirectly through improvement of root FW by improved nodule FW.

V. ACKNOWLEDGMENTS

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VI. DISCLOSURE

The author reports no conflicts of interest in this work

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