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Effect Carrier Materials of *Bradyrhizobium* sp. strain PZS_A08 on Growth of *Indigofera zollingeriana*

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ABSTRACT

Sustainable forage production is needed to meet animal feed needs as the livestock industry increases. The purpose of this study was to evaluate the effect of liquid and solid carrier material on the effectiveness and infectivity of *Bradyrhizobium* sp. strain PZS_A08 on the growth of *Indigofera zollingeriana* (*I. zollingeriana*). This study used two carrier materials consisting of zeolite (solid) and molasses (liquid). The five treatments given were P0 (50% NPK), P1 (50% NPK+liquid biofertilizer), P2 (50% NPK+sterile liquid biofertilizer), P3 (50% NPK+solid biofertilizer), P4 (50 % NPK+sterile solid biofertilizer), P5 (100% NPK). The research was conducted in a Completely Randomized Design (CRD) consisting of 6 treatments and four replications. Observations were made on the plant's height, number of leaves, number of nodules, upper parts and root wet biomass, root and upper parts dry biomass, root length, and microbial population). The use of liquid and solid carrier materials effectively affected the effectiveness and infectivity of *Bradyrhizobium* sp. strain PZS_A08 on *I. zollingeriana*. Inoculants *Bradyrhizobium* sp. strain PZS_A08 significantly increased plants' growth and reduced the use of 50% NPK fertilizer. Cold storage temperature (5ºC) effectively maintained *Bradyrhizobium* sp. strain PZS_A08 on liquid and solid carriers, while solid carriers showed better effectiveness at room temperature storage (30ºC). Through this research, solid carriers such as zeolite are recommended as carriers for *Bradyrhizobium* sp. filter PZS_A08.

Keywords: Biofertilizer, *Bradyrhizobium*, Carrier, I. zollingeriana, Nitrogen-fixing bacteria

INTRODUCTION

Continuous forage production is needed to meet livestock industry needs (Duan *et al*. 2019). Fluctuating quantity and quality of feed, especially during the dry season (Tinsley *et al*. 2019), decrease livestock productivity (Tabacco *et al.* 2018). It also impacts the high mortality rate and the low livestock growth rate. Therefore forage feed, a combination of grass and legume, is needed to complement the nutrients required by livestock (Duan *et al*. 2019; Koten *et al*. 2014). *I. Zollingeriana* feed plants are potential feed crops as supplementary feed ingredients to improve livestock's nutritional status on cattle, goats, and poultry (Devendra and Liang 2012; Faradillah *et al*. 2015). *Indigofera* can produce forages up to 7.9 tons in one harvest, with a protein content of around 24% per dry weight. *Indigofera* sp. can be a source of forage for ruminants because it has high nutritional quality and productivity (Hassen *et al*. 2008). This plant can be used as a protein and energy source because of the relatively high nitrogen content and digestibility of dry matter and organic matter. Simanuhuruk and Sirait (2009) reported that *Indigofera* sp. has the potential a basal feed in the grass for goats based on chemical composition analysis, consumption means of dry feed matter, live weight gain, and feed utilization efficiency. Tarigan and Ginting (2011) stated that the level of *Indigofera* sp. used in rations given to goats could increase the study's optimal response by 30-45%. Based on Nurhayu and Pasambe (2016), it was reported that *Indigofera* fed for beef cattle feed at the rate of 40%-60% increased the bodyweight of beef cattle and reduced

feed conversion. *Indigofera* sp. can also be processed into flour and used as a poultry ration mixture. It can improve the quality of eggs physically and chemically (Faradillah *et al*. 2015).

Although studies related to the potential and composition of *I. zollingeriana* have been widely carried out, the development to improve forage yield and biomass productivity has not been explored. Abdullah *et al*. (2011) have applied liquid urine as organic liquid fertilizer on *I. zollingeriana* plants to improve agronomic performance. Hutapea *et al*. (2018) have increased *I. zollingeriana* and increased the methionine level by inoculating nitrogen-fixing bacteria. This study obtained that *Bradyrhizobium* sp. strain PZS_A08 are the most effective bacteria in increasing *I. zollingeriana* biomass production by 27,9%. Although the positive effect of *Bradyrhizobium* sp. strain PZS_A08 has been reported, research on the formulation has not been carried out. One crucial factor in ensuring a good formulation process and producing high-quality biofertilizer is carrier material suitability. A suitable carrier will support bacteria viability and provide an optimum condition for preserving the bacteria population. A suitable carrier should be nontoxic, sterilizable, nonreactive, rich in organic matter, and have a solid moisture-holding capacity. So far, there are two carriers developed for biofertilizer formulations, consisted of solid and liquid carriers. Even though liquid biofertilizer formulations have been claimed to have better survival and nodulation, their use depends on the type of bacteria used and the methods used to colonize the soil and plants (Shravani *et al*. 2019). As carrier material, molasses and zeolite are often used for biofertilizer formulations, especially those containing N-fixing bacteria. Leggo (2015) has reported that zeolite use could support nitrification processes. The mineral surface of zeolite absorbed ammonium ions provided organic waste degradation processes, thus avoiding nitrogen loss to the atmosphere by volatilization. It allows more ammonium oxidation of nutrients by soil nitrifying microorganisms.

On the other hand, molasses as liquid biofertilizers are an excellent source of carbon, energy, and fermentative sugars, supporting bacteria multiplication (Garcha *et al*. 2019). Furthermore, the carrier media is used to pack the biological agent, prolong the shelf life of biological agents, grow the inoculum, maintain the bacteria's viability before the infection process, and form nodules (Brar *et al*. 2012). Thus, the carrier material aspect is highly critical in mass production to guarantee biofertilizer quality.

Therefore, the purpose of this study was to evaluate the effect of liquid and solid carrier material on the effectiveness and infectivity of *Bradyrhizobium* sp. strain PZS_A08 on the growth of *I. zollingeriana.*

MATERIALS AND METHODS

Study Site

This research was carried out in March 2018 until May 2018 at the Laboratory of Soil and Environmental Biotechnology, Department of Soil Science and Land Resources, Faculty of Agriculture IPB (Bogor Agricultural University) Cikabayan Experimental Greenhouse, IPB. *I. zollingeriana* plant seeds were obtained from the Laboratory of Feed Science and Technology, Faculty of Animal Husbandry, Bogor Agricultural University. Latosol soil was used as planting media in the experiment. In contrast, *Bradyrhizobium* sp. strain PZS_A08 was obtained from a previous study (Hutapea *et al*. 2018). The other material used consisted of essential fertilizers (Urea, Tri Calcium Phosphate (TSP), Potassium Chloride (KCl)), zeolite with 4% moisture content, and molasses.

Bradyrhizobium Isolates Preparation

Bradyrhizobium sp. strain PZS_A08 was rejuvenated using Yeast Extract Mannitol Agar (YEMA) media, consisting of 10 g of mannitol; 0.5 g of yeast extract; 0.5 g K_2HPO_4; 0.2 g $MgSO_4$, $7H_2O$; 0.1 g NaCl; 20 g of bacto agar in one liter of water. Then it was incubated for 24 to 48 hours at 30 °C. The growing isolates were subjected to a series of tests, including a hypersensitivity test using tobacco plants and a hemolysis test using blood agar.

Preparation of Biofertilizers

Biofertilizers containing microbial *Bradyrhizobium* sp. strain PZS_A08 were created aseptically in solid (zeolite) and liquid (molasses) carrier materials. The volume ratio between the carrier and bacteria inoculum used was 5:1. Before inoculating the carrier material, the microbes population was equated up to 10^{-7}. Each biofertilizer was incubated at 30 °C and refrigerator temperature of 5 °C to evaluate its viability level.

The planting medium used was Latosol soil taken from the Cikabayan experimental field, Agricultural University of Bogor. The soil was air-dried, then sieved using a two mesh sieve. The sifted soil was put into pots and added with essential fertilizer (Urea, TSP, KCl). Some samples were

taken for the initial soil analysis, water content, field capacity, and pH.

Planting and Maintenance

The one-month-old *I. zollingeriana* plant seeds that had grown well and had the same height were chosen and transferred to the prepared planting medium. Before planting, TPC (Total plate Count) calculation was conducted on the carrier material used. The population of *Bradyrhizobium* sp. strain PZS_A08 applied was 3.8 x 10^{11} CFU ml^{-1} on a molasses carrier material and 4 x 10^{11} CFU g^{-1} on zeolite carrier materials.

Maintenance activities included daily watering to keep the media moist. In addition, physical cleaning of weeds and pests was carried out. Finally, fertilization was done on the plants after 1 MST, and fertilization was given according to the treatment.

Plant Growth Observation

In this study, plant height measurements, the number of leaves, and the stems were conducted once a week for four weeks. In the final stage of observation, the plant height, number of leaves, number of branches, number of root nodules, upper and lower biomass, upper and lower dry weight, root length, and microbial population calculation (Total Plate Count) were conducted.

Experimental Design and Data Analysis

The research was conducted in a Completely Randomized Design (CRD) consisting of 6 treatments and four replications, so that the total units of the experiment were 24 units. The treatment used is shown in Table 1.

Table 1. Types of treatment used.

Treatment	Description
P0	50% (NPK)
P1	50% (NPK) +1 ml pot^{-1} liquid inoculant
P2	50% (NPK) - 1 ml pot^{-1} liquid inoculant
P3	50% (NPK) + 5 g pot^{-1} solid inoculant
P4	50% (NPK) - 5 g pot^{-1} solid inoculant
P5	100% (NPK)

The data obtained were then analyzed by variance. Finally, further analysis was carried out using Duncan Multiple Range Test (DMRT) with the SAS 9.4 program in the treatment.

RESULTS AND DISCUSSION

Based on preliminary and final soil analysis in Table 2, the organic matter content of the soil has increased in the treatment that gets the addition of biofertilizer, from 2.04% to 2.11% (zeolite carrier materials) and 2.12% (molasse carrier materials). This increase was caused by microbes' biomass as a source of organic matter to the soil, besides enzymatic reactions between plant roots could increase natural properties. The increased C/N value supports this in the soil after being given. However, the total measured N content in the soil also slightly increased. Oliveira *et al*. (2017) reported that biofertilizers positively impacted nutrient uptake

Table 2. Characteristics of the soil used in the experiment.

Parameter	Before treatment	After treatment (Inoculant in Zeolite)	After treatment (Inoculant in Molasses)
pH H_2O	5.44	5.48	5.45
pH KCL	4.80	4.92	4.89
Organic Carbon	2.04 %	2.11 %	2.12 %
Total N (N %)	0.18 %	0.27 %	0.21 %
C/N Ratio	11	13	13
P_2O_5 available	1.57 ppm	1.52 ppm	1.49 ppm
P_2O_5 potential	59.08 mg $100g^{-1}$	58.99 mg $100g^{-1}$	58.78 mg $100g^{-1}$
K_2O potential	6.76 mg $100g^{-1}$	6.71 mg $100g^{-1}$	6.81 mg $100g^{-1}$
K^+ exchangeable	0.10 cmol(+) kg^{-1}	0.12 cmol(+) kg^{-1}	0.10 cmol(+) kg^{-1}
Na+ exchangeable	0.02 cmol(+) kg^{-1}	0.02 cmol(+) kg^{-1}	0.02 cmol(+) kg^{-1}
Ca^{2+} exchangeable	3.12 cmol(+) kg^{-1}	3.19 cmol(+) kg^{-1}	3.12 cmol(+) kg^{-1}
Mg^{2+} exchangeable	0.47 cmol(+) kg^{-1}	0.48 cmol(+) kg^{-1}	0.48 cmol(+) kg^{-1}
Cation Exchange Capacity	16.04	16.01	16.04
Alkaline saturation	23.17 %	23.54 %	23.54 %
Al^{3+} exchangeable	0.99 cmol(+) kg^{-1}	0.87 cmol(+) kg^{-1}	0.97 cmol(+) kg^{-1}
H^+ exchangeable	0.54 cmol(+) kg^{-1}	0.47 cmol(+) kg^{-1}	0.52 cmol(+) kg^{-1}

efficiency. It also increased nutrient availability likes total N, nitrate-N, and ammonium-N. The following data indicate that the increase in the element C /N ratio is not caused by a decrease in the element N in the soil but a significant increase in organic carbon. Based on Dêbska *et al.* (2016), biofertilizer use increases the permanent humus compounds, correlated with increased soil organic matter stability. Consequently, the contribution of the organic matter fractions that are more resistant to decomposition is crucial for increasing soil carbon sequestration.

Meanwhile, other elements' content is relatively unchanged (phosphate, potassium, calcium, magnesium, and sulfate), including the CEC and soil pH. Thus, apart from the formulation of the carrier material, soil properties also influence the effect of biofertilizers on increasing plant production.

We are interested in this research because there has not been much data to discuss carriers' effect on adding *Bradyrhizobium* as biofertilizers. The carrier has a crucial role in delivering a suitable amount of microbes in good physiological conditions. As one of the microbes that have an essential role in implementing the N cycle in the soil, the formulation of N-fixing bacteria biofertilizer has been widely studied to obtain biological fertilizers, increasing the N content in the soil. Abd *El-Fattah et al*. (2013) have reported that the use of carriers and sterilization methods will affect the quality of biofertilizers containing *Azotobacter chroococcum*. The use of the type of carrier material also influences the effectiveness of biofertilizers in terms of the response of the plants produced (Khandare *et al*. 2015). Mukhtar *et al*. (2017) suggested that carriers in biofertilizers with phosphate solubilizing bacteria correlated with the increase in wheat yields. Microbes' effectiveness in increasing plant growth correlates with their colonized roots' ability. The suitability factor of the carrier with storage temperature is an essential factor in the production of biological fertilizers because it affects the stability of the population and the microbes' physiological conditions (Kaljeet *et al*. 2011).

Table 3 shows that the average growth of *I. zollingeriana* for 30 days after planting from 6 treatments tested mostly showed a significant effect on the observed variables. The parameter used in this study was the growth of the plant, which included: plant height, number of leaves, number of leaf stalks, the shoot and root biomass of the plant, dry weight of the upper and lower parts of the plant, number of root nodules, and root length. The treatment by giving the *Bradyrhizobium* sp. strain PZS_A08 (P1 and P3) showed a considerable influence on plants' vegetative growth compared with the absence of the *Bradyrhizobium* sp. strain PZS_A08. It indicates that *Bradyrhizobium* sp. strain PZS_A08 application can increase plants' height growth by 20-70% compared with giving 100% NPK fertilizer (P5) only. The positive effect of *Bradyrhizobium* sp. strain PZS_A08 is also supported by several data, including the number of effective root nodules formed and root biomass, which were higher compared without *Bradyrhizobium* sp. application. *Bradyrhizobium* sp. stimulates nodule

Table 3. Effect of *Bradyzobium* sp. PZS_A08 strain on the growth of *I. zollingeriana.*

Treatment	PH	NL	NB	RL	NEN	NNN	CB	RB	CDB	RDB
50% (NPK)	9.79c	19.30bc	5.80b	23.37bc	0.00c	1.75b	5.40b	1.45b	1.00b	0.35a
50% (NPK)+Liquid inoculant	13.53a	27.25a	7.60a	33.00a	7.00b	2.00ab	10.10a	2.87ab	2.25a	0.58a
50% (NPK)+ Liquid inoculant	10.25bc	19.15bc	6.2b	21.22c	0.00c	0.50b	7.80ab	1.67ab	1.52ab	0.27a
50% (NPK)+Solid inoculant	12.92a	26.95a	8.0a	32.12a	8.50a	3.00a	10.27a	3.02a	2.17a	0.48a
50% (NPK)+Solid inoculant	9.81 c	17.30c	6.1b	21.12c	0.00c	1.25ab	5.32b	1.45b	1.08b	0.20a
100% (NPK)	12.43ab	23.10ab	6.35b	31.25ab	1.00c	1.00ab	9.70a	2.77ab	2.12a	0.52a

Remarks: PH (plant height) (cm), NL (number of leaves) (sheets plant^{-1}), NB (number of branch) (sheets plant^{-1}), RL (root length)(cm), NEN (number of effective nodules), NNN (number of noneffective nodules). CB (canopy biomass) (g plant^{-1}), RB (root biomass) (g plant^{-1}), CDB (canopy dry biomass) (g plant^{-1}), RDB (root dry biomass) (g plant^{-1}). Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test.

formation, which affects the release of organic compounds into the soil and increases the amount of nitrogen (Gopalakrishnan *et al*. 2015; Lindström and Mousavi 2020). It also explains why the number of nodules affects the growth in the number of leaves, stems, and root length because of higher nitrogen accessibility.

Table 3 shows that adding *Bradyrhizobium* inoculant gave significant responses to the amount of biomass and dry weight of the *Indigofera zollingeriana*. Even though there is no significant difference between zeolite and molasses use, the number of nodules showed a different response because of carriers used. Zeolite's use stimulated better nodulation than molasses, which was revealed through more nodules and root biomass. Plant biomass includes all organic material of the plants from the unit area unity's photosynthesis at a particular time with units of g m^{-2}. In the P3 treatment, the number of effective nodules was more than the other treatments, indicating higher nitrogen fixation rates. It is related to nitrogen content in the soil and increases the *I. zollingeriana* metabolism.

Based on Figure 1, it can be seen that plant height and root length differ among different treatments between the *Bradyrhizobium* sp. strain PZS_A08 inoculum application and without application. It shows the influence of the application of *Bradyrhizobium* sp. strain PZS_A08 on plant height and root length. *Bradyrhizobium* sp. formed nodules symbiotically with *Indigofera zollingeriana* (*I. zollingeriana*), impacting plants' growth improvement because nitrogen availability increases. On the other hand, Maróti and Kondorosi (2014) state that the process of root elongation and nodule formation is influenced by the availability of carbohydrates produced from photosynthesis. In legume plants, high concentrations of nitrate and ammonium (>3mM) can inhibit the nodulation process, while at lower concentrations, it can stimulate nodule formation. Besides, N sources' availability in other forms plays an essential role in the differentiation of plant roots. Under N deficiency conditions, plants streamline the use of carbon sources and trigger plant root growth to explore nitrogen origin in the soil and initiate symbiosis with N-fixing bacteria (Radzman *et al.* 2013).

Based on the six treatments tested, most of the plants were able to produce root nodules, whereas in the treatment of liquid biofertilizers without *Bradyrhizobium* sp. strain PZS_A08 inoculant bacteria, there was only one non-effective root nodule which was formed from each repetition tested, and there was no effective root nodule. In treatment P1 and P3, the number of effective root nodules was more significant than P0, P2, P4, and P5. It means that *Bradyrhizobium* sp strain PZS_A08 application can increase the efficiency of effective formation of root nodules and increase the effectiveness of fixing N. It also confirmed that nodule formation was dominantly stimulated by added *Bradyrhizobium* sp. strain PZS_A08.

Nitrogen-fixing microbes could not permanently inhabit root nodules, even though they exist in the soil. Instead, it depends on microbes' infective ability and environmental factors such as water as providers and competition with other microbes. According to Pommeresche and Hansen (2017), an effective root nodule's characteristic will show pink to the brownish middle when cut transversely. This Leghemeglobin red pigment plays the most significant role in fixing N. The pigment is found in nodules between the bacteroids and the surrounding membrane. Therefore, the amount of leghemoglobin in the root nodules will directly relate to the nitrogen-fixed amount.

After harvesting, observations of nodules formed on the roots of *I. zollingeriana* in the addition of *Bradyrhizobium* sp. strain PZS_A08 with molasses and zeolite carrier treatment were conducted. Based on the observation results, the

Figure 1. Effect of inoculation with Bradyrhizobium on the growth of I. *zollingeriana* day 30. Remarks: P0 (50% NPK), P1 (50% NPK+1 ml pot^{-1} liquid inoculant), P2 (50% NPK-1 ml pot^{-1} liquid inoculant), P3 (50% NPK+5 g pot^{-1} solid inoculant), P4 (50% NPK-5 g pot^{-1} solid inoculant), P5 (100% NPK).

size of the root nodules formed ranged from 3 to 5 mm (Figure 2). This size is smaller than the Bradyrhizobium nodules in general, which have a length ranging from 5-10 mm for Indigofera (Bünger *et al*. 2021). Nodulations in other plants such as shrubby sophora (*Sophora flavescens*) and soybean (*Glycine max*) are smaller, about 1-3 mm long (Ledermann *et al*. 2021; Liu et al. 2018). This size is also influenced by the type and age of the plant, where Indigofera has a relatively long life. In this study, nodulation was observed in 2-month-old plants. It caused the growth of nodules to have not yet reached the maximum point.

The form of root nodules tended to be round with a rough surface. *Bradyrhizobium*, inoculated with molasses, had a spreading nodule located on the central axis and lateral roots. Meanwhile, *Bradyrhizobium* inoculated through zeolite carriers had relatively more nodules on the central axis than in the first-order lateral roots. The root nodulation was mainly adjacent to the central axis root and was in the lateral roots with a more extensive root diameter. However, the pattern of nodulation is affected by several factors, including plant cultivar and microbial strains. The dynamics of water movement in the soil consisting of filtration and percolation, can also affect soil bacteria colonization, affecting nodule formation (Czaban *et al.* 2007). Although there was no significant difference in affecting plant biomass production, carrier use was sufficient to influence the number of nodules and the nodule formation pattern. The observation results also showed that the color of effective nodules was red, and they had a relatively larger size than non-effective nodules. It is similar to the study of Nguyen *et al*. (2017), which shows the appearance of *Bradyrhizobium elkanii* nodules with a white outer layer and a red inner layer. The bacteria compatibility and genetic factors strongly influence the ability of *Bradyrhizobium* to infect. The active nodules in fixing N2 will be red because it contains many leghemoglobin (Larrainzar *et al*. 2020). It also confirmed that the inoculant, either using molasse or zeolite, can infect *I. zollingeriana.*

Another indicator that can confirm the success of *Bradyrhizobium* in colonizing the root zone of *Indigofera* is by observing the bacterial population in the root area. Based on the observations, the bacterial population with zeolite as a carrier could colonize the root area better than the molasses carrier. Inoculants with zeolite carriers could colonize the root area up to $29x10^6$ CFU ml^{-1}, while inoculants with molasses as carriers were up to $5x10^6$ CFU ml^{-1} (Table 4). Even though inoculation technology has several benefits, it also has a significant limitation on the inoculum survival rate in soil and rhizosphere (Santos *et al.* 2019). Hence maintaining physiological condition and microbe amount during storage and application period is essential.

The population growth of the *Bradyrhizobium* sp. strain PZS_A08 in the molasses carrier material was relatively high at room temperature (30 °C), up to 3.87×10^8 CFU ml^{-1} during the first week. However, there was a significant decrease at week six with a 0.31×10^8 CFU ml^{-1} population. Meanwhile, the bacteria on the molasses carrier material with a treatment temperature of 5 °C showed high population density at week six with a population of 3.65×10^8 CFU ml^{-1}. It indicates that the first week

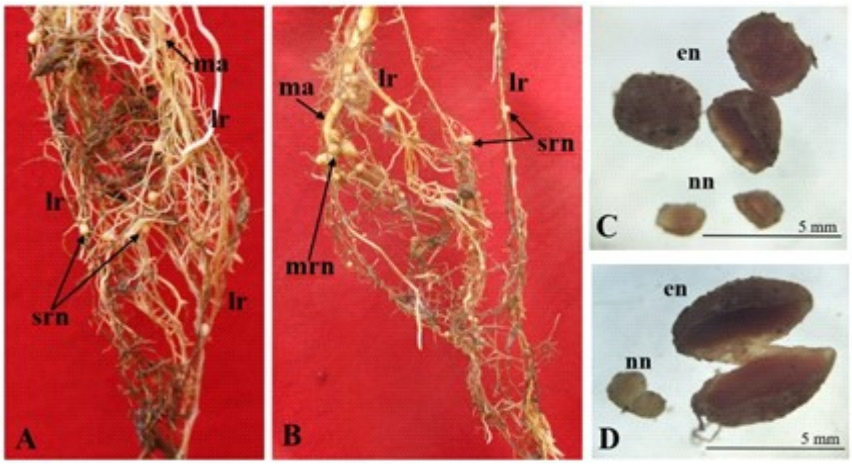

Figure 2. The appearance of *Bradyrhizobium* sp. strain PZS_A08 root nodules in soil with (a) a liquid carrier (molasses) and (b) a solid carrying material (zeolite).

Table 4. The population of Bradyrhizobium in the post-harvesting soil.

Treatment	Population (CFU ml^{-1})
P0 50% (NPK)	$< 10^5$
P1 50% (NPK)+Liquid inoculant	5×10^6
P2 50% (NPK)+ Liquid inoculant)	$< 10^5$
P3 50% (NPK)+Solid inoculant	29×10^6
P4 50% (NPK)+Solid inoculant)	$< 10^5$
P5 100% (NPK)	3.9×10^6

of microbial growth on the molasses medium had entered the exponential phase, and in the sixth week, it entered the phase of death. Store it at 5 °C was able to maintain the population of *Bradyrhizobium* sp. strain PZS_A08 up to more than 35 days. Microbes' growth in zeolite carriers exponentially increased in the third week with a population density of 3.91 $\times10^8$ CFU ml^{-1} at 30 °C and 3.19 $\times10^8$ CFU ml^{-1} at a temperature of 5 °C. While the population density decreased in the sixth week at 30 °C, the microbe population at 5 °C still increased to 3.65 $\times10^8$ CFU ml^{-1} (Figure 3). It shows that zeolite material could preserve inoculant population better-compared molasses, particularly at room temperature storage.

The level of viability affected the population of *Bradyrhizobium* sp. strain PZS_A08 applied to the plants and impacted the quality of biofertilizers. In addition, the type of carrier material and the proper environmental conditions significantly affected *Bradyrhizobium* sp. strain PZS_A08 to retain its population. Based on viability data, the bacteria population was relatively more conserve in low temperature, using molasse or zeolite as carrier material. Furthermore, the carriers' ability to maintain bacterial populations over a wide temperature range is essential because biofertilizers cannot always be stored at low temperatures when marketed to consumers (Mohamed *et al*. 2016). Therefore, based on this study, we recommend using zeolites better to maintain the *Bradyrhizobium* population at a temperature of 30°C.

CONCLUSIONS

The use of both liquid and solid carrier materials effectively influences the effectiveness and penetration of *Bradyrhizobium* sp. strain PZS_A08

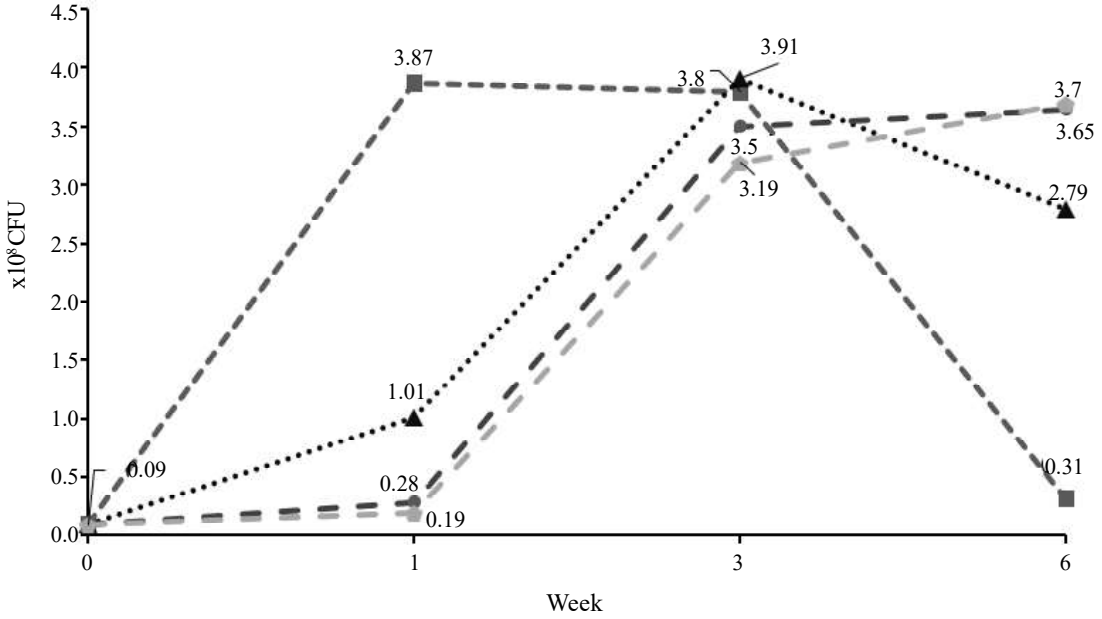

Figure 3. The viability of *Bradyrhizobium* sp. strain PZS_A08 on zeolite and molasses. : Molase 30 °C, : Zeolite 30 °C, : Molase 5 °C, : Zeolite 5 °C.

on *Indigofera zolingeriana*. Application of *Bradyrhizobium* sp. strain PZS_A08 has been shown to increase plant growth and decrease NPK fertilizer by up to 50%, using zeolite and molasses. Although it had no significant effect on increasing plant growth, the use of zeolites compared to molasses caused an increase in the number of nodules and root biomass. Also, molasses as a carrier material have a lower ability to maintenance microbial populations than the use of zeolites at room temperature. Although this is a relatively minor factor, zeolite as a carrier for *Bradyrhizobium* is more recommended for biofertilizer production than molasses used.

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REFERENCES

Abd El-Fattah Dalia A, WE Eweda, MS Zayed and MK. Hassanein. 2013. Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of azotobacter chroococcum inoculant. *Ann Agr Sci* 58: 111-18. http://dx.doi.org/10.1016/j.aoas.2013.07.001.

Abdullah L, DDS Budhie and AD Lubis. 2011. Pengaruh aplikasi urin kambing dan pupuk cair organik komersial terhadap beberapa parameter agronomi pada tanaman pakan *Indigofera* sp. *Pastura: J Trop Forage Sci* 1: 5-8. (in Indonesian).

Brar SK, SJ Sarma and E Chaabouni. 2012. Shelf-life of biofertilizers: an accord between formulations and genetics. *J Biofertil Biopestici* 3: 000e109. doi:10.4172/2155-6202.1000e109

Bünger W, A Sarkar, JL Grönemeyer, J Zielinski, R Revermann, T Hurek and B Reinhold-Hurek. 2021. Root . *Front Microbiol* 12: 611704. doi:10.3389/fmicb.2021.611704

Czaban J, A Gajda and B Wróblewska. 2007. The Motility of Bacteria from Rhizosphere and Different Zones of Winter Wheat Roots. *Pol J Environ Stud* 16: 301-308.

Dêbska B, J D³ugosz, A Piotrowska-D³ugosz and M Banach-Szott. 2016. The impact of a bio-fertilizer on the soil organic matter status and carbon sequestration-results from a field-scale study. *J Soil Sediment* 16: 2335-2343.

Devendra C and JB Liang. 2012. Conference summary of dairy goats in asia: current status, multifunctional contribution to food security and potential improvements. *Small Rumin Res* 108: 1-11. http://dx.doi.org/10.1016/j.smallrumres.2012.08.012.

Duan C, P Shi, N Zong, J Wang, M Song and X Zhang. 2019. Feeding solution: crop-livestock integration via crop-forage rotation in the southern tibetan plateau. *Agric Ecosyst Environ* 284: 106589. https://doi.org/10.1016/j.agee.2019.106589.

Faradillah F, R Mutia and L Abdullah. 2015. Substitution of soybean meal with indigofera zollingeriana top leaf meal on egg quality of cortunix cortunix japonica. *Media Peternakan* 38: 192-197. (in Indonesian).

Garcha S, R Kansal and SK Gosal. 2019. Molasses growth medium for production of rhizobium sp. based biofertilizer. *Indian J Biochem Biophys* 56: 378-383.

Gopalakrishnan S, A Sathya, R Vijayabharathi, RK Varshney, CLL Gowda and L Krishnamurthy. 2015. Plant growth promoting rhizobia: challenges and opportunities. *3 Biotech* 5: 355-377. http://dx.doi.org/10.1007/s13205-014-0241-x.

Hassen A, NFG Rethman, Z Apostolides and WA Van Niekerk. 2008. Forage production and potential nutritive value of 24 shrubby indigofera accessions under field conditions in South Africa. *Trop Grasslands* 42: 96-103.

Hutapea PS, L Abdullah, PDMH Karti and I Anas. 2018. Improvement of *Indigofera Zollingeriana* production and methionine content through inoculation of nitrogen-fixing bacteria. *Trop Anim Sci J* 41: 37-45.

Kaljeet S, F Keyeo and H Amir. 2011. The temperature on survivability of rhizobial inoculant. *Asian J Plant Sci* 10: 331-337.

Khandare RN, R Chandra, N Pareek and KP Raverkar. 2015. Effect of varying rates and methods of carrier-based and liquid azotobacter and psb biofertilizers on yield and nutrient uptake by wheat (*Triticum Aestivum* L.) and soil properties. *J Indian Soc Soil Sci* 63: 436-441.

Koten BB, R Wea, RD Soetrisno, N Ngadiyono and B Soewignyo. 2014. Konsumsi nutrien ternak kambing yang mendapatkan hijauan hasil tumpangsari arbila (*Phaseolus Lunatus*) dengan sorgum sebagai tanaman sela pada jarak tanam arbila dan jumlah baris sorgum yang berbeda. *J Ilmu Ternak* 1: 38-45. (in Indonesian).

Larrainzar E, I Villar, MC Rubio, C Pérez-Rontomé, R Huertas, S Sato, JH Mun and M Becana. 2020. Hemoglobins in the legume–rhizobium symbiosis. *New Phytol* 228: 472-484. doi: 10.1111/nph.16673

Ledermann R, B Emmenegger, JM Couzigou, N Zamboni, P Kiefer, JA Vorholt and HM Fischer. 2021. *Bradyrhizobium* diazoefficiens requires chemical chaperones to cope with osmotic stress during soybean infection. *mBio* 12: e00390-21. doi.org/10.1128/mBio.00390-21.

Leggo PJ. 2015. The efficacy of the organo-zeolitic biofertilizer. *Agrotechnology* 06: 1-4.

Lindström, Kristina and SA Mousavi. 2020. Effectiveness of nitrogen fixation in rhizobia. *Microb Biotechnol* 13: 1314-1335.

Liu YH, ET Wang, YS Jiao, CF Tiana, L Wang, ZJ Wang, JJ Guana, RP Singh, WX Chen and WF Chen. 2018. Symbiotic characteristics of bradyrhizobium diazoefficiens usda 110 mutants associated with shrubby sophora (*Sophora Flavescens*) and soybean (*Glycine Max*). *Microbiol Res* 214: 19-27. doi.org/10.1016/j.micres.2018.05.012.

Maróti G and É Kondorosi. 2014. Nitrogen-fixing rhizobium-legume symbiosis: are polyploidy and host peptide-governed symbiont differentiation general principles of endosymbiosis?. *Front Microbiol* 5: 1-6.

Mohamed ASA, AK Khider and S Muniandy. 2016. Effect of storage temperature, duration and types of biofertilizer carriers on survival and numbers of bacterial strains *Bacillus megaterium* var. *phosphaticum*, *Azotobacter chroococcum*, *Rhizobium leguminosarum* and *Transformant*, *Transconjugant B. megaterium* var. *phosphaticum.* International conference on agricultural, food, biological and health sciences (AFBHS-16), pp. 99-104. doi:10.17758/eap.eap816211.

Radzman N, MA Djordjevic and N Imin. 2013. Nitrogen modulation of legume root architecture signaling pathways involves phytohormones and small regulatory molecules. *Front Plant Sci* 4: 1-7.

Mukhtar S, I Shahid, S Mehnaz and KA Malik. 2017. Assessment of two carrier materials for phosphate solubilizing biofertilizers and their effect on growth of wheat (*Triticum Aestivum* L.). *Microbiol Res* 205: 107-117. http://dx.doi.org/10.1016/j.micres.2017.08.011.

Nguyen HP, H Miwa, T Kaneko, S Sato and S Okazaki. 2017. Identification of *Bradyrhizobium elkanii* genes involved in incompatibility with *Vigna radiate*. *Genes* 8: 374. doi:10.3390/genes8120374

Oliveira FLN, WS Oliveira, NP Stamford, EVN Silva, CERS Santos and ADS Freitas. 2017. Effectiveness of biofertilizer enriched in n by *Beijerinckia indica* on sugarcane grown on an ultisol and the interactive effects between biofertilizer and sugarcane filter cake. *J Soil Sci Plant Nutr* 17: 1040-1057.

Nurhayu A and D Pasambe. 2016. Indigofera sebagai substitusi hijauan pada pakan sapi potong di kabupaten bulukumba sulawesi selatan. *Seminar Nasional Peternakan 2*: 52-56. (in Indonesian).

Pommeresche R and S Hansen. 2017. Examining root nodule activity on legumes. *Fertil Crop Technical.* 1-4. Available at: http://orgprints.org/31344/1/tn-wp5-root-nodules_final_2017.pdf. (Accessed on May 5, 2021).

Santos MS, MA Nogueira and M Hungria. 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Express* 9. https://doi.org/10.1186/s13568-019-0932-0.

Shravani K, S Triveni, PC Latha and CK Damodara. 2019. Evaluation of shelf life and quality of carrier and liquid-based biofertilizers. *Int J Microbiol Res* 11: 1598-1601.

Simanuhuruk K and J Sirait. 2009. Pemanfaatan leguminosa pohon *Indigofera* sp. sebagai pakan basal kambing Boerka fase pertumbuhan. *Seminar Nasional Peternakan dan Veteriner 2009,* pp. 449-454. (in Indonesian).

Tabacco E, L Comino and G Borreani. 2018. Production efficiency, costs and environmental impacts of conventional and dynamic forage systems for dairy farms in Italy. *Eur J Agron* 99: 1-12. https://doi.org/10.1016/j.eja.2018.06.004.

Tarigan A and SP Ginting. 2011. Pengaruh taraf pemberian *Indigofera* sp. terhadap konsumsi dan kecernaan pakan serta pertambahan bobot hidup kambing yang diberi rumput *Brachiaria Ruziziensis*. *J Ilmu Ternak dan Veteriner* 16: 25-32. (in Indonesian).

Tinsley TL, S Chumbley, C Mathis, R Machen and BL Turner. 2019. Managing cow herd dynamics in environments of limited forage productivity and livestock marketing channels: an application to semi-arid Pacific Island beef production using system dynamics. *Agric Syst* 173: 78-93. doi.org/10.1016/j.agsy.2019.02.014.