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The Potential of *Azolla pinnata* Powder and Compost as a Carrier-base for Improving N-Fixing and P-Solubilizing Bacteria Performance to Increase Soybean Productivity

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ABSTRACT/ABSTRAK

Potensi tepung *Azolla pinnata* dan kompos sebagai bahan pembawa untuk meningkatkan kinerja bakteri penambat nitrogen dan pelarut fosfat dalam meningkatkan produktivitas tanaman kedelai

Effectiveness of *Azotobacter* sp. and *Bacillus* sp. inoculants often decreases when applied in acidic soils such as Inceptisol, so efforts are required to enhance their performance under these conditions. One strategy is to select appropriate inoculant carrier material such as *Azolla pinnata* powder and compost. The purpose of this study was to evaluate the population density of inoculants, the formation of root nodules, the dry weight of roots, the number of trifoliate leaf, the weight of 100 grain, and the number of pods in soybean plants treated with *A. pinnata* powder and compost. This research was conducted from August 2022 to January 2023 at the Soil Biology Laboratory and Ciparanje Experimental Garden, Department of Soil Science and Land Resources, Faculty of Agriculture, Universitas Padjadjaran. The study used a Factorial Randomized Block Design with two factors, i.e., application of NPK fertilizer with the dosages of 100% (300 kg/ha), 50% (150 kg/ha) and 0% (0 kg/ha) as the first factor while the second factor was the treatments of liquid culture inoculants, inoculants in compost, inoculants in *A. pinnata* powder, inoculants in compost mixture and *A. pinnata* powder which were repeated three times each. Results showed that liquid culture, culture in *A. pinnata* powder and compost increased the population of *Azotobacter* spp. and *Bacillus* spp. in the rhizosphere. The inoculant in the carrier base mixture of compost and *A. pinnata* powder produced highest dry root weight of the roots and the number of nodules significantly. Inoculation of *Azotobacter* sp. and *Bacillus* sp. liquid culture or in *A. pinnata* powder carrier resulted in pod yield of soybean equivalent to 150 kg/ha NPK application. The application of inoculants in *A. pinnata* powder, compost, or a mixture of both resulted in a weight of 100 grains that did not differ significantly, however, the weight of 100 grains was equivalent to a treatment using a NPK dose of 300 kg/ha in Inceptisol.

Efektivitas inokulan *Azotobacter* sp. dan *Bacillus* sp. sering kali berkurang ketika diaplikasikan di tanah asam seperti Inceptisol, sehingga diperlukan upaya untuk meningkatkan kinerjanya dalam kondisi ini. Salah satu strategi adalah memilih bahan pembawa inokulan yang tepat seperti tepung *Azolla pinnata* dan kompos. Tujuan penelitian ini adalah untuk mengevaluasi kepadatan populasi inokulan, pembentukan bintil akar, berat kering akar, dan

jumlah daun trifoliat pada tanaman kedelai yang diberi perlakuan tepung *A. pinnata* dan kompos sebagai *carrier* inokulan *Azotobacter* sp. dan *Bacillus* sp. Penelitian ini dilakukan pada Agustus 2022 hingga Januari 2023 di Laboratorium Biologi Tanah dan Kebun Percobaan Ciparanje, Departemen Ilmu Tanah dan Sumber Daya Lahan, Fakultas Pertanian, Universitas Padjadjaran. Penelitian menggunakan Rancanagan Acak Kelompok Faktorial dengan dua faktor yaitu faktor pertama berupa pemberian pupuk NPK dengan dosis 100% (300 kg/ha), 50% (150 kg/ha) dan 0% (0 kg/ha) dan faktor kedua adalah perlakuan inokulan kultur cair, inokulan dalam kompos, inokulan dalam tepung Azolla, inokulan dalam campuran kompos dan Azolla yang masing-masing diulang sebanyak tiga kali. Hasil penelitian menunjukkan bahwa *carrier* tepung Azolla dan kompos meningkatkan populasi *Azotobacter* spp. dan *Bacillus* spp. di rizosfer. Inokulan dalam campuran pembawa kompos dan tepung Azolla menghasilkan berat kering akar dan jumlah bintil tertinggi secara signifikan. Inokulasi kultur cair *Azotobacter* sp. dan *Bacillus* sp. atau inokulan padat dengan bahan pembawa tepung Azolla menghasilkan polong kedelai yang setara dengan aplikasi NPK 150 kg/ha. Aplikasi inokulan dalam tepung Azolla, kompos, atau campuran keduanya menghasilkan bobot 100 biji yang tidak berbeda nyata, namun, bobot 100 biji setara dengan perlakuan yang menggunakan dosis NPK 300 kg/ha di Inceptisol

INTRODUCTION

Soybean (*Glycine max* L.) is one of the third most important alternative foods after rice and corn. Soybean production in Indonesia in 2019 amounted to 424.19 thousand tons, with a harvested area of 285,265 ha and a productivity of 12.87 kg/ha with the highest soybean production provinces are East Java, Central Java and West Java (PDSIP, 2020). However, national soybean production is lower than soybean imports, which reached around 2.32 million tons in 2022 (BPS-Statistic Indonesia, 2023). The low national soybean production can be enhanced through appropriate cultivation techniques, suitable fertilization, effective pest and disease control.

One way to increase soybean productivity is through the use of inorganic fertilizers such as NPK. However, excessive use of chemical fertilizers can have negative impact including leaching, water pollution, soil acidification, reduced availability of trace elements, imbalance soil physical properties, and unsustainable crop production (Jayathilake *et al*., 2006; Titirmare *et al*., 2023). To reduce those negative impacts, we can decrease the amount of NPK fertilizer used by combining it with N fixing and P-solubilizing microbial inoculants. The N fixation and P-solubilizing microbial inoculants that we will focus on in this paper are *Azotobacter* sp. and *Bacillus* sp.

Azotobacter, a nitrogen-fixing bacterium, converts atmospheric nitrogen gas (N_2) into amonia (NH_3) that useble for plant (Cheng, 208). The *Azotobacter* genus belongs to the gram-negative, free-living bacteria group within the heterotrophic category (Martyniuk & Martyniuk, 2003). These microbes can fix N_2 from the air without needing to symbiotically associate with plants. *Azotobacter* spp. provides the advantage in immobilizing *Rhizobium* in the rhizosphere for the formation of nodules in soybean roots (Hindersah *et al*., 2017). During nitrogen fixation, it forms distinct structures and produces abundant exopolysaccharides and stimulates plant growth and resides in the rhizosphere (Bashan & de-Bashan, 2010; Gomare *et al*., 2011; Hindersah *et al*., 2020; Suryatmana *et al*., 2024). A few reports indicated that Nitrogen fixing bacteria (NFB) by free-living diazotrophs contribute estimated at up to 60 kg N/ha/year and provide N for plants (Vadakattu & Paterson, 2006; Reed *et al*., 2011). The agronomic features of *Azotobacter* that are likely an effective component of integrated plant nutrition strategy, which contributes positively to sustainable agricultural production (Aasfar *et al*., 2021). These bacteria also produce indole acetic acid (IAA) and gibberellins (GA), promoting the plant cell growth and seed germination. However, they are sensitive to acidic soil, high salt levels, and extreme temperatures (Jnawali *et al*., 2015). The viability and abundance of *Azotobacter* spp. in soil are influenced by factors such as soil pH, temperature, moisture, and other microbial communities (Kizilkaya, 2009).

Azotobacter spp. grows well within the optimal temperature range of 20–30 °C and thrives best in neutral to alkaline soil pH (pH 6.5–7.5), but performs poorly in acidic soil. The use of N-fixing inoculants often faces challenges in its functional effectiveness under acidic soil conditions or toxic stress, where nitrogenase activity sharply decreases (Suryatmana *et al*., 2024). Thus, careful consideration must be given to the application of these bacteria to address specific challenges like nutrient deficiencies and soil acidity.

Bacillus spp. is a phosphate-solubilizing bacterium and also capable of nitrogen fixation. It is Gram-positive, aerobic, rod-shaped with a diameter of 1.2–1.5 µm and length of 2.0–2.4 µm, cylindrical to oval, and has an optimum temperature for growth at 25 °C, 35 °C, and 37 °C (Holt *et al*., 1994). Phosphate-solubilizing bacteria can improve plant root growth because they play an important role to increase phosphate availability in soil. *Bacillus* sp. belongs to the group of plant growth-promoting rhizobacteria (PGPR) with significant potential because it can produce IAA, solubilize phosphate, secrete siderophores, and act as a biocontrol agent by inducing plant immunity systems and producing antibiotics (Compant *et al*., 2005; El-Saadony *et al*., 2022). *Bacillus* sp. is classified as an antagonistic bacterium capable of suppressing several plant diseases, can increase nitrogen availability by fixing N_2 from the atmosphere (Mrkovački *et al*., 2016). In addition to enhance N and P availability in the soil, *Bacillus* sp. can synthesize the phytohormone auxin in the form of Indole 3-Acetic Acid (IAA), which functions to promote root and shoot growth in plants (Vejan *et al*., 2016).

The phytohormone IAA influences plant development processes because endogenous IAA in plants can be altered by IAA secreted by soil microbes, resulting in increased plant IAA production (Glick, 2012). Soil and seed inoculation using phosphate-solubilizing bacteria, such as *Bacillus* sp., can solubilize phosphorus in the soil and convert it into an available form, thereby enhancing crop production. However, the efficacy of these bacteria often proves to be unstable under field conditions (Afzal *et al*., 2019). N-fixing bacteria are known to be highly sensitive to toxic stress and acidic conditions (Suryatmana *et al*., 2024). Because these N-fixing microbial cultures require protection from such extreme stresses, one method that can be employed is carrier technology, which serves to protect the cells, enhance viability, improve adaptability, and enhance effectiveness when applied in the field under slightly acidic soil conditions with low availability of N and P. Therefore, a carrier material is needed to protect *Bacillus* cells from changes in environmental conditions when applied in the field.

The mature compost contains various essential nutrients needed by plants (Sudrajat *et al*., 2014), so it has the potential to serve as a carrier for N-fixing bacteria because it contains the nutrients needed for microbial activity as well. Larasati *et al*. (2010) reported that compost originates from the decomposition process of organic materials by microorganisms under aerobic and controlled conditions. Compost contains organic materials used as energy sources for microorganisms, making *Rhizobium* bacteria in the soil highly effective (Santi & Goenadi, 2010). Compost and *Azotobacter* sp. can contribute to increasing the availability of N, P, and K, as well as the organic compounds needed by plants (Toago *et al*., 2017). *Azolla* is a potential organic material as an inoculant carrier. *Azolla pinnata* in Indonesia is commonly found in paddy fields with an average ambient temperature of 28–35 °C (Vidhya *et al*., 2014). This carrier material can provide easily degradable protein for bacteria (Datta, 2011). According to Setiawati *et al*. (2019), *Azolla pinnata* has a higher nitrogen content than other carrier materials, thus stimulating the growth of N-fixing bacteria added to it. The aquatic fern *A. pinnata* can fix nitrogen from the air because *Azolla* lives symbiotically with Cyanobacteria *Anabaena azollae*, which possess the enzyme nitrogenase. In this study, these two commonly used carriers (compost and *Azolla*) were tested along with Inceptisol soil. Amount of 33% of all farm land in Indonesia is Inseptisol land that can be used as a potential soil to increase soybean production in Indonesia. However, Inceptisols have low fertility because of acidic pH constraints, and easily leached surfaced layers (Sudirja, 2007).

Moreover, the acidity values (pH) of Inceptisols in Jatinangor are active acidity (pH H_2O) of 5.78 and potential acidity (pH KCl) of 4.44 (Sudirja *et al*., 2017). In this study, *A. pinnata* powder and compost were investigated as an inoculant base carrier to enhance soybean yield by evaluating the interactions between microbial inoculants (*Azotobacter* sp., *Bacillus* sp.) in base carriers (*A. pinata* and compost) combined with NPK fertilizers application on the production outcomes of Detap-1 soybean cultivated in Inceptisol.

MATERIALS AND METHODS

Time and Location

This research was conducted from August 2022 to January 2023 at the Soil Biology Laboratory and Ciparanje Experimental Garden, Department of Soil Science and Land Resources, Faculty of Agriculture, Universitas Padjadjaran. The location of research is a tropical area. During the research, greenhouse conditions were recorded, including air temperature, which fluctuated between 22.6 °C and 27.6 °C. Air humidity was recorded at an average of 91.7%.

Experimental and Treatment Design

The experimental design used was Randomized Complete Block Design (RCBD) with two factorials. The first factor was the application of NPK fertilizer dosage (a) at three levels: a_0 = 0% dose (0 kg/ha), a_1 = 50% dose (75 kg/ha), and a_2 = 100% dose (300 kg/ha). The second factor was the application of a mixture of *Azotobacter* sp. (nitrogen fixing bacteria/NFB) and *Bacillus* sp. (phosphate solubilizing bacteria/PSB) inoculants (b) in carrier materials: b_1 = NFB and PSB in compost carrier, b_2 = NFB and PSB in *A. pinnata* powder carrier, b_3 = NFB and PSB in compost + *A. pinnata* powder carrier, with three replications.

Figure 1. Dry *Azolla pinnata* powder

The inoculum dosage was 50 kg/ha or 25 g/polybag, with the cell density was 10^7 CFU/g carrier. Meanwhile, the NPK fertilizer used was 300 kg/ha (Roswy & Soediarso, 2022) or 1.5 g/polybag (100% recommended dosage) and 150 kg/ha or 0.75 g/polybag (50% recommended dosage). Inoculant application was performed during planting, while NPK was applied twice at planting time and four weeks after planting. The observations included the population density of *Azotobacter* spp. and *Bacillus* spp. that was calculated through serial dilution method of total plate count, number of trifoliate leaves, root dry weigh, number of nodule, weight of 100 grain and pod number per plant.

Experiment Implementation

Application of *Rhizobium* on soybean seeds was performed by soaking the seeds in *Rhizobium* liquid medium that mixed with a 1% gum Arabic solution as an adhesive in a sterile Beaker glass. The treatment was left for 1 hour and then planted in prepared media with one seed per hole. The recommended dosage of *Rhizobium* was 10.5 ml/kg of seed.

Preparation of NFB and PSB Inoculants and Application Method

Bacillus sp. and *Azotobacter* sp. inoculants were obtained from The Soil Biology Laboratory collection at the Faculty of Agriculture, Universitas Padjadjaran. Each inoculant was propagated in a 500 ml Erlenmeyer flask containing Ashby's liquid medium for *Azotobacter* sp. and Pikovskaya's liquid medium for *Bacillus* sp. at a volume of 200 ml. A 5% starter inoculum was used with a cell density of 10^7 CFU/ml and incubated for 72 hours in an incubator at a temperature of 30 °C with shaking at 110 rpm.

Preparing the Carrier Materials

The carrier base materials used were *A. pinnata* powder, compost, and a mixture of both. *A. pinnata* powder was obtained from *A. pinnata* cultivation pond at the Experimental Field in Jatinangor. *A. pinnata* biomass was dried to a moisture content approximately 20%. Subsequently,

it was crushed and sieved to a size of 2 mm. The compost used was obtained from the composting site of animal manure and straw at the Faculty of Animal Husbandry, Universitas Padjadjaran. Each carrier material was placed in heat-resistant plastic for sterilization in an autoclave at a temperature of 121 °C for 20 minutes. Microbial inoculation was then carried out into the carriers, with 10% of each liquid culture of *Azotobacter* and *Bacillus* being added. Additionally, 0.5 g of tapioca powder was added as an adhesive. Incubation was then conducted for seven days to allow *Azotobacter* sp. and *Bacillus* sp. multiplication. The density of *Azotobacter* sp. and *Bacillus* sp. consortia in the carrier was 10^8 CFU/ml. The inoculum dosage used was 25 g/pot. The inoculum application method used was soil dressing, at the time of soybean seed planting and application to the prepared planting holes.

Planting and Fertilization

Detap-1 soybean variety was planted in 10 kg soil in 40 × 40 cm polybag. Soybean seeds that had been soaked in *Rhizobium* culture were planted in the soil by making a hole of 5 cm in the center of the polybag. One seed was placed per polybag. The spacing between polybags was 20 x 20 cm. The fertilizers used in this study were inoculants and NPK Mutiara 16:16:16. Inoculant application was carried out during planting by making a 10 cm planting hole in the polybag next to the soybean seed with a distance of about 10 cm, and then the hole was covered with soil. The inoculant formula applied was 25 g/polybag. Three doses of NPK fertilizer were used, namely the recommended doses of 0%, 50%, and 100% equal to 0.75 g/polybag and 1.5 g/polybag. NPK fertilizer application was done twice, during planting and at 4 weeks after planting. The fertilizer was placed in 10 cm deep hole on 5 cm away from the planting hole, and covered with soil.

Observation and Statistical Analysis

The main observations in the study were growth components: including the number of trifoliate leaves, root dry weigh, root shoot ratio, number of nodule weight of 100 seeds, and pod yield per plant. Observation of the number of trifoliate leaves was conducted by counting the number of trifoliate leaves for each treatment at 2 weeks after planting (WAP), 3 WAP, 4 WAP, 5 WAP, and 6 WAP. Root dry weight calculations were conducted using a destructive method. Observation of nodule count was conducted when the plants entered the late vegetative stage by counting the number of nodules present on the soybean plant roots. Experimental data were analyzed using Statistical Product and Service Solutions (SPSS) version 15.0. Analysis of variance (ANOVA) was performed, and significant differences were assessed at a significance level of 5% ($p < 0.05$).

RESULTS AND DISCUSSION

Initial characteristics of the Inceptisol from Jatinangor are presented in Table 1. Based on the analysis results, the Inceptisols in Jatinangor had a pH of 5.69, which is classified as slightly acidic. Soybean plants thrive best in pH ranges of 5.5–7.0 and grow optimally at pH 6.0–6.5. Besides affecting the growth and development of soybean plants, pH is related to the growth of bacteria used in fertilizers. The population densities of *Azotobacter* spp. and *Bacillus* spp. in the soil were 3.04 x 10^6 and 6.15 x 10^6 CFU/g soil, respectively.

Table 1. Chemical, physical, and biological characteristics of Inceptisol

Parameters	Unit	Result	Criteria
pH : H2O	-	5.69	Slightly acid
pH : KCl 1N	-	4.51	-
C-organic	(%)	1.78	Low
N-total	(%)	0.21	Moderate
C/N	-	9	Low
P_2O_5 HCl 25%	(mg 100/g)	34.58	Moderate
P_2O_5 (Bray)	(ppm P)	4.48	Very low
K_2O HCl 25%	(mg 100/g)	12.58	Low
CEC	(cmol /kg)	37.8	High
Base saturation	(%)	2.35	Very low
Texture:			
Sand	(%)	13.45	
Dust	(%)	43.98	Dusty clay
Klay	(%)	42.56	
Azotobacter spp.	CFU/g soil	3.04 x 10^6	
Bacillus spp.	CFU/g soil	6.15 x 10^6	

Source: Results of Soil Fertility and Plant Nutrition Laboratory Analysis and Soil Microbiology Laboratory, Department of Soil Science, Faculty of Agriculture, Padjadjaran University, 2022. CEC = cation exchange capacity.

Important soil components include organic N at 1.78%, classified as low, total N at 0.21%, classified as moderate, and C/N ratio at 9, classified as low. The

Cation Exchange Capacity (CEC) value in this analysis is 37.8 cmol/kg, categorized as high. The CEC value is related to soil fertility status. Higher CEC values indicate higher soil fertility status. Soil texture analysis results show that sand has a value of 13.45 cmol/kg, silt has a value of 43.98 cmol/kg, and clay has a value of 42.56 cmol/kg, thus categorized as dusty clay.

Population Density of NFB and PSB

Data in Table 2 showed that the inoculation of NFB and PSB increased the population densities of *Azotobacter* and *Bacillus*. In the initial analysis, the Inceptisols in Jatinangor had densities of *Azotobacter* spp. and *Bacillus* spp. of 3.04 x 10^6 CFU/g soil and 6.15 x 10^6 CFU/g soil, respectively. The density of bacteria was not significantly different between treatments.

The density of *Azotobacter* spp. and *Bacillus* spp. had not significant value between treatments. But, a1b0, a2b0, a0b2a2b2, and a2b3 treatments tented to show the higher density of *Azotobacter* compared with other treatments, while the highest density of *Bacillus* populations was found in treatment a2b2 with a value of 8.42 x 10^9 CFU/g. The high or low density of bacterial populations in the soil can also be influenced by soil pH. According to Holt *et al.* (1994), *Azotobacter* can grow in pH ranges of 4.5–8.5, with the optimum pH for nitrogen fixation being 7.0–7.5. Meanwhile, according to Chen *et al.* (2020), the optimum pH for *Bacillus* sp. is 5–9. This indicated that *A. pinnata* powder and compost provided sufficient nutrition for the growth of *Azotobacter* spp. and *Bacillus* spp., as well as improved soil aeration and drainage to an optimum level. Microorganisms can thrive maximally under conditions of adequate aeration and drainage, and *A. pinnata* powder as a carrier enhanced the population of *Bacillus* spp. Meanwhile, *A. pinnata* powder and compost increased the population of *Azotobacter* spp. and *Bacillus* spp., reaching 1000 multiples of the population from 10^6 CFU/g soil to 10^9 CFU/g soil. This proved that compost and *A. pinnata* powder had a significant role in enhancing the viability of *Azotobacter* and *Bacillus*. This also occurred because compost served as a microhabitat for the inoculants, protecting them from environmental conditions. Inoculation treatments based on *A. pinnata* powder or compost carriers tended to increase in the population of *Azotobacter* spp. The function of the carrier is to provide the environment for microbes to sustain life and improve their performance when applied. It is reported elsewhere that the main role of the carrier is to provide a suitable chemical and physical environment, protect against the rapid decrease of cells during storage, and better adapt with native microflora in the carrier.

Table 2. Population density of *Azotobacter* spp. and *Bacillus* spp. at 12 WAP

Treatment		Population density (10^9 CFU/g soil)	
		Azotobacter spp.	*Bacillus* spp.
a_0b_0	NPK 0% + NFB and PSB liquid culture	4.60	5.00
a_1b_0	NPK 50% + NFB and PSB liquid culture	9.90	7.80
a_2b_0	NPK 100% + NFB and PSB liquid culture	9.77	4.96
a_0b_1	NPK 0% + NFB and PSB in compost carrier	7.60	7.58
a_1b_1	NPK 50% + NFB and PSB in compost carrier	5.10	4.31
a_2b_1	NPK 100% + NFB and PSB in compost carrier	6.05	7.61
a_0b_2	NPK 0% + NFB and PSB in *A. pinnata* powder carrier	9.25	4.35
a_1b_2	NPK 50% + NFB and PSB in *A. pinnata* powder carrier	8.85	4.98
a_2b_2	NPK 100% + NFB and PSB in *A. pinnata* powder carrier	9.30	8.42
a_0b_3	NPK 0% + NFB and PSB in compost + *A. pinnata* powder	7.35	7.20
a_1b_3	NPK 50% + NFB and PSB in compost + *A. pinnata* powder	8.30	6.93
a_2b_3	NPK 100% NFB and PSB in compost + *A. pinnata* powder	9.35	4.16

Note: CFU = *colony forming unit*, NFB = Nitrogen fixing bacteria, PSB = Phosphate solubilizing Bacteria. WAP = weeks after planting.

Number of Trifoliate Leaves

Based on the analysis of variance, there was no interaction effect between the NPK fertilizer dosage and the carrier of inoculant formula (Table 3). The independent effects of each inoculant treatment on the number of trifoliate leaves are presented in Table 3. In soybeans aged 2–3 WAP, it was found that the NPK fertilizer dosage and inoculant formula in the

carrier did not have a significant effect on the trifoliate leaves of Detap-1 soybeans. However, the NPK fertilizer treatment alone at 4, 5, and 6 WAP showed a significant effect in increasing the number of trifoliate leaves. The NPK fertilizer treatment at a dosage of 100% (300 kg/ha) resulted in higher yields compared to the NPK fertilizer treatments at dosages of 50% (150 kg/ha) and 0% (control treatment). This proved that the sufficient availability of N, P and K nutrients leads to increased plant growth (Murdaningsih & Kramat, 2014). Additionally, the application of 100% NPK fertilizer provided the best results for the number of Detap-1 soybean leaves because sufficient nitrogen supply can increase leaf numbers and enhance chlorophyll formation. The chlorophyll content of plants is closely associated with their nitrogen levels, resulting in darker green leaves (Fathi, 2022). The condition of the soybean plant is presented in Figure 2.

The inoculant treatments in compost and *A. pinnata* powder carriers did not show a significant difference in the number of trifoliate leaves, but they showed a tendency to be higher in the *A. pinnata* powder carrier and the mixture of *A. pinnata* powder and compost, at 21.56 and 20.89 leaves, respectively. This is attributed to the use of carrier bases that provide optimal nutrients and habitats, which can enhance fixation activity and phytohormone production. However, in this study, the increase in trifoliate leaf number was not significant. These results warrant further investigation, as it is possible that the nitrogen fixation activity and phosphate provision by the inoculant did not function optimally in acid soil.

Table 3. Effects of NPK fertilizer dosage and inoculant on the number of trifoliate leaves of Detap-1 soybean

Treatments	Number of trifoliate leaves				
	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
NPK dosage (a)					
a_0 (0%)	1.08	2.63	4.75a	9.79a	14.42a
a_1 (50%)	1.13	2.71	5.88b	11.83b	20.17b
a_2 (100%)	1.21	2.92	6.96c	14.25c	23.67c
NFB+PSB inoculant in carrier (b)					
b_0 (liquid culture)	0.89	2.33	5.22	10.89	17.67
b_1 (in compost)	1.11	2.44	6.00	11.00	16.00
b_2 (in *A. pinnata* powder)	1.22	2.89	6.22	13.33	21.56
b_3 (in compost + *A. pinnata* powder)	1.00	2.89	6.78	14.00	20.89

Note: Mean values followed by the same letter are not significantly different based on Duncan's multiple range test at the 5% significance level. WAP = weeks after planting.

Figure 2. Conditions of experimental soybean plants

Root Dry Weight

Root dry weight was measured at the late vegetative stage of soybean plants when the plants showed signs of flower buds. The analysis of variance indicated that there was an interaction effect between the NPK fertilizer dosage and the inoculant formula on root dry weight (Table 4). Application of 100% NPK with solid inoculant in mixture carrier resulted in highest root dry weight of soybean up to 6.61 g. This is because *A. pinnata* powder has a relatively higher nitrogen content compared to compost to enhance the activity of *Azotobacter* and *Bacillus* in the soil. These functional microorganisms play an important role in increasing the availability of nitrogen for plant roots. Meanwhile, compost is a decomposed organic material that improves the chemical properties and soil structure for improving the inoculant activity (Widodo & Kusuma, 2018), and serves as a habitat for bacteria to thrive in the soil. Therefore, the mixture of *A. pinnata* powder and compost is the best inoculant carrier material for enhancing root growth.

Table 4. Interaction effect of NPK fertilizer dosage and inoculant on root dry weight of soybeans at 7 WAP

Dry weight of roots			
NFB+PSB inoculant in carrier (b)	NPK dosage (a)		
	a_0 (0%)	a_1 (50%)	a_2 (100%)
b_0 (liquid culture)	2.75 a	1.87 a	4.54 a
	A	A	BC
b_1 (compost)	2.8 a	3.04 a	5.13 b
	A	AB	C
b_2 (*A. pinnata* powder)	2.69 a	3.23 a	4.33 a
	A	AB	BC
b_3 (compost + *A. pinnata* powder)	2.22 a	5.10 b	6.61 c
	A	C	D

Note: Mean values followed by the same letter do not differ significantly based on Duncan's multiple range test at the 5% significance level. Lowercase letters are read horizontally, while uppercase letters are read vertically. WAP = weeks after planting.

Nitrogen is an essential element in protein formation and influences root cell growth (Roswy & Sudiarso, 2022). Plants can develop larger and stronger root systems with sufficient nitrogen supply. Phosphorus affects root development by stimulating the formation of new root tissues, expanding the root system, and increasing nutrient uptake by the roots. Soybean plants treated with 50% recommended NPK fertilizer dosage (a_1) with inoculant in liquid culture (b0) resulted in low root dry weight. The phenomenon of low root dry weight is caused by the inoculant applied in liquid culture form without a carrier resulting less material protector for viability of inoculant in acid soil, resulting in low functional activity of inoculant. Due to the low functional activity of the inoculant, its contribution to providing N and P is also low, and there was even a tendency for competition between the inoculant and the plant in the use of N and P in the soil.

Number of Root Nodules

Based on the analysis of variance, the number of root nodules shows an interaction effect between the NPK fertilizer dosage and the inoculant formula in the carrier is shown in Table 5. Treatment a2b3 (100% recommended NPK dosage with inoculant formula in a carrier mixture of compost and *A. pinnata* powder resulted in an interaction effect and significantly increased the highest number of nodules, amounting to 94.67 compared to other treatments. This indicated that the application of the inoculant formula in a mixed carrier was the most effective treatment in enhancing the formation of root nodules. *A. pinnata* powder and compost carriers were proven to increase the viability and effectiveness of nitrogen-fixing and phosphate-solubilizing bacteria. According to Widawati *et al.* (2015), the contributions of *Azolla* and compost respectively contribute 1.33% and 30.08% of nitrogen, resulting supplying sufficient nitrogen for root development and nodule formation.

Table 5. Interaction effect of NPK fertilizer dosage and inoculant on the number of root nodules of soybeans at 7 WAP

Number of nodules			
NFB+PSB inoculant in carrier (b)	NPK dosage (a)		
	a_0 (0%)	a_1 (50%)	a_2 (100%)
b_0 (liquid culture)	42.00 a	48.67 a	83.33 a
	A	A	CD
b_1 (compost)	47.67 a	61.00 b	69.00 a
	A	AB	BC
b_2 (*A. pinnata* powder)	38.00 a	59.67 a	86.00 a
	A	AB	CD
b_3 (compost + *A. pinnata* powder)	51.33 a	50.33 a	94.67 b
	A	A	D

Note: Mean values followed by the same letter do not differ significantly based on Duncan's multiple range test at the 5% significance level. Lowercase letters are read horizontally, while uppercase letters are read vertically. WAP = weeks after planting.

Pod Number Per Plant

Based on the analysis of variance, the pod yield per plant showed no interaction between the NPK fertilizer dosage and the inoculant formula in the carrier regarding the pod yield per plant. The results of the Duncan multiple range test for the independent effect of each treatment on the pod yield per plant are presented in Table 6. The NPK fertilizer dosage and inoculant formula was independently significantly influenced the number of pods per plant. The results indicated that applying NPK fertilizer at 50% and 100% of the recommended dosage significantly caused higher pod number compared to the control treatment. Meanwhile, the inoculant treatments with *A. pinnata* powder or compost carriers alone did not increase the number of pods. Nevertheless, the inoculation of liquid cultures contained both bacteria or solid inoculant in *A. pinnata* powder produced pod yields a number equivalent to NPK application at 50% of the recommended dosage.

Table 6. Independent effect of NPK fertilizer dosage and inoculant on pod yield per plant of Detap-1 soybeans at 12 WAP

Treatments	Pod number/plant
NPK dosage (a):	
a_0 (0%)	29.9583 a
a_1 (50%)	45.7083 b
a_2 (100%)	56.7083 c
NFB+PSB inoculant in carrier (b):	
b_0 (liquid culture)	47.5556
b_1 (compost)	39.1111
b_2 (*A. pinnata* powder)	47.1111
b_3 (compost + *A. pinnata* powder)	44.8889

Note: Mean values followed by the same letter do not significantly differ based on Duncan's multiple range test at the 5% significance level. Mean values not followed by any letter did not undergo further testing because the treatment factor did not significantly influence the response based on the analysis of variance at the 5% significance level. WAP = weeks after planting.

The Weight of 100 Grain

Based on the analysis of variance, that there was no interaction effect between the NPK fertilizer dosage and the inoculant formula in the carrier on 100-grain weight, but both treatment independently influences-increased the weight of grain. The results of Duncan's multiple range test on the single effect of each treatment on the weight of 100 grain are presented in Table 7. The recommended 100% and 0% NPK dosages showed significantly differ from the 50% NPK dosage. The recommended dosage of NPK significantly increased the average weight of 100 grain was 17.7138 g compared to the 0% and 50% NPK dosages, which were 16.75 g and 15.38 g, respectively. The treatment of inoculant in carrier produced potentially higher weight compared to the

liquid, culture, although statistically not significant. According to Kementan (2023), the weight of 100 grain of Detap-1 soybean is 15.37 g but in this research revealed that the weight of 100 grain higher than previously reported. Inoculant in *A. pinnata* powder and mixed carrier application resulted weight of 100 grain equivalent to the 100% NPK application recommendation. The BPN and BPF inoculants stimulated by the presence of *A. pinnata* powder and compost as stimulants and nutrient providers to support the growth and activity of the inoculants. The activity of BPN and BPF contributes to supplying N, P, and phytohormones for plants. As reported by Sukmasari *et al*. (2021) and Nazirah (2019), BPF and BPN increase N and P levels in the soil and contribute to increasing soybean growth and yield.

Table 7. Independent effects of NPK fertilizer dosage and inoculant on the weight of 100 grain of Detap-1 soybeans at 12 WAP

Treatments	Weight of 100 grain (g)
NPK dosage (a)	
a_0 (0%)	16.7542 b
a_1 (50%)	15.3788 a
a_2 (100%)	17.7138 b
NFB+PSB inoculant in carrier (b):	
b_0 (liquid culture)	16.1522 b
b_1 (compost)	17.4278 b
b_2 (*A. pinnata* powder)	17.7500 b
b_3 (compost + *A. pinnata* powder)	17.7811 b

Note: Average values followed by the same letter do not significantly differ according to the Duncan multiple range test at the 5% significance level. WAP = weeks after planting.

CONCLUSION

Application of liquid inoculant and inoculant in *A. pinnata* powder and compost as carrier bases increased the populations of *Azotobacter* spp. and *Bacillus* spp. in the rhizosphere compared to the density of soil indigenous bacteria before treatment. The bacteria densities increased application of 1000 times compared to the initial soil population. The inoculant formula in the mixed carrier (compost and *A. pinnata* powder) resulted in highest dry root weights and root nodule counts compared to other treatments. The combination of *A. pinnata* powder and compost was the best carrier material in promoting root growth. Inoculation in liquid cultures of *Azotobacter* sp. and *Bacillus* sp., as well as inoculants in *A. pinnata* powder carrier, yielded the pod number of soybean equivalent to those of the 50% recommended NPK dosage application. Meanwhile, application of liquid culture, inoculants in *A. pinnata* powder carrier, compost, or mixed of both produced the weight of 100 grain were not significantly different. However, application inoculant in *A. pinnata* powder and mixed carrier quantitatively resulted the weight of 100 grain of soybean equivalent to treatments using 100% recommended NPK dosage. This research proved that *A. pinnata* powder material and mixed of both are a potential carrier in enhancing the effectiveness of inoculants, increasing the viability and activity of *Azotobacter* sp. and *Bacillus* sp. inoculants. Further research is needed using a mixture of carrier base materials with a more diverse composition. This diversity will allow each component to better protect and support the viability and activity of the inoculant.

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