Vol. 12, No. 4, pp. 897-906, November 2024 DOI: http://dx.doi.org/10.23960/jat.v12i4.8395

Jurnal Agrotek Tropika

P-ISSN: 2337-4993
E-ISSN: 2620-3138

Journal homepage: https://jurnal.fp.unila.ac.id/index.php/JA

APLIKASI AZOTOBACTER DAN PUPUK KIMIA UNTUK MEMPERBAIKI HASIL KACANG TANAH PADA INCEPTISOLS

APPLICATION OF AZOTOBACTER AND CHEMICAL FERTILIZER FOR IMPROVING GROUNDNUT YIELD IN INCEPTISOLS

Reginawanti Hindersah[1*], Agung Muhammad Yusuf[2], Dewikusuma Ikhsani Adhiningtyas[2], Diyan Herdiyantoro, Oviyanti Mulyani[1], Pujawati Suryatmana[1]

[1] Department of Soil Science, Faculty of Agriculture, Padjadjaran University, Indonesia
[2] Department of Agrotechnology, Faculty of Agriculture, Padjadjaran University, Indonesia
*Corresponding Author. E-mail address: reginawanti@unpad.ac.id

ARTICLE HISTORY:
Received: 31 December 2023
Peer Review: 03 April 2024
Accepted: 08 June 2024

KEYWORDS:
Azotobacter, nitrogen, nodulation, yield

ABSTRACT

The overuse of chemical fertilizer is posing environmental concerns and economic burdens, showing the need for sustainable alternatives. Azotobacter chroococcum (AC), a nitrogen-fixing bacterium, offers a promising strategy to enhance soil fertility and reduce chemical fertilizer dependency. Therefore, this study aimed to evaluate the effects of AC liquid inoculant combined with reduced doses of NPK fertilizer on nitrogen availability, uptake, nodulation, growth, and yield of groundnut cultivated in Inceptisols. A field experiment was conducted using a Randomized Block Design (RBD) with eight treatment combinations of AC (2 and 3 L ha^{-1}) and NPK fertilizer (75, 150, and 300 kg ha^{-1}). Duncan's Multiple Range Test (DMRT) was applied to assess treatment significance. The results showed that the combination of AC 2 L ha^{-1} + NPK 300 kg ha^{-1} increased soil NO_3^- levels by 37–53% compared to individual treatments. Although plant height was unaffected, this combination significantly enhanced nitrogen uptake (up to 169 mg $plant^{-1}$). The highest shoot dry weight (44.27 g) was observed with AC 2 L ha^{-1} + NPK 75 kg ha^{-1}. Specifically, the combination of AC 2 L ha^{-1} + NPK 150 kg ha^{-1} produced the highest nodule dry weight (0.49 g) and yield (2.62 kg $plot^{-1}$), outperforming the full NPK dose (300 kg ha^{-1}). This study shows that inoculation with AC can enhance groundnut yield by approximately 40% while enabling a 50% reduction in NPK fertilizer use to support more sustainable and cost-effective groundnut cultivation in Inceptisols.

KATA KUNCI:
Azotobacter, hasil, nitrogen, nodulasi

ABSTRAK

Inokulasi Azotobacter pemfiksasi nitrogen adalah pendekatan yang penting dan berkelanjutan untuk mengurangi dosis pupuk kimia. Percobaan lapangan ini bertujuan untuk menganalisis ketersediaan N, serapan N, nodulasi. serta pertumbuhan dan hasil kacang tanah yang ditanam dengan inokulan cair *A. chroococcum* (AC) dan pengurangan dosis pupuk NPK. Percobaan disusun dalam Rancangan Acak Kelompok untuk menguji delapan kombinasi perlakuan dosis inokulan AC (2 dan 3 L ha^{-1}) dan dosis pupuk NPK (75, 150 dan 300 kg ha^{-1}). Uji Jarak Berganda Duncan digunakan sebagai uji lanjut jika perlakuan mempengaruhi parameter yang diukur. Kadar NO_3^- tanah yang diberi AC 2 L/ha + NPK 300 kg ha^{-1} adalah 37% dan 53% lebih banyak daripada NO_3^- di tanah dengan Azotobacter dan NPK saja. Pemberian Azotobacter dan NPK tidak mempengaruhi tinggi tanaman tetapi AC 2 L ha^{-1} + NPK 300 kg ha^{-1} meningkatkan serapan N sampai 169 mg per tanaman. Bobot kering tajuk tertinggi yaitu 44.27 g diperlihatkan oleh tanaman yang diberi AC 2L ha^1 dan NPK 75 kg ha^{-1}. Namun, Bobot kering nodula dan hasil tertinggi masing-masing sebesar 0.49 g dan 2.62 kg/plot diperoleh dari tanaman dengan perlakuan AC 2L ha^{-1} + NPK 150 kg ha^{-1}. Inokulasi *A. chroococcum* disertai NPK 150 kg/ha meningkatkan hasil kacang tanah di Inceptisols sampai 40 % dibandingkan dengan pemberian NPK 300 kg ha^{-1} yang memperlihatkan kemampuan Azotobacter untuk menurunkan dosis pupuk NPK.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an export-import commodity and an important legume widely consumed as a source of protein. The national production of groundnut is 510,000 t of dry seeds per year, while the consumption reaches 658,000 t per year. According to Macro Statistic for Agricultural Sector (Komalasari, 2022), volume of processed groundnut importation to Indonesia in 2022 decreased to approximately 259,537 t from 318,177 t in 2018, while fresh groundnut was only 1.69 t compared to 14.17 t. Indonesia exported 5.44 t of fresh and processed groundnut in 2018, and the volume decreased by 2.67 t in 2022, showing the need for an increase in productivity. Despite the importance of groundnut for the food industry in the country, the average productivity in most agricultural land was less than 2 t/ha of pods, which is lower than the genetic potential. The Ministry of Agriculture stated that the yield of groundnut cultivars Kerinci and Tuban was 2.3 t ha^{-1} and 3.2 t ha^{-1} of dry pod, respectively.

The challenge in increasing productivity in the tropics is low nitrogen (N) and phosphorus (P) availability in soil, including Inceptisols (Yuniarti *et al*., 2019; Muslim *et al*., 2020). Therefore, most farmers use inorganic fertilizer to fulfill the needs of both major essential nutrients for maintaining plant yield. Environmental issues cause agricultural inputs to shift from inorganic fertilizer to organic and biofertilizers. Improper use of N fertilizer causes leaching and ammonia volatilization due to high rainfall and temperature in the tropics (Liu *et al*., 2020; Dari *et al*., 2021). Adsorption of P by aluminum and iron oxides in soil also limits the supply of P for plant uptake (Hanyabui *et al*., 2020).

Due to the nutrient and environmental limitations, biological methods such as legume-rhizobia symbiosis offer a promising alternative. Legume forms a symbiotic interaction in which the rhizobia in the root nodules fix N_2 to ammonia, catalyzed by nitrogenase. However, this enzymatic process will be inhibited by NH_3/NH_4^+ in nitrogenase (Einslea Reesb, 2020), showing the need for optimizing the nodule formation and N fixation to reduce inorganic fertilizer doses and costs. High doses of N fertilizer possibly inhibit root nodules formation and legume production (Herliana *et al*., 2019). Therefore, relying on N_2-fixer bacteria to provide N is suggested for groundnut cultivation in low-N soil. Inoculation of symbiotic N_2-fixer rhizobia increases macronutrient uptake by groundnut, as well as its grain yield and nutrient content (Naabe Yaro *et al*., 2021; Bitire *et al*., 2023).

In legume cultivation, adequate input of P is needed to provide the energy during N_2 fixation. This process requires a significant amount of chemical energy as adenosine triphosphate (ATP), since P deficiency in soil can reduce nitrogenase activity and nifH gene abundance (Qiu *et al*., 2023). Non-symbiotic N_2-fixer bacteria such as Azotobacter are able to provide inorganic P through phosphate solubilization (Nosrati *et al*., 2014). Despite the low abundance of Azotobacter (0.06%) in the rhizosphere microbiome (Hussen *et al*., 2020), the inoculation during legume cultivation is needed to increase population.

Azotobacter produces the common plant regulators indole-3-acetic acid (IAA), a species of auxin (Kerečki *et al*., 2022; Hindersah *et al*., 2020a) that has a significant role in the growth and the gravitropic movement of groundnut gynophore. The auxin signal is increased during peg elongation and gravitropic bending, which serves as lateral transport from lower to upper cortex and facilitates bending of groundnut peg (Kumar *et al*., 2019).

For more than four decades, the potential of Azotobacter to increase soybean nodulation has been reported (Thomas *et al*., 1981). Previous studies have shown that the formation and function of the legume–rhizobia symbiosis in soybean is improved by inoculating N_2-fixing Azotobacter or Azosprillum strains (Hindersah *et al*., 2019; Armendariz *et al*., 2019). For example, a pot experiment found that mixed inoculation of *B. japonicum* and Azotobacter led to the increment of weight of root nodules as well as the height and weight of soybean dry shoots and grains (Hindersah *et al*., 2019; Hindersah *et al*., 2020b).

Despite the numerous reports, the studies concerning Azotobacter inoculation to reduce chemical fertilizer in groundnut are limited. Exploring chemical fertilizer rate following Azotobacter inoculation is essential for sustainable agriculture. Therefore, this study was conducted to observe the ability of N-fixer AC in reducing the doses of NPK (15:15:15) in groundnut cultivation. The specific objective was to analyze N availability in soil, uptake by shoot, nodulation, as well as groundnut growth and yield after application of AC liquid inoculant and reduced doses of NPK compound fertilizer.

2. MATERIALS AND METHODS

2.1 Experimental Site and Soil Properties

This study was carried out at the experimental field of the Indonesian Fertilizer Company at Cikampek District, Karawang Regency, West Java. The field was located in a tropical area at an altitude of 25 m above sea level. The seeds of groundnut cv Tuban were grown on clay Inceptisol soil (17% sand, 37% silt, and 46% clay), with daily temperature and humidity in the range of 22°C–33°C and 62-89%, respectively.

The soil of the study area was collected with the composite method from 20-cm deep soil by using Auger, cleaned of debris, and subjected to chemical, physical, and biological analysis. The chemical analysis referred to Eviati and Sulaeman (2012). Texture determination was conducted using the Pipette Method, revisited by Mwendwa (2022). Meanwhile, Bradyrhizobium and Azotobacter were counted with the Serial Dilution Plate Method on N-free Yeast mannitol and Ashby Mannitol plate, respectively (Hungria *et al*., 2020; Hindersah *et al*., 2021).

Based on the test conducted, soil showed neutral acidity (pH 7.1), low organic C (1.6%), high total N (0.6%), low available P (12.07 ppm), very high potential P_2O_5 (64.34 mg 100 g^{-1}), and very low potential K_2O (3.86 mg 100 g^{-1}). The cation exchange capacity and base saturation of soil were average (23.49 me 100 g^{-1}) and very high (73.76%), respectively. Before the experiment, soil contained 2.41 x 10^7 CFU g^{-1} *Bradyrhizobium* spp. and 4.8 x 10^5 CFU g^{-1} *Azotobacter* spp. The experimental plots were amended with cow manure (Organic C of 24.9%, total N of 1.06%, C/N of 23, pH of 8.1) produced by Indonesia Fertilizer Company.

2.2 Experimental Design

The experiment was conducted using Randomized Block Design with four replications (Limbongan, 2021). A total of eight treatment combinations of AC liquid inoculant and combined NPK fertilizer were tested. The treatments were as follows (A) AC of 2 L/ha without NPK (Control), (B) Recommended dose of NPK compound fertilizer (NPK 300 kg/ha), (C) AC 2 L/ha, NPK 300 kg ha^{-1}, (D) AC 2 L/ha, NPK 150 kg ha^{-1}, (E) AC 2 L/ha, NPK 75 kg ha^{-1}, (F) AC 3 L ha, NPK 300 kg ha^{-1}, (G) AC 3 L/ha, NPK 150 kg ha^{-1}, (H) AC 3 L/ha, NPK 75 kg ha^{-1}.

The control treatments were the recommended dose of AC and NPK Phonska fertilizer (15:15:15). In this study, all groundnut seeds were treated with liquid inoculant of *Bradyrhizobium* sp. before sowing. Both inoculants with cell concentration of 10^8 CFU mL^{-1} were developed by Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran.

2.3 Experimental Establishment

A total of 32 experimental plots measuring 2 m x 3 m with a 50 cm distance between plots were made on the field. A continuous drainage trench of 50 cm wide and 30 cm deep was constructed around the experimental plot, with orientation from west to east. Land clearing was performed two days before soil tillage by removing all bushes, grass, sedges, and border leaves. Soil tillage was

conducted with a hoe as deep as 20 cm, and paraquat herbicide was sprayed on the soil surface with a concentration of 2.5 mL L^{-1}.

The A, C, D, and E plots received 1.50 mL single-strain Azotobacter liquid inoculant in the final (equivalent to 2 L ha_{-1}), while the F, G, and H plots were treated with 2.25 mL/plot (equivalent to 3 L/ha). The bacterial inoculant was diluted with 100 mL of distilled water, mixed with 3.75 kg of manure, and incubated for 3 hours before mixing with the topsoil of each plot. Groundnut seeds were sown seven days after manure application. Before sowing, seeds were soaked in a liquid inoculant of *Bradyrhizobium* spp. mixed with 1% of Arabic gum for 30 s. The 2 cm-deep holes with a distance of 40 cm between and 15 cm within rows were created by plugging the wooden stick in. A single seed was put in the holes and covered with the soil.

In all treatments, NPK fertilizer was applied in split, one week (50%) and three weeks after sowing (50%). This fertilizer was placed in the continuous band at the bottom of a 3-cm deep furrow at 5 cm from the stem and covered with soil. Manual weeding was conducted two times at 15 and 45 days after sowing when the gynophore entered the soil. The benomyl fungicide was sprayed at a concentration of 1 g L^{-1} to control the leaf spot diseases (*Cercospsora arachidicola*) that attacked at 30 days after sowing.

2.4 Parameters and Statistical Analysis

A total of six sampling plants were set in three center rows in a plot by the zig-zag method, each containing two plants. The border rows were not included in sampling plants determination. The soil samples were collected from the 15-cm depth, where the lateral roots were grown intensively, and kept at room temperature before available N analysis. Soil for Azotobacter enumeration was taken from rhizosphere of sampling plants, put in sealed plastic bag, and stored at 4^0C before the total Azotobacter count. Plant height, Ammonium (NH_4^+), and nitrate (NO_3^-), N uptake by shoot, root dry weight, number, and dry weight of root nodules were measured from the six sampling plants, and the average data were calculated. Subsequently, Azotobacter counts were measured at the late vegetative stage at day 25. The available N in soil and total N content in shoots were analyzed with the Kjeldahl method (Eviati and Sulaeman, 2019). The shoots were heated for 48h at 70^0C until constant weight to obtain the dry weight (Eviati and Sulaeman, 2019). N uptake of shoots was calculated by multiplying the dry weight and the total N content of the shoot. The dry weight of roots and nodules per plant was determined by the gravimetric method after heating at 70 ^{o}C for 48 h (Eviati and Sulaeman, 2019). Furthermore, Azotobacter population in the rhizosphere was counted by the serial dilution plate method in N-free Ashby media (Hindersah *et al*., 2021).

At harvest time, the number and weight of filled pods in all plots were measured. The plant productivity in a hectare was calculated based on pods weight in a plot. All data were subjected to analysis of variance at $p \leq 0.05$ to determine the significance of the treatment effect on parameters. When the analysis of variance had a significant effect on the parameter, Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ was performed. Data processing was performed using SPSS 17.0 software.

3. RESULTS AND DISCUSSION

3.1 Available N

Soil that received 2 L ha^{-1} of AC combined with 150 kg ha^{-1} of fertilizer and control treatment had higher NH_4^+ compared to others (Figure 1a). However, the highest levels of NO_3^- were found in soil that received 2 L ha^{-1} AC with 300 kg ha^{-1} fertilizer, which was 37% and 53% higher than the control of AC and NPK, respectively (Figure 1b). In this study, the application of 2 L ha^{-1} AC combined with 150 kg/ha NPK produced the highest N uptake in the shoot of an individual plant, which was

approximately 19.1–44.4% more than other treatments (Figure 1c). At the end of the vegetative stage of plant growth, the population of Azotobacter in the rhizosphere varied depending on the dose of biofertilizer and inorganic fertilizer. Application of 2 L/ha of liquid inoculant with 150 kg/ha of fertilizer clearly increased bacterial count in the rhizosphere close to 1.2 logs compared to the control (Figure 2d). In comparison, inorganic fertilizer amendment without biofertilizer had the lowest Azotobacter population.

The change of NH_4^+ and NO_3^- content in soil did not show a specific pattern related to fertilizer treatments. Statistically, the application of Azotobacter without NPK produced the same NH_4^+ and NO_3^- content as most Azotobacter-NPK combination treatments. This showed that in soil with high N (0.6% before the experiment), NPK fertilizer was considered not a dominant factor in increasing the N availability. It also showed that the NO_3^- content in soil with Azotobacter and NPK was similar. After the experiment, the NH_4^+ in all soil was less than 2 mg kg^{-1}, which was low according to the Indonesian classification. In Figure 2b, the NO_3^- content of 5.25 mg L^{-1} was shown by soil with AC 2 L ha^{-1} combined with NPK 300 kg ha^{-1} (C), showing medium-high (6-10 mg kg^{-1}) based on classification.

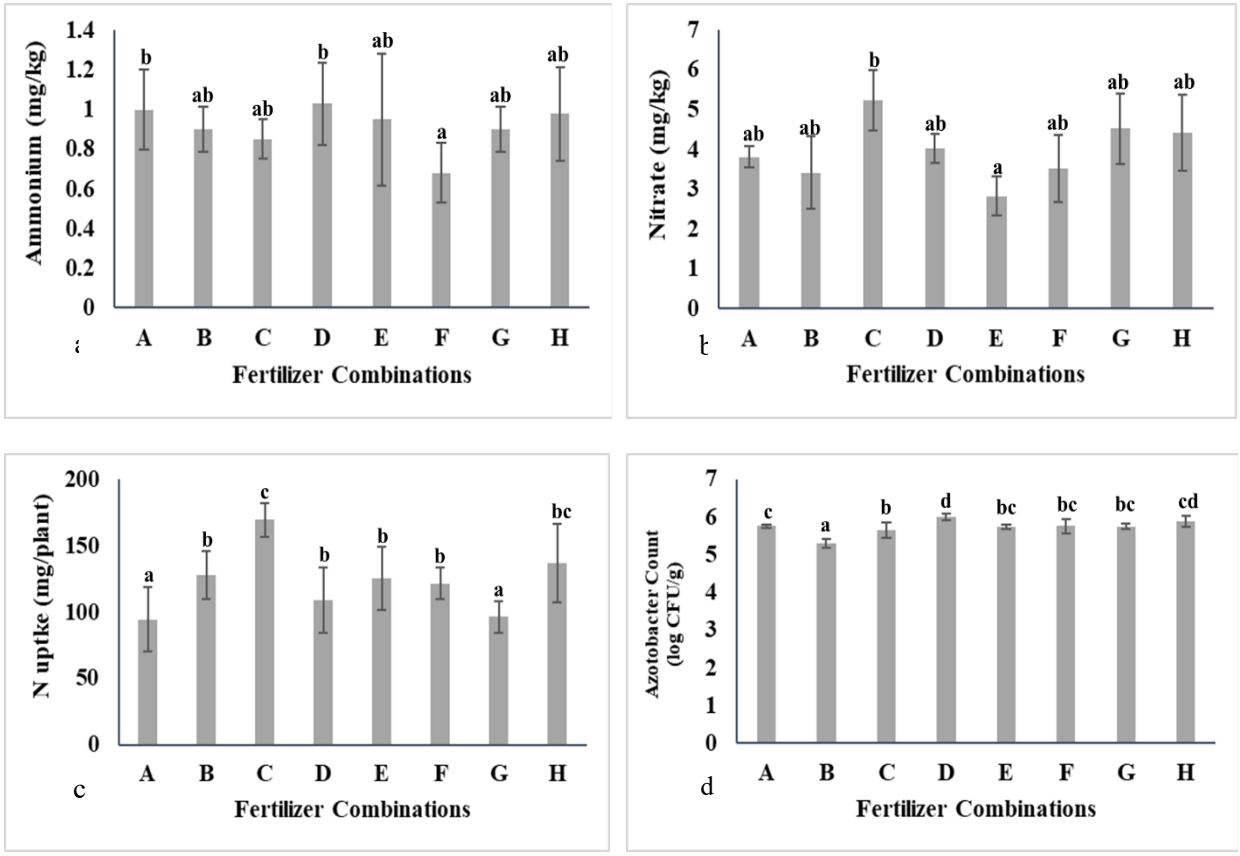

Figure 1. Effect of AC inoculation combined with NPK compound fertilizer on NH_4^+ (a) and NO_3^-; (b) in soil, N uptake by shoot; (c) and Azotobacter population in the rhizosphere of groundnut at 25 days after sowing; (d). A: *A. chroococcum* (AC) 2 L ha^{-1}), B: NPK 300 kg ha^{-1}, C: AC 2 L ha^{-1} + NPK 300 kg ha^{-1}, D: AC 2 L ha^{-1} + NPK 150 kg ha^{-1}, E: 2 L ha^{-1} + NPK 75 kg ha^{-1}, F: AC 3 L ha^{-1} + NPK 300 kg ha^{-1}, G: AC 3 L ha^{-1} + NPK 150 kg ha^{-1}, H: AC 3 L ha^{-1} + NPK 75 kg ha^{-1}. Graphs followed by the same letters were not significantly different based on DMRT with $p \leq 0.05$.

Since N dissolves in water and is highly mobile in soil, leaching might take place and reduce the available content. This shows that excessive NPK fertilization in high N soil can be inefficient, as surplus N is not effectively absorbed by plants and is leached out of soil through irrigation water and

rain (Sebilo *et al*., 2013). Moreover, during the dry season when the day temperature reaches 33^0C, volatilization can occur, contributing to N losses (Jadon *et al*., 2018).

N fixation in the early vegetative growth is inhibited by the abundance of content in soil since nitrogenase activity is reversibly susceptible to high N. Therefore, in early growth, plants adsorb ionic N from soil and fertilizer compared to fixation. Available N released from NPK is uptake by plants as well as used by the bacteria. The application of fertilizer generally provides nutrients for plant growth and microbial proliferation. In this study, Azotobacter inoculant of 3L/ha is considered to increase the population of Azotobacter more than the lower dose (2 L ha^{-1}) due to NPK provision. However, the increased population of nitrogen-fixing microbes tends to use the available N for metabolism, leading to a temporary suppression of nitrogenase activity. As soil N becomes depleted, the conditions gradually favor the activation of N fixation by Azotobacter, alongside the initiation of effective nodule formation.

3.2 Groundnut Nodulation

A combination of Azotobacter inoculation and NPK fertilizer increased the nodule number at the late vegetative stage of groundnut compared to fertilizer only. However, plants receiving 2 L ha^{-1} AC combined with 150 kg/ha and 3L ha^{-1} AC with 75 kg/ha NPK, had a higher number and weight of nodules compared to those treated with 2 L ha^{-1} of AC and 300 kg ha^{-1} of NPK fertilizer (Table 1). The use of 2 L ha^{-1} AC combined with 150 kg/ha NPK fertilizer produced a higher nodule dry weight of 0.49 g.

The experiment confirmed that the highest NPK fertilizer dose reduced nodulation due to repression of nitrogen fixation by elevated soil nitrogen levels. Azotobacter inoculation had a positive effect on nodulation, although a high dose of NPK was applied. The NPK provided nutrients for exogenous Azotobacter for population increment (Figure 1). Moreover, P from NPK contributed to chemical energy formation, serving as an energy source for to root system where nodulation occurred (Asante *et al*., 2020). Meanwhile, Azotobacter can provide available P through phosphate solubilization activity as reported for AC and *A. vinelandii* strains (Nosrati *et al*., 2014). The nodulation traits might reflect the increment of rhizobia proliferations and nitrogen fixation that requires a significant amount of chemical energy.

3.3 Plant Growth and Yield

A combination of various doses of AC liquid inoculant and NPK-compound fertilizer did not change groundnut height but increased the root and shoot dry weight. At 14 and 21 days after sowing, all treatments produced a similar shoot height (Table 2).

Table 1. Nodule Number and Dry Weight in Groundnut Root After AC Inoculation Combined with Various Doses of NPK Fertilizer.

Fertilizer Treatments	Nodule Number		Nodule Dry Weight (g $plant^{-1}$)	
A: AC* 2 L ha^{-1}	15.33±2.35	ab	0.033±0.008	b
B: NPK 300 kg ha^{-1}	8.92±1.19	a	0.015±0.003	ab
C: AC 2 L ha^{-1}, NPK 300 kg ha^{-1}	17.33±2.21	b	0.035±0.010	bc
D: AC 2 L ha^{-1}, NPK 150 kg ha^{-1}	20.58±3.45	b	0.049±0.014	c
E: AC 2 L ha^{-1} NPK 75 kg ha^{-1}	14.83±4.00	ab	0.029±0.008	ab
F: AC 3 L ha^{-1}, NPK 300 kg ha^{-1}	9.21±1.74	a	0.019±0.002	a
G: AC 3 L ha^{-1}, NPK 150 kg ha^{-1}	15.21±3.63	ab	0.035±0.008	bc
H: AC 3 L ha^{-1}, NPK 75 kg ha^{-1}	19.17±3.29	b	0.039±0.010	bc

Note: Means ± SE with the same letter were not significantly different based on DMRT with $p \leq 0.05$. *AC: *A. chroococcum*.

Table 2. Effect of AC Inoculation with Various Doses of NPK Compound Fertilizer on Height and Root Dry Weight of Groundnut at 21 Days After Sowing.

Fertilizer Treatments	Plant height (cm)		Dry weight at day 25 (g plant^{-1})	
	Day 14	Day 21	Roots	Shoots
A: AC* 2 L ha^{-1}	4.78±0.39 a	9.33±0.57 a	0.25±0.03 a	24.78±3.29 a
B: NPK 300 kg ha^{-1}	4.71±0.36 a	9.87±0.44 a	0.29±0.03 b	37.25±5.25 c
C: AC 2 L ha^{-1}, NPK 300 kg ha^{-1}	4.90±0.46 a	10.24±0.49 a	0.28±0.04 a	32.39±2.43 b
D: AC 2 L ha^{-1}, NPK 150 kg ha^{-1}	5.03±0.25 a	10.90±0.78 a	0.34±0.02 c	27.05±6.1 a
E: AC 2 L ha^{-1} NPK 75 kg ha^{-1}	4.57±0.27 a	9.81±0.84 a	0.24±0.04 b	44.27±8.3 d
F: AC 3 L ha^{-1}, NPK 300 kg ha^{-1}	4.68±0.26 a	9.97±0.63 a	0.26±0.03 a	34.52±3.37 bc
G: AC 3 L ha^{-1}, NPK 150 kg ha^{-1}	4.54±0.44 a	9.92±0.71 a	0.27±0.01 a	21.2±2.60 a
H: AC 3 L ha^{-1}, NPK 75 kg ha^{-1}	4.59±0.24 a	10.02±0.47 a	0.27±0.03 a	30.90±6.68 ab

Note: Means ± SE with the same letter were not significantly different based on DMRT with p ≤ 0.05. AC: *A. chroococcum*.

Table 3. Pod Number, Pod Dry Weight, and Yield of Groundnut After *A. chroococcum* Inoculation Combined with Various Doses of NPK Fertilizer.

Fertilizer Treatments	Pod Number per Plot	Pod Dry Weight (kg plot^{-1})	Yield (t ha^{-1})
A: AC* 2 L ha^{-1}	1,110±99.53 a	1.25±0.235 a	2.08±0.391 a
B: NPK 300 kg ha^{-1}	1,478±72.82 c	1.87±0.291 a	3.11±0.485 a
C: AC 2 L ha^{-1}, NPK 300 kg ha^{-1}	1,120±11.19 a	1.35±292 a	2.24±0.487 a
D: AC 2 L ha^{-1}, NPK 150 kg ha^{-1}	2,071±86.15 d	2.62±0.037 b	4.37±0.061 b
E: AC 2 L ha^{-1} NPK 75 kg ha^{-1}	1,219±68.2 a	1.53±0.295 a	2.54±0.491 a
F: AC 3 L ha^{-1}, NPK 300 kg ha^{-1}	1,273±113.4 a	1.64±0.462 a	2.74±0.770 a
G: AC 3 L ha^{-1}, NPK 150 kg ha^{-1}	1,164±67.02 a	1.43±0.143 a	2.39±0.239 a
H: AC 3 L ha^{-1}, NPK 75 kg ha^{-1}	1,331±111.84 b	1.60±0.177 a	2.65±0.295 a

Note: Means ± SE with the same letter were not significantly different based on DMRT with p ≤ 0.05. *AC: *A. chroococcum*.

Inoculation of 2 L ha^{-1} AC with 300 kg ha^{-1} or 150 kg ha^{-1} of fertilizer showed a potency to increase plant height by 9.7% and 16.8% compared to plants treated with Azotobacter only. This suggests that NPK fertilizer is needed in a rational dosage. The root dry weight of plants with 2 L ha^{-1} AC and 150 kg ha^{-1} NPK was 25.9-30.7% higher compared to other treatments. However, higher shoot dry weight (44.27 g) was shown in plants receiving 2 L ha^{-1} AC combined with 75 kg ha^{-1} NPK. This value was 78.6% and 18.8% greater than samples treated with AC and NPK fertilizer, respectively (Table 2).

The combination of fertilizer did not influence pod number per plot (Table 3). However, groundnut responded to 2 L ha^{-1} AC combined with 150 kg/ha NPK (D treatment) by producing higher pod and grain yield (Table 3). This treatment also produced a higher nodule number and dry weight (Table 1), suggesting that plants received sufficient N from rhizobia. The highest productivity (4.37 t ha^{-1}) was shown by plot with D treatment; approximately 40.5% greater than plants with the recommended NPK fertilizer dose (Table 3).

Azotobacter plays a role in Rhizobium-legume symbiotic establishment by improving root dry weight (Table 2). This shows a root growth stimulation by AC that produces plant hormones IAA (Kerečki *et al*., 2022; Hindersah *et al*., 2020a Hindersah *et al*., 2021). The results of this study correlate with the increase in root dry weight of soybeans in pot experiment (Hindersah *et al*., 2019) as well as in pod number and weight of 100 seeds (Hindersah *et al*., 2020b).

The positive effect of Azotobacter in this experiment corresponds to the capacity to synthesize the IAA. The synthesis and transport of IAA along with CK in the cortex is prominent in the nodule initiation and nodule primordia stages (Lin *et al*., 2020). In nodule, rhizobia fix N_2 to NH_3 and further change to NH_4^+, entering the nitrogen cycle to form asparagine, which is supplied to the host plant (Schwember *et al*., 2019). However, a recent study verified that seven hormones, including IAA, control the gynophore and nut development of groundnut (Wang *et al*., 2020). Since Azotobacter is a

heterotrophic rhizobacteria, the application of organic matter in all plots provides the organic carbon for proliferation.

The optimum nutrient uptake is required before the plant enters the anthesis stage. In this study, the flowering of groundnut started 24 days after sowing. The application of fertilizer combined with biofertilizer provided sufficient nutrients during shoot growth. The marketable pod shown in Table 3 excluded the broken and empty pods, suggesting that a combination of AC and a reduced dose of NPK could maintain groundnut yield. This experiment verified that in soil with high N before planting, a reduced dose could be applied by accompanying Azotobacter inoculation.

4. CONCLUSION

In conclusion, inoculation of AC with NPK compound fertilizer influenced available N in soil, uptake by shoot, root, and shoot dry weight, and root nodulation as well as the yield of groundnut. The highest NH_4^+ content of 1 mg kg^{-1} was in soil with AC 2 L ha-1 + NPK 300 kg ha^{-1} and control. The NO_3^- in soil treated with AC 2 L ha-1 + NPK 300 kg ha-1 were 37% and 53% higher than plots that received Azotobacter and NPK, respectively. Although the combination of Azotobacter and NPK did not affect plant height, AC 2 L ha^{-1} + NPK 300 kg ha^{-1} increased N uptake by approximately 169 mg per plant. The highest dry shoot weight (44.27 g) was obtained in plants treated with 2L ha^{-1} of AC and 75 kg ha^{-1} of NPK. However, the highest nodule dry weight (0.49 g) and yield (2.62 kg $plot^{-1}$) were obtained from plants treated with AC 2L ha-1 + NPK 150 kg ha^{-1}. Inoculation of 2L ha^{-1} of AC combined with NPK 150 kg ha^{-1} increased yields of groundnut grown in Inceptisols by 40% and 110% compared to plots treated with NPK 300 kg ha^{-1} and Azotobacter inoculation (control), respectively. These results showed that the use of Azotobacter could reduce the dose of NPK fertilizer.

5. ACKNOWLEDGEMENT

The authors are grateful to PT. Pupuk Kujang Cikampek of National Fertilizer Company for using the field and in-kind items during experiments.

6. REFERENCES

Armendariz, A.L., M.A. Talano, M.F.O Nicotra, L. Escudero, M.L. Breser, C. Porporatto, and E. Agostini. 2019. Impact of double inoculation with Bradyrhizobium japonicum E109 and *Azospirillum brasilense* Az39 on soybean plants grown under arsenic stress. *Plant Physiology and Biochemistry*. 138:26-35.

Asante, M., B.D.K. Ahiabor, and W.K. Atakora. 2020. Growth, nodulation, and yield responses of groundnut (*Arachis hypogaea* L.) as influenced by combined application of Rhizobium inoculant and phosphorus in the Guinea Savanna Zone of Ghana. *International Journal of Agronomy*. 2020: 8691757.

Bitire, T.D., M. Abberton, E.O. Tella, A. Edemodu, O. Oyatomi and O.O. Babalola. 2023. Impact of nitrogen-fixation bacteria on nitrogen-fixation efficiency of Bambara groundnut [*Vigna subterranea* (L) Verdc] genotypes. *Front. Microbiol*. 14:1187250.

Einslea, O., and D.C. Reesb. 2020. Structural enzymology of nitrogenase enzymes. *Chemical Review*. 120(12): 4969–5004.

Eviati dan Sulaeman. 2012. *Petunjuk Teknis Analisis Kimia Tanah, Tanaman, Air dan Pupuk*. Edisi 2. Badan Penelitian dan Pengembangan Pertanian, Kementerian Pertanian. 215 Hlm.

Hanyabui, E., S.O. Apori, K.A. Frimpong, K. Atiah, T. Abindaw, M. Ali, J.Y. Asiamah, and J. Byalebeka. 2020. Phosphorus sorption in tropical soils. *AIMS Agriculture and Food*. 5(4): 599-616.

Herliana, O., T. Harjoso, A.H.S. Anwar, and A. Fauzi. 2019. The effect of Rhizobium and N fertilizer on growth and yield of black soybean (*Glycine max* (L) Merril). *IOP Conference Series: Earth Environmental Science*. 255: 012015.

Hindersah, R., N. Rostini, A. Harsono, dan A.M. Kalay. 2019. Peran eksopolisakarida Azotobacter dan bahan organik untuk meningkatkan nodulasi dan biomassa kedelai pada dua ordo tanah. *Jurnal Agronomi Indonesia*. 47(2):156-162.

Hindersah, R., M.R. Setiawati, P. Asmiran, and B.N. Fitriatin. 2020a. Formulation of Bacillus and Azotobacter consortia in liquid cultures: Preliminary research on microbes-coated urea. *International Journal of Agriculture System*. 8(1): 1-10.

Hindersah, R., R.R. Risanti, I. Haikal, and M. Rachmadi. 2020b. Effect of Azotobacter application method on yield of soybean (*Glycine max* (L.) Merill) on dry land. *Agric*. 31(2):136-145.

Hindersah, R., I. Rahmadina, R. Harryanto, P. Suryatmana, and M. Arifin. 2021. Bacillus and Azotobacter counts in solid biofertilizer with different concentration of zeolite and liquid inoculant. *IOP Conference Series: Earth and Environmental Science*. 667:012010.

Hungria, M., M.A. Nogueir, L.J.M. Campos, P. Menna, F. Brandi, and Y.G. Ramos. 2020. Seed pre-inoculation with Bradyrhizobium as time-optimizing option for large-scale soybean cropping systems. *Agronomy Journal*. 112:5222–5236.

Kerečki, S., I. Pećinar, V. Karličić, N. Mirković, I. Kljujev, V. Raičević, and J. Jovičić-Petrović. 2022. *Azotobacter chroococcum* F8/2: a multitasking bacterial strain in sugar beet biopriming. *Journal of Plant Interactions*. 17(1): 719-730.

Komalasari, W.B. 2022. *Statistik Makro Sektor Pertanian*. Pusat Data dan Sistem Informasi Pertanian, Kementrian Pertanian. 246 hlm.

Kumar, R., M.K. Pandey, S. Roychoudhry, H. Nayyar, S. Kepinski, R.K. Varshney. 2019. Peg biology: Deciphering the molecular regulations involved during peanut peg development. *Frontier in Plant Sciences*. 10:1289.

Limbongan, Y. 2021. *Statistika dan Perancangan Percobaan*. Penerbit UKI Toraja Press. Makale Tana Toraja, Indonesia.

Lin, J., M. Frank, and D. Reid. 2020. No home without hormones: How plant hormones control legume nodule organogenesis. *Plant Communications*. 1(5):100104.

Liu, L., X. Zhang, W. Xu, X. Liu, Y. Li, J. Wei, Z. Wang, and X. Lu. 2020. Ammonia volatilization as the major nitrogen loss pathway in dryland agro-ecosystems. *Environmental Pollution*. 265:114862.

Muslim, R.Q., P. Kricella, S.M. Pratamaningsih, S. Purwanto, E. Suryani, and R. Sofyan. 2020. Characteristics of Inceptisols derived from basaltic andesite from several locations in volcanic landform. *SAINS TANAH – Journal of Soil Science and Agroclimatology*. 17(2): 115-121.

Mwendwa, S. 2022. Revisiting soil texture analysis: Practices towards a more accurate Bouyoucos method. *Heliyon*. 8(5): e09395. 10.1016/j. *Heliyon*. 2022: e09395.

Nosrati, R., P. Owlia, H. Saderi, I. Rasooli, and M.A. Malboob. 2014. Phosphate solubilization characteristics of efficient nitrogen fixing soil Azotobacter strains. *Iranian Journal of Microbiology*. 6(4): 285-95.

Qiu, L., F. Fan, Y. Li, Q. Yang, X. Gou, Y. Kong, S. Zhou, G. Chen, J. Xie, Y. Chen, L. Liu, Y. Tang, and L. Tu. 2023. Soil phosphorus drives variation in diazotrophic communities in a subtropical nitrogen-rich forest. *Forest Ecology and Management*. 544:121164.

Schwember, A.R., J. Schulze, A. Del Pozo, and R.A. Cabeza. 2019. Regulation of symbiotic nitrogen fixation in legume root nodules. *Plants*. 8(9):333.

Sebilo, M., B. Mayer, B. Nicolardot, G. Pinay, and A. Mariotti. 2013. Long-term fate of nitrate fertilizer in agricultural soils. *Proceedings of the National Academy of Sciences of the United States of America*. 110(45): 18185–18189.

Wang, H.X., M.L. Wang, X.Z. Wang, and Y.L. Ding. 2020. Detection of seven phytohormones in peanut tissues by ultra-high performance liquid chromatography-triple quadrupole tandem mass spectrometry. *Journal of Integrative Agriculture.* 19(3):700-708

Yaro, R.N., A.R. Mahama, J.X. Kugbe, and A. Berdjour. 2021. Response of peanut varieties to phosphorus and rhizobium inoculant rates on Haplic Lixisols of Guinea Savanna Zone of Ghana. *Frontier in Sustainable Food System.* 5: 616033.

Yuniarti, A., M. Damayani, and D.M. Nur. 2019. The effect of organic and N, P, K Fertilizers on organic C, Total N, C/N, N uptake, and yields od black rice on Inceptisols. *Jurnal Pertanian Presisi*. 3(2): 90-105.