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

AZOTOBACTER AND CHEMICAL FERTILIZER FOR IMPROVING GROUNDNUT YIELD IN INCEPTISOLS

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ABSTRACT

Inoculation of nitrogen-fixing Azotobacter is a sustainable approach for reducing chemical fertilizer dose. A field experiment was aimed to observe the N availability, N uptake, nodulation, and growth and yield of groundnuts grown with A. chroococcum (AC) liquid inoculant and reduced dose of NPK fertilizer. The field experiment was arranged in a Randomized Block Design to test eight combination treatments of A. chroococcum inoculant doses (2 and 3 L ha^{-1}) and NPK fertilizer doses (75, 150 and 300 kg ha^{-1}). Duncan Multiple Range Test was used when the effect of treatment was significant on the parameters. The NO_3^- levels in soil treated with AC 2 L ha^{-1} + NPK 300 kg ha^{-1} were 37% and 53% higher than that in soil with Azotobacter and NPK only. Application of various combination of Azotobacter and NPK did not affect plant height but AC 2 L ha^{-1} + NPK 300 kg ha^{-1} increased N uptake up to 169 mg per plant. The highest dry shoot weight (44.27 g) was shown by plants with 2L ha^{-1} of AC and 75 kg ha^{-1} of NPK. However, the highest nodule dry weight and yield (0.49 g and 2.62 kg plot^{-1} respectively) were obtained from plants treated with AC 2L ha^{-1} + NPK 150 kg ha^{-1}. This experiment showed that A. chroococcum combined with NPK 150 kg ha^{-1} increased yields of groundnut in Inceptisols by 40% compared to plots treated with NPK 300 kg ha^{-1}; which is Azotobacter enable to reduce the dose of NPK fertilizer.

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.

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PROOF

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an export-import commodities and important legume widely consumed as a source of protein; moreover. The national production of groundnuts is 510,000 t of dry seeds per year, while the national consumption reaches 658,000 t per year then fresh and processed groundnut must be imported. According to Macro Statistic for Agricultural Sector (Komalasari, 2022), volume of processed groundnut importation to Indonesia at 2022 were decreased up to 259,537 t compared to 318,177 at 2018 while that of fresh groundnuts were only 1.69 t compared to 14.17 at 2018. However, Indonesia exported 5.44 t of fresh and processed groundnuts at 2018 and the volume was decreased up to 2.67 at 2022. Those data verified that the groundnut productivity should be increased. Despite the importance of groundnuts for the food industry in Indonesia, the average groundnut productivity in most agricultural lands was less than 2 t/ha of pods; lower than their genetic potency. The Ministry of Agriculture stated for example that the yield of groundnut cultivars Kerinci and tuban was 2.3 t ha^{-1} and 3.2 t ha^{-1} of dry pod.

The challenge in increasing groundnut productivity in the tropics is low nitrogen (N) and phosphorus (P) availability in soil including Inceptisols (Yuniarti *et al*., 2019; Muslim *et al*., 2020) used in this experiment. The farmers utilize inorganic fertilizer in order to fulfill the need of both major essential nutrients for maintaining plant yield. Environmental issues cause agricultural inputs to shift from inorganic fertilizers to organic and biofertilizers. Improper use of N fertilizer causes N 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 limits the supply of P for plant uptake (Hanyabui *et al*., 2020). Currently, the price of fertilizer has increased elsewhere.

Legumes form a symbiotic interaction in which the rhizobia in the root nodules fix N_2 to ammonia catalyzed by nitrogenase. This enzymatic process will be inhibited by NH_3/NH_4^+ in nitrogenase (Einslea Reesb, 2020). Optimizing the nodule formation and N fixation is suggested to reduce inorganic fertilizer doses and costs. High doses of N fertilizer possibly inhibit root nodules formation and pods 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 increase macronutrient uptake by groundnuts, 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. The N_2 fixation requires a lot of chemical energy as adenosine triphosphate (ATP); P deficiency in soil reduce nitrogenase activity and nifH gene abundance (Qiu *et al*., 2023). The non-symbiotic N_2-fixer bacteria Azotobacter enables to provide inorganic P through phosphate solubilization (Nosrati *et al*., 2014). Therefore, dual inoculation of rhizobia and N_2 fixer bacteria Azotobacter is reported elsewhere but co-inoculation of Azotobacter and Bradyrhizobium is rarely studied. Despite the low abundance of Azotobacter (0.06%) in the rhizosphere microbiome (Hussen *et al*., 2020), the Azotobacter inoculation during legume cultivation is needed to increase their population.

Azotobacter produce tthe 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 the groundnut gynophore. The auxin signal is increased during peg elongation and gravitropic bending; which is lateral transport of auxin from lower to upper cortex help the gravitropic bending of ground nut peg (Kumar *et al*., 2019).

Azotobacter roles in soybeans nodulation and yield have been reported. For more than four decades, the potential of Azotobacter to increase soybean nodulation was reported (Thomas *et al*., 1981). 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). A pot experiment found that mixed inoculation of *B. japonicum* and Azotobacter led to the

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increment of weight of root nodules and the height and weight of soybean dry shoots and grains (Hindersah *et al*., 2019; Hindersah *et al*., 2020b).

The studies concerning Azotobacter inoculation to reduce chemical fertilizer in groundnut are limited. Saving chemical fertilizer rate following Azotobacter inoculation is prominent for sustainable agriculture. The general objective of this field experiment was conducted to observe ability of N-fixer *A. choroococum* in reducing the doses of NPK (15:15:15) in groundnut cultivation. The specific goal of the research was to analyze the N availability in soil, N uptake by shoot, nodulation as well as groundnut growth and yield after application of *A. chroococcum* liquid inoculant and reduced doses of NPK compound fertilizer.

2. MATERIALS AND METHODS

2.1 Experimental Site and Soil Properties

The experiment 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 the sea level. The seeds of groundnut cv Tuban were grown on clay Inceptisol soil (17% sand, 37% silt, and 46% clay). The daily temperature and humidity in the experimental site were 22 ^{o}C – 33 ^{o}C and 62-89% respectively.

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

The soil was neutral in acidity (pH 7.1), low in organic C (1.6%), high in total N (0.6%), low in available P (12.07 ppm); very high in potential P_2O_5 (64.34 mg 100 g^{-1}) but very low in 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 tested eight treatment combinations dose of *A. chroococcum* (AC) liquid inoculant and combined NPK fertilizer. The field experiment was set up in a Randomized Block Design with four replications (Limbongan, 2021). 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 current study, all groundnut seeds were treated with liquid inoculant of *Bradyrhizobium* sp. before sowing. Both inoculant 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

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around the experimental area. Plot orientation in the experimental field is from west to east. Land clearing was done two days before soil tillage by removing all bushes, grass, sedges and boarder leaves weeds from the land surface. 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 final (equivalent to 2 L ha_{-1}); and the F, G, and H plots treated with 2.25 mL/plot (equivalent to 3 L/ha). The bacterial inoculant was diluted with 100 mL distilled water. The diluted inoculant was mixed with 3.75 kg manure and incubated for 3 before mixing the manure with the topsoil of each plot. Groundnut seeds were sown seven days after manure application. Before sowing, the seeds were soaked in liquid inoculant of *Bradyrhizobium* spp. mixed with 1% of Arabic gum for 30 s. The 2 cm-deep holes with the distance 40 cm between row and 15 cm in row were created by plugging the wooden stick in. A single seed was put in the holes and covered with the soil.

The NPK fertilizer was applied in split, one week (50%) and three weeks after sowing (50%) in all treatments. The fertilizer was placed in the continuous band at the bottom of a 3-cm deep furrow at 5 cm away from the stem; then they were 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 the 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

Six sampling plants were set in three center rows in plot by zig-zag method so that each row has 2 sampling plants. The border rows did not include in sampling plant determination. The soil samples were collected from the 15-cm deep of soil where the lateral roots were grown intensively; and kept in room temperature before available N analysis. Soil for Azotobacter enumeration was taken from rhizosphere of sampling plant, put in sealed plastic bag and stored at 4^0C prior to 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. Azotobacter count were measured at the late vegetative stadia 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). Nitrogen uptake of shoots was calculated by multiplying the dry weight and the total N content of the shoot. The dry weight of roots and root nodules per plant was determined by the gravimetric method after heating at 70 ^{o}C for 48 h (Eviati and Sulaeman, 2019). The Azotobacter population in the rhizosphere was counted by serial dilution plate method in N-free Ashby media (Hindersah *et al*., 2021).

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

3. RESULTS AND DISCUSSION

3.1 Available Nitrogen

Soils treated with 2 L ha^{-1} of AC combined with 150 kg ha^{-1} of fertilizer, and soil received only 2 L ha^{-1} of AC (control treatment) had higher NH_4^+ compared to another treatments (Figure 1a). However, the highest levels of NO_3^- were found in soil with 2 L ha^{-1} AC with 300 kg ha^{-1} fertilizer;

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which is 37% and 53% higher than the control of AC and NPK respectively (Figure 1b). In the current study, the application of 2 L ha^{-1} AC combined with 150 kg/ha NPK resulted in the highest N uptake in the shoot of an individual plant; 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 contrast, 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 the fertilizer treatments. Statistically, the application of Azotobacter without NPK resulted in the same NH_4^+ and NO_3^- content with most Azotobacter-NPK combination treatments. This showed that in soil with high N (0.6% before the experiment), NPK fertilizer is considered not a dominant factor in increasing the N availability. It also showed that the NO_3^- content in soil with Azotobacter and NPK were similar. However, after the experiment the NH_4^+ in all soil was less than 2 mg kg^{-1}; which is low according to Indonesia's classification of NH_4^+ content. 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); which is medium-high (6-10 mg kg^{-1}) based on Indonesian classification.

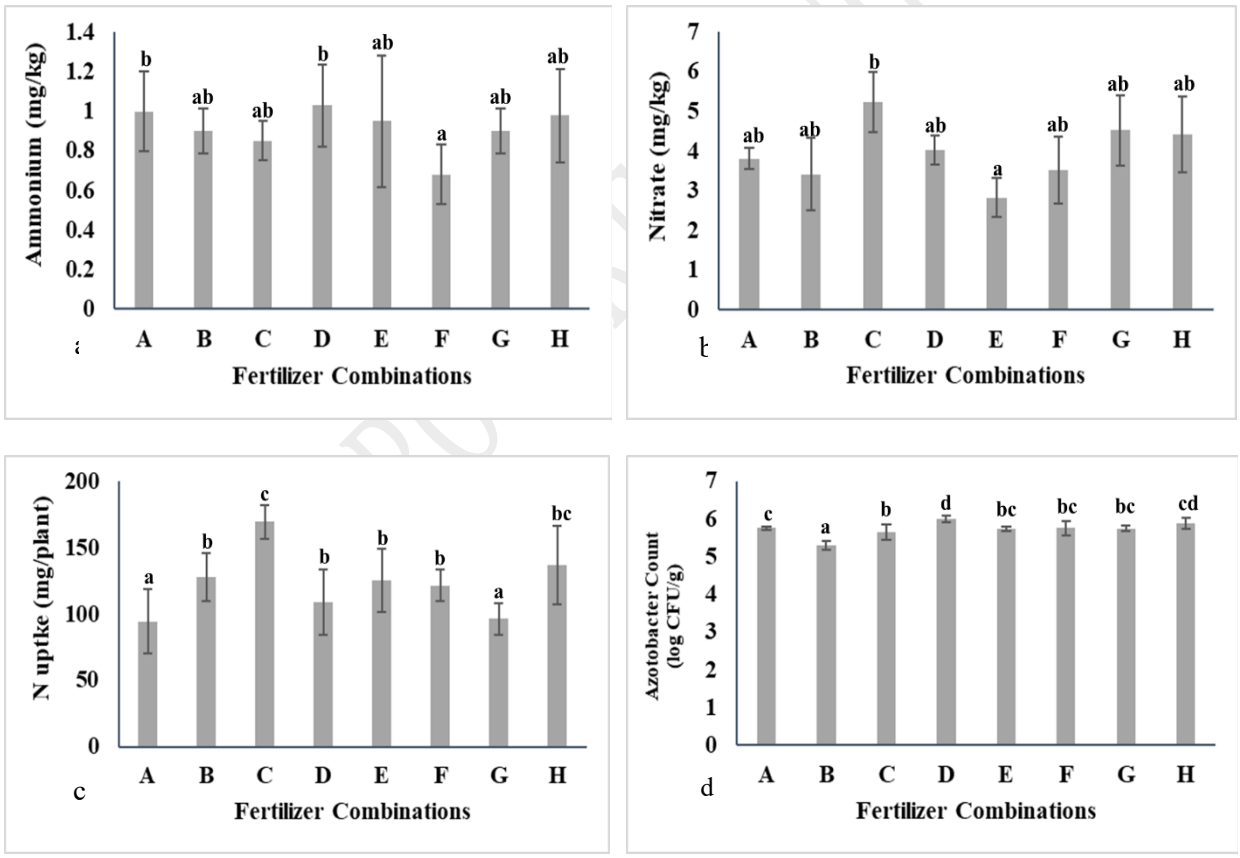

Figure 1. Effect of *A. chroococcum* inoculation combined with NPK compound fertilizer on NH_4^+ (a) and NO_3^- ; (b) in soil, N uptake by shoot; (c) and Azotobacter population in the rhizosphere of groundnut at 25 days after sowing; (d). A: *A. chroococcum* (AC) 2 L ha^{-1}), B: NPK 300 kg ha^{-1}, 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 $p \leq 0.05$.

Nitrogen dissolves in water and is highly mobile in soil so N leaching might take place and reduce the N available. Excessive NPK fertilization in high N soil is not effectively uptake and N shall be leached out of the soil through irrigation water and rain (Sebilo *et al*., 2013). Moreover, an experiment in the dry season when the day temperature reached 33^0C; therefore, induced the N volatilization (Jadon *et al*., 2018).

Nitrogen fixation in the early vegetative growth is possibly inhibited by abundance N in the soil since nitrogenase activity is reversibly susceptible to high N. Therefore, in early growth, plants adsorb ionic N from the soil and fertilizer instead of N fixation. Available N released from NPK is uptake by plants as well as utilized by the bacteria. Fertilizers do not only provide nutrients for plant growth but also for microbial proliferation. Azotobacter inoculant of 3L/ha is considered to increase the population of Azotobacter more than the lower dose (2 L ha^{-1}) due to N, P, and K provision. A higher population of N_2-fixer microbes in N-rich utilized available N for metabolisms and hence ceased nitrogenase. After that, the N available in soil will be reduced; and the N fixation by Azotobater as well as effective nodule formation will take place.

3.2 Groundnut Nodulation

In general, a combination of Azotobacter inoculation and NPK fertilizer increased the nodule number at the late vegetative stage of groundnut compared to NPK fertilizer only. However, plants with 2 L ha^{-1} AC combined with 150 kg/ha as well as 3L ha^{-1} AC with 75 kg/ha NPK, had a higher number and weight of nodules compared to plants 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 resulted in higher nodule dry weight i.e. 0.49 g.

The experiment verified that plants treated with the highest dose of NPK fertilizer reduced nodulation. This is related to the high soil N that represses N fixation. Azotobacter inoculation had a positive effect on nodulation even though a high dose of NPK was applied. The NPK provides nutrients for exogenous Azotobacter for population increment (Figure 1). Moreover, P from NPK contributes to chemical energy formation that serves energy source for to root system where nodulation takes place (Asante *et al*., 2020); meanwhile, Azotobacter enables to provide available P through phosphate solubilization activity as reported for *A. chroococcum* and *A. vinelandii* strains (Nosrati *et al*., 2014). The nodulation traits might reflect the increment of rhizobia proliferations and nitrogen fixation that needs a lot of chemical energies, ATP.

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 led to a similar shoot height (Table 2).

Table 1. Nodule Number and Dry Weight in Groundnut Roots After *A. chroococcum* 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*.

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Table 2. Effect of *A. chroococcum* 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 either 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 supposes that NPK fertilizer is needed in rational dosage. The roots 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. Nonetheless, but the higher shoot dry weight (44.27 g) was shown in a plant that received 2 L ha^{-1} AC combined with 75 kg ha^{-1} NPK, which is 78.6% and 18.8% higher than a plant with AC and NPK fertilizer respectively (Table 2).

In general, fertilizer combination did not influence pod number per plot (Table 3). However, the groundnut responded to 2 L ha^{-1} AC combined with 150 kg/ha NPK (D treatment) by producing more higher pod as well as grain yield (Table 3). This treatment also resulted in the higher nodule number and dry weight (Table 1) which is, plants were supposed to get more sufficient N from the rhizobia. The highest productivity (4.37 t ha^{-1}) was showed by plot with D treatment; approximately 40.5% higher than that of the plant 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 indicated a root growth stimulation by AC that produce plant hormones IAA (Kerečki *et al*., 2022; Hindersah *et al*., 2020a Hindersah *et al*., 2021). The results of the current experiment agree with the increase in the root dry weight of soybeans in pot experiment (Hindersah *et al*., 2019) and on pod number and weight of 100 seeds in field experiments was also verified (Hindersah *et al*., 2020b).

The positive effect of Azotobacter in this experiment corresponds to the capacity of Azotobacter 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). Rhizobia in nodule fix N_2 to NH_3 and further change to NH_4^+ that enter the nitrogen cycle to form asparagine; this amino acid is then supplied to the host plant (Schwember *et al*., 2019). However, recent study verified that seven hormones including IAA control the gynophore and nut development of groundnuts (Wang *et*

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al., 2020). Since the Azotobacter is a heterotrophic rhizobacteria, the application of organic matter in all plots provides the organic carbon for their proliferation.

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

4. CONCLUSION

Inoculation of *A. chroococcum* with NPK compound fertilizer influenced available N in soil, N uptake by shoot, root and shoot dry weight, and root nodulation as well as yield of groundnuts. 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 that in soil with Azotobacter and NPK respectively. Combination of Azotobacter and NPK did not affect plant height but AC 2 L ha^{-1} + NPK 300 kg ha^{-1} increased N uptake up to 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 *A. chroococcum* 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. This verified that Azotobacter enable to reduce the dose of NPK fertilizer.

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