Antagonistic Effect of Nitrogen Fertilizer and Rhizobium on Growth, Nodulation and Yield of Peanut (*Arachis hypogaea* L.) in Acidic Soil

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ABSTRACT

Acid soil is widely distributed in Indonesia but underexploited for agriculture due to limited nitrogen availability and aluminium toxicity. Nitrogen fertilizer and rhizobium are crucial to improving plant growth, especially in peanut cultivation. This study examines the antagonistic effects of nitrogen fertilizer and rhizobium on the growth, nodulation, and yield of peanuts cultivated in acidic soil. A factorial randomized complete block design with two factors: nitrogen fertilizer application (0, 50, 100, 150 kg ha^{-1}) and rhizobium inoculation (without rhizobium, rhizobium at 10 g kg^{-1} seed, and rhizobium sourced from peanut plantations). The combination of 100 kg ha^{-1} nitrogen and rhizobium from peanut plantations resulted in the highest leaf count (675.33 leaves per plant). A nitrogen dose of 50 kg ha^{-1} produced the highest effective number of nodules and total nodules. The optimum nitrogen fertilizer dose is 44 kg ha^{-1} for nodule growth. 50 kg ha^{-1} nitrogen dose produced the highest number of pods and seed weight, namely 48.67 pods and 407.79 g of seeds. These findings suggest that when applied at an appropriate dose, nitrogen fertilizer enhances peanut growth, nodulation, and yield in acidic soil. However, excessive nitrogen application may induce antagonism with the nodulation process, reducing overall yield.

Keywords: Legume plant; Nitrogen fixation; Nodules; Rhizobium

INTRODUCTION

Acidic soil covers 25% of Indonesia's total land area, about 45.79 million hectares, with 5.22 million hectares used for crop cultivation (BPS, 2020). Acidic soil is suboptimal, so harvest yields are below the national average each season. However, acidic soil is widely used for crop cultivation because the optimal area of agricultural land is decreasing. According to the BPS (2018), the conversion of rice fields reaches 100,000 to 150,000 hectares per year, which is not comparable to the creation of new rice fields, which is only 60,000 hectares per year. However, agricultural extensification efforts utilize suboptimal land, such as acid soil (Arista et al., 2023). However, soil acidity is considered a key variable in soil chemistry because of its significant impact on chemical reactions involving essential plant nutrients (Gerke, 2022; Javed et al., 2022; Raza et al., 2021; Sintorini et al., 2021).

Agricultural practices can accelerate the process of soil acidification during soil weathering (Bolan et al., 2023; Chen et al., 2022). Agricultural practices with the continuous addition of chemical

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fertilizers can increase the concentration of H+ ions in the soil (Han et al., 2021; Wan et al., 2021). An increase in H+ ions results in soil acidity, which hurts soil microbial and plant activity (Daba et al., 2021; Raza et al., 2020). Additionally, soil acidity makes metals more soluble and mobile, preventing plants from accessing vital nutrients (Alves et al., 2019; Wang et al., 2023a; Zhang et al., 2019). Low soil pH makes certain essential plant nutrients insoluble and less accessible, including phosphorus, calcium, magnesium, and molybdenum (Abdul Halim et al., 2018; Wang et al., 2023b) and can affect the decline in plant growth (Baccari & Krouma, 2023; Barrow & Hartemink, 2023). Planting plants from the Leguminaceae family is a strategy to utilize acidic soil, such as peanuts (Abd-Alla et al., 2023), because it is supported by the ability of legume plants to form a symbiotic relationship with soil microorganisms such as rhizobium (Yang et al., 2022). Legumes can fix nitrogen in the atmosphere and increase available nitrogen through biological nitrogen fixation (Basile & Lepek, 2021; Goyal & Habtewold, 2023; Ramoneda et al., 2021).

An estimated 1.75 × 1011 kilograms of nitrogen are fixed globally each year, with 8.0 × 1010 kg coming from legume symbiosis and an average of 20–200 kg of fixed N ha^{-1} year^{-1} (Kebede, 2021). Thus, legume-rhizobium symbiosis-mediated biological nitrogen fixation is an attempt to alter soil organism activity and boost nutrition availability (Goyal et al., 2021; Grzyb et al., 2021; Mesfin et al., 2020). The lack of rhizobium bacteria causes nodules to form on peanut roots, so plants cannot independently fix free nitrogen in the air through nitrogen fixation. Land that lacks nitrogen and does not contain rhizobium bacteria will result in the vegetative growth of peanut plants being hampered because they lack the nutrient nitrogen. The lack of rhizobium bacteria in the soil causes farmers to spend more on inorganic fertilizers to meet the need for nitrogen nutrients (Etesami, 2022; Vanlauwe et al., 2019). Rhizobium bacteria can infect the roots of peanut plants and create colonies to form nodules that trap free nitrogen in the air. Nitrogen available in the soil causes the rhizobium to be ineffective in collecting free nitrogen in the air; conversely, if nitrogen is not available in the soil, the rhizobium effectively increases free nitrogen in the air (Ramoneda et al., 2021). Optimal doses of nitrogen fertilizer are needed to ensure the presence of rhizobium bacteria so that mutualistic symbiosis with peanuts can occur in peanut growth. The research examines the effect of antagonism and nitrogen fertilizer on peanuts' growth, nodulation, and yield in sour planting.

MATERIALS AND METHODS

Study site and soil characteristics

At a height of 148 meters above sea level, the study was conducted at the Laboratory Experiment Field of the Faculty of Agriculture, Sebelas Maret University, Jumantono, Karanganyar Regency. It was situated at 7º37'48.82" South Latitude and 110º56'52.17" East Longitude. The research used alfisol soil with soil acidity characteristics of 5.6 (acid category); C-organic 0.65 % (very low); total nitrogen 0.06 % (deficient); P2O5 total 16 ppm (medium); K2O total 12.26 mg/100g (low); C/N ratio 4.84 (very low). Planting was carried out in polybags measuring 35 x 35 cm, and the distance between the polybags was 25 x 25 cm. The planting medium used is acid soil and cow dung fertilizer in a ratio of 1 : 1. The seeds used are Kancil variety peanuts. Basic fertilizer is applied twice before planting, using SP36 fertilizer at 200 kg ha^{-1} or 0.8 g polybag^{-1} and KCl fertilizer at 50 kg ha^{-1} or 0.2 g polybag^{-1}.

Experimental design

The research used a factorial complete randomized block design with two factors. The first factor is the dose of Nitrogen fertilizer with four levels, namely 0, 50, 100, and 150 kg ha^{-1}. The second factor is the application of rhizobium sources at three levels: without rhizobium, rhizobium dose of 10 g kg^{-1} seeds, and source of rhizobium in soil used from peanut planting. The research was repeated three times. Nitrogen fertilizer treatment was carried out at planting, and rhizobium treatment was carried out on peanut seeds before planting. Treatment of rhizobium from former peanut plantings was carried out by mixing the soil weighing 15 g into the planting medium.

Observation variables include growth variables, namely plant height four weeks after planting (WAP) and number of leaves at twelve WAP. The nodulation variables observed were the number of nodules and the effective number of nodules carried out at 10 WAP. The outcome variables are the number of pods and the weight of 1000 seeds. Outcome variables were observed at 90 days after planting.

Data Analysis

Analysis of Variant level 5% was used to examine the observational data. If it was significant, the 5% Duncan Multiple Range Test was used to determine whether there were significant differences between treatments. The ideal nitrogen dosage was found by regression.

RESULTS AND DISCUSSION

The research results showed that the combination of nitrogen fertilizer doses with the application of rhizobium sources affected peanut plant height four weeks after planting (Table 1). The optimum nitrogen fertilizer dose was 54.83 kg ha$^{-1,}$ with rhizobium inoculum from soil used for peanut plantations to produce the highest plant height, 15.70 cm, with a correlation coefficient of 0.99. The addition of nitrogen can stimulate the growth of rhizobium bacteria (Shome et al., 2022). Also, rhizobium bacteria from used peanut soil can form a symbiotic relationship with the perfect peanut root system to maximize the nitrogen fixation process (Boivin et al., 2020; Yang et al., 2022). The combination of nitrogen fertilizer doses with the application of rhizobium sources affects the number of leaves (Table 2). The dosage of 100 kg ha^{-1} of nitrogen fertilizer with rhizobium inoculum from soil used to plant peanuts showed the highest number of leaves, namely 675.33. However, the number of leaves in this treatment combination was similar to the treatment combination of 100 kg ha^{-1} of nitrogen and rhizobium. The compatibility of legin rhizobium and rhizobium from soil used for peanut plantations is high. Rhizobium can associate with the host to produce many nodules and fix nitrogen for plant growth (Fahde et al., 2023; Mathenge et al., 2019). The efficacy of nitrogen-fixing bacteria in peanuts will be increased by applying a specific amount of nitrogen fertilizer (Jaiswal et al., 2021). The availability of nitrogen will influence cell division in the apical meristem, resulting in the formation of tall leaves (Sun et al., 2020). Rapid leaf growth is impacted by cell division, which can also result in more leaves because it produces more new leaves (Sakakibara, 2021; Shi & Vernoux, 2022).

Table 1. Combination of nitrogen fertilizer doses and rhizobium sources on plant height four weeks after planting

Nitrogen Fertilizer Dosage (kg.ha^{-1})	Source of Rhizobium			Average
	Without Rhizobium	Rhizobium	Land Used by Peanut Plantings	
0	14.30abcde	15.33abc	13.00bcde	14.21a
50	13.13abcde	13.80abcde	15.73a	14.22a
100	15.70ab	13.53abcde	14.33abcd	14.52a
150	12.67cde	11.40de	8.50e	10.86b
Average	13.95a	13.52a	12.89a	+

Note: Numbers followed by the same letter notation in columns and rows are not significantly different in the DMRT test at the 5% level. (+): there is interaction

Table 2. Combination of nitrogen fertilizer doses and rhizobium sources on leaf number 10 weeks after planting

Nitrogen Fertilizer Dosage (kg.ha^{-1})	Source of Rhizobium			Average
	Without Rhizobium	Rhizobium	Land Used by Peanut Plantings	
0	606.00	552.00	629.00	595.67
50	555.67	569.33	571.33	565.44
100	598.33	636.00	675.33	636.56
150	529.67	543.67	427.00	500.11
Average	572.42	575.25	575.67	+

Note: Numbers followed by the same letter notation in columns and rows are not significantly different in the DMRT test at the 5% level. (+): there is interaction

Table 3. The role of nitrogen fertilizer dosage and rhizobium source on nodule growth

Treatment	Number of nodules	Effective number of nodules
Nitrogen Fertilizer Dosage (kg.ha^{-1})		
0	358.56a	18.89a
50	363.00a	19.33a
100	351.33a	18.67a
150	138.00b	13.67b
Source of Rhizobium		
No Inoculant	311.17	17.25
Rhizobium inoculant	301.17	17.67
Land Used by Peanut Plantings	295.83	18.00
Average	302.72	17.64
Interaction	-	-

Note: Numbers followed by the same letter notation in one column indicate that they are not significantly different in the DMRT test at the 5% level. (-): no interaction

The number of nodules and the number of effective nodules were shown to be influenced by the nitrogen fertilizer dosage (Table 3). The number of peanut nodules formed by a nitrogen dose of 50 kg ha^{-1} was 225, significantly different from 150 kg ha^{-1}. With a coefficient of 0.97, the most significant number of peanut nodules, or 391.56 grains, were produced by the optimal nitrogen fertilizer dose of 44 kg ha^{-1} (Figure 1). Peanuts are a family of legumes capable of forming nodules for nitrogen fixation. Low nitrogen fertilizer doses produce high nodules. Legumes have been proven to significantly increase the abundance of rhizosphere soil microorganisms (Malviya et al., 2021). Through nitrogen-fixing enzymes, nitrogen-fixing bacteria control the nitrogen-fixing process (Lai et al., 2022). Based on Abd-Alla et al. (2023), giving the highest dose of nitrogen fertilizer produces

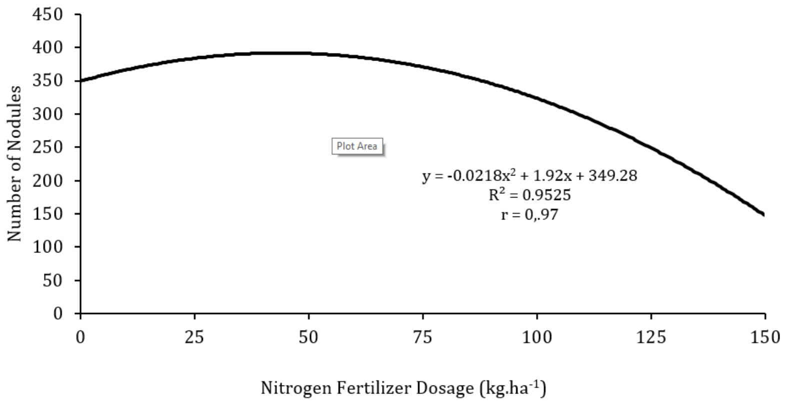

Figure 1. Regression test for the number of peanut nodules at 4 WAP with the administration of several doses of nitrogen fertilizer

Table 4. Effect of nitrogen fertilizer dose and rhizobium source on peanut yield

Treatment	Number of Pods	Weight of 1000 Seeds (g)
Nitrogen Fertilizer Dosage (kg.ha^{-1})		
0	36.11b	380.13a
50	48.67a	407.79a
100	46.33a	383.35a
150	38.56a	337.16b
Source of Rhizobium		
No Inoculant	45.58	380.90
Rhizobium inoculant	44.42	370.63
Land Used by Peanut Plantings	37.25	379.80
Average	42.42	377.11
Interaction	-	-

Note: Numbers followed by the same letter notation in one column indicate that they are not significantly different in the DMRT test at the 5% level. (-): no interaction

the lowest number of nodules because when there is excess nitrogen in the soil, rhizobium bacteria cannot fix nitrogen in the air. A nitrogen fertilizer dose of 50 kg ha^{-1} can produce 5.66 effective peanut nodules and more than a nitrogen fertilizer dose of 150 kg ha^{-1} (Table 3). The optimum nitrogen fertilizer dose of 49 kg ha^{-1} produced the highest number of effective peanut nodules, namely 19.93 grains, with a correlation coefficient of 0.98. Low nitrogen fertilizer doses can increase the activity of active rhizobium bacteria in the nodules to provide nitrogen for peanut plants. Peanut plants will produce enzymes in soil conditions low in nitrogen, so the rhizobium actively fixes nitrogen and results in effective active nodules (Etesami, 2022; Solanki et al., 2020).

The level of nitrogen use has an essential influence on rhizosphere microorganisms and changes in community growth and development (Li et al., 2022; Ren et al., 2020). Numerous studies have shown that the amount of nitrogen in the soil significantly impacts the makeup of the bacterial population and that short-term fertilization also alters the composition of the rhizobia community (Yu et al., 2021). Fertilization increases the abundance of nitrogen-fixing bacterial species (Wassermann et al., 2023). In Northeast China's black soil region, it helps to increase the quantity and diversity of nitrogen-fixing bacterial genes (Liu et al., 2021). However, the results showed that the source of rhizobium did not affect the number of nodules and the number of effective nodules (Table 3), which can be caused by rhizobium incompatibility. The availability of organic carbon can be a source of energy for rhizobium bacteria to work effectively. Previous research showed that the organic C content at the three research locations was low and produced the lowest rhizobium bacteria population compared to locations with moderate organic C content (Li et al., 2022). Therefore, it is necessary to add organic materials in the form of compost, manure, and green manure to increase the population of rhizobium bacteria in the soil. The soil used to plant peanuts contains rhizobium bacteria, which can infect roots and form nodules on legume plants (Jach et al., 2022). Rhizobium in soil used to harvest peanuts can still survive under environmental conditions supporting rhizobium bacteria growth (Neelipally et al., 2020).

The results showed that the dose of nitrogen fertilizer affected the number of pods and the weight of 1000 seeds (Table 4). A nitrogen fertilizer dose of 50 kg ha^{-1} significantly differs from a nitrogen fertilizer dose of 0 kg ha^{-1} regarding the number of pods and weight of 1000 peanut seeds. The optimum nitrogen fertilizer dose of 78.75 kg ha^{-1} produced the highest number of peanut pods, namely 49 pods, with a coefficient of 0.97 (Figure 1). The results of this research align with research by El-sherbeny et al. (2023) that the highest number of pods were produced in the treatment of urea and animal manure fertilizer. Peanut pod formation requires high nitrogen levels to form new cells composed of photosynthate. Forming peanut pods requires more nitrogen because it forms new cells composed of photosynthate. The ability of peanut plants to accumulate photosynthate when filling the pods is a factor that influences the formation of complete pods (Arsovski et al., 2018; Yavari et al., 2021). Nitrogen nutrients given during the pod-filling phase at the right dose have better pod yields with seeds inside. The ability of peanut plants to accumulate photosynthate when filling the pods is a factor that influences the formation of complete pods. Nitrogen is the structural component of protein and amino acids used by peanut plants for seed formation (Ahanger et al., 2021). Urea contains high nitrogen, namely 46%, to increase growth. However, the source of rhizobium did not affect the number of pods and the weight of 1000 seeds. Rhizobium bacteria use organic matter available in the soil for growth and metabolism so that the rhizobium can survive and increase its population (Santachiara et al., 2019).

CONCLUSION

The combination of 100 kg ha^{-1} of nitrogen with a source of used peanut rhizobium showed the highest number of plant leaves. The optimum nitrogen fertilizer dose is 44 kg ha^{-1} for nodule growth. The 50 kg ha^{-}1 nitrogen dose produced the highest pods and seed weight. Nitrogen fertilizer at the correct dose can increase peanuts' growth, nodulation, and yield in acid soil. However, a dose that is too high may cause antagonism with the nodulation process and reduced yield.

AUTHORS CONTRIBUTIONS

DS and S designed and conceived the experiments. DS and RNP experimented. DS, S, and RNP contributed to the preparation of samples and interpretation of the results. The manuscript was primarily composed by DS. All authors provided critical feedback and contributed to developing the research, analysis, and manuscript.

COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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