

The effects of organic and inorganic fertilizers on growth, activity of nitrate reductase and chlorophyll contents of peanuts (*Arachis hypogaea* L.)

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Abstract. The aim of this research was to study the growth and physiology of peanuts on different doses of cow manure and NPK 16 fertilizers. The study was carried out in research field of the Laboratory of Ecology and Plant Production, Agriculture Department, Faculty of Animal and Agricultural Sciences Diponegoro University Semarang Indonesia. The design used was Split plot. As the main plot was doses of cow manure (0, 5 and 10 tons / ha), subplots was NPK 16 fertilizer, namely 0, 25, 50, 75, and 100 kg N / ha, with three times replications. Parameters observed were plant height, number of leaves, activity of nitrate reductase (ANR) and chlorophyll contents. Parameters were observed, then analyzed for variance and continued with Duncan's multiple range test based on Steel and Torrie (1990). There was interaction between manure dosage and NPK 16 to total chlorophyll content of leaves. Dose of cow manure and NPK 16 each significantly affected for plant height, ANR and total chlorophyll but no significantly effect on number of leaves. Cow manure and NPK 16 affected on increasing of plant height, ANR and total chlorophyll contents of peanuts.

Keywords: organic fertilizer, cow manure, inorganic fertilizer, NPK 16, peanut plant

1. Introduction

Peanut (*Arachis hypogaea* L.) is one of world's largest legume crop which ranks second after soybeans. Peanut as one of the main commodities of Indonesia which its source of vegetable protein is important for food diversification in supporting national food security. Worldwide, peanut has its own importance due to largest source of edible oil and its high nutritional value of kernel as human food, and haulm as animal feed. In addition, peanut is world's largest source of edible oil, ranks 13th among the food crops and 4th among oilseed crop [1]. So that, it potential to be developed because it has high economic value and considerable domestic and international market opportunities. As a protein-producing plants, peanuts need sufficient amount of nitrogen. Fertilizer is applied to meet the needs of essential nutrient elements, such as nitrogen. Olajide and Igbeka, [2] reported that peanut is legume plants that are also able to fix air nitrogen, due to its symbiotic relation between peanut roots with several kind of N-fixation bacteria. Maximum nutrient absorption is expected to increase the peanut production. However, harvested area and peanut production in Indonesia have decreased in the 2013-2017 period. The harvested area has decreased by 8.36% and the average production has decreased by 9.06% [3]. Those average productivity is lower than its production potential and still cannot meet national consumption. Hence, efforts should be aimed to remove the constraints responsible for its poor productivity.

The decline in production is partly caused by reduced field area and insufficient supply of essential nutrient elements. The supply of essential elements is managed by applying fertilizer.

Deficiencies of essential nutrient elements have become the main factor that caused yield losses [4]. The elements needed as a key factor in increasing growth, yield, and quality and also as a factor that responsible for reducing the negative impact of environment [5]. It is well established fact that there is positive correlation between fertilizer use and productivity. About 50% increase in agricultural production in recent years can be attributed to fertilizers [6]. Therefore, supply of essential nutrient elements is considered as one of the basic needs to achieve the potential yield. Plants take nutrients mostly from the soil. It is also known that the optimal growth of plants is not only caused by the total amount of nutrients in the soil but also influenced by physico-chemical-biological properties of soil such as: soil texture, organic matter, cation exchange capacity, pH, electrical conductivity and activity of soil microbes [7]. Chemical fertilizers acts as beneficial input to get higher crop productivity, but high dose on chemical fertilizers is associated with reduction in some soil properties and crop yields over time [8]. While, the organic fertilizer, such as cow manure has positive effects in maintaining the soil properties.

Cow manure has capacity to improves soil conditions, such as increasing the pH in acid soils, increasing soil water-holding capacity, hydraulic conductivity and infiltration rate, and reducing soil bulk density. Manure also is a good source of plant nutrients and improves soil structure [9, 10, 11]. Moreover, Ould-Ahmed et al. [12] suggest that manure is an efficient compound for sandy soil with saline water irrigation. Hence, an integrated use of inorganic fertilizers with organic fertilizers; cow manure is a sustainable methods for efficient nutrient usage which enhances efficiency of the chemical fertilizers and also can improve the properties of soil [13]. The application of organic materials will improve physical, chemical and biological soil properties, while inorganic materials will improve the chemical soil properties, such as sufficing the supply of macro and micro essential nutrients to meet crop needs [14]. The availability of nutrients by organic and inorganic fertilizers is needed for promoting plant growth and production. Plant growth can be measured from several indicators including plant height, number of leaves, activity of nitrate reductase (ANR) and total chlorophyll content.

Recently, farmers tend to used chemical fertilizers individually for adequating the needs of plant nutrient elements. Due to they are more economical, affordable, easy to use and quick in response. While, organic fertilizer performed slower response on crop yield, eventhough they are good in maintaining soil properties. So, it is important to perform mid-way technique between organic and inorganic extremities that may sustain food availability without deteriorating soil fertility and/or productivity. Taking all these aspects in consideration, the present study was therefore conducted to evaluate the effects of application of cow manure and NPK 16 fertilizer on growth, yield and physiological responses of peanut plants.

2. Materials and Methods

A field experiment was conducted at the experiment field (Lat. 110°16'20"- 110°30'29"N and Long. 6°55'34"- 7°07'04" E, altitude approximately 125 m above mean sea level) and laboratory of plant ecology and production, agriculture department, faculty of animal and agriculture sciences, Diponegoro university. Daily climate parameters were recorded by automatically wheather system (AWS) located at the field area. The average annual rainfall varies from 450 mm to 500 mm. The mean daily maximum and minimum temperatures during the growing season of peanut fluctuated between 29.7 to 37.9⁰ C and 14.6 to 26.6⁰ C, respectively. Similarly, mean daily relative humidity ranged between 45 to 91%.

2.1 Research materials

The materials used in the experiments were peanut seeds Var. Jerapah, cow manure, NPK 16 fertilizer, total chlorophyll and ANR reagents. The tools used were hoes, digital scales, mortar, and spectrophotometer.

2.2 Crop establishment and experimental design

Peanut seeds were planted in the 500 m² field. The experiment field were divided into 45 plots, each plot is 2 x 2 m in size with 0.5 m in space between each plot. NPK 16 fertilizer and cow manure were used during fertilization as plant treatments. Cow manures were obtained from the waste of cattle farms of Diponegoro University. Peanut seeds were planted 1 week after applying cow manure. The plant spacing is 20x20 cm with 2 seeds in each hole. The experimental design used in this study is split plot; with the cow manure treatment as main plot and NPK 16 treatment as sub plot.

2.3 Fertilizer treatments

Two different sources of fertilizer were used as treatments, organic source was a cow manure and inorganic source was a NPK 16. Fertilization using cow manure was conducted one week before planting with the doses of 0, 5, 10 ton/ha. While, fertilization using NPK 16 was applied twice; at one week after planting and the end of vegetative phase (21 DAP) with the doses of 0, 25, 50, 75 and 100 kg/ha.

2.4 Observed parameters and statistical analysis

Observed parameters were plant height, number of leaves, total chlorophyll content and activity of nitrate reductase. The plant height and number of leaves were observed at 49 DAP, while total chlorophyll content and activity of nitrate reductase were measured during vegetatif phase (25 DAP). The data obtained were processed according to variance analysis to show the effects of treatment on the observed parameters and continued with Duncan's Multiple Area Test to see differences between treatments [15].

3. Results and Discussion

3.1 Plant growth responses as affected by organic and inorganic fertilizer

3.1.1 Plant height

Separately, the application of organic and inorganic fertilizer showed significant effect on plant height. While, the interaction between those treatment had not significant effect on plant height. Under organic fertilizer; cow manure treatment, plants improved its height. Among the doses level of cow manure, 10 ton/ha dose generated the highest plant height. However this responses were not significant different with those 5 ton/ha dose treatment (Table 1). So that, under lower dosage which is 5 ton/ha, could increase the plant height. On the other hand, differential responses of plant height also performed by inorganis fertilizer; NPK 16 treatments. Under 75 kg/ha dose, plants generated the highest plant height. While, under the higher dose which is 100 kg/ha, the plants height were not significant different with those 75 kg/ha dose treatment (Table 1). Similar results have been carried out in rice-wheat cropping system [16] and in soybean [17]. Generally, plants increase the growth by application of fertilizer treatments. Improvement of plant growth was significantly found by the application of both organic and inorganic source of mineral nutrition.

Table 1. Plant height of peanut plants due to application of cow manure and NPK 16 fertilizer at 7 weeks after planting

Cow Manure	NPK 16					Mean
	0 kg/ha	25 kg/ha	50 kg/ha	75 kg/ha	100 kg/ha	
	----- cm -----					
0 ton/ha	6,6	7,1	6,4	7,0	7,1	6,84 ^b
5 ton/ha	6,7	7,2	7,7	8,7	8,6	7,78 ^a
10 ton/ha	8,2	7,0	7,8	8,3	8,0	7,86 ^a
Mean	7,16 ^c	7,10 ^c	7,30 ^{bc}	8,00 ^a	7,9 ^{ab}	

* Mean values followed by same letter within column did not differ significantly according to Duncan's multiple range tests (P < 0.05).

Application of cow manure could enhanced the soil organic matters. Soil organic matters improve soil structure and at the same time, increased the availability of nutrient source. Availability of organic matter also contribute to crop growth and yield directly by supplying nutrients and indirectly by modifying soil physical properties such as stability of aggregates and porosity that can improve the root growth, rhizosphere and stimulate plant growth [18]. Moreover, manure application also affects to accumulation of macro-aggregate protected carbon and nitrogen. Plant growth rates are influenced by elements of nitrogen (N), phosphorus (P) and potassium (K). N element as a constituent of chlorophyll, division and enlargement of cells in the apical meristem. Activity of the apical meristem generates the shoot growth, so that affects to the increasing of plant height.

3.1.2 Number of leaves

Application of cow manure and NPK 16 were not significant on number of leaves. Similarly, the interaction between those treatments also had no significant effect on number of leaves (Table 2). Similar result also showed in *Brassica napus* L. that supplemented with organic fertilizer. A non-significant organic fertilizer treatment effect was revealed from weeks 1 to 8 [19].

Table 2. Number of leaves of peanut plants due to application of cow manure and NPK 16 fertilizer at 7 weeks after planting

Cow Manure	NPK 16					Mean
	0 kg/ha	25 kg/ha	50 kg/ha	75 kg/ha	100 kg/ha	
	----- blade -----					
0 ton/ha	16	19	16	17	17	17
5 ton/ha	16	17	16	15	16	16
10 ton/ha	16	15	15	17	14	15,4
Mean	16	17	15,67	16,33	15,67	

Naturally, cow manure provide the organic matters to soil. Mineralization process of the organic matters release the nutrient elements source that can be used to generate plant growth. However, mineralization process take long time. Organic fertilizers releases nutrients more slowly but store them longer in the soil [20], thereby ensure a long residual effect [21]. Application of cow manure is able to provide nutrients for leaf formation [22]. The nutrients from organic fertilizer support rapid root development [23] which might have enhanced leaf growth towards the end of plant life. Since organic fertilizer constitutes a slow release source of plant nutrients [24, 25, 26], so that its nutrient elements had not shown a positive effects on the number of leaves. Plant tend to utilized N elements that available from the soil.

3.2 Physiological change of peanut plant as affected by organic and inorganic fertilizer

3.2.1 Activity of nitrate reductase

The activity of nitrate reductase enzyme (NR; EC1.6.6.1), the first enzyme involved in the assimilation of N-NO₃⁻, is crucial for the development of plants and plays a key role in the assimilation of N [27]. Application of cow manure and NPK 16 significantly effects the activity of nitrate reductase. However, the interaction between those treatments had no significant effect on it. Cow manure treatment effect on differential activity of plant nitrate reductase. The highest activity of nitrate reductase are generated by application 5 ton/ha cow manure dosage. Under 5 ton/ha dose could improved the activity of nitrate reductase as high as 4,339 micromol NO₂/g/hour. Separately, NPK 16 also could improved the activity of nitrate reductase. Application 75 kg/ha dose of NPK 16 resulted the highest activity of nitrate reductase, however this result were not significant different with those 25 kg/ha dose (Table 3). Under 25 kg/ha dose could improved the activity of nitrate reductase as high as 3,710 micromol NO₂/g/hour. Giving small amount of N fertilization can be more beneficial for

stimulating plant growth. Since, small amount of N able to improve plant growth and photosynthetic rates a without inhibition of root nodule formation. root nodule formation [28].

Table 3. Activity of nitrate reductase of peanut plants due to application of cow manure and NPK 16 fertilizer

Cow Manure	NPK 16					Mean
	0 kg/ha	25 kg/ha	50 kg/ha	75 kg/ha	100 kg/ha	
	----- micromol NO ₂ /g/hour -----					
0 ton/ha	2,720	2,962	1,849	2,791	2,251	2,515 ^c
5 ton/ha	4,583	4,415	4,383	4,378	3,935	4,339 ^a
10 ton/ha	2,912	3,753	3,586	4,539	3,080	3,498 ^b
Mean	3,405 ^b	3,710 ^{ab}	3,273 ^b	3,903 ^a	3,089 ^b	

* Mean values followed by same letter within column did not differ significantly according to Duncan's multiple range tests (P < 0.05).

Cow manure contributed to the accumulation of nitrate reductase activity. Mineralization of organic matters from cow manure increase the availability of N elements. Positive effects of organic fertilization on activity of nitrate reductase also be found in soybean and shorgum plants that applied with several kind of manures in vertisols of semi-arid tropics [29] and in saladette tomato that applied with vermicompost tea [30]. Activity of nitrate reductase depends on the supply of carbohydrates as a final result of photosynthesis. Then it further be used in the respiration process to generate energy. Nitrate reductase biosynthesis is affected by availability of nitrogen in the cultivation media, and its activity is induced by the present of nitrate in the leaves [31]. An increase in the amount and activity of NR leads to a corresponding increase in the potential for nitrate reduction and confers a greater capacity for general aminoacid synthesis, protein synthesis, or total N assimilation [32].

3.2.2 Accumulation of total chlorophyll content

Different doses of cow manure and NPK 16 had significant effect on accumulation of total chlorophyll content. The interaction between this two treatments also showed significant effect on it. There were not significant effects on total chlorophyll content under no application of cow manure and NPK 16. Total chlorophyll content significantly decreased under application of 5 ton/ha cow manure and NPK 16. Among 5 ton/ha cow manure treatments, the highest chlorophyll content was generated by application of 5 ton/ha without NPK 16 fertilizer, with an increase of 0.410 mg/g. This result was significantly different from the rest NPK 16 treatment; 25, 50, 75 and 100 kg/ha. Under 10 ton/ha cow manure treatment, application of NPK 16 up to 75 kg/ha decreased the total chlorophyll content. However, NPK 16 by 100 kg/ha dose significantly increased the chlorophyll content of leaves with an increase of 0.540 mg/g. This result which was significantly different from NPK 16 under the doses of 25, 50 and 75 kg/ha. Similar experiment was conducted in peanut at seedling stage, the chlorophyll content in the seedling stage increased with the increase of the peanut biochar-based fertilizer application[33]. Another similar result also be found in maize and shorgum plants [34] and in soybean [35], total chlorophyll content was increased by increasing dosage of inorganic fertilizer that supplemented with organic fertilizer. Application of cow manure without NPK 16 could not adequate the nutrient supply that responsible for chlorophyll biosynthesis. However, supplementation of cow manure on the application of NPK 16 significantly improved the nutrient absorption, so that chlorophyll biosynthesis was optimized. A high amount of chlorophyll is required to maintain photosynthetic pigments, and synthesize the enzymes that take part resulting in increased growth and yield of peanut plants.

Table 4. The total chlorophyll content of peanut plants due to application of cow manure and NPK 16 fertilizer

Cow Manure	NPK 16					Mean
	0 kg/ha	25 kg/ha	50 kg/ha	75 kg/ha	100 kg/ha	
	-----mg/g-----					
0 ton/ha	0,409 ^b	0,335 ^{bc}	0,320 ^{bcd}	0,341 ^{bc}	0,353 ^{bc}	0,3516 ^a
5 ton/ha	0,410 ^b	0,222 ^d	0,252 ^{cd}	0,287 ^{cd}	0,270 ^{cd}	0,2882 ^b
10 ton/ha	0,301 ^{cd}	0,312 ^{bcd}	0,343 ^{bc}	0,345 ^{bc}	0,540 ^a	0,3682 ^a
Mean	0,3733 ^{ab}	0,289 ^c	0,305 ^c	0,3153 ^{bc}	3,3877 ^a	

* Mean values followed by same letter within interaction column did not differ significantly according to Duncan's multiple range tests ($P < 0.05$).

Application of cow manure with NPK 16 improved soil physical, chemical and biological properties, hence the plant nutrients absorption was optimized [14]. The suitability and usefulness of organic fertilizers has been attributed to high availability of NPK content [36], which capable to enhance soil fertility [37]. They also act as a substrate for soil microorganisms which lead to increase microbial activity. High microbial activity result in increasing the rate of organic material decomposing and releasing nutrient for plant uptake.

Synergistic application of both fertilizer may served adequate nutrition that involved in chlorophyll biosynthesis. Chlorophyll biosynthesis are influenced by light, carbohydrate, temperature, genetic and nitrogen availability. The addition of NPK nutrients can increase plant growth and production because these elements can stimulate roots, strengthen plant stems and increase photosynthesis rates. N element contributes for high growth and production of plant leaves. Availability of N is sufficient to increase chlorophyll biosynthesis, leaf organ formation and assimilation [38].

4. Conclusion

It was concluded that different doses of cow manure and NPK 16 affected the growth and physiology of peanut plants. Application of 10 ton/ha cow manure and 100 kg/ha NPK 16 fertilizer showed the highest plant height, total chlorophyll content and ANR. Hence, might be used as recommendation dosage in peanut plants cultivation.

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