Jurnal Ilmu Pertanian Indonesia (JIPI), January 2026
(Indonesian Journal of Agricultural Sciences)
ISSN 0853-4217 EISSN 2443-3462

Vol. 31 (1): 17−22
http://journal.ipb.ac.id/index.php/JIPI
DOI: 10.18343/jipi.31.1.17

Effect of Ammonium Sulfate on Flower and Pod Development of Soybean [*Glycine* max (L.) Merr.]

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(Received April 2024/Accepted September 2025)

ABSTRACT

Soybean [*Glycine max* (L.) Merr.] is a food crop rich in vegetable oil and protein, with increasing demand and economic worth. Increasing soybean production can be accomplished by providing nutrients nitrogen and sulfur, hence increasing the percentage of bloom development into pods. Applying ammonium sulfate at the start of planting can fulfill nitrogen and sulfur fertilizer requirements. The purpose of this study was to examine and determine the ideal concentration of ammonium sulfate for optimal flower and pod formation, as well as the percentage of flower development into pods and leaf sulfur content. Ammonium sulfate was applied in an experimental garden by mixing it into the planting material in polybags under controlled settings. This study employed a single-factor fully randomized design with four doses of ammonium sulfate. The treatments were ammonium sulfate [$NH_4)_2SO_4$] at various concentrations of 0, 100, 200, and 300 kg/ha administered at the start of planting, with five replications. The research factors were the quantity of flowers, pods, percentage of flower development into pods, and sulfur level in the leaves. The sulfur content of the leaves was determined using an atomic absorption spectrophotometer. Data were analyzed using ANOVA at a significance level of 5%, followed by DMRT if the results were significant. The results revealed that ammonium sulfate 300 kg/ha produced the highest number of flowers, pods, percentage of flower development into pods, and sulfur content in leaves.

Keywords: ammonium sulfate, nitrogen, productivity, soybean, sulfur

INTRODUCTION

Soybean (*Glycine max* [L.] Merr.) is a crop that produces vegetable oil and is a key source of protein with great economic value (Liu *et al.* 2020). It is a popular staple food crop grown all over the world (Pagano & Miransari 2016). Soybeans continue to be in high demand due to their nutritional content and health benefits, are rich in protein, oil, carbohydrates, sugars, sucrose, fatty acids, fiber, vitamins, and triglycerides (Sharma *et al.* 2014). Over the last 15 years, soybean demand has increased due to rising needs in both domestic and foreign markets (Nair *et al.* 2023).

Tyner and Francis (2017) attribute low soybean productivity to inadequate soil fertility and a low percentage of blooms maturing into pods (Sufianto 2011). They found that only 6.6−10% of flowers grow into pods. Incomplete pollination, asynchronous flowering, significant flower output above the fourth node, and underground pod formation all contribute to this low proportion. These issues can be addressed through fertilization, which includes the use of ammonium sulfate. Tyner and Francis (2017) described ammonium sulfate ($(NH_4)_2SO_4$) as an inorganic salt that effectively fertilizes soil. However, no specific studies have confirmed its usefulness on soybean plants. Because ammonium sulfate is water-soluble, plants can easily access nitrogen (ammonium) and sulfur (sulfate) early in the growth cycle. It has 21% nitrogen and 24% sulfur. Sulfur is an essential ingredient for plant growth, development, and physiological functions such as respiration, photosynthesis, nitrogen fixation, and protein and lipid synthesis (Saleem *et al.* 2023). Sulfur can be applied by using ammonium sulfate (Powlson & Dawson 2022). Nitrogen is a vital ingredient for plant growth and influences soil fertility. Nitrogen is easily lost from the soil due to its highly mobile nature, which limits its availability. As a result, early nitrogen fertilizer is critical in soybean production since the topsoil lacks moisture, and the roots have not yet developed optimally (Puja *et al.* 2020).

This study used soybean plants, with the expectation that the addition of ammonium sulfate as a base fertilizer would improve soybean productivity. The aim of this experiment was to analyze the effect of ammonium sulfate fertilization on flower production, pod development, and the percentage of flower-to-pod conversion, as well as to determine the optimal sulfur concentration for soybean plants. The application of ammonium sulfate at the beginning of planting is expected to enhance soybean productivity. This study used soybean plants, with the idea that adding

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ammonium sulfate as a base fertilizer would increase soybean yield. The purpose of this study was to investigate the impact of ammonium sulfate fertilization on flower output, pod development, and the percentage of flower-to-pod conversion, as well as to establish the best sulfur content for soybean plants. The application of ammonium sulphate at the start of planting is expected to increase soybean productivity.

METHODS

This study was carried out from February to June 2023 at the experimental field and the Laboratory of Plant Structure and Function Biology, Department of Biology, Faculty of Science and Mathematics, Diponegoro University. The sulfur content of soybean leaves was measured at Gadjah Mada University's (UGM) Integrated Research and Testing Laboratory (LPPT) in Yogyakarta. The study used a Completely Randomized Design (CRD) with a single factor and four ammonium sulfate treatment levels (0, 100, 200, and 300 kg/ha), each replicated seven times.

Materials and Equipment

This study utilized 30 × 45 cm polybags, labels, weighing scale, laboratory glassware, ziplock plastic bags, freezer, test tubes, filter paper, sieve, blender, UV-Vis spectrophotometer, and atomic absorption spectrophotometer (AAS). The materials used in this research included Grobogan soybean seeds obtained from the Indonesian Legumes and Tuber Crops Research Institute (Balitkabi), planting media, ammonium sulfate solution $(NH_4)_2SO_4$, 80% acetone, distilled water, concentrated nitric acid (HNO_3), hydrochloric acid (HCl), potassium sulfate (K_2SO_4), copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), and boric acid (H_3BO_3).

Seed Selection and Nursery Preparation

Seed selection for the Grobogan soybean variety involved selecting seeds that were plump, not shriveled, free of flaws, clean of dirt, disease-free, and somewhat consistent in size. The seeds employed in this investigation were viable, as demonstrated by their capacity to sink in water (Azmi *et al.* 2020). The selected seeds were steeped in water for about an hour before being placed in seedling trays. Following germination, the seedlings were given 7 days to grow before being transplanted. Seedlings were chosen for consistency in height (about 4 cm) and leaf count (5 leaves) to guarantee consistent growth across experimental units.

Planting Media Preparation

The planting media was made up of soil, compost, and rice husk in a 2:1:2 ratio. The components were properly combined and sieved to ensure a homogeneous texture. Polybags were labelled with treatment codes, and each bag contained 5 kg of the prepared planting medium. Wahyudi *et al.* (2022) used ammonium sulfate ($(NH_4)_2SO_4$) treatments in powder form at doses of 0, 100, 200, and 300 kg/ha, 7 days before planting.

Planting, Maintenance, and Observation

Seedlings were transplanted into 30 × 45 cm polybags holding the planting media, with a depth of around 2 cm. Weeding, watering, and additional fertilizing were all part of the maintenance tasks. Weeding was done at least twice or as needed based on the weed conditions, and watering every two days with 300 mL of water per polybag. Fertilizers used included SP-36 at 75 kg/ha, KCl at 50 kg/ha, and urea at 50 kg/ha, administered at planting and again at blooming. Fertilization was accomplished by creating small holes between the soybean plants, inserting fertilizer into the holes, and then covering them with soil. The quantity of flowers was counted manually when blossoming commenced. The number of pods was carefully counted when pod formation began.

Analysis of Sulfur Content in Soybean Leaves

The analysis was carried out at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University (UGM) in Yogyakarta. Dried leaf samples were pulverized into a fine powder and placed in an Erlenmeyer flask. To digest the sample, a 4:1 solution of 65% HNO_3 and 37% HCl was added (5 mL total). The mixture was heated on a hot plate to 160 °C until it turned transparent. The digested sample was then diluted with distilled water to a final volume of 10 mL and put to a 15-mL conical tube. The content of sulfur in the sample was measured using AAS. The sulfur content in the leaves was determined in mg/g of dry weight by multiplying the measured sulfur absorbance by the dilution volume (L) and dividing by the sample's dry weight (g), as stated by Nugroho and Frank (2012).

Data Analysis

The acquired data were analyzed using Analysis of Variance (ANOVA) with a significance level of 5%. If the ANOVA results showed significant differences, Duncan's Multiple Range Test (DMRT) was used with the same 5% significance threshold.

RESULTS AND DISCUSSION

Number of Flowers

Table 1 shows the number of soybean flowers produced at different ammonium sulfate concentrations. According to the ANOVA results, ammonium sulfate application had a significant effect on the quantity of flowers in soybean plants. The treatment with 300 kg/ha ammonium sulfate yielded the highest average number of flowers. DMRT analysis revealed that the 300 kg/ha treatment differed considerably from the 0, 100, and 200 kg/ha treatments. These findings suggest that 300 kg/ha of ammonium sulfate is the best dose for boosting blossom yield. The number of flowers increased in proportion to the ammonium sulfate concentration. According to Bagale (2021), excessive ammonium sulfate application might have a deleterious impact on soybean plants, causing nutritional poisoning. The number of flowers in treated plants (100, 200, and 300 kg/ha) increased from 20% to 111% when compared to the control. This improvement is most likely due to the delivery of vital nutrients during the blossoming period.

The use of ammonium sulfate, which includes both nitrogen and sulfur, enhanced the number of flowers in soybean plants. The addition of nitrogen in the form of ammonium is absorbed by the roots and converted into amino acids (Wang *et al.* 2014). According to Mastur *et al.* (2016), amino acids are converted into proteins, which subsequently become components of protoplasm in cells that create plant organs such as stems. These cells differentiate, leading to increasing stem height. The apical meristem is responsible for plant elongation, as well as the development of branches and leaves. When there is enough nitrogen, environmental changes and some internal conditions can cause the apical meristem to differentiate into floral buds. According to Zhang *et al.* (2021), nitrogen promotes flowering. Nitrogen is absorbed as ammonia and nitrates. Nitrogen assimilation produces glutamine (Gln), which affects the expression of the nitrogen-mediated heading date-1 gene. Increased Gln levels increase the expression of Nhd1, which directly activates the florigen gene heading date 3a (Hd3a), encouraging blooming. This also inhibits the production and activity of Fd-GOGAT, resulting in a negative feedback loop in nitrogen assimilation regulation.

Annisa and Gustia (2018) described that flowering is affected by both internal and extrinsic stimuli. Internal factors include those inherent in the plant, such as phytohormones and heredity, whereas external factors include environmental conditions including light, humidity, light intensity, temperature, and nutrient availability. Putriantari and Santosa (2014) found that nitrogen fertilization promotes vegetative development, including an increase in the number of branches. A plant's ability to produce blossoms increases as its number of branches grows. This finding is consistent with study by Kartika *et al.* (2016), that nitrogen administration boosted blossom production in basil (*Ocimum basilicum* L.).

Sulfur also helps with meristem differentiation and flower formation because of its role in protein synthesis and general plant growth. However, its impact on meristem differentiation and flower production is less pronounced than that of nitrogen, because sulfur predominantly stimulates the formation of essential plant components such as enzymes and proteins (Saleem *et al.* 2019). Yue *et al.* (2020) discovered that eggplant plants treated with sulfur (CS_2) flowered earlier than untreated plants by the 10th day, indicating that sulfur has a function in increasing flower production.

Number of Pods

Table 1 also shows the number of soybean pods at different ammonium sulfate concentrations. According to ANOVA, ammonium sulphate application had a significant effect on pod numbers. The treatment with 300 kg/ha ammonium sulfate produced the highest average number of pods. DMRT analysis revealed that the 300 kg/ha treatment differed considerably from the 0, 100, and 200 kg/ha treatments. As a result, 300 kg/ha of ammonium sulfate is thought to be the ideal concentration for enhancing soybean pod production.

It is anticipated that 300 kg/ha ammonium sulfate provides enough nitrogen and sulfur nutrients in the planting medium to promote pod development. The data revealed that ammonium sulfate had a considerable effect on pod development. The 300 kg/ha treatment produced the largest average number of pods per plant. These findings are comparable with those of Puspasari *et al.* (2018), that nitrogen fertilizer could boost soybean pod yield. The rise in pod number corresponds to the increased nitrogen supply. Lošák *et*

Table 1 Number of flowers, number of pods, and percentage of flower-to-pod development at different ammonium sulfate concentrations

Ammonium sulfate (kg/ha)	Number of flowers	Number of pods	Flower to pod development (%)
0	27.60±0.82^{c}	15.80±1.09^{d}	57.25±0.47^{cd}
100	33.00^{c}±0.71^{c}	22.20±0.87^{c}	67.27±0.90^{c}
200	37.80±1.30^{b}	26.60±1.00^{b}	70.37±0.82^{b}
300	41.20±3.42^{a}	32.00±1.47^{a}	77.67±0.96^{a}

Remarks: Values followed by the same letter in the same column are not significantly different based on Duncan's Multiple Range Test (DMRT) at a 5% significance level.

al. (2018) found that nitrogen treatment affects the chemical composition of seeds, with lower levels increasing oil content and greater levels increasing protein. Sulfur also affects pod development. According to Barczak *et al.* (2014), vitamins, amino acids, and proteins all include sulfur. Its presence promotes protein manufacturing in the seed, resulting in normal pod growth.

Percentage of Flower to Pod Development

The ideal dose for promoting flower-to-pod growth was 300 kg/ha of ammonium sulfate, which reached 77.67% (Table 1). DMRT results revealed that the 300 kg/ha treatment differed considerably from the 0, 100, and 200 kg/ha treatments. The percentage of flower-to-pod conversion rose as the ammonium sulfate concentration increased. This enhancement was related to the presence of nitrogen and sulfur during the pod formation phase. Pods are the organs responsible for producing soybean seeds, which reflect the soybean plant's final yield. Lewar *et al.* (2020) found that the number of pods produced is affected by certain nutrients involved in bloom development. These nutrients help plants grow flowers, mature seeds, produce proteins, and synthesize bioactive chemicals. Lehar *et al.* (2016) discovered that nitrogen (N) and sulfur (S) play critical roles in pod growth. The inclusion of macronutrients and micronutrients in the planting medium increases soil fertility and overall plant productivity.

Sulfur Content in Leaves

Figure 1 depicts the sulfur content of soybean leaves at different ammonium sulfate concentrations. ANOVA revealed that the administration of ammonium sulfate had a significant effect on sulfur content in soybean plant leaves. The DMRT results showed that the 300 kg/ha ammonium sulfate treatment differed considerably from the 0, 100, and 200 kg/ha treatments. Thus, the 300 kg/ha concentration was deemed best for enhancing sulfur content in the leaves of the Grobogan soybean variety. This is probably because ammonium sulfate contains 24% sulfur (Tyner & Francis 2017). The highest sulfur concentration was found in the 300 kg/ha treatment, at 1814.51 mg/kg, while the lowest was in the 0 kg/ha treatment, at 1544.10 mg/kg. The treated plants' leaf sulfur content increased by 7% to 18% as compared to the control. This data is similar with Gyori (2005), that the average sulfur concentration in 13 winter wheat cultivars was around 1500 mg/kg, with varietal variances of up to 120 mg/kg. These findings are consistent with the current investigation, in which the average sulfur concentration in control soybean leaves was 1544.10 mg/kg.

Conferring to this study, the plants did not exhibit any undesirable signs caused by high sulfur deposition, such as leaf yellowing or premature leaf drop. The sulfur accumulating in the plants had no harmful effect on growth, even though the total sulfur content in the leaves varied greatly between the highest and lowest treatment levels. Rakesh *et al.* (2020) revealed that plants' sulfur requirements differ based on their genetic features. Even within the same plant species, sulfur requirements might vary greatly amongst cultivars. Sulfur concentration is typically higher in oilseed crops and legumes, such as soybeans. The average yield increase in legumes owing to sulfur application ranges between 168 and 428 kg/ha.

CONCLUSION

The application of 300 kg/ha ammonium sulfate significantly boosted soybean plant output, including the number of flowers, pods, percentage of flower-to-pod development, and the optimum sulfur content in

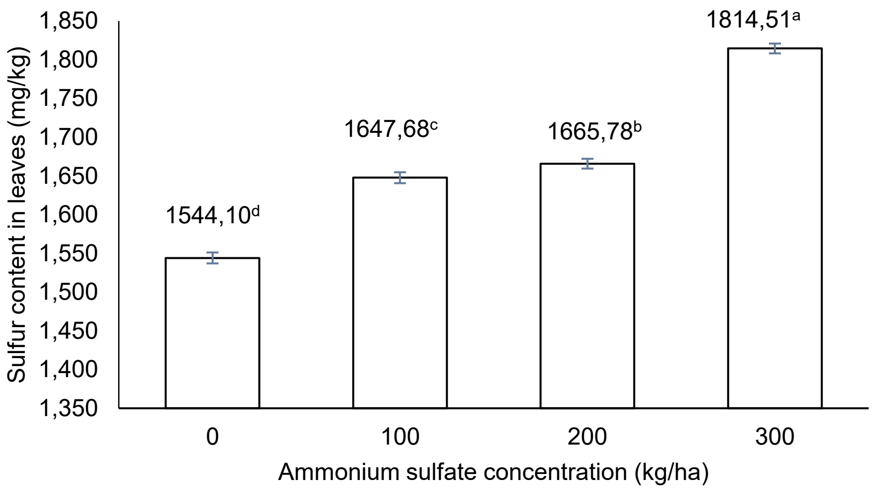

Figure 1 Sulfur content in soybean leaves at different ammonium sulfate concentrations.

the leaves. The recommended ammonium sulfate concentration for soybean cultivation is 300 kg/ha.

ACKNOWLEDGEMENTS

The author would like to thank Diponegoro University's Faculty of Science and Mathematics for funding research under the 2023 State Budget (APBN) Research Grant, Fiscal Year 2023, under Grant Number: 24.C/UN7.F8./PP/II/2023.

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