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Equal Substitution of Synthetic Nitrogen Fertilizer with Azolla Compost on Growth, Yield, and Nitrate Content of Green Onion in Ultisols

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ABSTRACT

The impact of Azolla compost (AC) substitution for synthetic N fertilizer (NF) on nitrate leaf content and agronomic performances of green onion in Ultisols have not been well understood. Therefore, this study aimed to assess the effects of equal replacing NF with AC on growth, yield, and nitrate accumulation in the green onion leaf. The polybag trial was performed in June 2020 on the crop research farm of the Agriculture Faculty in a completely randomized design with six treatments in five replications. Green onion was subjected to five 92 kg ha^{-1} N equal combinations of NF and AC, i.e., NF_{100} (100% NF+0% AC), NF_{75} +AC_{25} (75% NF+25% AC), NF_{50} +AC_{50} (50% NF+50% AC), NF_{25} +AC_{75} (25% NF+75% AC), and NF_0 +AC_{100} (0% NF+100 % AC) and no NF and AC as a control. Results demonstrated that NF+AC treatments significantly improved plant height, leaf number, tiller number, stem diameter, and plant fresh weight. Treatment of NF_{25}+AC_{75} resulted in similar responses to the recommended dose of N fertilizer, as evidenced by plant height, number of green leaves, root fresh weight, shoot dry weight, tiller numbers per hill, stem diameter, shoot fresh weight, and plant fresh weight. Lower leaf nitrate contents occurred under increased substitution of NF with AC. Thus, partial substitution of NF with AC could be a sustainable option for improving growth and yield while reducing nitrate accumulation in the leaf of green onion in Ultisols.

INTRODUCTION

Green onion, also known as spring onion or scallion (*Allium fistulosum* L.), is one of the most widely cultivated Allium species. All parts of this plant are edible and have antibacterial and therapeutic properties beneficial to health (Lesjak *et al*., 2018). In addition, due to its distinctive taste, green onion is widely used as a seasoning component in various foods. This condition makes the demand for green onions in Indonesia continue to increase. The increase is predicted to be about 1.2% annually and is estimated to reach 1.7 million metric tons by 2026.

Meanwhile, its production decreased by almost 6.1% in 2022 in comparison to the previous year, which could reach 627,853 tons (Indonesian Statistic Agency, 2019). In Bengkulu, the decline was more pronounced due to the shift of green onion cultivation areas from the highlands to the lowlands mostly dominated by Ultisols acid dry land as compensation for reduced production areas in

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the highlands. The shift of green onion cultivation areas occurred because Ultisols acid dry land is the largest land area in Bengkulu reaching almost 4.57 million ha or accounting for more than 20% of the Bengkulu province area and also in Indonesia covering about 45.8 million ha or around 25% of Indonesia's land area (Subagyo *et al*., 2000).

In general, Ultisols are characterized by high soil acidity (pH 3.5-5.5), low organic matter content (less than 2%), low cation exchange capacity (CEC), and low nutrient content, mainly N, P, and K, and high Al saturation (Brady and Weil, 2008). Meanwhile, green onion is more susceptible to nutrient deficiencies than most leafy vegetables because of its shallow and unbranched root characteristics. This root characteristic is also the cause of the high N requirement (Amare, 2020; Brewster, 1994; Ghodke *et al*.,2018). To compensate for low and imbalanced nutrients, especially N, N fertilizer is generally applied at a higher dose than N required for plant growth and maximum yield (Amare, 2020), meanwhile, the continuous overuse of chemical fertilizers results in serious environmental problems in the long term. The issues included increased soil acidification, soil organic matter depletion, nutrient imbalance, increased salt accumulation, decreased cation exchange capacity in the soils, and ion toxicity especially Al_3^+ (Yang *et al*., 2021), conditions that inhibit cell division rate in roots as well as growth and yield of green onion (Amare, 2020). Therefore, reducing the use of inorganic N fertilizers to the lowest possible rates and compensating them with organic amendments such as Azolla compost is an environmentally friendly approach for crop fertilization that has been well documented by many researchers (Seleiman and Abdelaal, 2018). They further reported that applying composted organic matter increases the solubility and availability of natural P in soil. Organic acids in organic matter are responsible for this mechanism by making Ca-P, Al-P, and Fe-P soluble in protons and complex cations. Besides being able to reduce the use of N fertilizer, previous research reported that Azolla compost-based fertilization increased soil organic matter, acquisition of plant nutrients, and soil microbial activity, which ultimately changes soil characteristics and increases crop yields (Tejada *et al*., 2009).

Azolla (*Azolla microphylla*) is a type of small aquatic fern that thrives in swamps, ponds, and lakes where the water is stagnant. It can bind atmospheric nitrogen by maintaining a symbiotic relationship with Cyanobacteria (i.e., *Anabaena Azollae*) found in the dorsal lobes of its leaves (Singh, 1990). Previous research reported that Azolla compost is very potential for N because of its high nitrogen content (2.41%) (Khozuei *et al*., 2022). Therefore, its addition to the soil is expected to increase N availability, increasing crop productivity (Braun-Howland and Nierzwicki-Bauer, 2018).

Previous research reported that using Azolla compost combined with synthetic N fertilizer could reduce the use of synthetic fertilizers without a significant reduction in crop growth and yield. Applying Azolla compost as an individual organic fertilizer or in combination with synthetic N fertilizer could reduce the N fertilizer use by up to 60% without significantly disrupting rice growth and productivity (Seleiman *et al*., 2022). They further reported that the addition of 40% NPK + 60% Azolla compost resulted in comparable rice grain yield (10.76 t ha^{-1}) as well as N, P, and K content and uptake with the recommended total dose of NPK fertilizer (100% NPK). Another report by Marwanto *et al*. (2023) stated that using Azolla compost-based organomineral fertilizer (AOF) promoted green onion growth, biomass yield, and N uptake. Applying AOF at 2.23 g $plant^{-1}$ plus 50% of N recommended dose produced the highest plant fresh weight per clump (81.48 g). Thus, it is hypothesized that Azolla compost can be used as an alternative fertilizer for the green onion to compensate for the reduced use of synthetic N fertilizer with comparable results.

Excessive use of chemical fertilizers causes the accumulation of nitrates and nitrites in vegetables, causing health risks. The risk of nitrate and nitrite contamination in vegetables can be reduced by applying organic fertilizer. Previous research reported that using organic manure reduced the risk of exposure to nitrate and nitrite contamination in vegetables (Mora *et al*., 2021).

Most studies primarily have been dealing with cereal crop yields. Still, research on the impact of substituting equal amounts of Azolla compost for chemical N fertilizer on green onion is limited. Therefore, the main objective of this study was to assess the effects of replacing equal amounts of synthetic N fertilizer with Azolla compost on the growth, yield, dry matter (DM), and accumulation of nitrate in the leaf of green onion through a polybag trial.

MATERIALS AND METHODS

Experimental Site, Weather Conditions, and Seed Material

A polybag trial was carried out in research area of the Agronomy Department located on the outskirts of the University of Bengkulu campus, Indonesia (Latitude: 30 45"–30 59" S and Longitude: 102022" E) and 15 m above the mean sea level during the summer season of 2020 to assess the effects of replacing equal amounts of synthetic N fertilizer (NF) with Azolla compost (AC) on the growth, yield, and the accumulation of nitrate in the leaf of green onion

Before sowing, initial soil fertility was determined by taking some soil samples collected from acidic surface soil (0–15 cm) belonging to the order Ultisol and analyzing them in the laboratory for the determination of N, phosphorus (P), potassium (K), organic C (OC), and pH using the standard procedures of soil testing. The soil characteristics were determined on bulk soil samples by the Walkley and Black method for OC, the Khejdhal method for total N, the Bray method for available P, flame photometer for exchangeable K, electrometry method for soil pH, the ammonium acetate method for CEC, and EC meter for EC. The initial soil chemical properties are described in Table 1.

Table 1. Initial chemical characteristics of soil

Properties	Values	Criteria
Total-N (%)	0.29	Medium
Available P (ppm)	3.02	Very low
Exchangeable K (me 100 g^{-1})	0.15	Very low
CEC (cmol(+)/kg)	9.70	Low
EC (dS m^{-1})	0.31	Normal
Organic-C (%)	4.09	High
pH H_2O	4.89	Acid

The research area was located under a humid-tropical climate. The climatic data affecting green onion growth during the trial, such as maximum and minimum temperatures, rainfall, and air relative humidity (RH) during the growing seasons of 2020 (June–August), were collected from the local Meteorology, Climatology, and Geophysics Station (ID WMO: 96255). The hybrid F_1 onion seedlings cultivar Fragnant with uniform age and size obtained from the local farmer was used to maintain the maximum uniformity in the trial.

Production and Characteristics of Azolla Compost

Azolla microphylla was collected from the swamps and pools around the Bengkulu University campus, cleaned with running water, air-dried for 24 hours, then oven-dried at 55°C until the moisture content reached about 50%. Approximately 13 kg of the oven-dried Azolla was piled on a plastic mat to a thickness of 10 cm, added with a mixture of 650 g of rice bran and 1300 g of rice husk charcoal, sprayed with a combination of 5.2 ml of EM4 solution, 3.25 g of brown sugar and 1 liter of water and then the plastic mat was covered tightly. Composting was carried out for 14 days, and every two days the plastic mat cover was opened, and the pile was manually turned over. At the end of the composting period, a sample of Azolla compost was taken, and its nutrient content was analyzed in the laboratory to determine N, P, K, OC, and pH using the standard testing procedures (Balittanah, 2005). The Azolla compost chemical characteristics are presented in Table 2.

Experiment Design

The polybag trial was performed in June 2020 at the crop research area of the Agriculture Faculty located on the outskirts of the Bengkulu University campus. In this trial, green onion was subjected to five 92 kg N ha^{-1} equal combinations of NF and AC and no NF and AC as a control. The treatments were as follows: (1) P_0 (control or no NF and AC), (2) P_1 (NF_{100}+AC_0 or containing 92 kg ha^{-1} N from NF or 200 kg ha^{-1} urea or 0.76 g $polybag^{-1}$ urea), (3) P_2 (NF_{75} +AC_{25} or containing 69 kg

Table 2. The analysis of azolla compost used during the polybag trial.

Properties	Values	Criteria
Total-N (%)	4.06	High
Available P (%)	0.35	Very low
Exchangeable K (%)	0.62	Very low
Organic-C (%)	15.66	High
CEC (meq/100 g)	55.00	High
pH H_2O	6.90	High

ha^{-1} N from NF or 150 kg ha^{-1} urea or 0.57 g $polybag^{-1}$ urea and 23 kg ha^{-1} N from AC or 566.5 kg ha^{-1} AC or 2.15 g $polybag^{-1}$ AC), (4) P_3 (NF_{50} +AC_{50} or containing 46 kg ha^{-1} N from NF or 100 kg ha^{-1} urea or 0.38 g $polybag^{-1}$ urea and 46 kg ha^{-1} N from AC or 1,133 kg ha^{-1} AC or 4.31g $polybag^{-1}$ AC), (5) P_4 (NF_{25} +AC_{75} or containing 23 kg ha^{-1} N from NF or 50 kg ha^{-1} urea or 0.19 g $polybag^{-1}$ urea and 69 kg ha^{-1} N from AC or 1,699 kg ha^{-1} AC or 6.47 g $polybag^{-1}$ AC), and (6) P_5 (NF_0+AC_{100} or containing 92 kg ha^{-1} N from AC or 2,266 kg ha^{-1} N from AC or 8.63 g $polybag^{-1}$ AC). The six treatments were arranged in a completely randomized design with five replications and each replication consisted of three polybags.

Crop Cultivation and Management

Implementation of the polybag trial was started by preparation of growing media. The growing media comprises a homogenous mixture of soil and rice husk charcoal (4:1 v/v). Soil for the growing media was collected from topsoil at depths of 0–20 cm, crushed manually with a milling device, and then passed through a 2-millimeter sieve. The soil was then mixed uniformly with milled rice husk charcoal. Lime (dolomite) was applied at 2 tons ha^{-1} (10 g $polybag^{-1}$) during growing media preparation and incorporated into the soil.

Healthy green onion seedlings from local farmers were used as seed material. Each seedling was characterized by an age of 2.5 months, a length of 12.5 cm, a stem diameter of 2.5 cm, strong roots, a stem diameter of 1.0 cm, and a weight of 15-20 g. The seedling at the age of 2.5 months was then planted into polybags at 5 cm depth in June during the summer season of 2020, and there was only one seedling for each polybag. The seedlings were grown in polybags (10 kg soil; 40 cm x 25 cm) and treated with inorganic N fertilizer, azolla compost, and their combinations. Each polybag was spaced 30 cm x 30 cm apart in the plastic house.

Before transplanting, each polybag was added with the recommended synthetic fertilizers (triple superphosphate (TSP) at 150 kg ha^{-1} or 0.68 g $polybag^{-1}$ and potassium chloride (KCl) at 100 kg ha^{-1} or 0.45 g $polybag^{-1}$, respectively. Meanwhile, the dosage of urea and AC were applied according to the treatments. Urea was applied twice in two exact dosages. The first half dose was given at basal application with the total amount of TSP and KCl one day before sowing, while the rest (50%) was applied at plant age of 3 weeks after sowing (WAS). Azolla compost was applied once at basal application with other chemical fertilizers, mixed, and integrated with the soil of each polybag. Polybags with non-amended soil (no urea and Azolla compost) were used as controls.

Seven days after sowing until harvesting, weeding was performed manually once a week, and no herbicide was used. After the sowing time, the insecticide carbofuran (3% doses at a rate of 20 kg ha^{-1}) was used to control *Agrotis ipsilon* (black cutworm). After that, all pests were physically controlled by removing them from the polybags. Soil upraising was conducted once a week after sowing until harvesting. Plant watering was performed manually twice a day when there was no precipitation until the soil growing medium reached field capacity, with an indication when the water started dripping out of the bottom hole of the polybag. Harvesting was carried out manually at 7 WAS by uprooting all parts of the crop. Harvesting was done as soon as the plants indicated yellow and dry leaves at the tip, reached about 15 to 20 cm tall, and were as wide as a pencil.

Data Collection and Analysis

Three plants for each replicate were used to record the data. Using measuring tape, plant height (cm) was determined from the ground level up to the tip of the longest leaf. The number of green leaves was determined by counting fully developed leaves, and the average was computed for each plant. Stem diameter (mm) was measured at the widest circumstance of the stem using a Vernier caliper. The number of tillers per hill was calculated by counting the number of stem-borne roots at harvesting. Root and shoot dry weight was measured by drying the sample in open dry air for three days, then in an oven for 48 h at 70^0C until constant weight was attained. The average root and shoot fresh weight per plant (g $plant^{-1}$) was calculated as the fresh root or shoot weight at harvesting. Average shoot fresh weight per plant (g $plant^{-1}$) was calculated as the fresh shoot weight without roots at harvesting. The average plant fresh weight per hill (g $hill^{-1}$) was calculated as the fresh shoot weight with roots at harvesting.

The obtained data from the effects of Azolla compost, synthetic fertilizers, and their combinations on the growth and yield of green onion, as well as on dry matter content, were subjected to analyses of variance (ANOVA) according to Steel *et al*. (1997) using SPSS (v. 22, IBM Inc., Chicago, IL, USA). The means of different traits were calculated and compared using the Least Significant Test. The ANOVA did not consider leaf nitrate content data because the samples were insufficient due to badly damaged leaves.

RESULTS AND DISCUSSION

Soil characteristics and climatic conditions during the growing period

Soil analysis results showed that the soil for the experiment was acidic with a pH of 4.89, and low N, P, and K content (Table 1). These characteristics were unfavorable for green onion growth. Efforts to increase the availability of soil nutrients were carried out through the application of N fertilizer (NF) combined with azolla compost (AC) with high N (4.06). In addition, AC also had high OC, pH, and CEC (Table 2). With these characteristics, adding AC improves the soil characteristics and eventually supports green onion growth.

According to Rao (2016), green onion is highly sensitive to water stress due to shallow and short-rooted properties and heat stress. In this study, green onion did not experience moisture stress due to high and well-distributed rainfall during its growing period (Table 2) and frequent manual watering to maintain high soil moisture during low or no precipitation, as recommended by Hedge (1986), who stated that continuous watering is necessary to maintain high green onion yields when rainfall is low or absent at all. Meanwhile, the humidity (RH) level during the growing period was also increased with a monthly average of above 80%. The RH is not critical for green onion production as it does not profoundly affect the physiological parameters and the green biomass yield (Uzo and Currah, 1990).

Temperature variations significantly influence the green onion vegetative growth rate, and the optimal temperatures for onions range from 15–20^{o}C during early development (Rao, 2016). In this study, daily averages of ambient temperatures were above 27^0C. These conditions were less favorable for green onion growth. The research report by Rao (2014) provided evidence of the adverse effects of high averages of daily air temperature. He stated that an increase in air temperature of 3^{o}C promoted a decrease in the agronomic performance of green onion.

Growth and dry matter production

Tables 3 and 4 contain the records of growth and dry matter content as a response to the treatments of reduction of mineral fertilizer (NF) with Azolla compost (AC) under the same N levels. For the growth at early stages (2 and 4 WAS), fertilizer substitution treatments slowly and insignificantly ($p>0.05$) improved plant height and number of green leaves. After that (6 WAS), along with the rapid growth of green onion, the treatments promoted a significant ($p < 0.05$) increase in the two growth traits.

At harvest, the variations among the treatments on the growth and dry matter-related traits were significantly ($p < 0.05$) different (Table 4). In terms of plant height, P_1 treatment (i.e., adding 100% NF or the total recommended dose of N fertilizer) caused the significantly ($p<0.05$) highest rise in the

Table 3. Plant height and number of green leaves of green onion under different treatments of equal substitution of synthetic N fertilizer (NF) with Azolla compost (AC).

Treatments	Plant height (cm)			Number of green leaves		
	2 WAS	4 WAS	6 WAS	2 WAS	4 WAS	6 WAS
P_0 (Control)	22.13 a	31.36 a	26.38 d	2.66 a	6.13 a	11.33 b
P_1 (NF_{100} +AC_0)	23.60 a	33.30 a	37.46 a	2.87 a	7.33 a	12.93 ab
P_2 (NF_{75} +AC_{25})	23.00 a	32.76 a	35.79 ab	3.00 a	7.07 a	13.20 a
P_3 (NF_{50} +AC_{50})	22.30 a	32.63 a	33.30 abc	3.07 a	8.53 a	14.59 a
P_4 (NF_{25} +AC_{75})	24.53 a	33.19 a	31.06 bcd	2.89 a	7.93 a	13.46 a
P_5 (NF_0 +AC_{100})	23.03 a	32.53 a	29.36 cd	2.86 a	7.00 a	12.80 ab

Note: Means within the same column followed by different small letters demonstrate significant differences at $p < 0.05$, according to LSD's test. WAS=week after sowing. Control: no NF and AC; NF_{100} (containing 92 kg ha^{-1} N from NF); NF_{75} +AC_{25} (containing 69 kg ha^{-1} N from NF and 23 kg ha^{-1} N from AC); NF_{50} +AC_{50} (containing 46 kg ha^{-1} N from NF and 46 kg ha^{-1} N from AC); NF_{25} +AC_{75} (containing 23 kg ha^{-1} N from NF and 69 kg ha^{-1} N from AC); AC_{100} (containing 92 kg ha^{-1} N from AC).

parameter, which was an average 27% and 43% higher than P_5 treatment (i.e., adding 100% AC) and control, respectively, but showed statistically ($p>0.05$) identical performance with P_2, P_3, and P_4 treatments (25%, 50%, and 75% substitution of NF with AC at equivalent N rate, respectively). For the number of green leaves and plant dry weight, P_3 treatment produced the highest values of these two traits, which were significantly ($p< 0.05$) increased by 13% and 39%, respectively compared to P_5 treatment and 34% and 101%, respectively contrasted to the control treatment. However, the values of the two parameters of the P_3 treatment were not significantly (p>0.05) different to those of P_1, P_2, P_3, and P_4 treatments. Regarding root fresh weight, P_1 treatment gave the best effect on increasing this trait, which was 23 % and 31% greater compared to P_5 and the control treatment, but offered equal impact with P_2, P_3, and P_4 treatments on this trait.

These results demonstrate that equal combined application of NF and AC (P_2, P_3, and P_4 treatments) gave similar results in all growth and dry matter-related traits (except root dry weight) with the application of the total recommended dose of N fertilizer (P_1 treatment). These findings mean that, like the P_1 treatment, these three treatments were able to supply the required N rates sufficiently enough to support the growth and dry matter production of green onion. In addition, NF could be partly replaced (up to 75%) by AC input under the same N levels without reducing significant growth performances of green onion, agreeing with those of previous works by Geng *et al*. (2019) and Zai *et al*. (2022) in corn and Yang *et al*. (2020) in winter wheat. A possible reason for these results is

Table 4. Root growth and dry matter accumulation of green onion under different treatments of equal substitution of synthetic N fertilizer (NF) with Azolla compost (AC).

Treatments	Plant height (cm)	Number of green leaves	Root fresh weight (g)	Root dry weight (g)	Plant dry weight (g)
P_0 (Control)	27.65 d	16.22 c	5.75 c	0.92 a	3.27 c
P_1 (NF_{100} +AC_0)	39.48 a	20.53 a	7.53 a	1.01 a	6.58 a
P_2 (NF_{75} +AC_{25})	38.42 ab	20.60 a	7.50 a	0.90 a	6.29 a
P_3 (NF_{50} +AC_{50})	35.65 ab	21.80 a	7.25 a	1.13 a	6.94 a
P_4 (NF_{25} +AC_{75})	35.00 ab	21.06 a	7.29 a	1.17 a	5.79 a
P_5 (NF_0 +AC_{100})	31.09 c	19.33 b	6.11 b	0.99 a	4.75 b

Note: Means within the same column followed by different small letters demonstrate significant differences at $p < 0.05$, according to LSD's test. Control: no NF and AC; NF_{100} (containing 92 kg ha^{-1} N from NF); NF_{75} +AC_{25} (containing 69 kg ha^{-1} N from NF and 23 kg ha^{-1} N from AC); NF_{50} +AC_{50} (containing 46 kg ha^{-1} N from NF and 46 kg ha^{-1} N from AC); NF_{25} +AC_{75} (containing 23 kg ha^{-1} N from NF and 69 kg ha^{-1} N from AC); AC_{100} (containing 92 kg ha^{-1} N from AC).

that by adding AC in the soil under reduced N fertilizer application, the availability of balanced and sufficient N in the soil might have been reestablished due to its high content of readily available nutrients, especially N (Table 2), organic C, and exchangeable cations (Razavipour *et al*., 2018). A good supply of N enhances plant growth and development, such as increased root growth and development due to the essential properties of this mineral element for root growth (Marschner, 1995), increased plant height as well as the number of green leaves because of the critical role of N on the synthesis of amino acids, proteins, chlorophylls, and other compounds crucial for growth, photosynthesis, and cellular metabolism (Brewster, 1994), and high dry matter content owing to the N key role on photo assimilation (Marschner, 1995).

The better green onion growth in the soil treated with combined application of AC and NF in this study was probably associated with better root growth. According to Min *et al*. (2014), better root growth, which was generally influenced by better nutrient (especially N) availability in the rhizosphere, was subsequently followed by better nutrient and water acquisition by plant roots.

When 100% NF was replaced with AC (the P_5 treatment), all the tested variables of the treatment decreased and significantly ($p < 0.05$) lower than those of the P1, P2, P3, P4, even though higher than those of the control treatment. According to Geng *et al*. (2019), the possible explanation for this response was that synthetic N fertilizer such as urea has a fast-release characteristic; therefore, the released nutrient is absorbed by plants right after application (Castro *et al*., 2003). Inversely, organic N fertilizer is delivered slowly, meaning that nutrients in AC must first be microbiologically converted in the soil into chemical forms accessible to plant roots. According to Amare (2020), the slowly released nutrients have little-known effects on plant growth in the short term.

The lowest values of plant height, the number of green leaves, root fresh weight, and plant dry weight were observed in the control treatment probably due to less N availability. If the availability of N in the soil is insufficient, plant growth will be greatly retarded because of the critical nature of this mineral element for its development (Amare, 2020).

Yield and its components and nitrate content in the leaf

Identical to the growth response, crop yield (shoot and plant fresh weight), and its components (tillers number per hill and stem diameter) differences were significant ($p<0.05$) among treatments. The P_2 treatment produced the highest tillers number per hill and stem diameter, which was 38% and 16% higher than the P_5 treatment and 171% and 34% greater than the control treatment. Still, it was statistically ($p>0.05$) similar when compared to the P_1, P_3, and P_4 treatments (Table 5). In terms of crop yield, the P_3 treatment had the highest shoot and plant fresh

Table 5. Green onion yield, its components, and the leaf nitrate content under different treatments of equal substitution of synthetic N fertilizer (NF) with Azolla compost (AC)

Treatments	Tiller number	Stem diameter (mm)	Shoot fresh weight (g)	Plant fresh weight (g)	Nitrate content (mg kg^{-1})*
P_0 (Control)	1.33 d	6.61 c	41.53 c	49.80 d	1,048.29 ± 83.83
P_1 (NF_{100} +AC_0)	3.59 a	8.19 ab	57.33 a	65.33 ab	1,828.62 ± 68.77
P_2 (NF_{75} +AC_{25})	3.60 a	8.85 a	54.33 a	64.73 ab	1,666.00 ±21.29
P_3 (NF_{50} +AC_{50})	3.26 ab	8.55 a	57.67 a	74.53 a	1,661.39± 67.26
P_4 (NF_{25} +AC_{75})	3.06 ab	7.79 ab	55.84 a	65.40 ab	1,445.82 ± 26.99
P_5 (NF_0 +AC_{100})	2.60 c	7.60 b	50.40 b	60.13 c	1,401.65 ± 14.58

Note: Means within the same column followed by different small letters demonstrate significant differences at $p < 0.05$, according to LSD's test. Control: no NF and AC; NF_{100} (containing 92 kg ha^{-1} N from NF); NF_{75} +AC_{25} (containing 69 kg ha^{-1} N from NF and 23 kg ha^{-1} N from AC); NF_{50} +AC_{50} (containing 46 kg ha^{-1} N from NF and 46 kg ha^{-1} N from AC); NF_{25} +AC_{75} (containing 23 kg ha^{-1} N from NF and 69 kg ha^{-1} N from AC); AC_{100} (containing 92 kg ha^{-1} N from AC). *Data are presented as the mean ± standard deviation (SD) of two replicates.

weight, which was higher by 14% and 24%, respectively, when compared to those of the P5 treatment and by 39% and 50% when contrasted to the control treatment, but showed statistically (*p>0.05*) identical to the P_1, P_2, and P_4 treatments. These results imply that when combined with synthetic N fertilizer, Azolla compost could reduce the applied N by up to 75% without decreasing green onion yields. The results of this current study were consistent with those of previous works by Seleiman *et al*. (2022) in rice and Marwanto *et al*. (2023) in green onion. Seleiman *et al*. (2022) reported that applying Azolla in combination with synthetic N fertilizer could create a balanced availability of nutrients, primarily N, and subsequently reduce the N fertilizer use by up to 60% without significantly disrupting rice growth and productivity. They further reported that the addition of 40% NPK + 60% Azolla compost resulted in comparable rice grain yield (10.76 t ha^{-1}) as well as N, P, and K content and uptake with the recommended total dose of NPK fertilizer (100% NPK). Another report by Marwanto *et al*. (2023) stated that using Azolla compost-based organomineral fertilizer (AOF) promoted green onion growth, biomass yield, and N uptake. Applying AOF at 2.23 g $plant^{-1}$ plus 50% of N recommended dose produced the highest plant fresh weight per hill (81.48 g). A possible explanation for these responses was that AC might have been able to revive the adequacy of nutrients in the soil when the use of synthetic fertilizers was reduced. The improved green onion yields in these treatments might also have been related to its more vigorous growth, as previously reported by Iqbal *et al*. (2019). With the reduced use of chemical N fertilizer, negative environmental impacts such as eutrophication, gas emission, water contamination, and leaching because of leaking out the unutilized N from overuse of chemical fertilizer from the farming systems in various N forms can be minimized. The cause of the reduced N application might have been associated with the ability of AC to compensate for the reduced N supply from urea and then re-establish the balanced availability of nutrients in the soil under reduced amounts of synthetic fertilizers applied due to AC ability to supply of abundantly various nutrients (such as N and P nutrients), characterized by gradual release, organic matter, and exchangeable cations (Razavipour *et al*., 2018). On the other hand, synthetic fertilizer with a high content of N has the property of rapid nutrient release into the soil, facilitating green onion with an immediate supply of sufficient N nutrients.

Table 5 shows that the P5 treatment (complete substitution of N fertilizer with AC) yielded lower yields than the other N substitution treatments but was higher than the control treatment. These findings indicate that appropriate organic substitution benefits yield, but excessive organic fertilizer substitution will lead to insufficient N and yield reduction. Xin *et al*. (2017) also reported that mineral fertilizer is essential for raising crop yields, and the complete substitution of the fertilizer with organic fertilizer (where N fertilizer was 100% replaced by compost to match the same rate of N) resulted in decreased production. Most of the N in organic fertilizers and composts is in the organic form and requires to be mineralized first to plant-available ammonium and oxidized to nitrate by soil microorganisms. In other words, the release of nutrients from organic manure is slow and persistent, resulting in a low level of available N in the soil Herencia *et al*. (2011). This nutrient release mechanism explains the lower shoot and plants fresh weight in the P5 treatment.

The lowest values of shoot fresh weight and plant fresh weight were observed in the control treatment due to N deficiency in the soil as supported by the analysis results of soil fertility used in this study (Table 1). If the availability of N in the soil is insufficient, plant growth and yield will be greatly retarded. This response was because of the critical nature of this mineral element for its growth and creation (Amare, 2020). Unfortunately, residual soil nitrogen (NH_4^+-N + NO_3-N) concentration and soil pH after harvest were not evaluated. The N content and pH in post-harvest soil reflect the sustainable nutrients supply to the soil. It was reported by Zaman *et al*. (2018) that the combined application of organic and inorganic fertilizer treatment enhanced the soil structure and hence improved the macro- and micro-nutrient availability, resulting in increased crop yield.

In contrast, the sole inorganic treatment resulted in significantly lower nutrient values. Meanwhile, adding organic matter improved soil pH by increasing the buffering capacity (Ogbodo, 2011). Under favorable soil pH and nutrient availability, the microbial activity in the soil was enhanced and subsequently might cause the increased availability of nutrients.

Excessive use of nitrogenous fertilizers leads to the absorption and accumulation of soil nitrogen as nitrate in plants (Almasi *et al*., 2018). In this study, nitrate content in the leaf differed in the reduced N fertilization treatments (Table 5). A gradual declining trend in nitrate contents with increased substitution of AC for NF was found, and the highest was observed in the P1. The reduced N fertilization treatments can be sorted based on the decrease in nitrate content as follows: P1 > P2 > P3 > P4 > P5 > P0. The nitrate contents of all treatments were in the range of 1,000–2,000 mg kg^{-1}, categorized as high content (Santamaria, 2006), but were still below the permitted quantification for nitrates in vegetables set by the legislation of the European Union in Regulation 1258/2011. These findings mean that the high nitrate content in this present study is attributed mainly to the excessive application of chemical N fertilizers, consistent with previous works by Herencia *et al*. (2011), who reported that shortly after applying chemical fertilizers, mineral nitrogen and other nutrients are rapidly released into the soil, and nitrate is quickly taken up and temporarily stored in crop vacuoles, thus making it extremely high in leafy vegetables (Gadallah *et al*., 2022; Kiani *et al*., 2022). This response explains the higher nitrate content of the P1 treatment. However, the release of nutrients from organic manure is slow and persistent, resulting in a low level of rapidly available nitrogen in the soil (Herencia *et al*., 2011). This occurrence explains the lower nitrate content in the P5. Also, for this reason, nitrate is usually less accumulated in the edible part of crops under the organic farming system (Dangour *et al*., 2009).

CONCLUSIONS

Applying Azolla compost to provide green onion with sufficient nutrients in Ultisols reduced mineral N input. Azolla compost achieved a synthetic N fertilizer substitution level of no more than 75% without affecting green onion yield loss in the greenhouse production scale. A 75% reduction in the chemical fertilizer combined with a 25% input of the Azolla compost under the same rate of N fertilizer produced similar responses to the total recommended dose of N fertilizer, as evidenced by plant height, number of green leaves, root fresh weight, shoot dry weight, tiller numbers per hill, stem diameter, shoot fresh weight, and plant fresh weight. Further assessments of the effectiveness of replacing synthetic N fertilizer with Azolla compost should be carried out in the open field production system and accompanied by measurements of residual soil fertility, mainly the soil N mineral (NH_4^+-N + NO_3-N) concentration and soil pH after harvest.

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