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Research article

Morphological and physiological plasticity of tomato in response to Azolla fern, a novel organic fertilizer of environmentally friendliness

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ABSTRACT

Tomatoes are highly valued vegetable crops due to their excellent nutritional content. However, production remained low due to an incorrect combination of organic and inorganic soil nutrition. A pot experiment was conducted under shaded conditions with Azolla and inorganic nitrogen fertilization to determine the morphological and physiological plasticity of tomatoes. The study used a factorial combination of four levels of Azolla (0, 25, 50, and 75 g per pot) and four levels of nitrogen (0, 0.23, 0.46, and 0.69 g per pot) in a complete randomized design with three replications. We collected data on chlorophyll content (Chl a, Chl b, TChl), photosynthetic rate, water use efficiency, transpiration rate, stomata number, branch number (primary and secondary), plant height, leaf area, stomata conductance, relative water content, and number of leaves. Analysis of variance was employed to analyze the data, and the means were separated using the least significant differences test at a 5 % significance level. The results showed that primary and secondary branches, stomata number, transpiration rate, and water use efficiency were highly plastic due to the higher nitrogen levels and Azolla alone. The interaction effect of Azolla and nitrogen had a significant influence on chlorophyll content, photosynthesis rate, stomatal conductance, relative water content, number of leaves, plant height, and leaf area. It can be concluded that a balanced combination of organic and inorganic fertilizers remains essential for optimal tomato growth and physiology, emphasizing that the exclusive use of organic farming methods may not be the ideal solution.

1. Introduction

Tomato is a versatile, nutritious food high in minerals, vitamins, lycopene, and antioxidants, eaten raw or cooked worldwide [1]. Tomato paste, tomato juice, ketchup, and whole peeled tomatoes are processed goods produced for both domestic and international markets [2]. It had also nutritional, economic and health benefits to the societies, however, its production and productivity were low in

Abbreviations: Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; *E*, transpiration rate; EC, electrical conductivity; gs, stomatal conductance; RWC, Relative water content; SOC, soil organic carbon; TChl, Total chlorophyll content; TN, total nitrogen.

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developing countries and particularly in Ethiopia [3]. However, the application of conventional fertilizer has an impact on the development of tomato plants [4], which may ultimately lead to a reduction in fruit yield and fruit quality. Moreover, the widespread use of chemical fertilizers in intensive agriculture has negative effects, including nutrient loss, greenhouse gas emissions, and water pollution. This can cause decreased food safety and lower vegetable quality, with issues like nitrate accumulation in plants [5]. Furthermore, excessive chemical fertilizer application can result in decreased food safety and lower vegetable quality, such as nitrate accumulation in plants [6].

Nitrogen is vital for plant growth, contributing to leaf expansion, biomass production, root growth, nutrient uptake, and dry mass production, and hence insufficient amounts of N for plants can hinder their growth and development [7]. Nitrogen fertilization is known to improve plant growth and biomass production [8]. It is also the most important nutrient for tomato's growth and cultivation [9]. In tropical countries like Ethiopia, problems such as high cost, scarcity, nutrient imbalance, and soil acidity arise from improper use of inorganic fertilizer like nitrogen. Proper nitrogen source and combination are crucial for tomato plant growth and development [10]. Organic farming methods cause relatively lower environmental damage compared with conventional farming and organic crop products are considered tasty and healthy [11]. Nowadays, using organic fertilizers is an efficient method to achieve sustainable agricultural development [12].

In sustainable agriculture, a new trend is to reduce the use of different inputs, especially chemical inputs, and thereby reduce the adverse effects on the environment [13]. Furthermore, there has been a significant increase in market demand for organic products [14]. Improvement in soil fertility and productivity are also reported through the use of organic fertilizer sources [15,16]. Gao et al. [12] also reported that application of organic fertilizers enhances tomato productivity and fruit quality, impacting by variables such as soil organic matter, total soil nitrogen, and the type of organic fertilizers used. Applying high-efficiency organic fertilizers can help both long-term food security and environmental protection since they can boost crop output without compromising soil quality [17]. Therefore, applications of Azolla can be optimal alternatives to synthetic fertilizers to improve the morphology and physiology of tomato plants. Azolla is a free-floating aquatic fern in the Salviniaceae family, genus Azolla [18], but can grow on moist soils as long as the moisture persists in the soil. The tiny aquatic fern Azolla lives in waters of tropical and subtropical regions [19].

Azolla is gaining attention as organic fertilizer due to its high macro- and micronutrient content, particularly nitrogen, reducing methane emissions and increasing crop yields to combat global warming [20]. Azolla contains essential amino acids (nitrogen 5.08 %, phosphorus 4.8 g/kg). Minerals such as calcium, phosphorus, potassium, magnesium, copper and zinc are also abundant in Azolla. Its dry weight contains rich (25–35 %) in protein [16]. To ensure the growth and physiology of tomatoes that result in high quality fruits, it is crucial to have an optimal combination of organic and inorganic fertilizers in developing countries like Ethiopia, where the supply of chemical fertilizer is entirely dependent on imports and higher costs to use. However, limited research has been done so far on the effects of applying Azolla as organic fertilizers on the growth and physiological plasticity of tomatoes. Therefore, the aim of this study was to improve the growth and physiology of tomato plants through the combined use of Azolla as a novel organic fertilizer and nitrogen as an inorganic fertilizer.

2. Materials and methods

Experimental site description: A pot experiment with shade house conditions was conducted at the Hawassa University College of Agriculture experimental site in 2022–2023. The shade house was enclosed with metal wire and covered with transparent polycarbonate. The tomato variety "Galilama" was used in this study. It was selected for its excellent fruit quality, high yield and diverse disease resistance, making it ideal for climate adaptation and bush and stacking production. The fruit is oval, firm and weighs between 100 and 170 g.

Composition of the inorganic and organic fertilizers: The source of inorganic nitrogen was urea, which contains 46 % nitrogen. The reason for using urea as a nitrogen source is its accessibility in its form. The sources of inorganic fertilizer used in this experiment were urea (46 % N) at varying concentrations. The amount of nitrogen treatment was determined based on the recommended urea dose for tomatoes, approximately 200 kg per ha of urea (92 kg per ha of nitrogen). The values of urea and nitrogen per hectare were 0, 100 (46 N), 200 (92 N), 300 (138 N). To facilitate application, the nitrogen treatments (0, 0.23, 0.46 and 0.69 g) in each experimental pot was converted to urea in the following amounts: 0, 0.5, 1 and 1.5 g per pot. Azolla was used as the source of the organic fertilizer [16].

Experimental design and data collection: A factorial combination of four levels of inorganic nitrogen (0, 0.23, 0.46 and 0.69 g per pot) and four levels of Azolla (0, 25, 50 and 75 g per pot) was tested in a completely randomized design with three replications. Nitrogen and Azolla values of zero (0) were used as controls [16].

The leaf chlorophyll content was measured on fully expanded leaves in the vegetative stage. After being put in an aluminum foil-sealed bag, the leaves were brought to the Hawassa University crop physiology lab. To avoid light-induced chlorophyll degradation, fresh leaf discs (0.5 g) were put in 15-mL tubes filled with 95 % alcohol, and the glass were sealed with Parafilm. After the sample mixture was homogenized, it was centrifuged for 15 min at 20 °C at 10,000 ppm. The supernatant was separated, and 0.5 ml of each concentration level was analyzed in triplicate for chlorophyll *a* and chlorophyll *b* at an absorbance of 663 nm and 646 nm wavelength, respectively, in a UV-2450 spectrophotometer (Hitachi Tokyo, Japan) [21].

$$\text{Chl } a \ [\mu g \ g^{-1} \ (\text{DM})] = 12.21 \ (A_{663}) - 2.81 \ (A_{646})$$

$$\text{Chl } b \ [\mu g \ ml^{-1} \ (\text{DM})] = 20.13 \ (A_{646}) - 5.03 \ (A_{663})$$

$$\text{Total chlorophyll content } [\mu g \ ml^{-2}] = \text{Chl } a + \text{Chl } b$$

Where; A = Absorbance.

Photosynthetic rate [μmol (CO_2) m^{-2} s^{-1}], transpiration rate [μmol (H_2O) m^{-2} s^{-1}], and stomatal conductance [μmol m^{-2} s^{-1}] was measured from the three randomly selected 3rd young and fully expanded leaves at the vegetative growth stage in each treatment using an open system LCA-4 (LCA-4 Software Version 1.04) ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). The measurement was done between 10:00 h and 12:00 h by maintaining the following specifications: leaf surface area was 6.54 cm^2, ambient carbon dioxide concentration was 390 μmol mol^{-1}, leaf chamber mass flow rate was 300 μmol s^{-1}, atmospheric pressure was 840 bar and photosynthetic active radiation [μmol m^{-2} s^{-1}] was manually fixed at 1200 μmol m^{-2} s^{-1}. Water use efficiency was determined as the ratio between the net CO_2 assimilation rate and transpiration rate. Relative water content (%) was determined in three fully expanded topmost leave collected from each treatment at the vegetative stage and leaf discs (9 mm in diameter) were immediately weighted (leaf fresh weight) and recorded after the sample was immediately hydrated to full turgidity for 24 h by immersing the leaf disk in distilled water in a closed 2615-ml falcon tube under room temperature. The weight of the turgid leaf disk was measured to determine the turgid mass (leaf turgid weight) after the water droplet was removed by using tissue paper. Samples were oven-dried for 24 h at 75 °C to obtain dry mass (leaf dry mass). Relative water content (RWC) was calculated by the methods developed by Pieczynski et al. [22].

$$\text{Relative Water Content (RWC)(\%)} = \left(\frac{\text{Fresh Mass} - \text{Dry Mass}}{\text{Turgid Mass} - \text{Dry Mass}}\right) \times 100$$

Stomata anatomy was measured from fully expanded leaves for each treatment [23]. A thin layer of transparent nail polish was uniformly stained on the lower surface of fresh intact leaves about [1.5 cm × 1.5 cm] and waited for 10 min until the nail polish dried to capture the epidermal imprint of the leaves, afterward, a thin layer covering a surface on the leaves was peeled off using transparent tape and attached on the microscope slide. The resulting molds were examined with an Automated Upright Leica Microscope DM5000 B with a 40x magnification lens fixed to a digital Leica DFC425/DFC425C image process camera. For each sample, stomata number was measured. Number of leaves per plant was recorded by counting the total number of leaves produced by each treatment at 50 % flowering. The number of branches extending from the main stem was counted and recorded on each treatment at the flowering stage from each pot. Number of secondary branches extended from primary branches were counted and recorded from each treatment at flowering stages. Plant height [cm] was measured from the soil surface to the topmost growth points of the above-ground plant part. The measurement was taken as the length from all plants of each pot at the final harvesting time. Leaf area [cm^2] was measured from three leaves of each treatment at 50 % flowering stage by using a portable area meter model Ll3000A (LiC or Lincoln, Nebraska, USA).

Statistical analysis: Data were subjected to analysis of variance using the General Linear Model in SAS (ver. 9.4, SAS Institute, Cary, NC). The means were separated by using the least significant differences test at the 5 % level of significance. If the interaction was significant, it was used to explain results. If the interaction was not significant, means were separated using the least significant difference (LSD).

Table 1

Interaction effect of nitrogen and Azolla for chlorophyll *a* and *b*, total chlorophyll and photosynthesize rate.

Nitrogen [g per pot]	Azolla [g per pot]	Chlorophyll *a* [μg g^{-1}] (DM)	Chlorophyll *b* [μg g^{-1}] (DM)	Total chlorophyll	Photosynthetic rate [μmol m^{-2} s^{-1}]
0	0	9.48^{l}	7.04^{o}	17.90^{m}	1.45^{o}
	25	11.43^{k}	7.91^{n}	19.62^{l}	1.89^{n}
	50	11.78^{jk}	8.80^{m}	22.09^{k}	2.12^{m}
	75	13.47^{hi}	10.03^{i}	26.44^{hi}	3.48^{i}
0.23	0	12.29^{j}	9.21^{l}	25.23^{j}	2.45^{l}
	25	12.91^{i}	9.46^{k}	26.04^{i}	2.88^{k}
	50	14.06^{fg}	10.50^{h}	26.93^{gh}	3.88^{gh}
	75	14.84^{e}	11.08^{f}	27.66^{f}	4.11^{f}
0.46	0	13.12^{i}	9.71^{j}	26.25^{i}	3.12^{j}
	25	14.49^{ef}	10.88^{g}	27.18^{fg}	2.88^{k}
	50	15.79^{cd}	11.98^{d}	29.56^{d}	3.88^{gh}
	75	16.23^{c}	12.17^{c}	30.18^{c}	4.11^{f}
0.69	0	13.83^{gh}	10.13^{i}	26.59^{hi}	3.74^{h}
	25	15.46^{d}	11.54^{e}	28.85^{e}	4.55^{e}
	50	17.47^{b}	12.66^{b}	30.81^{b}	5.46^{b}
	75	18.50^{a}	12.89^{a}	31.39^{a}	6.33^{a}
LSD (0.05)		0.29	0.09	0.30	0.11
CV (%)		2.45	1.00	1.34	3.58

Mean followed by the same letter (s) in the same column are not significantly different at 5 % level of significance. LSD and CV stand for least significance difference and coefficient of variation, respectively.

3. Results and discussion

3.1. Physiological plasticity

Chlorophyll content: The findings presented in Table 1 demonstrated that the interaction between Azolla and nitrogen had a significant ($P \leq 0.001$) impact on the content of chlorophyll in leaves, including Chl *a*, Chl *b*, and total chlorophyll (Table 1S). Applying 0.69 g per pot of nitrogen and 75 g per pot of Azolla together led to the highest chlorophyll levels (Chl a: 18.50; Chl b: 12.89; TChl: 31.39), while the control treatment had the lowest chlorophyll content. Table 1 shows the lowest chlorophyll content that was measured from the control. Nitrogen availability from Azolla and nitrogen were thought to be the causes of the increase in chlorophyll contents. Since nitrogen is a crucial component of chlorophyll molecules, enough nitrogen can increase the amount of chlorophyll that is produced. N is a crucial element in the development of the photosynthetic apparatus in plants because it is a component of chlorophyll [5]. A leaf's ability to photosynthesize is directly correlated with its chlorophyll content, which is higher when there is more nitrogen present. Similarly, Zaituniguli et al. [24] also discovered long-term fertilization boosts chlorophyll content in crop leaves, enhancing photosynthesis intensity and water use efficiency to ensure higher crop yields. The chlorophyll content also increases significantly in the okra plant when nitrogen is applied, both from organic and inorganic sources [25]. Peng et al. [26] also found the significant impact of nitrogen-containing nutrient sources on leaf chlorophyll contents. Zhang et al. [27] confirmed higher chlorophyll in rice with increased nitrogen fertilizer, supporting present study's findings. The study shows Azolla and nitrogen boost chlorophyll content in leaves together at specific levels. However, more research is needed on the biochemical and physiological mechanisms and long-term effects on plant health and productivity. Further research is needed to explore these aspects to optimize the use of Azolla and nitrogen in sustainable agricultural practices.

Photosynthetic rate (A): The results showed that the nitrogen and Azolla interaction effect had a significant influence ($P \leq 0.001$) on the photosynthesis rate (Table 1S). The combined application of 0.69 g per pot of nitrogen and 75 g per pot of Azolla gave the maximum photosynthesis rate, while the control gave the lowest result (Table 1). This observation can be explained by the fact that nitrogen could accelerate the uptake of carbon by photosynthetic organisms because it increases gas exchange capacity and the number of photosynthetic pigments. Nitrogen sourced from inorganic fertilizer and Azolla has a significant component of chlorophyll and protein in plants. The photosynthetic apparatus of plants consists mainly of N, a widely used fertilizer in plants [5]. Peng et al. [26] also found that the content of photosynthetic pigments, photochemical efficiency and the open area fraction of the reaction center can be increased by applying nitrogen in the right amounts. These properties support the quantum efficiency, and photosynthesis rate of PSII in plants and help in the efficient use of light energy captured by the plant during photosynthesis [28]. Furthermore, the higher rate of photosynthesis observed at higher nitrogen levels also reflect an increase in both the photochemical phase and the biochemical phase [29]. Moreover, Sonowal et al. [5] also found that adequate soil nitrogen boosts protein and amino acid production, enhancing chlorophyll synthesis, plant productivity, and photosynthesis efficiency. The study highlights the positive effect of nitrogen and Azolla combination on photosynthesis, but details on the underlying mechanisms are lacking. The impact of organic versus inorganic nitrogen sources on photosynthesis and chlorophyll-protein complex formation in plants requires further investigation across different species and environments for optimizing sustainable agricultural practices.

Stomatal conductance (gs): Stomatal conductance was significantly ($P \leq 0.001$) affected by the interaction effect of nitrogen and Azolla (Table 1S). The maximum stomatal conductivity was measured from the combined applications of 0.69 g per pot and 75 g, while the minimum value of stomatal conductivity was determined from the control (Fig. 1). Nitrogen, which can be obtained from organic and inorganic sources, could help send the necessary signals to plant cells to open stomata and maintain cation and anion balance within cells, ultimately increasing stomatal conductance. The relationship between stomatal conductance and soil nitrogen has been extensively studied. Stomatal conductivity increased with the addition of soil nitrogen [30], which is consistent with the present

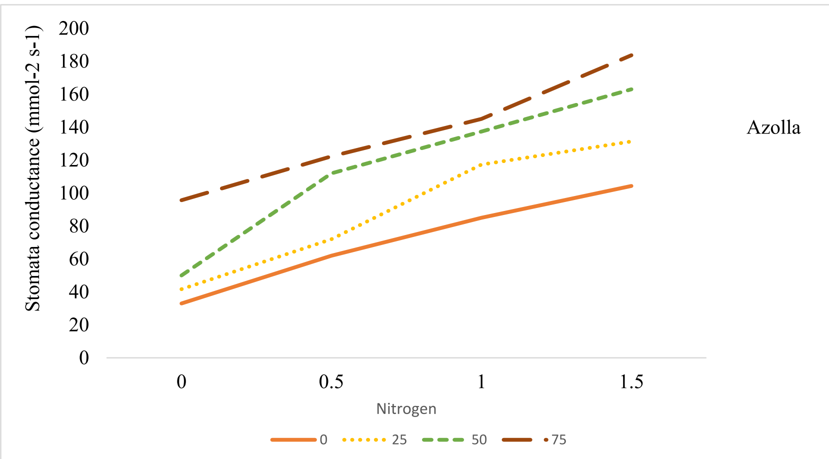

Fig. 1. Interaction effects of nitrogen and Azolla on stomata conductance.

findings showing significantly higher stomatal conductivity due to higher nitrogen content from inorganic nitrogen and Azolla. Zhu et al. [31] also conducted an experiment on the effects of nitrogen additions on mesophyll and stomatal conductance in Manchurian ash and Mongolian oak and found that soil nitrogen addition resulted in a significant increase in stomatal conductance. Similarly, Ye et al. [32] also found that organic fertilizer affects stomatal conductance in green jujube leaves, impacting photosynthetic rate and yield.

Relative water content (RWC): The interactions of nitrogen and Azolla had a significant influence ($P \leq 0.001$) on the RWC (Table 1S). The combined application of 0.69 g of nitrogen per pot and 75 g of Azolla per pot gave the highest RWC (95.69 %), while the control plot gave the lowest value (44.86 %) (Fig. 2). This result could be attributed to the use of both organic and inorganic fertilizers, which help increase soil organic matter and improve the soil's ability to absorb water. Lower water stress in both dry and wet weather would increase the water absorption and holding capacity of the soil. Organic and inorganic fertilizers work best together for improving soil and enhancing soybean growth in central India's Vertisols. Relying only on inorganic fertilizers may harm soil and water-use efficiency in the long run [33]. Organic fertilizer enhances soil quality, water retention, and plant health, unlike inorganic fertilizers, which may reduce relative water content. It is essential for promoting plant growth and root development [34], which has a positive effect on the relative water content in the leaves of the plant. Zhu et al. [35] studied the impact of nitrogen inputs on gas exchange in Manchurian ash and Mongolian oak. Their findings show that nitrogen addition can boost stomatal and mesophyll conductance, enhancing photosynthesis and growth. However, increased stomatal conductance may lead to more water loss, increasing vulnerability to water stress in dry conditions. A study comparing organic and inorganic fertilizers found that combining them improves photosynthesis in maize by increasing nutrient uptake efficiency. Organic fertilizers enhance root growth and soil moisture retention, while inorganic fertilizers support chlorophyll production and enzyme activity for better growth [36]. Ojo et al. [37] found that organic fertilizers improve soil water-holding capacity, benefiting plant water content, while inorganic fertilizers focus on plant growth. Similarly, Morsi et al. [38] discovered that organic fertilizers enhance soil structure and water retention, maintaining plant RWC under stress. Manzoor et al. [39] also showed that a blend of organic and inorganic fertilizers enhances soil properties, water retention, and plant growth, especially in tea plants.

Water use efficiency (WUE): The analysis of variance results revealed that water use efficiency was significantly ($P \leq 0.001$) affected by the main effects of nitrogen and Azolla (Table 1S). Maximum water use efficiency was measured at the highest nitrogen fertilizer rates (0.69 g per pot) and was lowest at 0.23 g nitrogen per pot and in the control (Table 2). The result further explained that the tomato plant treated with a high level of nitrogen fertilizer had 140.86 % higher water use efficiency compared to the control. The WUE value, which increased steadily with increasing N amounts, could be due to the contribution of nitrogen to the formation of more leaves, which helps to reduce water evaporation from the soil layer through dense plant cover. Azolla also significantly affected the water use efficiency of tomato plants. WUE was increased as Azolla content increased from 0 to 75 g per pot. The maximum WUE (2.58 g pot^{-1} mm^{-1}) was recorded at 75 g Azolla per pot and was lowest in the control plot (1.27 g pot^{-1} mm^{-1}) (Table 2). This could resolve a situation where most of the soil beneath the plant canopy is wetted and therefore can be shaded, thereby retaining a significant amount of water. Organic fertilizers improve soil conditions, such as soil structure, moisture retention, and nutrient availability. These improvements promote healthier plant growth and optimal stomatal development, as plants can better regulate water loss and gas exchange [40]. Similarly, Defeng [41] also found that combined application of organic and inorganic fertilizers significantly improved the water use efficiency of maize by 59.2 % compared with the control.

Transpiration rate (E): The result showed that the transpiration rate was significantly ($P \leq 0.001$) influenced by the main effects of nitrogen and Azolla (Table 1S). Application of 0.69 g of nitrogen per pot increased the transpiration rate by 43.80 % compared to the control (Table 2). The increased soil fertility due to a higher nitrogen supply increased the leaf area from which water transpires. The rate of perspiration is also significantly influenced by the use of different amounts of Azolla. Plants treated with the highest amount (75 g per pot) of Azolla increased their leaf transpiration rate by 107.43 % compared to the control (Table 2). Plants treated with 25

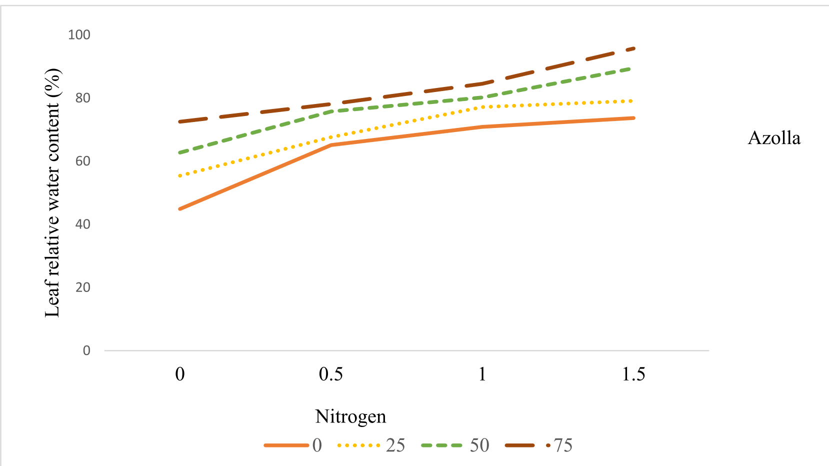

Fig. 2. Interaction effects of nitrogen and Azolla on leaf relative water content.

Table 2
Effect of nitrogen and Azolla on water use efficiency transpiration rate, stomata number of tomato plant.

Nitrogen [g per pot]	Water use efficiency [g per pot mm^{-1}]	Transpiration rate [μmol m^{-2} s^{-1}]	Stomata number per mm^2
0	1.15^c	2.26^c	5.92^d
0.23	1.34^c	2.75^b	7.25^c
0.46	1.97^b	2.85^b	8.58^b
0.69	2.77^a	3.25^a	13.83^a
LSD (0.05)	0.24	0.32	1.23
Azolla [g per pot]			
0	1.27^d	1.75^c	6.90^c
25	1.55^c	2.73^b	6.92^c
50	1.82^b	3.01^b	9.08^b
75	2.58^a	3.63^a	11.83^a
LSD (0.05)	0.24	0.32	1.23
CV (%)	15.96	13.98	16.59

Mean followed by the same letter (s) in the same column are not significantly different at 5 % level of significance. LSD and CV stand for least significance difference and coefficient of variation, respectively.

and 50 g of Azolla per pot showed no significant effect on transpiration rate. For optimal plant growth, the application of a sufficient amount of fertilizer is required, and the present experiment showed that the application of the highest Azolla concentration promoted vegetative growth, an increase in leaf area, and photosynthesis, which may be associated with a higher transpiration rate. Saneoka et al. [42] also showed that the application of nitrogenous fertilizers plays an important role in improving photosynthesis, stomatal conductance, and transpiration rate by increasing leaf area expansion and leaf chlorophyll content. The use of both organic and inorganic fertilizers can regulate tomato plant transpiration rate by improving soil structure and water retention, reducing excessive water loss through transpiration. This controlled transpiration helps maintain plant turgor, supporting consistent growth and yield [43].

Stomatal number: The analysis of variance revealed that stomata number was significantly ($P \leq 0.001$) affected by the main effects of nitrogen and Azolla (Table 1S). The highest number of stomata (13.83) were obtained by applying the highest amounts of nitrogen fertilizer (0.69 g per pot), while the lowest number of stomata (5.92) was obtained by unfertilized plants (Table 2). The application of different Azolla concentrations ($P \leq 0.001$) also significantly influences the stomata number of the tomato plant (Table 1S). The highest number of stomata (11.83) was obtained from the plant treated with 75 g of Azolla per pot, while the smallest number of stomata (6.90) was recorded in the control (Table 2). The reason for this result could be that improving soil fertility for healthy leaf growth leads to an increase in the leaf thickness of the layer. The plant treated with 25 g of Azolla per pot and the control plant showed no significant effect on the number of stomata. The greater the nitrogen dosage was the thicker the cuticle, the leaf, and the palisade. As for stoma size, the higher the nitrogen dosage was the smaller it was. The higher the nitrogen dose, the thicker the cuticle and palisades of the leaves. This result could be due to the fact that as nitrogen content increases, upper epidermis cuticle thickness, lower epidermis cuticle thickness, leaf thickness, palisade thickness, and stomatal size increase, thereby increasing stomatal density [44]. Shewangizaw ***et al.*** [45] reported that optimal nitrogen application improved tomato growth and yield by enhancing nutrient availability for photosynthesis and metabolic processes, leading to increased vegetative growth, fruit size, and biomass production. Excessive nitrogen levels, however, did not further improve yield and risked nitrogen leaching. Studies indicate that organic fertilizers contribute to a more balanced development of stomata. For instance, research by Zahara et al. [46] demonstrated that organic fertilizers improve soil structure and nutrient availability, supplying plants with steady nutrients for healthier, robust leaf

Table 3
Main effect of nitrogen and Azolla on the number of primary and secondary branch of tomato plant.

Nitrogen [g per pot]	Primary branch	Secondary branch
0	4.67^c	7.50^c
0.23	6.08^b	9.08^b
0.46	6.50^b	9.50^b
0.69	10.00^a	13.00^a
LSD (0.05)	1.1	1.10
Azolla [g per pot]		
0	5.25^c	8.08^c
25	6.42^b	9.42^b
50	6.50^b	9.50^b
75	9.08^a	12.08^a
LSD (0.05)	1.10	1.10
CV (%)	19.36	13.54

Mean values followed by the same letter (s) in the same column are not significantly different at 5 % level of significance. LSD and CV stand for least significance difference and coefficient of variation, respectively.

structures and optimal stomatal functioning. The use of organic matter might also contribute to a more stable or favorable microclimate at the soil-root interface, enhancing leaf physiology and stomatal performance. Understanding these effects can enhance cultivation practices for this important plant.

3.2. Morphological plasticity

Branch (primary and secondary) and leave number: The analysis of variance result revealed that the main effects of nitrogen and Azolla significantly ($P \leq 0.001$) influenced the number of branches. Whereas, the interaction effect showed a significant ($P \leq 0.001$) effect on the number of leaves per plant (Table 1S). According to the data presented in Table 3, the primary and secondary branches were increased by 114.13 % and 73.33 %, respectively, when the maximum amount of nitrogen (0.69 g per pot) was applied compared to the control. Nitrogen supply strengthened the tomato branch, which could be due to the positive stimulation of meristematic growth and new branches and leaves. Similarly, Rao et al. [47] reported that increasing nitrogen levels supported chloroplast function, thereby increasing the growth of a plant. The primary and secondary branches of tomato plants are also significantly influenced by the application of Azolla. The highest values of primary and secondary branches were measured at the highest level of Azolla and were lowest at the control plot. The application of Azolla increases the number of primary and secondary branches by 72.95 % and 49.50 %, respectively, compared to unfertilized plants (Table 3). Azolla contains several macro- and micro-nutrients that stimulate cell division and elongation, which stimulates the branching number of tomato plants. Zeleke et al. [16] found that Azolla, rich in macro- and micronutrients, enhances nutrient availability in the root zone, boosting plant uptake and increasing tomato branch numbers.

The combined application of the highest amount of nitrogen (0.69 g per pot) and Azolla (75 g per pot) gave the maximum number of leaves per plant, while the untreated plants gave the lowest values (Fig. 3). The nitrogen fertilizer obtained from organic and inorganic sources increases protein synthesis and contributes to the formation of new tissues, especially leaf tissues in plants. N increases vegetative growth and plant branching by increasing cell division and plant meristem cells. In return, more leaves are produced in the plant, which increases photosynthesis performance. Organic fertilizer activates substrate nutrients, improves soil properties, stimulates plant nutrient uptake, boosts nutrient content, supports dry matter accumulation, and promotes vegetative and reproductive growth like branch number, leaf area and leaf number [48]. These finding is also aligned with the conclusions drawn by Kumari et al. [49], highlighting the significant benefits of using organic fertilizers in particular to promote the vegetative growth and development of beetroot plants (leaf number and size), which in turn improves crop quality and production.

Plant height (cm): Plant height was significantly ($P \leq 0.001$) affected by the interactions of nitrogen and Azolla (Table 1S). The longest plant height was measured with the combined application of 0.69 g and 75 g of Azolla per pot and increased by 208.59 % compared to the control (Table 4). The increase in plant height due to an increase in Azolla and nitrogen is due to the provision of immediately available macro- and micronutrients from the two-fertilizer source. Plant height may have increased due to readily available nitrogen from organic and inorganic fertilizer, which may have promoted greater vegetative growth and development. This also explains why N fertilizer ensures favorable conditions for stem elongation with optimal vegetative growth. Taranet et al. [50] also found an increase in plant height with an increase in the application of organic and inorganic fertilizers due to their contribution to cell size, elongation, and division, which in turn improve plant growth. Azolla has significant nutritional value for the growth and development of plants during the growth and development phase, as well as a high nitrogen content, which can be used as a replacement for inorganic nitrogen fertilizer [16]. In addition, Al-Bdairi and Kamal [51] also found a significant effect of the interaction between Azolla and nitrogen fertilizer application on plant height. Yu et al. [52] also found that bio-organic fertilizers can also effectively promote the vegetative growth and reproductive growth of plants, and improve the quality of plants. Moreover, Adekiya et al. [53] found organic and inorganic fertilizers increased growth and yield of tomato and cucumber compared to control.

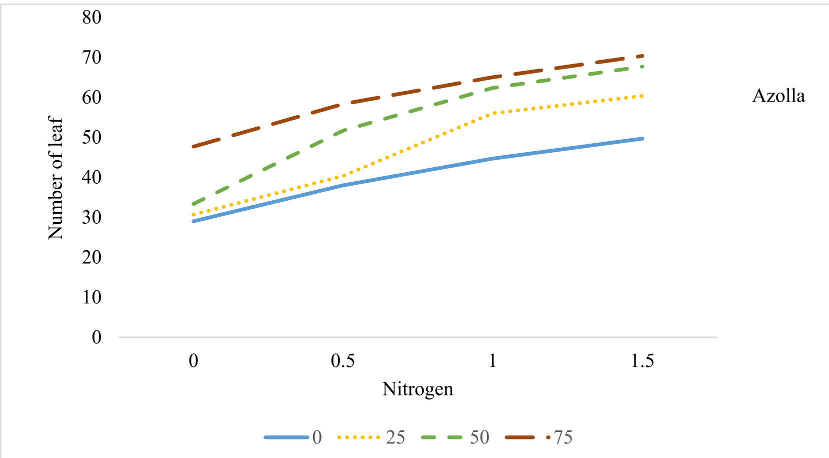

Fig. 3. Interaction effects of nitrogen and Azolla on the number of leaves.

Table 4
Interaction effect of nitrogen and Azolla for plant height and leaf area of tomato plant.

Nitrogen [g per pot]	Azolla [g per pot]	Plant height [cm]	Leaf area [cm^2 per leaf]
0	0	38.67^{l}	8.73^{m}
	25	40.33^{l}	13.25^{l}
	50	46.67^{k}	14.19^{kl}
	75	67.67^{h}	18.43^{i}
0.23	0	56.33^{j}	14.96^{jk}
	25	60.33^{ij}	15.92^{j}
	50	77.00^{f}	21.54^{g}
	75	84.67^{e}	23.46^{f}
0.46	0	64.33^{hi}	17.32^{i}
	25	81.00^{ef}	22.74^{f}
	50	97.67^{c}	27.14^{d}
	75	108.33^{b}	28.58^{c}
0.69	0	72.33^{g}	20.11^{h}
	25	92.00^{d}	25.07^{e}
	50	118.00^{a}	30.24^{b}
	75	119.33^{a}	33.92^{a}
LSD (0.05)		2.02	0.57
CV (%)		3.17	3.24

Mean values followed by the same letter (s) in the same column are not significantly different at 5 % level of significance. LSD and CV stand for least significance difference and coefficient of variation, respectively.

Leaf area [cm^2]: The interactions of Azolla and nitrogen fertilizer on leaf area were highly significant ($P \leq 0.001$) (Table 1S). Application of 75 g of Azolla per pot and 0.69 g of nitrogen per pot together produced the largest leaf area (33.92 cm^2), while the control plot produced the smallest (8.73 cm^2) leaf area per plant (Table 4). This may be because both organic and inorganic fertilizers help slow the rate of cell division, which in turn promotes leaf elongation and expansion. Different scholars reported that the integrated application of organic and inorganic fertilizers in crop production has been reported to increase growth more than either alone. Similarly, the application of organic fertilizer in combination with inorganic fertilizer has been reported to increase the absorption of N, P, and K in plants compared to the application of organic or inorganic fertilizers alone [54], which increases stem elongation and leaf expansion. Similarly, Zhang et al. [55] found that the application of fertilizer can boosts leaf growth and chlorophyll content, while more nitrogen leads to bigger cells, increased cell numbers, and faster expansion, which was consistent with our findings. Increased cell division and the formation of additional branches thus contribute to the leaf area per plant. Increasing cell size, number, or changes in leaf architecture could be the cause of a larger individual leaf area with more N [54]. Pyne et al. [56] also observed higher leaf area index and chlorophyll with increasing nitrogen. Applying a suitable combination of organic and inorganic fertilizers increases the amount of nitrogen in the soil, which in turn promotes vegetative growth and thereby increases leaf area, which in turn increases the rate of photosynthesis [57].

4. Conclusion

Tomatoes are globally consumed due to their high nutritional value, but their production in developing countries like Ethiopia remains low. Conventional chemical fertilizers, especially nitrogen, play a critical role in tomato cultivation, yet their misuse leads to environmental degradation, including nutrient loss, greenhouse gas emissions, and water pollution. Sustainable agriculture seeks to reduce chemical inputs and promote organic alternatives like Azolla, an aquatic fern that shows promise as an organic fertilizer. The study investigated the effects of combining nitrogen and Azolla fertilizers on the growth and physiological development of tomato plants using the "Galilama" variety. Various doses of nitrogen and Azolla were tested, and the results showed significant improvements in chlorophyll content, photosynthesis, stomatal conductance, water retention, and water use efficiency, plant height, and leaf area when nitrogen and Azolla were applied together in higher quantities. This synergy between the organic and inorganic fertilizers promoted overall plant health, water efficiency, and productivity. The combined application of 0.69 g of nitrogen and 75 g of Azolla per pot demonstrated the highest improvements in several key parameters, such as chlorophyll content, photosynthetic rate, stomatal conductance, relative water content, water use efficiency, and plant height. The integration of these fertilizers improved photosynthesis, water efficiency, plant height, and vegetative growth, making it a promising strategy for increasing tomato productivity in sustainable agriculture. This approach also reduces the negative environmental impacts associated with conventional chemical fertilizers. Therefore, the combined use of Azolla and nitrogen could serve as an optimal fertilization strategy for tomato cultivation, especially in regions with challenging soil conditions and limited access to chemical inputs.

CRediT authorship contribution statement

Yenetiru Getaw Zeleke: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Ashenafi Haile:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Alemayehu Kiflu:** Writing – review & editing, Validation, Project administration, Methodology, Data curation, Conceptualization. **Habtamu Alemayehu:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Data curation.

Data availability statement

Data will be made available on request.

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Declaration of 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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Appendix A. Supplementary data

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