



## Effects of Humic Acid and Nitrogen Levels on Growth, Yield, and Nutrient Uptake of Flax (*Linum usitatissimum* L.) in Calcareous Soil

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**Abstract:** Flax is one of the earliest plants that humans have domesticated. Due to its high omega-3 and fatty acid content, its cultivation and consumption have increased as a healthy oil resource. It was tested in the fields of Qaladza city, Sulaimani governorate, Iraq, during the growing season of 2022–2023. Four different amounts of humic acid (0, 50, 100, and 150 kgHA ha<sup>-1</sup>) and four different amounts of nitrogen fertilizer (0, 100, 200, and 300 kg N ha<sup>-1</sup>) were used to see how they affected the flax variety Thorshansity 72, which is a cultivar from Poland. Results indicated that from a nutritional point of view, humic acid and nitrogen are two critical elements in plant growth. There were significant differences in the plant height, number of fruiting branches, and number of capsules per plant based on the humic acid and nitrogen fertilizer rates. Differences were also observed in the number of seeds per capsule and seed yield. There were also differences in the nutrients found in the soil, shoots, and roots, and the amount of oil in the seeds of a flax plant.

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## 1. Introduction

Flax, scientifically known as *Linum usitatissimum* L., is a traditional cash crop cultivated for its versatile usage in producing both seeds and fibers, which are utilized in the production of linen. The oil is consumable and is used in the production of paints, varnishes, printing inks, linoleum and soaps due to its quick staining properties. To increase flax output, one might cultivate high-yielding cultivars and employ appropriate fertilizer (Elsorady et al., 2022). Currently, the advantages of flax have surpassed all predictions. There are notable variations in both the quantity and quality of flax crops, as well as the amount of oil they contained (Kocjan and Trdan, 2008).

Humic acid (HA) is a crucial organic fertilizer and a vital constituent of humic compounds. Humus is a crucial component of organic matter in soils and municipal waste compost. It plays a fundamental role in the cycling of many environmental elements and soil ecological processes (Senesi et al., 1996). Multiple studies have documented the positive impact of humates on nutrient provision, rendering them a promising strategy for enhancing nutritional equilibrium and plant vigor (Boehme et al., 2005). Humic compounds enhance plant development, boost crop yield, and enhance the overall quality of several plant species by facilitating the absorption of nutrients and regulating their release (Atiyeh et al., 2002). In addition, humic substances stimulate shoot and root growth and nutrient uptake in vegetable crops (Cimrin and Yilmaz, 2005). Moreover, humates have an impact on respiration and the levels of sugars, amino acids, and nitrate that are accumulated (Boehme et al., 2005).

Plant nutrition and adequate levels of nutrient elements are prerequisites for maximum yield and quality. Surveys show that the use of chemical fertilizers causes an increase in food production by about 50% (Aliari, 2006). When it comes to plant productivity, N fertilizers have a greater impact compared to other fertilizers. However, these fertilizers have limited efficiency and sometimes lead to lodging and environmental contamination (Marschner, 2011). The determination of crop fertilizer requirements is influenced by several factors, such as climate conditions, plant species, cultivars, and soil fertility levels (Casa et al., 1999). The addition of nitrogen fertilizer affects the quantity and quality of flax yield, which increases the rate of photosynthesis by enhancing chlorophyll and protein synthesis (Dordas, 2010). Rahimi et al. (2011) stated that nitrogen increases the seed yield, the percentage of oil in the seeds, and the type of compounds that increase with the concentration of nitrogen added to the plant (Al-Obady and Shaker, 2022).

It is essential to conduct a comprehensive study on the growth of flax and its mineral and nutrient uptake. Flaxseed is widely used to alleviate constipation and improve digestive health. Additionally, flaxseed may reduce the risk of heart-related diseases by lowering total blood cholesterol and low-density lipoprotein (LDL, or "bad") cholesterol levels. It is crucial to ensure the growth of flax to reap these benefits. Flax has a complex root system that enables it to capture limited and unevenly distributed water and nutrient resources from the soil. These resources are then allocated to the energy-delivering, above-ground parts of the plant (Nowak and Jeziorek, 2023).

The aim of this study is to find out how humic acid and nitrogen fertilization affect the shape, function, and chemical makeup of flax plants grown in calcareous soil, as well as their yield and quality.

## 2. Materials and Methods

To assess the study's objectives, the experiment was conducted at research farms of Raparin University, College of Agricultural Engineering Sciences (45° 42' 58" E, 35° 22' 56" N, and 578 m above sea level) in Qaladza city, Sulaimani governorate, Iraqi Kurdistan region, as shown in (Figure 1), during the spring growing season of November 15, 2022, to May 31, 2023.

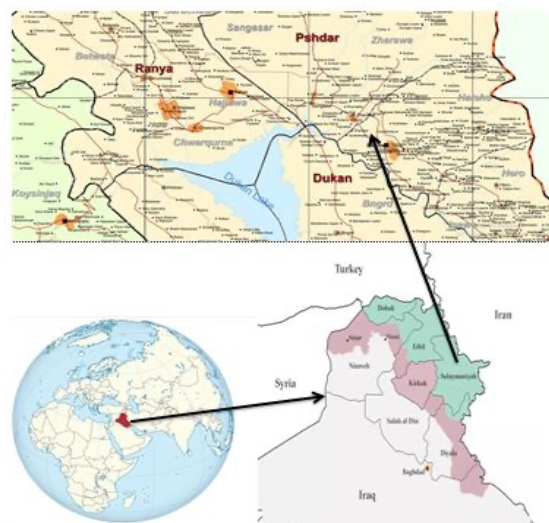


Figure 1. Location of study area.

The average climatological data for the experiment field location is shown in Figure 2. The climatological data for 2022 and 2023 showed notable differences in temperature, precipitation, and relative humidity. In January, 2023 was warmer (5.1 °C compared to 2.6 °C in 2022) but recorded lower precipitation (92.2 mm versus 125.4 mm) and relative humidity (57.0% compared to 66.0%). In March, temperatures increased to 11.5 °C in 2023 from 6.9 °C in 2022, accompanied by higher precipitation (153.6 mm versus 133.6 mm), while relative humidity slightly decreased. During the summer months (June-August), precipitation remained minimal, and relative humidity was low, with slightly lower temperatures in 2023. Notably, November 2022 experienced higher precipitation (159.4 mm compared to 106.8 mm in 2023), while December 2023 saw increased rainfall (117.2 mm compared to 43.4 mm in 2022). These differences highlighted the interannual variability across the climate parameters (Figure 2).

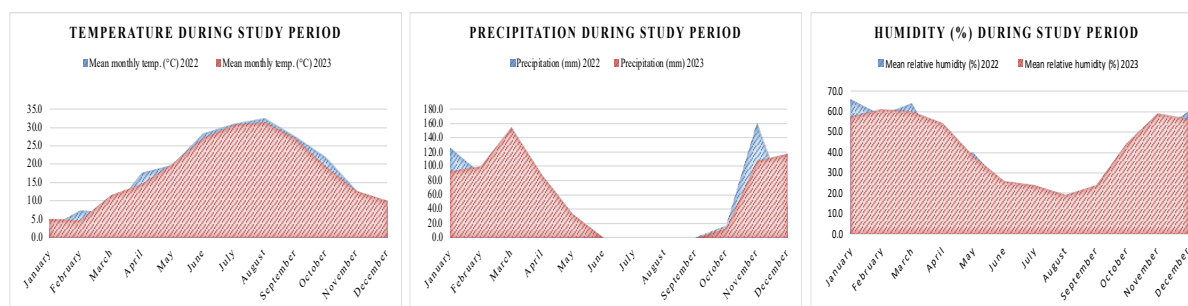


Figure 2. Climatological data of the experiment field location, in 2022 and 2023.

All necessary management protocols were implemented promptly, and conventional methods were employed for the suppression of unwanted vegetation. A soil sample was collected from a depth of 0 – 40 cm before planting in the field experiment. The collected soil samples were dried by exposure to air, then sifted through a sieve with a 2 mm mesh size, and stored in a plastic container until they were examined. Data in Table 1 provides a comprehensive representation of the main chemical and physical characteristics of the soil. Soil samples from the rhizosphere were gathered from the regions under study. A total of four soil samples were obtained for each treatment. These samples were properly mixed, homogenized, air-dried, and then sieved to exclude bigger particles. The specimens were individually packed in new polythene bags, labeled, transported to the laboratory, and stored at a temperature of 4 °C until examination. Micronutrients in the rhizosphere soil were determined by the diethylenetriaminepentaacetic (DTPA) test. Lindsay (1978) developed this test to assess the availability of micronutrients such as Zn, Mn, Fe, and Cu.

Table 1. Main soil chemical and physical characteristics of the field experiment

Soil Properties	Value	
Particle size distribution (PSD) g kg <sup>-1</sup>	Sand	56.1
	Silt	514.3
	Clay	429.6
Textural class	SiC	
Bulk density Mg m <sup>-3</sup>	1.41	
pH	7.61	
EC dS m <sup>-1</sup> at 25 °C	0.73	
Soluble ions mmol L <sup>-1</sup>	Ca <sup>2+</sup>	1.65
	Mg <sup>2+</sup>	0.77
	Na <sup>+</sup>	0.38
	K <sup>+</sup>	0.19
	HCO <sub>3</sub> <sup>-</sup>	3.10
	SO <sub>4</sub> <sup>2-</sup>	0.81
Organic matter (OM) g kg <sup>-1</sup>	21.4	
equivalent CaCO <sub>3</sub> g kg <sup>-1</sup>	Total %	23.7
Total N mg g <sup>-1</sup> soil		0.72
Ava P µg g <sup>-1</sup> soil (Olsen method)		6.92

In the study, Thorshansity 72 flax variety supplied from Poland was used as material. The experiment incorporates four levels of humic acid, specifically Bio Health WSG (Humic acid 75%) manufactured in Germany (H0 = 0, H1 = 50, H2 = 100, and H3 = 150 kg HA ha<sup>-1</sup>), and four levels of nitrogen, specifically urea, which contains 46% N (N0 = 0, N1 = 100, N2 = 200, and N3 = 300 kg N ha<sup>-1</sup>) and is incorporated into the soil at a depth of 5 cm at sowing time. There were 16 experimental units of three replicas each. The size of each experimental unit was one square meter (1 m<sup>2</sup>). Each experimental plot consisted of five lines, each with a length of one meter. The spacing between these lines was 0.20 meters (Çoban and Önder, 2014), while the spacing between individual plants inside the lines was 0.10 meters, resulting in an average plant density of 550 000 plants per hectare. The experimental units were spaced 0.5 meters apart, while the blocks were spaced one meter apart. The experimental treatments were arranged in a factorial experiment using the complete randomized block design (CRBD). The treatments administered were as in Table 2.

Table 2. The description and code of the treatments

Treatment	Description	Code
T1	Control	H0N0
T2	100 kg N ha <sup>-1</sup>	H0N1
T3	200 kg N ha <sup>-1</sup>	H0N2
T4	300 kg N ha <sup>-1</sup>	H0N3
T5	50 kg HA ha <sup>-1</sup>	H1N0
T6	50 kg HA ha <sup>-1</sup> + 100 kg N ha <sup>-1</sup>	H1N1
T7	50 kg HA ha <sup>-1</sup> + 200 kg N ha <sup>-1</sup>	H1N2
T8	50 kg HA ha <sup>-1</sup> + 300 kg N ha <sup>-1</sup>	H1N3
T9	100 kg HA ha <sup>-1</sup>	H2N0
T10	100 kg HA ha <sup>-1</sup> + 100 kg N ha <sup>-1</sup>	H2N1
T11	100 kg HA ha <sup>-1</sup> + 200 kg N ha <sup>-1</sup>	H2N2
T12	100 kg HA ha <sup>-1</sup> + 300 kg N ha <sup>-1</sup>	H2N3
T13	150 kg HA ha <sup>-1</sup>	H3N0
T14	150 kg HA ha <sup>-1</sup> + 100 kg N ha <sup>-1</sup>	H3N1
T15	150 kg HA ha <sup>-1</sup> + 200 kg N ha <sup>-1</sup>	H3N2
T16	150 kg HA ha <sup>-1</sup> + 300 kg N ha <sup>-1</sup>	H3N3

The physiological parameters were measured in the following manner: Upon reaching maturity, five plants from each plot were marked, and data on both reproductive and vegetative development metrics were collected. Measurements were collected for different yield components, such as plant height, the quantity of fruiting branches per plant, the number of seed capsules per plant, and the number of seeds per capsule. The crop was harvested on May 31, 2023, once it had reached the mature physiological stage. At this stage, plants exhibit symptoms of maturity. Six plants were collected from each treatment, as previously agreed upon. The plants were cleansed using tap water, followed by three rinses with distilled water. Subsequently, they were subjected to air-drying in a dry cabinet room at a temperature of 70 °C until they attained a stable weight. Next, the plants were finely diced to facilitate thorough drying and then subjected to oven-drying at a temperature of 70 °C until a consistent weight was obtained. The seeds obtained from each harvested region were measured in terms of weight.

The weight of seeds for each experimental unit, which covered an area of one square meter, was measured. The yield was then calculated in metric tons per hectare (Mg ha<sup>-1</sup>). The extraction of flax seed oil was performed using the method described by Kates and Eberhardt (1957). The N, P, and K content in the roots, shoot, and seed of flax were determined by ICP-AES (Varian, Vista Axial Simultaneous) as described by Soltanpour et al. (1979). A random subset of seeds was chosen from the grain yield of each experimental unit. The seed yield (ton ha<sup>-1</sup>) was calculated in tons per hectare using the following equation:

$$\text{Seed yield (ton ha}^{-1}\text{)} = \text{Plant density ha}^{-1} \times \text{Seed yield kg plant}^{-1} \quad (1)$$

$$\text{Nutrients uptake kg ha}^{-1} = \text{Nutrients content} \times \text{yield kg ha}^{-1} \quad (2)$$

## 2.1. Statistical analysis of the data obtained in the study

The data of the study were analyzed using the IBM SPSS software (v.23.0) within the framework of a Completely Randomized Block Design. Separate variance analyses were conducted for each parameter, and Duncan's multiple comparison test was applied to compare and group the means.

## 3. Results and Discussion

### 3.1. Effect of humic acid, nitrogen fertilizer levels, and their interactions on some vegetative growth criteria of flax plant

#### 3.1.1. Plant height (cm)

Table 3 reveals that both nitrogen fertilizer and humic acid significantly influenced plant height ( $P \leq 0.05$ ). Plant height increased with higher humic acid levels, ranging from 93.67 cm in the control group to a maximum of 106.00 cm in H3. Similarly, nitrogen fertilization peaked at 101.42 cm in N3, while the control (N0) measured 97.33 cm. Combined application of humic acid and nitrogen (H3N3) resulted in the tallest plants (109.33 cm), whereas the control (H0N0) had the shortest (91.67 cm; Table 3). These findings are in agreement with studies by Bakry et al. (2013) and Bakry et al. (2014) who reported improved crop yields with humic acid, and research by Zedan et al. (1999) and Omar (2020) who showed that nitrogen enhances plant height by boosting photosynthesis and protein synthesis (Rahimi and Bahrani, 2011).

Table 3. Effect of humic acid, nitrogen, and their interactions on the vegetative growth criteria of flax

Humic acid Levels	Nitrogen Levels								Effect of Humic acid **	
	N0	*	N1	*	N2	*	N3	*		
<b>Plant height (cm)</b>										
H0	91.67±0.02	fg	93.33±3.06	fg	92.67±1.53	fg	97.00±2.00	de	93.67	<i>D</i>
H1	95.33±0.58	ef	99.33±0.58	cd	100.00±0.00	b-d	99.00±1.00	cd	98.42	<i>C</i>
H2	100.00±1.00	b-d	100.67±2.08	bc	100.00±1.73	b-d	100.33±0.58	b-d	100.30	<i>B</i>
H3	102.33±1.53	bc	103.33±2.08	b	109.00±2.00	a	109.33±2.52	a	106.00	<i>A</i>
Effect of Nitrogen ***	97.33	<i>C</i>	99.17	<i>B</i>	100.42	<i>AB</i>	101.42	<i>A</i>		
<b>Number of fruiting branches plant<sup>-1</sup></b>										
H0	1.67±0.58	d	1.33±0.58	d	2.00±0.00	cd	2.00±1.00	cd	1.75	<i>C</i>
H1	2.00±0.00	cd	2.67±0.58	bc	2.00±0.00	cd	2.67±0.58	bc	2.33	<i>B</i>
H2	3.00±0.00	b	3.00±0.00	b	3.00±1.00	b	3.33±0.58	ab	3.08	<i>A</i>
H3	3.33±0.58	ab	3.00±0.00	b	4.00±0.00	a	3.33±0.58	ab	3.42	<i>A</i>
Effect of Nitrogen	2.5		2.5		2.75		2.83			
<b>Number of capsules plant<sup>-1</sup></b>										
H0	80.00±7.55	g	83.00±4.36	g	73.33±15.57	g	88.33±19.30	e-g	81.17	<i>D</i>
H1	111.33±2.08	d	85.33±10.97	fg	133.00±18.19	cd	114.33±12.86	cd	111.00	<i>C</i>
H2	107.33±4.73	de	104.67±6.66	d-f	119.67±1.53	cd	182.00±16.70	ab	128.40	<i>B</i>
H3	172.33±3.21	b	186.00±15.72	ab	185.67±10.02	ab	197.67±4.16	a	185.40	<i>A</i>
Effect of Nitrogen ***	117.75	<i>C</i>	114.75	<i>C</i>	127.92	<i>B</i>	145.58	<i>A</i>		

\*: There is no statistically significant difference at the 5% level between the means shown with the same lowercase letter in the same column for each feature.

\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital and italic letter in the same column for each feature.

\*\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital letter in the same row for each feature.

#### 3.1.2. Number of fruiting branches and capsules per plant

The application of humic acid and nitrogen fertilizers significantly impacted the number of fruiting branches and capsules per flax plant ( $P \leq 0.05$ ). Humic acid treatments notably increased fruiting branches, with the highest value (3.42) observed at H3 and the lowest (1.75) in the control. While nitrogen fertilizers alone had limited influence on fruiting branches, their interaction with humic acid showed significant effects, with values ranging from 1.33 to 4.00, the highest being at H3N2. Capsule

production also improved with both treatments; humic acid at H3 yielded the highest capsule count (185.42), while the control recorded the lowest (81.17). Nitrogen fertilizer at N3 produced the highest capsule count (145.58), with significant variations observed in the interaction treatments, ranging from 73.33 to 197.67 capsules (Table 3). These findings align with previous studies by Bakry et al. (2014), Parhizkar et al. (2012), and Homayouni et al. (2013), which reported positive effects of humic acid and nitrogen on flax growth and yield.

### 3.2. Effect of humic acid, nitrogen fertilizer levels, and their interactions on some reproductive growth criteria of flax plant

#### 3.2.1 Number of seeds per capsule

Table 4 displays the impacts of humic acid and nitrogen fertilization rate, as well as their interactions, on the quantity of seeds per capsule. The application of humic acid fertilizer had a significant impact on the seed count at a significant level of  $P \leq 0.05$ . The rate of humic acid (H3) exhibited the highest average number of seeds. The first capsule (9.83) had the highest recorded value, whereas the control had the lowest mean value (7.33). The impact of nitrogen fertilization rate on seed quantity has a significant difference at a significant level of  $P \leq 0.05$ . The nitrogen level (N3) had the highest mean value (9.17) compared to the other nitrogen values. Furthermore, the humic acid and nitrogen quantities applied exhibited a significant interaction at a significant level of  $P \leq 0.05$ . The upper limit of the quantity of seeds. The mean value of capsule<sup>-1</sup> was 10.33, which was the result of the interaction effect caused by H3N3. The lowest mean value of 7.00 was seen with H0N2 and the control group (Table 4). Similar results were found by Bakry et al. (2014) for humic acid; they found that the application of humic acid significantly affected the number of seeds per flax capsule. The study conducted by Homayouni et al. (2013) showed that nitrogen rates had a substantial impact on the quantity of seeds per capsule of flax.

Table 4. Effect of humic acid, nitrogen, and their interactions on the reproductive growth criteria of flax

Humic acid Levels	Nitrogen Levels								Effect of Humic acid **	
	N0	*	N1	*	N2	*	N3	*		
<b>Number of seeds capsule<sup>-1</sup></b>										
H0	7.00±0.00	f	7.33±0.58	ef	7.00±0.00	f	8.00±1.00	c-f	7.33	<i>C</i>
H1	7.67±0.58	d-f	8.00±1.00	c-f	8.67±0.58	b-e	8.67±1.53	b-e	8.25	<i>B</i>
H2	9.00±1.00	a-d	9.33±1.15	a-c	10.30±0.58	a	9.67±0.58	ab	9.58	<i>A</i>
H3	9.67±1.15	ab	10.00±1.00	ab	9.33±0.58	a-c	10.33±0.58	ab	9.83	<i>A</i>
Effect of Nitrogen ***	8.33	B	8.67	AB	8.83	AB	9.17	A		
<b>Seed yield Mg ha<sup>-1</sup></b>										
H0	2.80±0.02	d-f	3.26±0.05	cd	3.08±0.16	d	3.03±0.17	d	3.04	<i>D</i>
H1	3.81±0.14	bc	3.76±0.06	bc	4.16±0.35	b	3.77±0.48	bc	3.88	<i>C</i>
H2	3.86±0.45	bc	4.32±0.56	bc	5.28±0.66	a	5.41±0.21	a	4.72	<i>B</i>
H3	5.23±0.19	a	5.62±0.12	a	5.79±0.24	a	5.78±0.25	a	5.61	<i>A</i>
Effect of Nitrogen ***	3.92	C	4.24	BC	4.58	A	4.50	AB		

\*: There is no statistically significant difference at the 5% level between the means shown with the same lowercase letter in the same column for each feature.

\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital and italic letter in the same column for each feature.

\*\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital letter in the same row for each feature.

#### 3.2.2. Seed yield (Mg ha<sup>-1</sup>)

The results in Table 4 demonstrate that seed yield (Mg ha<sup>-1</sup>) was significantly influenced by humic acid, nitrogen fertilization rates, and their interaction ( $P \leq 0.05$ ). The highest yield (5.79 Mg ha<sup>-1</sup>) was achieved with the H3N2 treatment, while the lowest (2.797 Mg ha<sup>-1</sup>) occurred with H0N0. Humic

acid (H3) alone produced the highest average yield (5.606 Mg ha<sup>-1</sup>), and nitrogen (N2) also significantly increased yield (4.577 Mg ha<sup>-1</sup>) compared to controls (Table 4). Humic acid acts as a biostimulant, enhancing water and nutrient uptake and promoting growth by stimulating cell division and elongation. These findings align with studies by Bakry et al. (2014), Canellas et al. (2015), and Nardi et al. (2016). Additionally, nitrogen improves enzyme activity, protein synthesis, and chlorophyll formation, supporting plant growth, as reported by Marschner (2011).

### **3.3. Effect of humic acid, nitrogen fertilizer levels, and their interactions on some nutrient contents in the rhizosphere soil of flax plant**

#### **3.3.1. Nitrogen (N) content in the soil rhizosphere (mg kg<sup>-1</sup>)**

The nitrogen concentration in the soil rhizosphere significantly increased with humic acid application ( $p \leq 0.05$ ). The highest N content (78.500 mg kg<sup>-1</sup>) was observed at the H3 humic acid level, notably exceeding the control (44.750 mg kg<sup>-1</sup>; Table 5). These results align with evidence that humic acids enhance N retention and soil organic matter (Trevisan et al., 2010). Nitrogen fertilizer levels significantly influenced N concentration in the rhizosphere ( $p \leq 0.05$ ). The highest concentration (55.083 mg kg<sup>-1</sup>) occurred at N3, compared to the control (50.083 mg kg<sup>-1</sup>; Table 5). These findings suggest that higher N levels boost nutrient availability in soil. A significant interaction between humic acid and nitrogen fertilizer was observed ( $p \leq 0.05$ ). The maximum N concentration (99.333 mg kg<sup>-1</sup>) was recorded at the H3N2 combination, while the control treatment showed the lowest value (42.667 mg kg<sup>-1</sup>; Table 5). This synergistic effect underscores the role of humic acids and N fertilizers in enhancing soil N availability (Canellas et al., 2015). The findings emphasize the potential of combining humic acids (H3) and N fertilizers (N3) to improve nitrogen concentration in the rhizosphere. These practices can support sustainable agriculture by optimizing nutrient use efficiency and promoting soil health.

#### **3.3.2. Phosphorus (P) content in the soil rhizosphere (mg kg<sup>-1</sup>)**

As shown in Table 5, the application of humic acid significantly affected P concentrations in the rhizosphere ( $p \leq 0.05$ ). The highest P content (4.747 mg kg<sup>-1</sup>) was observed under the H3 humic acid level, while the control treatment recorded the lowest value (3.214 mg kg<sup>-1</sup>). Nitrogen levels also significantly influenced P availability. The N3 treatment produced the highest P concentration (4.069 mg kg<sup>-1</sup>), compared to the control treatment (3.786 mg kg<sup>-1</sup>). A significant interaction between humic acid and nitrogen was observed. The combination of H3 and N2 resulted in the highest P content (5.967 mg kg<sup>-1</sup>), which was substantially greater than the control (H0N0, 3.193 mg kg<sup>-1</sup>). The findings indicate that humic acid (H3) enhances P solubility and microbial activity in the rhizosphere, aligning with prior research (Canellas et al., 2015). Similarly, the lowest P concentration (3.214 mg kg<sup>-1</sup>) in the control underscores the importance of humic substances in mobilizing soil P reserves. The synergy between humic acid and nitrogen fertilizers emphasizes their critical role in sustainable soil fertility management.

#### **3.3.3. Potassium (K) content in the soil rhizosphere (%)**

Table 5 demonstrates that humic acid significantly influenced K concentration in the rhizosphere ( $p \leq 0.05$ ). The highest K concentration (0.129%) was observed at the H2 humic acid level, compared to 0.122% in the control. This effect is attributed to the chelating properties of humic acids, which enhance soil structure, cation exchange capacity, and the release of K ions into the soil solution (Atiyeh et al., 2002; Bakry et al., 2013). Potassium concentration in the rhizosphere was also significantly affected by nitrogen levels ( $p \leq 0.05$ ). The highest concentration (0.133%) was recorded at N3, compared to 0.122% in the control. This result aligns with findings by Dordas (2010) and Zedan et al. (1999), who reported that nitrogen fertilization enhances root growth and nutrient transport efficiency, thereby improving K uptake. The interaction between humic acid and nitrogen levels significantly influenced K concentration ( $p \leq 0.05$ ). The highest concentration (0.138%) was recorded in the H3N3 treatment, while the control exhibited 0.117%. This synergistic effect reflects the combined benefits of humic acids and nitrogen fertilization on root activity and nutrient availability (Atiyeh et al., 2002; Bakry et al., 2013).

Table 5. Effect of humic acid, nitrogen, and their interactions on some nutrient contents in the rhizosphere soil of flax

Humic acid Levels	Nitrogen Levels								Effect of Humic acid	
	N0	*	N1	*	N2	*	N3	*	**	**
<b>Nitrogen (mg kg<sup>-1</sup>) contents</b>										
H0	42.67±5.69	ef	56.00±6.24	c	29.67±1.15	i	50.67±3.79	cd	44.75	<i>B</i>
H1	37.67±4.16	f-h	51.00±2.65	cd	47.33±2.08	de	40.67±2.89	fg	44.17	<i>B</i>
H2	51.67±3.06	cd	36.33±6.43	gh	30.00±2.65	i	34.33±6.11	hi	38.08	<i>C</i>
H3	68.33±2.52	b	51.67±3.06	cd	99.33±1.53	a	94.67±4.04	a	78.50	<i>A</i>
Effect of Nitrogen ***	50.08	B	48.75	B	51.58	B	55.08	A		
<b>Phosphorus (mg kg<sup>-1</sup>) contents</b>										
H0	3.19±0.02	ef	3.36±0.11	e	3.43±0.08	de	2.88±0.06	ef	3.21	<i>C</i>
H1	4.50±0.10	bc	3.37±0.06	e	2.75±0.04	f	3.42±0.06	de	3.51	<i>B</i>
H2	3.42±0.07	de	2.66±0.10	f	4.02±0.02	c	4.97±0.11	b	3.77	<i>B</i>
H3	4.03±0.05	c	3.98±0.06	cd	5.97±0.04	a	5.01±0.05	b	4.75	<i>A</i>
Effect of Nitrogen ***	3.79	B	3.35	C	4.04	AB	4.07	A		
<b>Potassium (%) contents</b>										
H0	0.117±0.00	f-h	0.117±0.00	ef	0.123±0.00	b-f	0.129±0.00	b	0.122	<i>B</i>
H1	0.121±0.01	c-f	0.120±0.00	d-f	0.136±0.00	a	0.128±0.00	bc	0.127	<i>A</i>
H2	0.120±0.00	d-f	0.135±0.00	a	0.125±0.00	b-d	0.136±0.01	a	0.129	<i>A</i>
H3	0.128±0.00	bc	0.124±0.00	b-e	0.120±0.00	d-f	0.138±0.00	a	0.128	<i>A</i>
Effect of Nitrogen ***	0.122	C	0.124	BC	0.126	B	0.133	A		
<b>Iron (mg kg<sup>-1</sup>) contents</b>										
H0	26.47±2.59	c	23.00±0.10	de	15.90±0.20	h	16.13±0.50	hi	20.38	<i>C</i>
H1	29.73±0.40	b	19.63±0.90	fg	24.17±0.90	cd	19.80±1.47	fg	23.33	<i>B</i>
H2	20.37±0.75	e-g	18.27±1.80	gh	18.07±0.93	gh	29.73±1.31	b	21.61	<i>C</i>
H3	22.10±4.00	d-f	18.23±1.00	gh	31.47±0.55	b	58.37±1.44	a	32.54	<i>A</i>
Effect of Nitrogen ***	24.67	A	19.78	C	22.40	B	31.01	B		
<b>Manganese (mg kg<sup>-1</sup>) contents</b>										
H0	10.43±0.32	gh	10.20±0.20	gh	10.17±0.15	gh	10.23±0.06	gh	10.26	<i>C</i>
H1	10.40±0.20	gh	10.77±0.35	fg	11.43±0.15	ef	14.13±0.25	a	11.68	<i>B</i>
H2	14.00±0.22	a	12.93±0.64	b	12.43±0.67	b-d	12.67±0.50	bc	13.01	<i>A</i>
H3	9.97±0.15	h	11.73±0.47	de	11.83±0.25	de	11.97±0.40	c-e	11.38	<i>B</i>
Effect of Nitrogen ***	11.20	B	11.41	B	11.47	B	12.25	A		
<b>Zinc (mg kg<sup>-1</sup>) contents</b>										
H0	4.17±0.05	d	4.31±0.02	cd	4.24±0.04	d	4.42±0.03	cd	4.30	<i>B</i>
H1	4.66±0.26	c	3.70±0.32	e	3.49±0.05	ef	5.37±0.10	b	4.30	<i>A</i>
H2	4.39±0.47	cd	2.57±0.04	i	3.24±0.03	fg	2.54±0.05	i	3.20	<i>D</i>
H3	3.01±0.10	gh	2.72±0.28	hi	5.15±0.09	b	6.89±0.41	a	4.40	<i>C</i>
Effect of Nitrogen ***	4.10	B	3.30	C	4.00	B	4.80	A		

\*: There is no statistically significant difference at the 5% level between the means shown with the same lowercase letter in the same column for each feature.

\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital and italic letter in the same column for each feature.

\*\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital letter in the same row for each feature.

### 3.3.4. Iron (Fe) content in the soil rhizosphere (mg kg<sup>-1</sup>)

Humic acid application significantly influenced Fe concentration in the rhizosphere ( $p \leq 0.05$ ), as shown in Table 5. The highest Fe concentration (32.542 mg kg<sup>-1</sup>) was observed with the H3 treatment, while the lowest (20.375 mg kg<sup>-1</sup>) occurred in the control group. This result aligns with previous findings



that humic substances chelate Fe, enhancing its mobility and availability in alkaline soils where Fe is often limited (Atiyeh et al., 2002; Canellas et al., 2015). Similar studies by Bakry et al. (2013) also demonstrated increased Fe availability under humic acid treatments in flax plants. Nitrogen (N) levels significantly impacted Fe concentration ( $p \leq 0.05$ ). The highest Fe concentration ( $31.008 \text{ mg kg}^{-1}$ ) was recorded with the N3 treatment, while the control yielded a lower concentration ( $24.667 \text{ mg kg}^{-1}$ ). This result supports the role of nitrogen fertilizers in enhancing Fe solubility by promoting rhizosphere acidification through root exudates (Dordas, 2010; Marschner, 2011). Parhizkar et al. (2012) also reported improved Fe uptake under high nitrogen levels in flax plants.

The interaction between humic acid and nitrogen significantly influenced Fe concentration in the rhizosphere ( $p \leq 0.05$ ). The H3N3 treatment produced the highest Fe concentration ( $58.367 \text{ mg kg}^{-1}$ ), while the control recorded  $26.467 \text{ mg kg}^{-1}$  (Table 5). These findings highlight the synergistic effect of humic acid and nitrogen fertilizers on Fe bioavailability, emphasizing their role in improving soil micronutrient dynamics and plant uptake.

### 3.3.5. Manganese (Mn) content in the soil rhizosphere ( $\text{mg kg}^{-1}$ )

The application of humic acid significantly influenced Mn concentration in the rhizosphere ( $p \leq 0.05$ ). The H2 treatment exhibited the highest mean Mn concentration ( $13.008 \text{ mg kg}^{-1}$ ), whereas the control treatment (H0) had the lowest mean value ( $10.258 \text{ mg kg}^{-1}$ ; Table 5). These results align with findings by Bakry et al. (2013), demonstrating that humic acids enhance micronutrient availability by chelating metal ions and improving their solubility in the soil. Nitrogen levels also significantly affected Mn concentration in the rhizosphere. The N3 treatment achieved the highest mean Mn concentration ( $12.250 \text{ mg kg}^{-1}$ ), while the control treatment (N0) recorded the lowest ( $11.200 \text{ mg kg}^{-1}$ ; Table 5). This increase can be attributed to nitrogen's role in enhancing plant metabolic activity, facilitating active transport of Mn (Marschner, 2011). The interaction of humic acid and nitrogen levels resulted in the highest Mn concentration ( $14.133 \text{ mg kg}^{-1}$ ) under the H1N3 treatment, compared to all other combinations. The control treatment (H0N0) recorded the lowest Mn concentration ( $10.433 \text{ mg kg}^{-1}$ ; Table 5). These findings suggest that combining humic acid and nitrogen fertilizers enhances Mn bioavailability through synergistic effects, as humic substances stimulate microbial activity and improve nutrient solubility (Canellas et al., 2015). The significant increase in Mn concentration under H2 and H1N3 treatments highlights the potential of humic acid and nitrogen fertilization for improving Mn availability. Mn is critical for plant physiological processes, including photosynthesis and enzyme activation, making its bioavailability essential for optimal growth (Marschner, 2011; Senesi et al., 1996).

### 3.3.6. Zinc (Zn) content in the soil rhizosphere ( $\text{mg kg}^{-1}$ )

The application of humic acid significantly influenced Zn concentration in the rhizosphere ( $p \leq 0.05$ ). The H3 treatment recorded the highest mean Zn concentration ( $4.443 \text{ mg kg}^{-1}$ ), significantly higher than the control (H0), which recorded  $4.287 \text{ mg kg}^{-1}$  (Table 5). Nitrogen levels also significantly affected Zn concentration ( $p \leq 0.05$ ). The N3 treatment exhibited the highest mean Zn concentration ( $4.804 \text{ mg kg}^{-1}$ ), while the lowest concentration was observed at N1 ( $3.324 \text{ mg kg}^{-1}$ ; Table 5). The interaction of H3 and N3 resulted in the maximum Zn concentration ( $6.887 \text{ mg kg}^{-1}$ ), significantly higher than all other treatments. In contrast, the control interaction (H0N0) recorded the lowest concentration ( $4.173 \text{ mg kg}^{-1}$ ; Table 5). Humic substances increase Zn solubility and mobility in the rhizosphere by forming soluble complexes with metal ions, enhancing bioavailability to plants (Atiyeh et al., 2002; Canellas et al., 2015). Additionally, humic acids promote root growth and activity, improving root-soil interactions and Zn acquisition (Nardi et al., 2016).

## 3.4. Effect of humic acid, nitrogen fertilizer levels and their interactions on some nutrient contents in the shoot of flax plant

### 3.4.1. Nitrogen, Phosphorus, and Potassium content in the shoot (%)

Table 6 highlights the significant effects of humic acid and nitrogen fertilizer levels on the nitrogen (N), phosphorus (P), and potassium (K) content in flax shoots. Nitrogen content in the shoot exhibited significant variation ( $p \leq 0.05$ ), with the highest mean (2.872%) observed under H3 ( $150 \text{ kg HA ha}^{-1}$ ) and the lowest (2.112%) in the control (H0). Similarly, nitrogen fertilizer application yielded the highest mean N content (2.842%) under N3 ( $300 \text{ kg N ha}^{-1}$ ) and the lowest (2.161%) in the control.

Table 6. Effect of humic acid, nitrogen, and their interactions on some nutrient contents in the shoot of flax

Humic acid Levels	Nitrogen Levels								Effect of humic acid **	
	N0	*	N1	*	N2	*	N3	*		
<b>Nitrogen (%) contents</b>										
H0	1.707±0.06	g	2.020±0.04	f	2.293±0.17	e	2.427±0.12	e	2.112	<i>C</i>
H1	1.573±0.11	g	3.080±0.31	ef	2.270±0.07	ef	2.947±0.06	a-c	2.468	<i>B</i>
H2	2.527±0.09	de	2.900±0.08	cd	2.737±0.06	cd	2.880±0.27	a-c	2.761	<i>A</i>
H3	2.837±0.06	bc	2.403±0.17	a	3.133±0.13	a	3.113±0.29	ab	2.872	<i>A</i>
Effect of Nitrogen ***	2.161	C	2.601	B	2.608	B	2.842	A		
<b>Phosphorus (%) contents</b>										
H0	0.438±0.03	h	0.251±0.05	j	0.366±0.02	hi	0.346±0.01	hi	0.350	<i>D</i>
H1	0.408±0.01	hi	0.317±0.02	ij	0.316±0.01	ij	0.748±0.15	bc	0.447	<i>C</i>
H2	0.547±0.03	g	0.572±0.01	e-g	0.565±0.03	fg	0.661±0.04	c-e	0.586	<i>B</i>
H3	0.711±0.04	b-d	0.854±0.06	a	0.793±0.02	ab	0.643±0.07	d-f	0.750	<i>A</i>
Effect of Nitrogen ***	0.526	B	0.499	C	0.510	cd	0.599	A		
<b>Potassium (%)</b>										
H0	1.157±0.07	d	1.190±0.01	d	1.272±0.05	cd	1.151±0.11	d	1.193	<i>B</i>
H1	1.307±0.02	cd	1.150±0.11	d	1.187±0.07	d	1.267±0.06	cd	1.228	<i>B</i>
H2	1.173±0.03	d	1.213±0.02	d	1.173±0.03	d	1.183±0.11	d	1.186	<i>B</i>
H3	1.580±0.21	ab	1.423±0.10	bc	1.483±0.20	b	1.673±0.12	a	1.540	<i>A</i>
Effect of Nitrogen ***	1.304	A	1.244	A	1.279	A	1.318	A		
<b>Iron (mg kg<sup>-1</sup>)</b>										
H0	124.0±2.00	d	86.9±2.52	g	120.7±0.58	de	193.3±8.50	b	131.2	<i>A</i>
H1	116.0±7.00	de	142.7±1.15	c	87.8±4.05	g	98.9±0.82	f	111.4	<i>S</i>
H2	200.0±1.00	ab	85.7±5.69	g	91.7±0.55	fg	121.3±2.52	de	124.7	<i>C</i>
H3	135.3±3.06	c	121.3±0.36	de	113.0±10.58	e	207.7±9.29	a	144.3	<i>B</i>
Effect of Nitrogen ***	143.8	B	109.2	C	103.3	D	155.3	A		
<b>Manganese (mg kg<sup>-1</sup>)</b>										
H0	34.57±1.89	fg	31.17±0.25	h	31.63±0.45	h	39.27±0.74	e	34.16	<i>C</i>
H1	31.37±0.29	h	35.23±2.02	fg	35.73±1.64	f	30.40±0.50	h	33.18	<i>C</i>
H2	32.97±1.03	gh	53.97±2.40	ab	55.23±2.74	a	51.40±0.82	bc	48.39	<i>B</i>
H3	45.80±3.06	d	51.47±0.81	bc	51.00±0.44	c	52.07±0.15	bc	50.08	<i>A</i>
Effect of Nitrogen ***	36.18	B	42.96	A	43.40	A	43.28	A		
<b>Zinc (mg kg<sup>-1</sup>)</b>										
H0	175.0±5.29	h	174.3±2.52	h	216.3±3.79	g	214.3±3.06	g	195.0	<i>D</i>
H1	211.7±8.05	g	252.7±5.51	f	255.0±4.58	f	256.7±4.51	f	244.0	<i>C</i>
H2	256.7±1.53	f	280.3±18.15	e	287.7±3.51	e	311.0±7.94	d	283.9	<i>B</i>
H3	337.0±11.27	c	365.3±14.43	b	377.0±4.58	b	391.3±8.62	a	367.7	<i>A</i>
Effect of Nitrogen ***	245.1	D	268.2	C	284.0	B	293.3	A		

\*: There is no statistically significant difference at the 5% level between the means shown with the same lowercase letter in the same column for each feature.

\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital and italic letter in the same column for each feature.

\*\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital letter in the same row for each feature.

Interaction effects were also significant, with maximum N content (3.113%) under combined H3N3 and H3N2 treatments. The significant increase in shoot nitrogen content with higher levels of

humic acid and nitrogen fertilizers corroborates reports by (Canellas et al., 2015) and (Adani et al., 1998), who demonstrated that humic substances improve nutrient availability by enhancing root growth and nutrient absorption. Additionally, nitrogen fertilizers are well-known to directly supply nitrogen, a vital component of amino acids and proteins, as supported by Zhang et al. (2015).

Phosphorus content in the shoots significantly increased with humic acid levels, peaking at 0.750% under H3 compared to 0.350% in the control. Nitrogen fertilizer also enhanced P content, with the highest mean (0.599%) under N3 and the lowest (0.547%) in the control. Interaction effects were notable, with the highest P content (0.793%) under H3N2 and the lowest (0.251%) under H0N1 (Table 6). The observed increase in phosphorus uptake with humic acid applications can be attributed to their ability to chelate and solubilize phosphorus, making it more available to plants. The interaction between nitrogen and phosphorus is critical, as nitrogen availability often promotes root expansion, enabling greater phosphorus uptake (Marschner, 2011).

For potassium content, humic acid application significantly increased K levels, with a maximum mean (1.540%) under H3 compared to 1.193% in the control. However, nitrogen fertilizer alone did not show a significant effect on K content. Interaction analysis revealed the highest K content (1.673%) under H3N3 and the lowest (1.150%) under H1N1 (Table 6). These results underline the synergistic effects of humic acid and nitrogen fertilizer in enhancing nutrient content in flax shoots. The significant rise in potassium content with increased humic acid levels aligns with studies by (Piccolo et al., 1997), who noted that humic acids improve soil cation exchange capacity, thereby increasing potassium availability. Although nitrogen application did not significantly affect potassium levels in isolation, the interaction between humic acid and nitrogen fertilizers resulted in the highest potassium content, highlighting the synergistic effect.

#### **3.4.2. Iron, Manganese and Zinc content in the shoot ( $\text{mg kg}^{-1}$ )**

The data in Table 6 shows that both humic acid and nitrogen fertilizer levels significantly influenced the concentrations of iron (Fe), manganese (Mn), and zinc (Zn) in flax plant shoots ( $p \leq 0.05$ ). For iron, the highest concentration ( $144.333 \text{ mg kg}^{-1}$ ) was observed at H3, significantly higher than the control ( $131.225 \text{ mg kg}^{-1}$ ). Nitrogen application at N3 led to the highest Fe content ( $155.308 \text{ mg kg}^{-1}$ ), significantly different from the control ( $143.833 \text{ mg kg}^{-1}$ ). The interaction of humic acid and nitrogen at H3N3 resulted in the highest Fe content ( $207.667 \text{ mg kg}^{-1}$ ), compared to the control ( $124.000 \text{ mg kg}^{-1}$ ; Table 6).

For manganese, the highest concentration ( $50.083 \text{ mg kg}^{-1}$ ) was found at H3, with the lowest at H1 ( $33.183 \text{ mg kg}^{-1}$ ). Nitrogen at N2 increased Mn content to  $43.400 \text{ mg kg}^{-1}$ , higher than the control ( $36.175 \text{ mg kg}^{-1}$ ). The interaction of humic acid and nitrogen at H3N3 showed the highest Mn content ( $55.233 \text{ mg kg}^{-1}$ ), compared to the control ( $34.567 \text{ mg kg}^{-1}$ ; Table 6). This supports the findings of (Bakry et al., 2014), who also noted the positive effects of these treatments on manganese uptake.

Zinc content in the shoots was similarly affected, with the highest value ( $367.667 \text{ mg kg}^{-1}$ ) observed at H3, significantly higher than the control ( $195.000 \text{ mg kg}^{-1}$ ). Nitrogen at N3 resulted in  $293.333 \text{ mg kg}^{-1}$ , also higher than the control ( $245.083 \text{ mg kg}^{-1}$ ). The highest Zn content ( $391.333 \text{ mg kg}^{-1}$ ) was observed at H3N3, while the lowest was at H0N1 ( $174.333 \text{ mg kg}^{-1}$ ; Table 6).

### **3.5. Effect of humic acid, nitrogen fertilizer levels, and their interactions on some nutrient contents in the root of flax plant**

#### **3.5.1. Nitrogen, Phosphorus, and Potassium content in the root (%)**

The results in Table 7 show that both humic acid and nitrogen fertilizer rates significantly influenced nitrogen (N) content in the roots of flax plants ( $p \leq 0.05$ ). N content increased with higher humic acid levels, with the highest value (1.179%) recorded at H2 and the lowest (0.959%) at the control. For nitrogen fertilizer, the highest N content (1.249%) was observed at N3, significantly higher than the control (1.036%). The interaction between humic acid and nitrogen rates produced the highest N content (1.327%) at H3N3, compared to the control (0.987%). Humic acid has been shown to enhance nitrogen uptake by improving root structure and soil nutrient availability (Canellas et al., 2015). Similarly, nitrogen fertilizers directly increase nitrogen content in plant tissues, as observed in studies by Marschner (2011), emphasizing their vital role in metabolic processes and growth.

Table 7. Effect of humic acid, nitrogen, and their interactions on some nutrient contents in the root of flax

Humic acid Levels	Nitrogen Levels								Effect of Humic acid **	
	N0	*	N1	*	N2	*	N3	*		
<b>Nitrogen (%)</b>										
H0	0.987±0.02	f-h	0.770±0.10	j	0.860±0.07	ij	1.220±0.05	a-c	0.959	<i>C</i>
H1	1.030±0.11	e-h	0.977±0.06	g-i	1.260±0.10	ab	1.190±0.06	b-d	1.114	<i>B</i>
H2	1.197±0.08	b-d	1.073±0.04	d-g	1.187±0.02	b-d	1.260±0.04	ab	1.179	<i>A</i>
H3	0.930±0.06	hi	1.127±0.06	c-e	1.107±0.11	c-f	1.327±0.05	a	1.123	<i>AB</i>
Effect of Nitrogen***	1.036	C	0.987	C	1.103	B	1.249	A		
<b>Phosphorus (%)</b>										
H0	0.242±0.00	e	0.268±0.01	de	0.258±0.00	de	0.245±0.02	de	0.253	<i>B</i>
H1	0.157±0.00	g	0.311±0.03	bc	0.178±0.04	fg	0.312±0.01	bc	0.239	<i>A</i>
H2	0.255±0.02	de	0.192±0.02	f	0.280±0.01	cd	0.312±0.02	bc	0.260	<i>B</i>
H3	0.243±0.02	de	0.245±0.01	de	0.342±0.02	b	0.386±0.04	a	0.304	<i>C</i>
Effect of Nitrogen***	0.224	C	0.254	B	0.264	B	0.314	A		
<b>Potassium (%)</b>										
H0	0.733±0.04	a-e	0.790±0.07	a	0.600±0.04	f	0.723±0.04	a-e	0.712	<i>B</i>
H1	0.590±0.06	f	0.747±0.04	a-d	0.803±0.02	a	0.680±0.03	c-f	0.705	<i>B</i>
H2	0.597±0.14	f	0.600±0.07	f	0.650±0.07	ef	0.673±0.01	d-f	0.630	<i>C</i>
H3	0.683±0.04	b-f	0.777±0.02	ab	0.783±0.03	a	0.773±0.06	a-c	0.754	<i>A</i>
Effect of Nitrogen***	0.651	B	0.728	A	0.709	A	0.713	A		
<b>Iron (mg kg<sup>-1</sup>)</b>										
H0	241.7±7.57	ef	286.0±13.75	cd	265.0±32.08	de	279.0±8.54	cd	267.917	<i>C</i>
H1	346.3±23.03	ab	342.7±4.51	ab	301.0±10.00	c	354.3±6.51	ab	336.083	<i>A</i>
H2	293.3±14.15	cd	220.7±19.66	f	332.7±25.72	b	350.7±18.77	ab	299.333	<i>B</i>
H3	277.3±22.81	cd	282.3±20.01	cd	284.3±14.05	cd	366.7±22.90	a	302.667	<i>B</i>
Effect of Nitrogen***	289.7	B	282.9	B	295.8	B	337.7	A		
<b>Manganese (mg kg<sup>-1</sup>)</b>										
H0	4.167±0.05	g	4.810±0.04	ef	2.927±0.09	j	6.343±0.12	b	4.562	<i>B</i>
H1	5.070±0.06	e	2.897±0.23	j	5.523±0.36	d	6.947±0.07	a	5.109	<i>A</i>
H2	3.843±0.031	hi	3.153±0.05	j	5.847±0.16	c	5.663±0.06	cd	4.627	<i>B</i>
H3	3.523±0.08	i	4.240±0.14	g	4.770±0.21	f	5.923±0.09	c	4.614	<i>B</i>
Effect of Nitrogen***	4.151	C	3.775	D	4.767	B	6.219	A		
<b>Zinc (mg kg<sup>-1</sup>)</b>										
H0	42.90±0.30	e-g	46.27±0.34	e-g	48.77±0.51	e	48.33±0.42	e	46.567	<i>B</i>
H1	46.80±0.46	ef	43.10±0.20	e-g	40.93±0.15	g	57.53±4.61	d	47.092	<i>B</i>
H2	47.40±3.80	ef	42.07±8.93	fg	44.27±0.59	e-g	60.23±1.27	cd	48.492	<i>B</i>
H3	63.63±0.25	c	80.27±0.76	b	98.83±0.85	a	80.73±0.35	b	80.867	<i>A</i>
Effect of Nitrogen***	50.18	D	52.93	C	58.20	B	61.71	A		

\*: There is no statistically significant difference at the 5% level between the means shown with the same lowercase letter in the same column for each feature.

\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital and italic letter in the same column for each feature.

\*\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital letter in the same row for each feature.

Similarly, phosphorus (P) content in the root increased with higher humic acid levels. The maximum P content (0.304%) was recorded at H3, and the minimum (0.239%) at H1. Nitrogen fertilizer also significantly affected P content, with the highest value (0.314%) at N3 and the lowest (0.224%) at the control. The interaction between humic acid and nitrogen resulted in the highest P content (0.386%)

at H3N3, and the lowest (0.157%) at H1N0 (Table 7). Higher levels of humic acid improve phosphorus availability by chelating soil micronutrients and reducing P fixation (Trevisan et al., 2010). This is complemented by nitrogen fertilization, which boosts root growth, enhancing phosphorus uptake (Rengel and Marschner, 2005).

Regarding potassium (K), significant increases in root K content were observed with the application of humic acid. The maximum K content (0.754%) was recorded at H3, higher than the control (0.712%). The highest K content for nitrogen fertilizer (0.713%) was recorded at N3, compared to the control (0.651%). The interaction of humic acid and nitrogen rates showed the highest K content (0.803%) at H1N2 and the lowest (0.590%) at H1N0 (Table 7). Humic acid application increases potassium absorption due to improved cation exchange capacity and root permeability (Rose et al., 2016). The role of nitrogen fertilizers in stimulating root biomass also indirectly contributes to greater potassium uptake (López-Bucio et al., 2003).

### 3.5.2. Iron, Manganese, and Zinc content in the root ( $\text{mg kg}^{-1}$ )

Table 7 highlights that the application of humic acid and nitrogen fertilizer significantly influenced the concentrations of iron (Fe), manganese (Mn), and zinc (Zn) in flax roots, with notable interactions between the two factors. Specifically, the highest Fe concentration ( $336.083 \text{ mg kg}^{-1}$ ) was recorded at the H1 treatment, while the control treatment had the lowest Fe content ( $267.917 \text{ mg kg}^{-1}$ ). Similarly, nitrogen treatment N3 showed the highest Fe concentration ( $337.667 \text{ mg kg}^{-1}$ ), significantly higher than the control ( $289.667 \text{ mg kg}^{-1}$ ). The interaction between H3N3 resulted in the highest Fe concentration ( $366.667 \text{ mg kg}^{-1}$ ), whereas the control had the lowest ( $241.667 \text{ mg kg}^{-1}$ ).

For Mn content, the highest concentration ( $5.109 \text{ mg kg}^{-1}$ ) was recorded at H1, compared to the control ( $4.562 \text{ mg kg}^{-1}$ ). The nitrogen treatment N3 produced the highest Mn concentration ( $6.219 \text{ mg kg}^{-1}$ ), significantly greater than the control ( $4.151 \text{ mg kg}^{-1}$ ). The interaction between humic acid and nitrogen at H1N3 led to the highest Mn content ( $6.947 \text{ mg kg}^{-1}$ ), while the lowest Mn was found at H1N1 ( $2.897 \text{ mg kg}^{-1}$ ). For Zn, increasing humic acid application significantly raised root Zn content.

The highest Zn concentration ( $80.867 \text{ mg kg}^{-1}$ ) was seen at H3, compared to the control ( $46.567 \text{ mg kg}^{-1}$ ). Nitrogen treatment N3 resulted in the highest Zn concentration ( $61.708 \text{ mg kg}^{-1}$ ), while the lowest concentration ( $50.183 \text{ mg kg}^{-1}$ ) was observed in the control. The interaction between H3N2 resulted in the highest Zn concentration ( $98.833 \text{ mg kg}^{-1}$ ), significantly higher than the control ( $42.900 \text{ mg kg}^{-1}$ ). These findings are consistent with previous studies. For instance, Bakry et al. (2014) demonstrated that humic acid significantly enhances nutrient uptake in plants, including minerals such as iron and manganese. The significant increase in Mn and Zn content with humic acid and nitrogen treatments, as observed in the current study, aligns with these earlier findings, which underscore the positive impact of these treatments on mineral nutrition in flax.

### 3.6. Effect of humic acid, nitrogen fertilizer levels, and their interactions on the oil content in the seed and linolic and lenolinic acid in the seed oil of flax plant

The data in Table 8 show that both humic acid and nitrogen fertilizer significantly affected seed oil content ( $p \leq 0.05$ ). The highest oil content (37.706%) was recorded with the H3 treatment, while the lowest (28.089%) was in the H0 treatment. For nitrogen, the maximum oil content (33.532%) was observed at N3, significantly higher than the control (32.154%). The interaction between humic acid and nitrogen fertilizer also significantly influenced oil content, with the highest value (39.577%) observed at H3N3.

Additionally, significant effects were found for linoleic acid percentage in seed oil ( $p \leq 0.05$ ). The highest linoleic acid content (15.740%) was observed at H3, while the lowest (14.544%) was in the control. For nitrogen, N3 resulted in the highest linoleic acid content (15.611%) compared to the control (15.030%). The interaction of humic acid and nitrogen fertilizer also significantly affected linoleic acid, with the highest value (16.160%) at H1N3.

Significant increases in linolenic acid content were also noted with the application of humic acid and nitrogen. The highest linolenic acid percentage (50.362%) was recorded at H3, significantly higher than the control (48.833%). For nitrogen, the highest value (50.038%) was observed at N3, compared to the control (49.039%). The interaction of humic acid and nitrogen fertilizer led to the maximum linolenic acid content (50.507%) at H1N3, with the minimum value (48.203%) at the control. These

findings are consistent with previous studies by Bakry et al. (2014) who reported that both humic acid and nitrogen fertilizer significantly enhance oil and fatty acid content in flax seeds.

Table 8. Effect of humic acid, nitrogen, and their interactions on the oil content in the seed and Linolic and Lenolinic acid in the seed oil of flax

Humic acid Levels	Nitrogen Levels								Effect of Humic acid **	
	N0	*	N1	*	N2	*	N3	*		
<b>Oil Seed %</b>										
H0	26.31±3.52	hi	30.20±0.67	e-h	27.34±5.57	g-i	28.50±1.43	f-i	28.09	<i>B</i>
H1	30.96±2.23	e-g	27.12±2.28	g-i	25.31±2.30	i	29.29±0.60	f-i	28.17	<i>B</i>
H2	32.41±2.24	d-f	31.42±3.97	a	34.37±1.34	de	36.76±0.82	b-d	36.24	<i>A</i>
H3	38.93±0.41	ab	34.08±2.63	de	36.24±1.75	cd	39.58±1.24	a-c	37.71	<i>A</i>
Effect of Nitrogen ***	32.15	AB	30.71	A	30.81	B	33.53	A		
<b>Linolic acid - Seed %</b>										
H0	14.07±0.06	h	14.71±0.23	ef	14.82±0.08	e	14.58±0.08	ef	14.54	<i>D</i>
H1	15.33±0.04	cd	14.47±0.13	fg	14.29±0.06	gh	16.16±0.24	a	15.06	<i>C</i>
H2	14.69±0.03	ef	16.03±0.10	a	15.14±0.05	d	15.71±0.33	b	15.39	<i>B</i>
H3	16.03±0.13	a	15.57±0.06	bc	15.37±0.11	cd	15.99±0.13	a	15.74	<i>A</i>
Effect of Nitrogen ***	15.03	C	15.19	B	14.90	D	15.61	A		
<b>Lenolinic acid - Seed %</b>										
H0	48.20±0.20	g	49.29±0.08	d	49.17±0.06	d	48.67±0.41	ef	48.83	<i>D</i>
H1	49.80±0.01	c	48.60±0.02	ef	48.47±0.12	fg	50.51±0.40	ab	49.35	<i>C</i>
H2	48.95±0.06	de	50.10±0.10	a	49.86±0.31	c	50.47±0.24	ab	49.99	<i>B</i>
H3	50.40±0.22	a	50.14±0.18	bc	50.00±0.30	c	50.50±0.15	ab	50.36	<i>A</i>
Effect of Nitrogen ***	49.04	C	49.68	B	49.38	C	50.04	A		

\*: There is no statistically significant difference at the 5% level between the means shown with the same lowercase letter in the same column for each feature.

\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital and italic letter in the same column for each feature.

\*\*\*: There is no statistically significant difference at the 5% level between the means shown with the same capital letter in the same row for each feature.

## Conclusions

This study presents a comprehensive evaluation of the effects of different levels of humic acid and nitrogen on flax plant growth and oil properties over the course of one year. The assessment focused on vegetative growth parameters (plant height, number of fruiting branches per plant, and number of capsules per plant), reproductive growth parameters (seed yield, number of seeds per capsule), and nutrient content in the rhizosphere soil, shoot, and root. Various factors influencing plant development and oil quality were examined to determine how humic acid and nitrogen fertilization impacted flax growth and oil composition.

Key findings from the study include:

Significant differences were observed in most flax characteristics, except for the number of fruiting branches, which was unaffected by nitrogen.

Nutrient content in the rhizosphere soil, shoot, and root varied significantly with different humic acid and nitrogen rates, with the exception of potassium content in the shoot, which was not influenced by nitrogen.

The oil content in flax seeds, along with the levels of linoleic and linolenic acids in the seed oil, showed significant variation based on the application of different humic acid and nitrogen levels.

Flax plants demonstrated optimal vegetative and reproductive growth at nitrogen levels below 300 kg ha<sup>-1</sup>, suggesting that higher nitrogen applications were unnecessary for this cultivar.

These results highlight the significant role of humic acid and nitrogen fertilization in enhancing flax plant growth and oil quality.

## Ethical Statement

Ethical approval is not required for this study because only plant materials are used in this study.

## Conflict of Interest

The authors declare that there are no conflicts of interest.

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## Author Contributions

Azad Salih Abdullah Krusk contributed to project management, conceptual design, methodology, and data analysis; Aso Hashm Husamaldin contributed to data collection, literature review, results evaluation, and writing; Muhamad Tahsen Maruf contributed to experimental work, data validation, and editing; and Tamer Eryiğit contributed to results interpretation, manuscript revision, and final approval of the manuscript.

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