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MUTUAL EFFECTS OF HUMIC ACID CONTENT AND NITROGEN SOURCES FOR VEGETATIVE DEVELOPMENT AND FLOWERING OF SNAPDRAGON (*Antirrhinum majus* L.)

Nurdilek Gulmezoglu[1], Çiğdem Yücel[2], Sibel Yigiter-Saricam[3]✉

[1] Eskisehir Osmangazi University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, 26160 Eskisehir, Turkiye

[2] Republic of Turkiye Ministry of Agriculture and Forestry Eskisehir Directorate of Provincial Agriculture and Forestry, 26160 Eskisehir, Turkiye

[3] Eskisehir Osmangazi University, Faculty of Agriculture, Department of Horticulture, 26160 Eskisehir, Turkiye

ABSTRACT

Snapdragon (*Antirrhinum majus* L.), a garden plant cherished for its unique and colorful flowers, is widely used in bouquets and wreaths as a cut flower. The purpose of this study is to examine the effects of nitrogen sources (ammonium sulfate, AS; ammonium nitrate, AN; and urea) and humic acids from lignite sources (TKI-Humas and HUM-Zn) on the growth and flower production of snapdragon that are grown in pots under controlled conditions. It has been observed that plants started to flower during applications of HUM-Zn with AN and urea, whereas they remained at the vegetative stage during the application of HUM-Zn with AS. Furthermore, it has been determined that the two humic acid sources with AS usage prolonged the vegetative development and did not induce flowering of plants. Simultaneous application of humic acid and nitrogen sources has caused an increase in the leaf width, peduncle diameter, floret weight, chlorophyll content, and biomass of the snapdragon. Results show that the application of HUM-Zn with AN and urea has been effective on the plant's vegetative organs, flowering, and dry weight. It indicated that HUM-Zn contains zinc, which is effective in flowering and biomass development. In conclusion, it was concluded that the simultaneous application of humic acid with AN or urea rapidly affected the flowering process of snapdragon.

Key words: *Antirrhinum majus* L., snapdragon, humic acid, nitrogen, zinc, urea

INTRODUCTION

Snapdragon (*Antirrhinum majus* L.) is a plant that is used as a cut flower and a seasonal bedding plant, following in popularity after roses, carnations, chrysanthemums, and gladiolus [Mohamed and Elagabain 2021]. Having a large spectrum of colors, magnificent spikes, and a relatively long vase life contributes to its high production [Abdulhadi et al. 2022]. *Antirrhinum*, a genus native to the Western Mediterranean region, comprises approximately 28 species [Liberal et al. 2014]. Some species of the genus are annual, whereas the others are perennials and may have herbaceous or woody stems.

Snapdragon prefers soils that are rich in organic matter, have high water permeability, and are sandy and lime. It requires the application of extra fertilizers in order to increase the flowering of snapdragon. It is essential to fertilize the plants in order to increase yield and quality. While mineral fertilizers increase

✉ ssaricam@ogu.edu.tr

crop yield, they can lead to adverse environmental effects, especially the accumulation of heavy metals in the soil, if used excessively [Atafar et al. 2010]. Therefore, nutritional resources that have the most negligible environmental effects on plants and lead to the highest yield must be preferred. Excessive use of chemical fertilizers impairs water availability and does not result in crop yield increase [Ye et al. 2020].

In order to decrease the use of chemical fertilizers, the combined and coherent application of inorganic, organic, and biological plant nutrient sources should be studied, and the plant nutrient dynamics within the soil-plant system should be understood [Aulakh and Grant 2008]. Chemical or inorganic inputs can be used more efficiently when combined with appropriate organic/biological sources [Adesemoye et al. 2008]. The difficulty of this method is to find the most suitable combinations of organic and biological sources that result in a positive synergy with chemical fertilizers and in decreasing the adverse environmental effects of chemical fertilizers.

Research studies have shown that humic acid can have beneficial effects for improving plant growth, and according to Kutlu and Gulmezoglu [2023], humic acid reduces the damage of salt stress. Incorporating humic acids into the soil offers a means of reducing the quantity of chemical fertilizers required by plants while simultaneously enhancing their efficient nutrient uptake [Delfine et al. 2005]. It is essential to understand the plant-humic acid relationship properly in order to increase plant nutrient uptake [Peoples and Craswell 1992, Zhu et al. 2001]. Recent studies have demonstrated that the concurrent application of humic acid and zinc can exert a positive influence on plants, leading to potential enhancements in yield and yield-related parameters [Hatami 2017, Kutlu and Gulmezoglu 2020]. Insufficient zinc during development leads to a 50–70% drop in photosynthesis, varying with plant type and soil Zn levels. Additionally, zinc plays vital roles in water use efficiency, root growth, flowering, and grain formation [Alloway 2008].

Nitrogen is an inseparable part of plant tissue and contributes significantly to plant yield [Erisman et al. 2008, Liu et al. 2013]. Nitrogen is one of the most essential mineral elements for indoor plants and directly affects their growth and flowering behavior [Delgado et al. 1998]. Wilkinson et al. [2020] have pointed out that the effect of nitrogen on plant growth and productivity is often ignored. However, Luo et al. [2020] explained that studies regarding the effects of nitrogen forms and concentrations on the root system of a plant are researched more often compared to the studies on their effects on a plant's vegetative and generative growth. Humic matters can integrate nitrogen into their structure directly with chemical reactions or indirectly with microbial activities and then through the process of disintegration of microbial biomass. It has been reported that there are very few studies on the nitrogen requirements and source of the snapdragon [Verma et al. 2019]. Moreover, there are also few studies on indoor plants' reactions to the combined effects of humic acid and chemical fertilizers. In this study, the effects of applying different nitrogen forms with humic acid on the growth, flowering, and flower yield of the snapdragon have been examined.

MATERIALS AND METHOD

In this study, 'Tetra Mixed' snapdragon seed was used, which is one of the commercial varieties. 'Tetra Mix' is a variety that can grow up to 90 cm in height with diverse and beautiful colors. The experiment was set based on the factorial experiment pattern in random blocks with three repetitions. In a single repetition, three plants are used. Humic acid from two commercial liquid lignite sources (TKI-Humas and HUM-Zn), as given in Table 1, as well as three nitrogen forms [ammonium sulfate (AS; 21% N; 24% S), ammonium nitrate (AN; 17% NH_4-N + 8% NO_3-N) and urea (46% N)] were used.

The soil used in the experiment was taken from 0–30 depth from an agricultural field, dried, and filtered. The texture of the soil samples was determined with the hydrometer method [Bouyoucos 1962]. The pH and EC of the soil samples were measured in a 1 : 2.5 (soil : water) suspension. Organic matter modified by Walkley Black method [Nelson and Sommers 1983], lime by Scheibler calcimeter [Nelson 1983], available phosphorus by 0.5 M sodium bicarbonate (pH 8.5) extraction method [Olsen and Dean 1965], available K by 1 N ammonium acetate (pH 7.0) extraction method [Pratt 1965], and micro element (Fe, Mn, Cu and Zn) levels were determined by extraction method with DTPA+TEA (pH 7.3) [Lind-

Table 1. Some chemical properties of humic acids used in the research

	pH	Organic matter (%)	Total humic + fulvic acids (%)	Water soluble	
				K_2O (%)	Zn (mg kg^{-1})
HUM-Zn	9	14	13	2.0	5000
TKI-Humas	11	5	12	1.6	2.4

HUM-Zn – humic acid containing zinc; TKI-Humas – Türkiye Coal Enterprises production humic acid

Table 2. Physical and chemical properties of the soil

Texture	pH	EC (dS m^{-1})	Lime (%)	Organic matter (%)	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Fe (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})
Loamy	7.61	0.82	2.52	2.13	136.7	1251.3	7.77	2.09	0.75	12.07

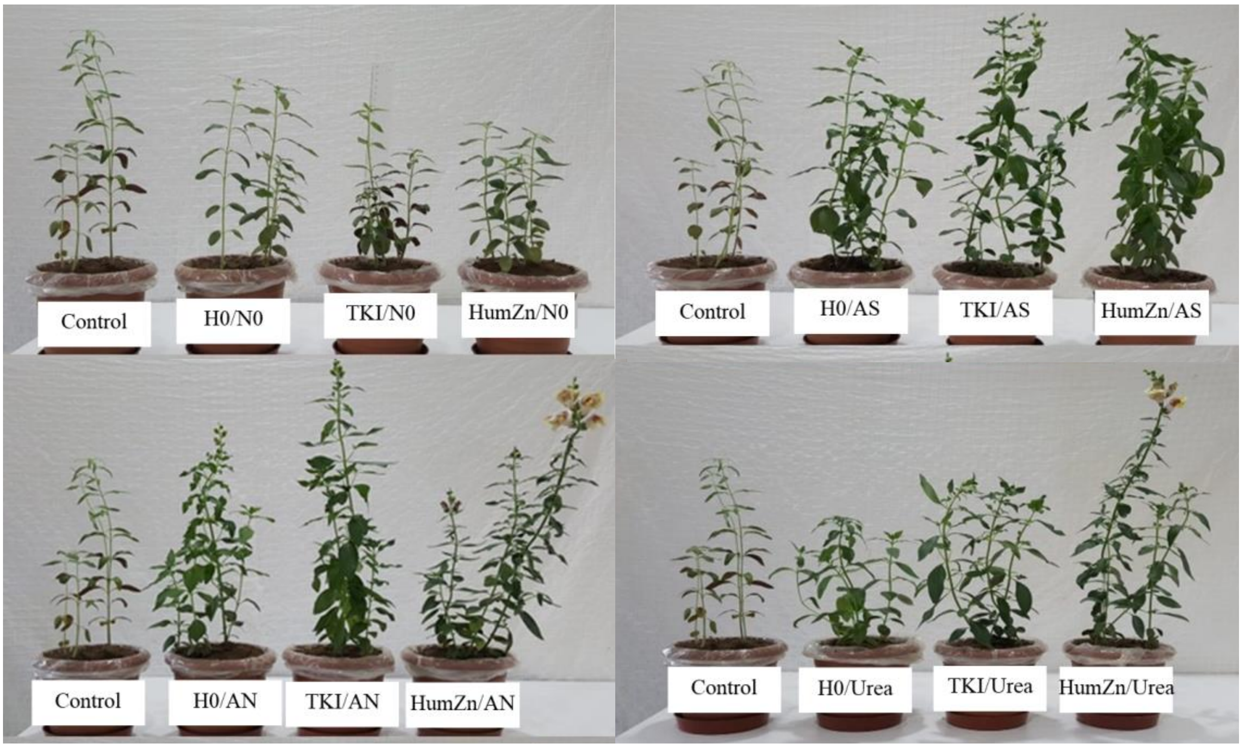
say and Norvell 1978]. Some physical and chemical components of the soil used in this experiment are given in Table 2. The soil was loamy, mildly alkaline, and limy.

Set-up and management of the experiment

The study was conducted between the beginning of July and the end of October 2020, and the plants were kept in a controlled growth chamber during the vegetative period and in plastic greenhouses during the budding period. In the study, plastic pots capable of holding 2.5 kg of soil were filled with a mixture of 1500 g of soil, 500 g of washed and dried sand, and 10 g of perlite.

Before sowing, 200 mg N kg^{-1} from nitrogen sources (for AN, AS, and urea) and main nutrient elements (100 mg P kg^{-1} and 125 mg K kg^{-1} for KH_2PO_4; 2.5 mg Fe kg^{-1} for Fe-EDTA and 5 mg Zn kg^{-1} for $ZnSO_4.7H_2O$) were added as a solution to the soil before planting. Humic acids were calculated for each pot of soil considering the suggested amount (60 L humic acid for one hectare) for soil by producers, then watered down and mixed with the soil. Total 36 pots (12 combinations: 1. control (no fertilizer-no HA); 2. H0/AS (200 mg N kg^{-1} used AS); 3. H0/AN (200 mg N kg^{-1} used AN); 4. H0/urea (200 mg N kg^{-1} used urea); 5. TKI/N0 (only TKI-Humas); 6. TKI/AS (TKI-Humas + 200 mg N kg^{-1} used AS); 7. TKI/AN (TKI-Humas + 200 mg N kg^{-1} used AN); 8. TKI/urea (TKI-Humas + 200 mg N kg^{-1} used urea); 9. HUM-Zn/N0 (only HUM-Zn); 10. HUM-Zn/AS (HUM-Zn + 200 mg N kg^{-1} used AS); 11. HUM-Zn/AN (HUM-Zn + 200 mg N kg^{-1} used AN); 12. HUM-Zn/urea (HUM-Zn + 200 mg N kg^{-1} used urea) × 3 repetitions) with applications of humic acid, nitrogen, and other main fertilizers (P_2O_5, K_2O, Fe, and Zn) other than nitrogen were left to incubate in a plant growing chamber for three weeks. Only the main fertilizers (P, K, Fe, and Zn) were applied to HA0/N0 groups to investigate the impacts of nitrogen fertilizers and humic acids. The other groups were arranged by applying the interactions of humic acids and nitrogen sources, all of which were applied basic fertilizers.

At the end of the three-week incubation period, ten snapdragon seeds were planted in each pot. After the seeds were germinated, the thinning process took place to have three snapdragon plants per pot, and controlled conditions, 16/8 light/darkness period was implemented, 25/20°C, 60% humidity, and 10 Klux light intensity were conducted. The plants were watered



Control – no humic acid, N and chemical fertilizers (the control pots in the figure demonstrate the snapdragon's development under conditions of cultivation without any fertilizer application), HA0/N0 – only essential fertilizers (P_2O_5, K_2O, Fe, and Zn) applied, HA0 – no humic acid, N0 – no nitrogen sources, HUM-Zn – humic acid containing zinc, TKI-Humas – Turkiye Coal Enterprises production humic acid, AS – ammonium sulfate, AN – ammonium nitrate

Fig 1. Visualization of the snapdragon plants after eight weeks with the application of humic acid and nitrogen

with de-ionized water based on their needs. Closely 25 days after the initial sowing, in the vegetative phase of the plants, nitrogen fertilizers of the identical quantity (200 mg N kg^{-1}) and type were once again administered. Before the snapdragon plants started to flower, they were transferred from a growing cabinet to a high greenhouse with a plastic cover and were grown there until harvest. Budding was observed after the plants were 30–35 days old, and flowering was completed within three weeks. Before the plants were taken to the greenhouse, they were photographed to show the differences between the applications (Fig. 1).

The snapdragon plants were harvested by cutting from the soil surface three months after planting (after flowers started to dry out). Plant height, floret width, and length were measured in centimeter (cm) with a ruler before harvest. Peduncle diameter, leaf width, and leaf length measurements were completed in millimeter (mm) with a ruler. Chlorophyll contents (unit is CM: chlorophyll-meter) were determined using a portable chlorophyll meter (SPAD; Spectrum Field-Scout CM 1000) on three fully developed leaves per plant, originating from the upper section immediately prior to the harvest. Harvested plants were weighed to determine their fresh weight, then were dried in an oven at 65°C till they reached a consistent weight, and weighed for their dry weight [Kacar 1972].

Quantitative values variance analysis based on factorial experiment patterns in random blocks was completed using IBM SPSS 20 (Statistical Package for the Social Sciences – IBM®), and the differences between applications were numbered according to the LSD test.

RESULTS AND DISCUSSION

Different responses of snapdragon to humic acids or nitrogen supplies

The final stages of vegetative development of snapdragons treated with humic acid and nitrogen fertilizers are given in Figure 1. It was seen that the internodes were long, and the leaves were underdeveloped in plants grown without any mineral fertilizer (Control-C) and plants grown with main fertilizers (P, K, Fe, and Zn) without nitrogen or humic acid (HA0/N0). Similarly, it was observed that humic acid applications did not positively affect plant growth without nitrogen fertilizer application. Among the nitrogen sources, plants treated with AN budded earlier than plants treated with other nitrogen sources (H0/AN in Fig. 1). Humic acids and nitrogen were co-applied, leading to a manifestation of favorable growth in the plants. However, there was a difference in the development of the plants depending on the humic acid × nitrogen fertilizer (HA × NF) interaction. Plants treated with AS and HUM-Zn remained in the vegetative stage, whereas those treated with HUM-Zn/AN or HUM-Zn/urea transitioned to the blooming stage (Fig. 1). On the other hand, it was seen that bud formation had just started when TKI was applied only with AN.

Among the essential plant nutrients, nitrogen is primarily required for average growth, leaf expansion, root growth, and biomass production [Quaggiotti et al. 2004, Leite et al. 2020]. Nitrogen deficiency decreases plant growth, yield, and flower quality [Haj Seyed Hadi et al. 2015, Fageria and Baligar 2005]. Nitrate and ammonium are the primary nitrogen sources available to plants. These nitrogen sources elicit distinct physiological responses in plants, a divergence likely rooted in genetic variability. Consequently, variations in absorption and assimilation processes occur depending on the specific nitrogen source [Li et al. 2006]. Also, nitrogen sources affect the vegetative and generative development of the plant, as well as nitrogen distribution in various organs and nitrogen utilization efficiency [Luo et al. 2020]. Bernstein et al. [2005] have reported that most plants can utilize either or both ammonium and nitrate and that the effects of the form of nitrogen applied depend on the ontogenic development of the plant, the plant species, and the nitrogen concentration supplied.

The present study also supports the argument that nitrogen sources make a difference in the growth stages of plants, and this present study revealed that the application of HUM-Zn with AN and urea fertilizers was more effective than that of AS in promoting flowering, increasing plant growth, and net assimilation rate. Because HUM-Zn contains zinc and there is a synergistic effect between nitrogen and zinc, it contributed to the development of snapdragon plants.

The effect of humic acid on plant growth can be attributed to a humic acid source, application rate, application method, solubility, and soil structure, as mentioned in some studies [Nikbakht et al. 2008, Ampong et al. 2022]. Some studies report that nitrogen and zinc fertilizers positively affect plant growth by combining humic substances and mineral nutrients [Ozkutlu et al. 2006, Ibrahim and Ramadan 2015]. Garcia-Mina et al. [2004], supporting the results of the present study, have reported that humic substance + Zn compounds were highly effective in promoting plant growth. Also, zinc promotes the transport and distribution of nitrogen to the leaves [Ji et al. 2022], and this interaction (HA × NF × Zn) enhanced the flowering of snapdragon plants, as shown in the present study.

Plant growth factors

Humic acid treatments were effective ($P < 0.05$ and 0.01) on leaf width, chlorophyll content, and dry weight (Tab. 3). Leaf width and chlorophyll content increased the most with TKI-Humas, and dry weight increased with HUM-Zn application (Tab. 4 and Fig. 2).

The utilization of nitrogen-based fertilizers on snapdragon plants produced statistical significance ($P < 0.05$ and 0.01) in other traits except for floret width (Tab. 3). Among the nitrogen sources, AN provided the highest increase in plant height and floret length, urea in leaf width and length, AS in peduncle diameter, urea in chlorophyll content, and AN in fresh and dry weight (Tab. 4).

The interaction of HA × NF sources significantly affected the other traits except for peduncle diameter and fresh weight. Among the various interactions of HA × NF, the most significant distinctions were observed as follows: plant height with TKI-Humas + AS, flower length with HUM-Zn + urea, leaf width with TKI-Humas + urea, chlorophyll content with TKI-humas + urea, and dry weight of the plant with HUM-Zn + AN.

Table 3. Variance analysis results of snapdragon plant

SV	Plant height	Leaf width	Leaf length	Peduncle diameter	Floret length	Floret width	Chlorophyll content	Fresh weight	Dry weight
HA	4.39ns	50.24**	54.32ns	0.042ns	0.03ns	0.06ns	631.23*	35.73ns	2.17*
NF	690.9**	55.09**	1044.56**	1.345**	0.17*	0.03ns	15355.9**	2219**	87.6**
HA × NF	15.77*	19.29**	117.71*	0.103ns	0.32*	0.35**	1367.7**	33.92ns	2.39**

SV – sources of variance, HA – humic acid, NF – nitrogen sources, * – 5%, ** – 1% at significance level, ns – non significant

Table 4. The means of morphological components and chlorophyll content of snapdragon

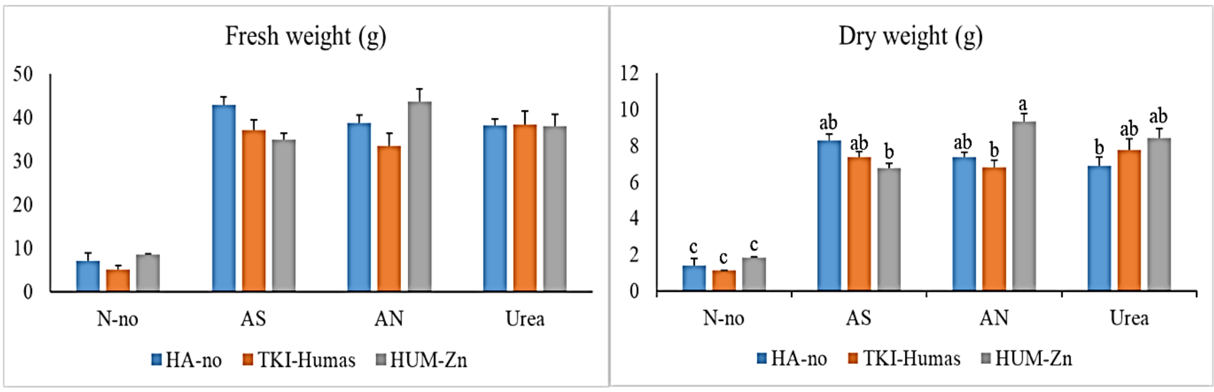
HAs	NS	Plant height (cm)	Leaf width (mm)	Leaf length (mm)	Peduncle diameter (mm)	Floret length (cm)	Floret width (cm)	Chlorophyll content (cm)
HA-0	N-0	22.33b	18.35c	50.16b–d	2.25	5.16a	3.51ab	156.7def
	AS	36.35a	21.31bc	57.44a–c	2.87	4.85a	4.11a	180.1cd
	AN	38.63a	21.22bc	50.70b–d	2.46	4.66b	3.62ab	186.2cd
	urea	38.81a	24.85ab	75.04a	3.30	4.80a	3.29b	213.8a–c
TKI-Humas	N-0	18.74b	18.50c	34.90d	2.18	4.94a	3.62ab	133.7ef
	AS	40.42a	20.51bc	60.35ab	2.80	4.58b	3.40ab	237.2ab
	AN	38.34a	20.36bc	57.94a–c	3.00	5.12a	3.82ab	163.3de
	urea	33.87a	28.84a	64.01ab	3.01	4.53b	3.39ab	248.7a
HUM-Zn	N-0	20.06b	16.58c	40.37cd	2.28	4.51b	3.64ab	125.8f
	AS	36.87a	20.57bc	64.58ab	2.93	4.41b	3.38ab	202.1bc
	AN	38.29a	18.18c	53.75b–d	2.96	5.02a	3.69ab	163.7de
	urea	39.29a	17.64c	61.88ab	2.17	5.20a	4.07ab	237.8a
HA	HA-0	34.03	21.43a	58.34	2.72	4.87	3.63	184.2b
	TKİ	32.84	22.05a	54.30	2.75	4.80	3.56	195.7a
	HUM-Zn	33.63	18.24b	55.14	2.59	4.79	3.70	182.3b
N source	N-0	20.37b	17.81c	41.81c	2.24b	4.87ab	3.59	138.7d
	AS	37.88a	20.80b	60.79a	2.87a	4.61b	3.63	206.5b
	AN	38.42a	19.92b	54.13b	2.81a	4.93a	3.71	171.1c
	urea	37.32a	23.78a	66.98a	2.83a	4.85ab	3.59	233.4a

Statistically signifiant characters are lettered, and means followed by the same letter in the columns are not significantly different at $P \leq 0.05$
HAs – humic acid sources, NS – nitrogen sources, HUM-Zn – humic acid containing zinc, TKI-Humas – Turkiye Coal Enterprises productior humic acid, AS – ammonium sulfate, AN – ammonium nitrate, N – nitrogen, HA – humic acid

The floret width of snapdragon plants was determined to be the highest (4.11 cm) with AS fertilizer without humic acid application.

Some research was conducted on the effect of different doses of humic acid applications on flower yield, quality improvement, and flower growth of plants. Previous studies have reported that ornamental plants like *Zinnia elegans*, *Tagetes patula*, and *Impatiens wallariana* demonstrate an increased bud count, higher flower yield, and extended vase life of flowers through the application of humic acid [Dudley et al. 2004, Nikbakht et al. 2008, Mohammadipour et al. 2012, Esringü et al. 2015]. Memon and Khetran [2014] have reported that humic acid application positively affected flower development in snapdragons, and flower length was shortened by decreased amount of humic acid. Aslan and Sarıhan [2021] determined that the application of humic acid and AN (33%) fertilizer doses had different effects on the yield and quality of the lavender plant in the two years examined. Humic acid and nitrogen application did not affect the essential oil rate; however, it increased flowering and plant growth, and also, the effect of humic acid was higher on plant growth in the second year. The present study determined that humic acid positively affects the development of plants by balancing the nitrogen needed by plants for vegetative and generative growth.

The data showed that chlorophyll content increased more with urea application compared to other nitrogen fertilizers (Tab. 4). Between 50–70% of the total nitrogen content in leaves is involved in chloroplast-related enzymes [Lana et al. 2012]. Silva Júnior [2013] reported an elevation in chlorophyll content among urea-treated orchid plants compared to control groups, indicating that urea fosters the growth of photosynthetic tissues by inducing modifications in both mesophyll structure and chlorophyll levels. Nitrogen sources were equally effective on the fresh weight of snapdragon plants, but the highest value was obtained from AN application (Fig. 2). Significant effects of treatments and their interactions on dry weight were determined. HUM-Zn + AN application caused the highest increase in fresh and dry weight values. At the same time, urea, HUM-Zn, and TKI-Humas treatments increased plant fresh and dry weights (Fig. 2). This result indicated that humic acids are effective in increasing the biomass of plants depending on the nitrogen source to which they are applied. Previous studies have shown that adding urea to humic acids produced from lignite can increase plant yield [Zhang 2019], indicating a synergistic effect between the two compounds. However, little is known about the mechanism by which the application of lignite humic acids with urea improves plant growth. Based on the studies conducted for this mechanism, two possible mechanisms are proposed for lignite-derived humic acid. These are either that some of the NH_4 produced from urea mineralization combines with the humic



Statistically significant characters are lettered, and means followed by the same letter in the columns are not significantly different at $P \leq 0.05$

Fig 2. Fresh and dry weight of plants (g plant^{-1})

acid, which reduces the net loss from evaporation, or that lignite humic acids inhibit the activity of urease, which decomposes urea to NH_3, resulting in hydrolysis of a lower amount of urea. This reduced rate of hydrolysis can reduce NH_3 loss by increasing urea availability to plants [Dong et al. 2006, Dong et al. 2009]. However, when applied directly with urea or other chemical fertilizers, humic acids can reduce the adverse effects on soil bacteria or fungi [Boguta et al. 2021]. The increase in fresh and dry weight of snapdragon and the application of urea and AN fertilizers with the two lignite-derived humic acids we examined can be attributed to these mechanisms.

Our results suggest that humic acid and nitrogen sources can be effective agronomic indicators to improve the growth and flowering of snapdragon plants. The application of HUM-Zn with urea or AN fertilizer was found to promote early flowering and development of plants. It can be explained by the fact that due to the zinc content of HUM-Zn, nitrate and urea nitrogen promote flowering. At the same time, humic acid supplemented with zinc may contribute positively to the development of plants grown in zinc-deficient soils. The use of AN and urea fertilizers and zinc-containing humic acid will be necessary for the early flowering of ornamental plants.

AUTHORS' CONTRIBUTIONS

NG contributed to the statistical analysis, experiment planning, implementation, and conversion into articles. CY contributed to the experiment's application and execution. SYS, experiment planning, design, and writing of the article. The authors declare that they have no conflict of interest.

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