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Evaluation of nutrient management strategies on chicory (*Cichorium intybus* L.) growth and physiological traits in Arak's climate

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ABSTRACT

Purpose: This study investigated the effects of phosphorus, potassium, and humic acid fertilizers on growth, yield, and physiological traits of chicory under Arak's climatic conditions using a factorial experiment. **Research Method:** The experiment was conducted in a randomized complete block design with three replications. Experimental factors included levels of phosphorus fertilizer (0, 8 and 12 $kgha^{-1}$), potassium fertilizer (0, 10 and 15 $kgha^{-1}$), and humic acid (0, 0.5 and 2 $kgha^{-1}$). **Findings** The results showed that the highest Leaf Area Index was observed in the treatment of 12kg/ha phosphorus+10 kg/ha potassium sulfate+2 kg/ha humic acid, with a mean of 2.9. The highest number of flowers and the highest total chlorophyll content were obtained in 12 kg/ha phosphorus + 10 kg/ha potassium sulfate + 0.5 kg/ha humic acid treatment with averages of 49.11 per plant and 39.5µg/g FW respectively. The highest flower yield was in 10 kg/ha potassium sulfate treatment with an average of 330.6 $kgha^{-1}$. The highest content of free proline was obtained in 8 kg/ha phosphorus+15kg/ha potassium sulfate+2 kg/ha humic acid treatment with 0.97µmol/g FW. Combined phosphorus, potassium, and humic acid application significantly improved chicory growth, yield, and physiology, increasing leaf area index, flower number/yield, shoot yield, biomass, and chlorophyll content, while decreasing free proline. **Research limitations:** No significant limitations were identified in this study. **Originality/Value:** To maximize chicory growth and yield in the specific climatic conditions of this study, a balanced fertilizer application is crucial, as excessive fertilization can be harmful. This study suggests a combination of 8 kg/ha of superphosphate, 10kg/ha of potassium sulfate, and 0.5 kg/ha of humic acid powder. Future research should focus on understanding how these nutrients affect chicory at a mechanistic level and on evaluating the long-term consequences of various fertilization programs for soil fertility and environmental health.

Keywords:
Flower yield, Leaf area index, Photosynthetic pigments, Proline

INTRODUCTION

Medicinal herbs play a vital role in promoting human health and well-being, serving as both therapeutic agents and preventive measures against diseases (Ritala et al., 2014). These plants are rich sources of bioactive compounds, though their production is often influenced by environmental factors in addition to genetic processes (Adediran et al., 2005).

Cichorium intybus L., commonly known as chicory, is a widely distributed medicinal herb characterized by its blue, composite flowers and classification within the Asterales order, Asteraceae family. The family Asterales is alternatively known as Compositae, Astraticus, or Asteraceae. Chicory is a vascular, flowering, and dicotyledonous plant. Most dark chicory species are herbaceous; however, a remarkable number of these herbs are generally gramineous, annual or perennial and rarely small shrubs or trees. Leaves are simple or more or less blade cut with different shapes. Phyllotaxis is usually alternate generally with capitule flowers. Cousinas Race (Compositae) encompasses 1620 genera and about 20,000 species; hence, it is the largest family of vascular plants worldwide. Chicory is also economically highly important and encompasses many species with nutritional, medicinal, and ornamental usage. Out of 1620 chicory genus in the world, 146 genera with about 1123 species have been reported in Iran, 406 of which are exclusive to Iran (Anju et al., 2020; Tengo & Akbari, 2016).

Chicory is used as fodder for livestock in many regions of the world since it produces fodder with desirable quality and quantity in the hot seasons. The plant needs cool, sunny, or less shade climates. Chicory cannot tolerate the intense summer heat. Under desirable management conditions, chicory produces acceptable dry matter yield (7-15 tons/ha) (Neciu et al., 2017). This plant flowers in summer from July to September. In Iran, it is cultivated in the mountainous regions of Khorasan, Gilan, Mazandaran, Zanjan, Tehran, Azerbaijan, Isfahan, and others (Shad et al., 2013).

Chicory leaves contain chicory acid, sulfate, sodium phosphate, magnesium, potassium, and potassium nitrate. Its roots contain 11-15% inulin and different sugars such as glucose, fructose, and sucrose, as well as chicoric acid, pectin, sesquiterpene, and tartaric acid. Chicory flowers also contain chicory acid. The chicory rootsare used in treating liver and biliary diseases and wound healing and are consumed an appetizer, bile stimulant, digestive aid, diuretic, and antipyretic (Jaiswal et al., 2011). Some other remarkable medicinal compounds of chicory are aesculin, coumarin, terpenoid, and flavonoid (Fathi et al., 2018).

Given that Iran is one of the dry and semi-arid countries, dry farming in water-scarce regions is unavoidable (Andersson et al., 2003).

Since the biological and environmental side-effects of chemical fertilizers have a negative effect on the life cycle and sustainability of agricultural systems, further methods to promote the production of agricultural products should be considered. Regarding the environmental considerations, there has been an increase in the use of organic fertilizers, including animal manure, humic acid, folic acid, and vermicomposts. Using organic composites as fertilizers enhances the carbon levels of the soil and has direct and indirect effects on soil biological products (Prakash et al., 2007).

Regarding the beneficial effects of organic materials on the physical, chemical, biological, and fertility properties of soil, they are considered as one of the main determinants of soil fertility. Organic fertilizers enhance soil organic composites and pH, and due to the improvement of cationic exchange capacity, they increase the yield of microorganisms, access to nutrients, and soil fertility (Kaur et al., 2008).

Humic Substances are complex organic macromolecules formed from the biological and chemical decomposition of plant and animal residues in soil (Guo et al., 2019). They are a primary component of soil organic matter and play a crucial role in improving soil physical, chemical, and biological properties. Humic Substances mainly consist of humic acid, fulvic acid, and humin, accounting for 60-80% of soil organic matter (Yang et al., 2021).

The unique properties of HS, such as their high capacity for binding ions, heavy metals, and organic substances, make them valuable soil amendments for improving soil structure, water holding capacity, and fertility (Li et al., 2019). Additionally, HS play a significant role in stabilizing contaminants and enhancing groundwater quality.

Rezvanimoghadam et al. (2019) examined the effect of mycorrhizal inoculation and organic fertilizers on the yield of the functional components of *Cichorium pumilum*. They noticed that mycorrhizal inoculation increased the number of lateral branches, the height of the plant, the number of flowers, the number of seeds, and the number of seeds per plant. Among different levels of biological fertilizers, mycorrhizal fungi (*Glomus mosseae*) caused a 29% increase in grain yield compared to the control. Organic fertilizers also decreased the seed yield of *Cichorium pumilum*; hence, cow manure enhanced the production of lateral stems and the plant height, and humic acid also enhanced the production of flowers, the number of seeds per plant, and the seed yield. Furthermore, humic acid significantly increased the root growth of gerbera (*Gerbera jamesonii* L.) grown in nutrient solution. Moreover, humic acid significantly increased the content of phosphorus, magnesium, iron, and potassium in the leaves and the number of flowers (Rezvanimoghadam et al., 2019). Previous studies have documented that phosphorus is an ion that becomes unusable for the plant under dry conditions since the ion is strongly absorbed by soiled clays, and only a small portion of phosphorus is soluble. Under dry conditions, nitrogen absorption is decreased not only because of its low solubility, but also because of the reduced root absorption rate (Kafi & Mahdavi Damghani, 2000). Some studies show that, drought conditions, organic fertilizers, and their interaction have significant effects on potassium absorption ($p=0.01$). Accordingly, the highest level of potassium was obtained for the crop irrigation capacity of 90% along with the use of potassium nanocholate fertilizer; however, the lowest level was observed for severe drought and the control fertilizer treatment (Rezaienia et al., 2018).

According to the literature, the present study mainly aimed to examine the effect of different sources of humic acid on yield and yield components and the qualitative traits of chicory. Moreover, the study was to investigate the effect of different sources of phosphorus and potassium fertilizers on the quantitative and qualitative traits of chicory and determine the most favorable combination of fertilizer treatment to reach the highest flower yield in chicory using organic (humic acid) and chemical (phosphorus and potassium) fertilizers. Given the importance of using organic fertilizers along with chemical fertilizers in reducing the consumption of these fertilizers, the novelty of this study is conducting extensive research on different sources of fertilizers and determining the optimal combination of fertilizer type regarding climate conditions and type of cultivation.

MATERIALS AND METHODS

Location of experiment
This research aimed to investigate the effect of potassium, phosphorus, and humic acid fertilizers on some growth, functional, and physiological traits of chicory under the climatic conditions of Arak, Markazi province, in 2017.

The experiment included phosphorus fertilizer levels from single superphosphate (P) (0, 8, and 12 kg/ha), potassium fertilizer from potassium sulfate (K) (0, 10, and 15 kg/ha), and powder humax humic acid (H) (0, 0.5, and 2 kg/ha). These were arranged in 81 experimental plots, each measuring 4 × 3 meters. The distance between plots and blocks was one meter.

Experiment procedure
The soil samples were taken before planting. For this purpose, the samples were randomly taken from several different farm areas at a 0-30 cm depth, poured into separate envelopes, and covered, and transported to the laboratory. The results showed the electrical conductivity of 0.92 dS/m, the total nitrogen of 0.08%, and the absorbable phosphorus and potassium of 11.6 and 300 ppm. It was also revealed that the soil texture was silt loam. Moreover, land preparation operations, including initial p lowing, land leveling, and plotting was performed in late March 2016. The chicory seeds (seed ID: IBRC-PHS100-34567) were obtained from Pakan Seed Company and cultivated in experimental 4 3 meter plots traditionally. The final density of 20 plants per square meter (the distance of each row) was considered to be 10 cm (Asilbekova et al., 2005). The first irrigation followed planting the seeds. The experimental plots were irrigated every other seven days according to the regional norm.

When the plant reached the final stage and was harvested (early July), five plants were randomly picked up from each plot, and their features were examined. Since chicory has unlimited growth and flowers gradually, flower harvesting was performed four times during a week. To this end, the flowers were picked up from the middle lines of the plots by removing the margins and dried in the shade and away from direct sunlight. After drying, they are weighed, and the yield per hectare was calculated. To calculate the shoot yield (flowerless aerial organs) at the end and for required sampling, all plants on a plot, except for the margins of the plot, were harvested and weighed after drying as such the yield per hectare was measured. All fertilizers were obtained from Bazargan Kala Company and were used according to the manufacturer's instruction. The seed required for cultivation was purchased from Pakan Seed Company. Quantitatively measured traits were as follows:

Plant height
To measure plant height, five plants were randomly selected from the central part of each plot. A meter was used to measure the height from the crown to the apex of each plant.

Flower diameter
It was measured in centimeters using a caliper.

Number of leaves
It was counted and recorded immediately after each sampling.

Dry weight

The dry weight of aerial organs such as leaves, stems, and flowers was measured and recorded after drying in an oven using a sensitive scale 0.001 mg.

Chlorophyll a, chlorophyll b, and carotenoid content measurement

The levels of chlorophyll a, b, and total chlorophyll as well as the carotenoid content were measured using Lichtenthaler and Well burn's method (Lichtenthaler & Wellburn, 1983), according to which 0.25 grams of the plant leaves were completely homogenized in a Chinese mortar containing 5 ml of acetone 80%. Then the resulting solution was poured into the cuvette and read with a spectrophotometer at wavelengths of 663, 646 and 470. The levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were calculated using the following equations (Equation 1-4) (Lichtenthaler & Wellburn, 1983):

$$Chl\,a = 12.7_{A_{663}} - 2.69_{A_{645}} \times V/1000W \qquad \text{Eq. 1}$$

$$Chl\,b = 22.9_{A_{645}} - 2.69_{A_{663}} \times V/1000W \qquad \text{Eq. 2}$$

$$Chl\,b = 22.9_{A_{645}} - 2.69_{A_{663}} \times V/1000W \qquad \text{Eq. 3}$$

$$Cartenoid = 7.6_{A_{480}} - 14.9_{A_{510}} \times V/1000W \qquad \text{Eq. 4}$$

Where, C is the concentration in mg/FW of leaves, V is the extract volume, W is the sample weight, A stands for the level of light absorption, and D is the dilution ratio.

Proline measurement

Bates et al.'s method (Bates et al., 1973) was used to measure the level of free proline, according to which 0.5 mg of leaves from each sample was added to 10 ml of 3% sulfosalicylic acid aqueous solution, and the resulting mixture was completely homogenized in a Chinese mortar. Then the homogenized mixture was filtered by Whatman grade 2 qualitative filter papers. Then, 2 ml of this solution was mixed with 2 ml of ninhydrin reagent, for whose preparation 1.25 g of ninhydrin was dissolved in 30 ml of acetic acid and 20 ml of 6 M phosphoric acid. In the next phase, 2 ml of acetic acid was added to each tube. After that, the samples were placed in a bain-marie bath at 100℃ for one hour. Finally, when taken out of the bain-marie bath, they were immediately placed in an ice bath for a few minutes.

In the following, 4 ml of toluene was added to each test tube and the samples were vortexed for 15-20 seconds until they were completely uniformed. Then the tubes were placed in the laboratory environment for a while. During the same period, the upper and lower phases inside the test tube were completely distinguishable, and the upper phase was used to determine the concentration of proline according to the standard proline curve in the spectrophotometer at a wavelength of 520 nm. The unit of measurement for proline in this experiment is µmol/g FW.

Statistical Analysis

Data normality was verified prior to analysis of variance (ANOVA) using SAS 9.1. Treatment means were compared via Duncan's test (*p* ≤ 0.05). Figures were generated in Excel, and the manuscript was prepared in Microsoft Word.

RESULTS AND DISCUSSION

Plant height

The main effects of phosphorus, potassium, and humic acid fertilizers, as well as the double and triple interactions of phosphorus × potassium × humic acid, had a significant influence on chicory plant height at *p* = 0.01 (Table 1). Mean comparisons indicated that the tallest plants were observed in treatments P1K2H1 (48.82 cm), P1K3H1 (49.63 cm), P2K3H1 (51.44 cm), P1K2H2 (48.8 cm), and P1K3H2 (49.59 cm). These treatments exhibited increases of 30.7%, 31.8%, 34.2%, 30.6%, and 31.7%, respectively, compared to the control. In contrast, the lowest plant height (13.9 cm) was recorded for the combined treatment P3K3H1 (Fig. 1a).

Under the same environmental conditions, the provision of nutrients to the plant using different fertilizers can promote the plant growth and subsequently increase the bush height (Bahmani et al., 2015). Potassium fertilizer and humic acid (nitrogen source) had the highest effect on enhancing the plant height, which can be mostly justified by considering the capabilities of these fertilizers, including promoting plant nutrition and increasing the water retention capacity in the soil since the optimal level of these compounds promotes plant growth and enhances potassium and nitrogen nutrients in soil (Rezaei Moadab et al., 2014; Gorzi et al., 2024).

Table 1. Variance analysis for effects of different levels of phosphorus, potassium and humic acid fertilizers on some morphological traits, yield, and yield components of chicory.

S.O.V	df	Mean Squared							
		Bush height	Number of secondary stems	Leaf area index	Number of flowers	Flower yield	Shoot yield	Biological yield	Flower harvest index
Block	2	8.91^{ns}	0.45^{ns}	0.004^{ns}	10.9^{ns}	300^{ns}	1081.4^{ns}	2060^{ns}	2.07^{ns}
Phosphorus (P)	2	1924.2**	89.7**	1.04**	227.3**	12729.6**	12232.1**	21405.1*	206.2**
Potassium (k)	2	441.7**	19.5**	0.1^{ns}	510.7**	3377.9^{ns}	4129.3*	6807.7^{ns}	64.3^{ns}
Humic acid (H)	2	57.9**	0.45^{ns}	0.06^{ns}	50.4^{ns}	202.8^{ns}	6985.4**	5481.1^{ns}	51.6^{ns}
Phosphorus (P) × Potassium (k)	4	83.4**	4.87**	0.29^{ns}	584**	3728.7*	8407**	6565.1^{ns}	130.3**
Phosphorus (P) × Humic acid (H)	4	184.3**	3.08**	0.04^{ns}	35.9^{ns}	2063*	981.1^{ns}	2953.5^{ns}	27.4^{ns}
Potassium (k) × × Humic acid (H)	4	160.3**	1.99**	0.3^{ns}	152.6**	2939.7*	4315.2*	9917.7^{ns}	33.1^{ns}
Phosphorus (P) × Potassium (k) × Humic acid (H)	8	192.6**	6.79**	0.85*	125.8**	3914.5*	8611.6**	13139**	71.4*
Error	52	10.73	0.49	0.15	40.8	1059.7	1338	4367.1	33.7
CV (%)	-	9.04	10.95	16.3	17.96	20.2	10.59	10.75	13.29

Note: ns, * and ** are not significant and significant at p=0.05 and p=0.01, respectively.

Phosphorus is known as the main nutrients of plants. Most agricultural soils encompass general reserves of phosphorus; however, the stabilization and precipitation of phosphorus causes the deficiency or ineffectiveness of phosphorus, thereby limiting the growth of crops (Kohler et al., 2007; Bijeh Keshavarzi & Omidi, 2025). Phosphorus deficiency directly impacts plant growth by decreasing root hydraulic conductivity. This limitation in water and nutrient transport to aboveground tissues hinders cellular processes including cell expansion and photosynthesis. As a result, visible symptoms such as reduced leaf size, shortened stems, and chlorosis become apparent (Hawkesford et al., 2012). On the other hand, phosphorus deficiency increases abscisic acid transport in the wood vessel, which may be the result of disturbance in the plant's water relations. Nutrient deficiencies in plants increase their sensitivity to environmental stress. The process may occur indirectly as such the general decrease in the plants' growth, development, and competition power caused by nutritional deficiency further decreases the dry matter production under environmental stress conditions (Hajiboland, 2012; Ansarifard et al., 2024). In some cases, the effect of nutritional deficiency on increasing sensitivity to environmental stress is direct as such due to the special role of an element in metabolism and the function of a specific enzyme pathway, the deficiency of the concerned element directly increases the sensitivity to specific environmental stress (Hajiboland, 2012). Some researchers have documented the significant effect of foliar application of nano phosphorus fertilizer on the height of Baobab and soybean plants, and this finding is consistent with the findings of the present study (Bhaskaran et al., 2010; Liu & Lal, 2014).

In this study, potassium fertilizer had a positive effect on plant height. Potassium is the most abundant cation in the cytoplasm, and potassium salts create a suitable osmotic potential inside the cells and tissues of glaucophytes (Bhat & Murthy, 2008). The role of potassium in the enlargement of cells is a part of the cell growth process, and the processes regulated by turgor operation are associated with the concentration of this element in vacuoles (Malekooti & Homaei, 2004).

Number of secondary stems

The main effects of phosphorus, potassium, and humic acid fertilizers and the double and triple phosphorus interactions of phosphorus × potassium × humic acid on the number of secondary stems were significant at p=0.01. Regarding the comparison of the means of the three interactions, the highest number of secondary stems was observed in the treatments P2K2H2 (with an average of 10.66 secondary stems per plant) and P1K3H2 (with an average of 9.66 secondary stems per plant), which revealed the increases of 37.5 and 31.0, compared to the control treatment. The smallest number was obtained in the P3K3H1 treatment with an average of 2.33 secondary stems per plant (Fig. 1b).

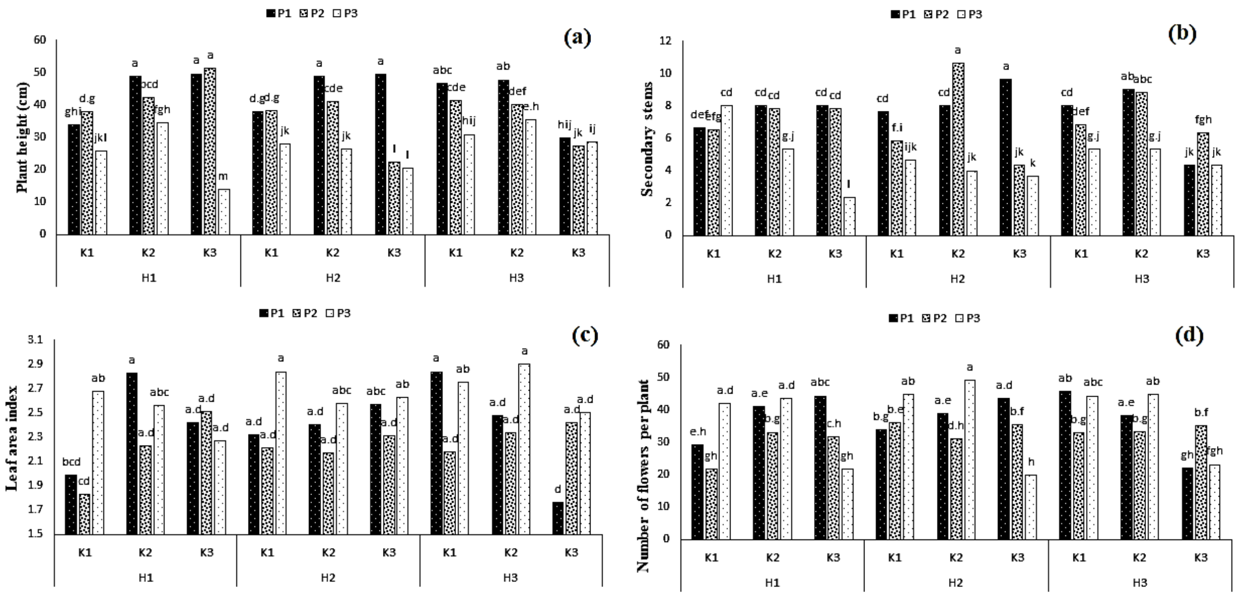

Fig. 1. Comparing mean interaction effects of phosphorus × potassium × humic acid on plant height (a), number of secondary stems (b), leaf area index (c) and number of flowers (d)
(P1, P2, P3: levels of phosphorus fertilizer (0, 8, and 12 kg/ha); K1, K2, K3: Sulphate potassium fertilizer (0, 10, and 15 kg/ha); H1, H2, H3: Powder Humax humic acid (0, 0.5, and 2 kg/ha)).

In this study, of 0.5 kg of powder Humax humic acid significantly increased the number of secondary stems in the plant. In this regard, organic fertilizers further enhance the plant growth and development, including the number of secondary stems, by providing high and low-consumed elements needed by the plant, improving the physical, chemical and biological traits of the soil, increasing the water retention capacity in the soil, expanding the plant's root system properly through improving the soil structure and increasing the soil porosity, producing plant hormones by bacteria, and reinforcing the absorption and transfer of minerals (Fatma et al., 2014).

In an experiment, the researchers examined the effect of humic acid on the yield of spring wheat and noticed that humic acid increased access to phosphorus and other nutrients and also significantly increased the yield (Sabzevari & Khazaie, 2009). Accordingly, one of the reasons making the high concentrations of these three fertilizers have negative and reducing effects is an increase in the solubility of the elements and their higher availability, resulting in further toxicity and the loss of morphological and developmental traits. Wang et al. (2007) added humic acid to alkaline soil with phosphorus fertilizer to wheat under field conditions and observed that phosphorus uptake and wheat yield increased by 25%.

Leaf area index

The leaf area index (LAI), defined as the ratio of the total one-sided leaf area to the ground surface area, was significantly influenced by phosphorus fertilizer (*p* = 0.01) and the triple interaction of phosphorus × potassium × humic acid (*p* = 0.05) (Table 1). Mean comparisons of the triple interactions showed that the highest LAI values were recorded for treatments P1K2H1, P3K1H2, P1K1H3, and P3K2H3, with increases of 29.6%, 29.9%, 29.9%, and 31.3%, respectively, compared to the control. The lowest LAI (1.76) was observed in treatment P1K3H3 (Fig. 1c).

The distribution of dry matter implies the allocation of assimilates obtained from the photosynthesis process to vegetative and storage organs. Although not all parts of the plant have economic consumption, the presence of vegetative parts such as leaves and stems before the formation of the economic organ is necessary for the optimal formation of the economic parts of any crop (Khadempir, 2014).

Leaf area index is a key variable in physiological studies to examine plant growth, light absorption, photosynthetic efficiency, evaporation and transpiration, and plant response to fertilizers and irrigation, and it is also a direct and acceptable indicator of crop yield (Lizaso et al., 2003).

Number of flowers per plant

The variance analysis results showed that the effects of phosphorus and potassium fertilizers and the interaction effects of phosphorus fertilizers on potassium and potassium on humic acid, and the triple interaction effect of phosphorus × potassium × humic acid on the number of flowers were significant at p=0.01 (Table 1). The highest number of flowers was obtained in the treatment P3K2H2 with the mean of 49.11 per plant, revealing a 40.6% increase compared to the control treatment. The lowest mean was observed for the treatment P3K3H2 with the mean of 19.97 per plant (Fig. 1d).

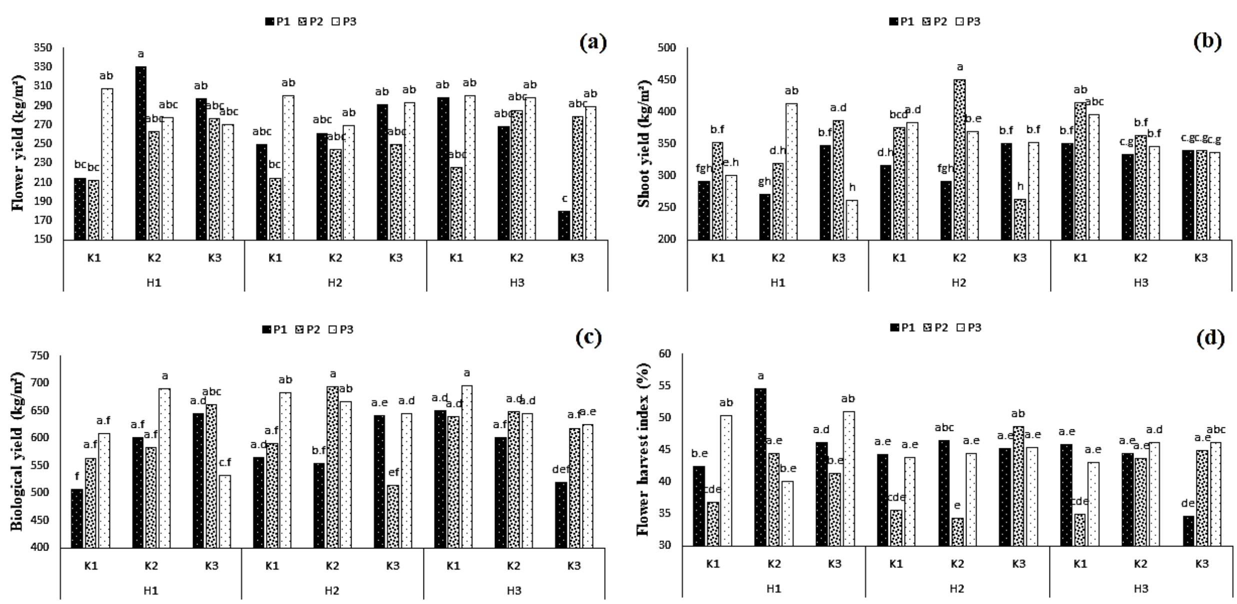

Fig. 2. Comparing mean interaction effects of phosphorus × potassium × humic acid on flower yield (a), shoot yield (b), biological yield (c) and flower harvest index (d)
(P1, P2, P3: levels of phosphorus fertilizer (0, 8, and 12 kg/ha); K1, K2, K3: Sulphate potassium fertilizer (0, 10, and 15 kg/ha); H1, H2, H3: Powder Humax humic acid (0, 0.5, and 2 kg/ha)).

Soil health is one of the key factors in determining crop yield. Humic substances in soil have multiple effects, one of the main components of which is humic acid that have effects on the growth of crops by affecting the physical, chemical and biological traits of soil. In a greenhouse study, Sangeetha and Singaram (2007) examined the effect of humic acid on the absorption of soil nutrients and onion yield. The treatments included the application of NPK and different levels of humic acid as soil treatment along with foliar spraying. The results indicated that 20 kg/ha of humic acid along with 100% NPK resulted in the highest onion yield and a 12 percent increase in the NPK absorption. After adding humic acid and the fertilizer, access to P, N and K increased from 105 to 199.3, 7.9 to 12.63, and 132 to 139 mg per kilogram of soil during the test period. This factor is also one of the reasons for the positive effects of humic fertilizer in the present study.

Flower yield

The results indicated that the effects of phosphorus and potassium fertilizers and the interaction effects of phosphorus fertilizers on potassium and potassium on humic acid, and the triple interaction effect of phosphorus × potassium × humic acid on the flower yield were significant (Table 1). The highest flower yield was obtained in the treatment P1K2H1 with the mean of 330.6 kg/ha, showing a 35.0-percent increase compared to the control treatment. The lowest flower yield was also obtained in the treatment P1K3H3 with the mean of 180 kg/ha (Fig. 2a).

The soil application of potassium sulfate improved vegetative growth and increased yield and the yield components of wheat. The foliar application of potassium fertilizer affected all growth and yield traits of wheat. The application of potassium fertilizer during tillering and tillering + stemming stages of wheat significantly increased economic yield. The effect of foliar spraying and the soil application of potassium nano-fertilizer on the yield and the yield components of wheat were also significant (Boveiri Dehsheikh et al., 2017). Aghazadeh-Khalkhali et al. (2015) investigated effect of potassium fertilizer and reported that the application of 3 grams per liter of potassium nano-fertilizer, compared to the other treatments, had the highest effects on the dry weight yield of aerial organs, grain yield (the most critical quantitative trait), and mucilage yield (the most critical qualitative traits) in psyllium. Accordingly, the application of potassium fertilizer by the constant supply of low- and high-use nutrients can improve the growth and yield of psyllium.

Potassium consumption may also improve plant growth due to its role in establishing the balance of electric charge in plant tissues and maintaining cell mass (Ihtisham et al., 2018). This implies that potassium improves plant yield by increasing the length of the greening period (Kamaluddin et al., 2007). Besides increasing yield and improving product quality, potassium makes plants resistant to environmental stress (Valadabadi & Aliabadi Farahani, 2010).

Shoot yield

The results showed that the effects of phosphorus and potassium fertilizers and the interaction effects of phosphorus fertilizers on potassium and potassium on humic acid, and the triple interaction effect of phosphorus × potassium × humic acid on shoot yield were significant (Table 1). The comparison results for the means of the three interactions revealed the highest shoot yield in the treatment P2K2H2 with the mean of 450.6 kg/ha, suggesting an increase of 35.3% compared to the control treatment. The lowest mean of shoot yield was observed in the treatments P3K3H1 and P2K3H2 with the means of 261.3 and 264 kg/ha, respectively (Fig. 2b).

In a study, the application of humic acid by enhancing Rabisco's activity increased the photosynthetic activity of the plant and promoted its yield. Using humic acid as a solution or powder in the soil increased the length and weight of carrot roots and generally enhanced plant growth (Delfine et al., 2005). Ampong et al. (2022) noticed that the humic acid concentration of

100 mg in 1 liter significantly increased the root growth of Gerbera grown in the nutrient solution, and that humic acid significantly increased the level of phosphorus, potassium, magnesium, and iron in the leaves and the number of flowers in the plant (Carvalho et al., 2005).

Biological yield

Variance analysis indicated significant effects of phosphorus fertilizer (*p* = 0.05) and the triple interaction (phosphorus × potassium × humic acid) (*p* = 0.01) on biological yield (Table 1). Mean comparisons of the triple interactions showed the highest biological yields in treatments P3K2H1 (690 kg/ha), P2K2H2 (694.6 kg/ha), and P3K1H3 (695.3 kg/ha), corresponding to increases of 26.6%, 27.1%, and 27.2%, respectively, relative to the control. The lowest biological yield (506 kg/ha) was observed in the unfertilized control treatment (Fig. 2c).

Dry matter reflects the net photosynthesis of the plant. The produced dry matter is either consumed for the plant growth or accumulates in storage organs, which can determine the yield of agricultural plants (Sajadi Nik et al., 2011; Bostani et al., 2025). Lack of access to supplementary sources of nitrogen, phosphorus and potassium in the growth stages, at the level of the control treatment, decreased the production of photosynthetic material and resulted in low biological weight because of the lower growth of aerial organs. The results of experiments on different plants showed that humic acid increased plant growth both indirectly and directly and had increasing effects on different plants' yield at different levels (Ulukan, 2008). Humic acid had direct and positive effects on the growth of wheat (Vaughan & Linehan, 1976) and chicory (Valdrighi et al., 1996). The response curve of plant growth in the treatment with humic acid showed that plant growth increased with increasing concentration of humic acid; however, a decrease in growth was noticed at extremely high concentrations (Chen & Aviad, 1990). This stimulating effect in low concentrations can be more associated with the direct action on the plant, i.e., the effect of natural hormones, and the indirect action on the metabolism of soil microorganisms, the dynamics of nutrient absorption from the soil, and the physical conditions of the soil. Humic acid spraying also increased the yield by 29% in safflower (Karimi et al., 2016). The elements involved in the photosynthetic operation of the plant increase the level of sap produced in the plant, and if the photosynthetic export to the plant organs in the flowering stage is performed well, it increases yield components in the plant and ultimately promotes yield.

Flower harvest index

The results indicated the significant effects of phosphorus fertilizer at p=0.01, the interaction effect of phosphorus and potassium at p=0.01, and the triple interaction effect of phosphorus × potassium × humic acid at p=0.05 on the flower harvest index (Table 1). Compared to the mean of the interaction effect, the highest flower harvest index was noticed in the treatment P1H2H1 with the mean of 54.5%, revealing an increase by 22.2% compared to the control treatment. The lowest flower harvest index was observed in the treatment P2K2H2 with the mean of 34.3% (Fig. 2d). The flower harvest index indicates how the cultivated material is distributed between the vegetative parts of the plant and the economic part. Some researchers have highlighted the role of the flower harvest index in the distribution of photosynthetic materials to the economic part, suggesting that this trait is influenced directly by genes and indirectly by the environment (Blum, 1988).

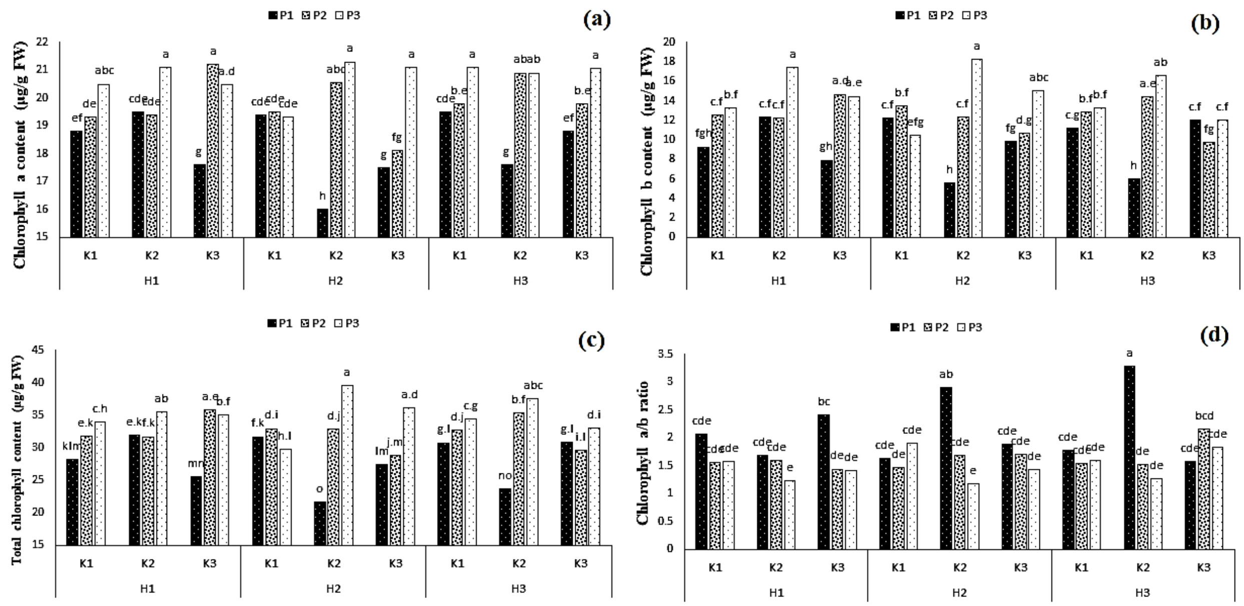

Fig. 3. Comparing mean interaction effects of phosphorus × potassium × humic acid on chlorophyll a content (a), chlorophyll b content (b), total chlorophyll content (c) and chlorophyll a/b ratio (d)
(P1, P2, P3: levels of phosphorus fertilizer (0, 8, and 12 kg/ha); K1, K2, K3: Sulphate potassium fertilizer (0, 10, and 15 kg/ ha); H1, H2, H3: Powder Humax humic acid (0, 0.5, and 2 kg/ ha))

Chlorophyll a content

The data analysis results suggested that the effect of phosphorus fertilizers and humic acid and the interaction effects of phosphorus in potassium and phosphorus × potassium × humic acid on the content of chlorophyll a were significant p=0.01 (Tables 2).

Regarding the comparison of the mean values of the three interaction effects, the highest levels of chlorophyll a were observed a in the treatments P3K2H1, P2K3H1, P3K2H2, P3K3H2, P3K1H3, and P3K3H3 (with the mean values of 21.1, 21.2, 21.3, 21.1, 21.2, and 21.06 µg/g FW, respectively), showing increases by 10.9, 11.3, 11.7, 10.9, 10.9 and 10.7% compared to the control treatment. However, the lowest level was obtained in the treatment P1K2H2 with the mean of 16 µg/g FW (Fig. 3a).

The application of phosphorus fertilizer increased the level of chlorophyll a, compared to its non-application. A decrease in the concentration of chlorophyll in phosphorus-deficient plants implies that, despite the decrease in the weight (Lynch et al., 1991) and surface area of the leaves, which is common in phosphorus-deficient plants and is also observed in the present study, chlorophyll did not accumulate in the fresh matter of the plant; however, it decreased (Celik et al., 2004; Mousavi et al., 2025). This reveals a further decrease in chlorophyll synthesis compared to the growth of leaves. Although phosphorus does not play a direct role in the synthesis and structure of chlorophyll, unlike elements such as magnesium or iron, which are chlorophyll components or interfere in the biosynthesis stages of this compound, its decreased biosynthesis can be due to ATP or the general reduction of metabolism and less access to reduce the synthesis of chloroplast membranes, i.e., the chlorophyll site. In some species, the concentration of chlorophyll does not decrease under phosphorus deficiency conditions;

however, it showed an increase due to a more severe decrease in leaf growth, represented by the presence of darker leaves (Hawkesford et al., 2012).

Chlorophyll b content

In this study, phosphorus, phosphorus × potassium, and phosphorus × potassium × humic acid had significantly different effects on Chlorophyll b (Tables 2). Regarding the comparison of the mean values of the three interaction effects, the highest levels of chlorophyll a were observed a in the treatments P3K2H1 and P3K2H2 with the mean values of 17.46 and 18.22 µg/g FW, respectively, showing increases by 46.9 and 49.1% compared to the control treatment. The lowest chlorophyll b content was also observed in the treatments P1K2H2 and P1K2H3 with the mean values of 5.6 and 5.97 µg/g FW, respectively (Fig. 3b).

Chloroplast, the first light-absorbing pigment in the leaf, plays a critical role in a plants' biochemical and physiological operations, including the synthesis of amino acids, fatty acids, starch, and many secondary metabolic compounds, photosynthesis, and also plays a key role in the plant's response to stress (Jiang & Zhang, 2002; Kadkhodaei, 2013; Pakbaz et al., 2024). Karakurt et al. (2009) investigated the effect of five concentrations of humic acid on the yield and quality of pepper fruits. The treatments were applied at the beginning of the fourth week after cultivation. It was revealed that humic acid had no significant effect on fruit stability, length, and diameter. Moreover, humic acid was significantly effective in enhancing the chlorophyll content in leaves and mainly affected the chlorophyll b content in leaves. In this study, 20 milliliters of humic acid per liter of water, as foliar spraying and soil application, caused the highest chlorophyll content in leaves. Furthermore, humic acid significantly increased fruit weight and total yield compared to the control.

Table 2. Variance analysis for effects of different levels of phosphorus, potassium, and humic acid fertilizers on chlorophyll in chicory

S.O.V	df	Mean Squared					
		chlorophyll a	chlorophyll b	Total chlorophyll	chlorophyll a/b	Carotenoids	Proline content
Block	2	2.33**	3.7ns	11.9ns	0.27ns	1.19ns	0.008
Phosphorus (P)	2	41.1**	166.9**	373.8**	3.11**	11.45**	0.11*
Potassium (k)	2	0.3ns	7.01ns	9.24ns	0.13ns	0.25ns	0.22**
Humic acid (H)	2	4.11**	4.29ns	11.1ns	0.2ns	0.3ns	0.02ns
Phosphorus (P) × Potassium (k)	4	4.27**	39.6**	61.4**	1.1**	3.37*	0.18**
Phosphorus (P) × Humic acid (H)	4	0.46ns	0.98ns	1.69ns	0.002ns	0.53ns	0.02ns
Potassium (k) × × Humic acid (H)	4	0.53ns	4.94ns	7.5ns	0.28ns	0.55ns	0.03ns
Phosphorus (P) × Potassium (k) × Humic acid (H)	8	4.36**	22.1**	44.1**	0.68**	1.14ns	0.08**
Error	52	0.4	4.42	4.47	0.2	1.28	0.02
CV (%)	-	3.24	17.21	6.62	25.7	11.68	21.1

Note: ns, * and ** are not significant and significant at p=0.05 and p=0.01, respectively.

Total chlorophyll content

The effects of phosphorus treatments, phosphorus in potassium, and the triple interaction effect of phosphorus × potassium × humic acid on the total chlorophyll content in chicory leaves were significant atp = 0.01 (Table 2). Regarding the comparison results of the treatments, the highest total chlorophyll content was obtained in the treatment P3K2H2 with the mean of 39.5 µg/g FW, indicating an increase by 28.7% compared to the control treatment. The lowest total chlorophyll content was noticed in the treatment P1K2H2 with the mean of 21.6 µg/g FW (Fig. 3c).

Potassium accelerates the transport of materials resulting from photosynthesis, and this may be associated with the photophosphorylation processes. In this regard, an increase in photophosphorylation and photosynthetic electron transfer is observed in plants well supplied with potassium (Salardini, 2004). Potassium consumption also improves plant growth because of its role in establishing a balance in the electric charge of plant tissues and maintaining cell mass (Khayyat et al., 2018).

Humic acid beings about many benefits for crop production and directly and indirectly affects plant growth. The indirect effects of humic acid on yield have been attributed to improvement in the physical, chemical and biological conditions of the soil. Its decomposition on clay and compacted soils facilitates the transfer of micronutrients from the soil to the plant, increases water retention, enhances the germination rate, improves nutrient absorption and plant growth, and stimulates the development of microorganisms. It has direct effects on plant growth by increasing the chlorophyll content, accelerating the respiration process, responding to growth hormones, increasing the penetration of substances into the plant membrane, affecting the production of dry matter, and promoting the absorption of nutrients by plants. The indirect effects of humic compounds on plant growth can be attributed to the improved physiological conditions of the plant; however, its direct effect is attributed to the increased chlorophyll content, the accelerated respiration process and hormonal responses, the increased permeability of the plant membrane, or the interaction of these factors (Khaled & Fawy, 2011). In the present study, the foliar application of humic acid increased the chlorophyll content (Khattab et al., 2014). By increasing nitrogen content, humic acid preserves the photosynthetic tissue and increases chlorophyll synthesis in plants, and by accelerating the growth of photosynthesizing surfaces, it improves photosynthetic pigments. Besides taking part in the structure of amino acids and enzymes, nitrogen is one of the main elements of the tetra-pyrrole ring of chlorophyll, whose increase enhances the chlorophyll content in the plant by promoting the content of ammonium, glutamate synthetase, and glutamine synthetase enzymes involved in the chlorophyll production (Boveiri Dehsheikh et al., 2017).

Chlorophyll a/b ratio

The data analysis results revealedthe significant effect of phosphorus, phosphorus × potassium, and phosphorus × potassium × humic acid treatments on the chlorophyll a/b ratio at p=0.01 (Table 2). The highest chlorophyll a/b ratio was observed in the treatment P1K2H3 with the mean of 3.29, showing an increase by 37.3% compared to the control treatment. The lowest chlorophyll a/b ratio was observed in the treatment P2K2H3 with the mean of 1.17 (Fig. 3d).

Carotenoid content

The data analysis revealed significant effects of phosphorus, the phosphorus × potassium interaction, and the triple interaction (phosphorus × potassium × humic acid) on the chlorophyll a/b ratio at *p* = 0.01 (Table 2). The highest chlorophyll a/b ratio (3.29) was observed in treatment P1K2H3, representing a 37.3% increase compared to the control. Conversely, the lowest ratio (1.17) was recorded for treatment P2K2H3 (Fig. 4). Carotenoids, as antioxidants and a protective system against oxidative stress, are the victims of induced oxidative stress (Di Cagno et al., 1999).

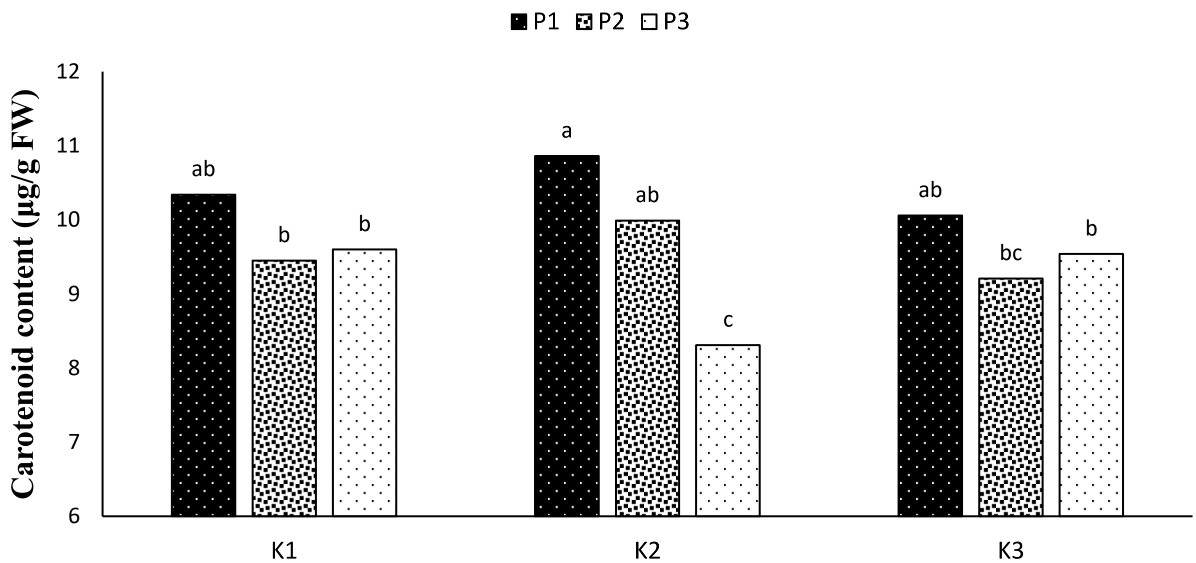

Fig. 4. Comparing mean interaction effects of phosphorus × potassium × humic acid on carotenoid content (P1, P2, P3: levels of phosphorus fertilizer (0, 8, and 12 kg/ha); K1, K2, K3: Sulphate potassium fertilizer (0, 10, and 15 kg/ ha)).

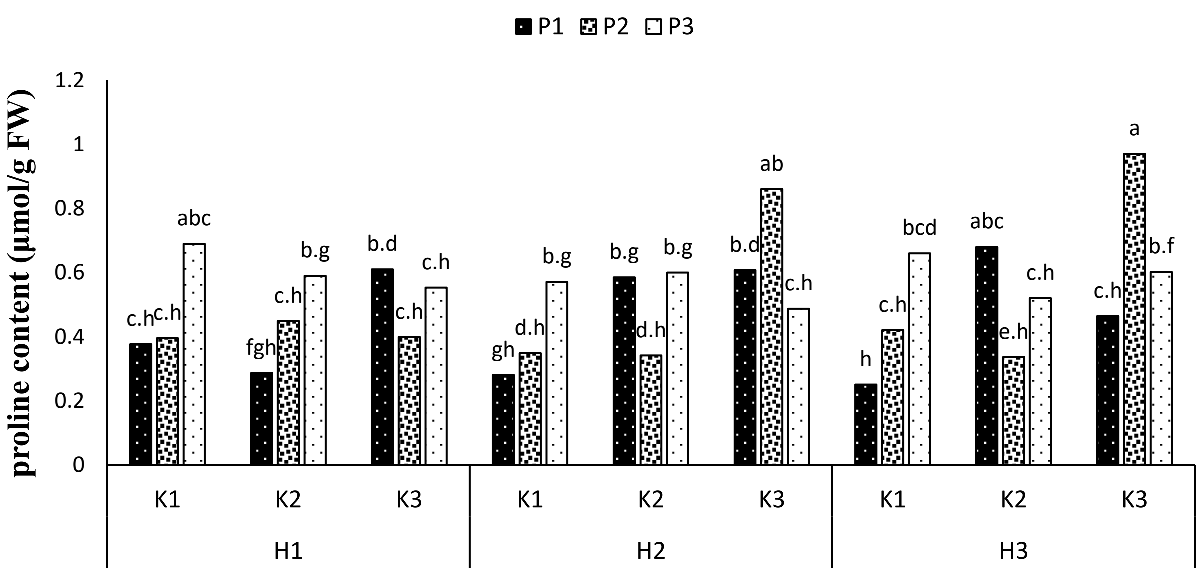

Fig. 5. Comparing mean interaction effects of phosphorus × potassium × humic acid on proline content (P1, P2, P3: levels of phosphorus fertilizer (0, 8, and 12 kg/ha); K1, K2, K3: Sulphate potassium fertilizer (0, 10, and 15 kg/ ha); H1, H2, H3: Powder Humax humic acid (0, 0.5, and 2 kg/ ha)).

Proline content

The effects of phosphorus, potassium, phosphorus in potassium and phosphorus × potassium × humic acid treatments on the proline amino acid were significantly different. According to the results, the highest free proline content was observed in the treatment P2K3H3 with a mean of 0.97 µmol/g FW, showing an increase by 61.2% compared to the control treatment. The lowest free proline content was noticed in the treatment P1K1H3 with the mean of 0.251 µmol/g FW (Fig. 5).

Increased proline concentration in plants under stress is a kind of adaptation to overcome stress conditions (Manivannan et al., 2007). Proline is an amino acid, whose increased concentration is the most common and general response observed under stress conditions (Suriyan & Chalermpol, 2009). It seems that high levels of concerned fertilizers and their high solubility arouses toxicity and stress in the plant; hence, an increase in the proline content is observed at high levels. Free amino acids were accumulated in the leaves of plants suffering from phosphorus deficiency and also under drought stress. However, in the root, only phosphorus deficiency resulted in the accumulation of free amino acids, and drought did not have such an effect. The decreased protein synthesis under phosphorus deficiency conditions results in the accumulation of free amino acids, as reported for nitrogen and potassium deficiency conditions (Hawkesford et al., 2012).

Potassium deficiency increases the storage of hydrocarbons in the plant, which is caused by the delay in protein production. According to some reports, potassium is involved in and directs the last phase of the protein production process. Accordingly, when the potassium content decreases in the plant, the protein content also decreases, and galactamides and amino acids increase (Malekooti & Homaei, 2004).

Simple correlation of traits

Pearson correlation analysis (Table 3) revealed significant positive relationships between flower yield and leaf area index (0.35), biological yield (0.71), and harvest index (0.80). Total chlorophyll content, as a key physiological parameter influencing growth and productivity, showed significant positive correlations with plant height (0.38), number of secondary stems (0.41), shoot yield (0.33), biological yield (0.35), chlorophyll a content (0.83), and chlorophyll b content (0.97). Conversely, significant negative correlations were observed between total chlorophyll content and both the chlorophyll a/b ratio (-0.82) and carotenoid content (-0.72). An increase in leaf area index enhanced the number of photosynthetic pigments per unit area, thereby enhancing the assimilate production and promoting the plant's growth and yield (Khadempir, 2014).

Table 3. Simple correlation between yield and physiological traits of chicory in different fertilizer treatments.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1												
2	0.81**												
3	0.02	-0.09	1										
4	0.3*	0.14	0.44*	1									
5	0.07	-0.04	0.35*	0.27	1								
6	0.16	0.18	0.06	0.13	-0.03	1							
7	0.17	0.1	0.3*	0.28	0.71**	0.69**	1						
8	-0.06	-0.15	0.24	0.15	0.8**	-0.61**	0.15	1					
9	0.4*	0.38*	0.18	-0.09	0.15	0.28	0.31*	-0.04	1				
10	-0.34*	-0.38*	0.11	-0.01	0.15	0.32*	0.33*	-0.07	0.68**	1			
11	0.38*	0.41*	0.14	-0.03	0.16	0.33*	0.35*	-0.07	0.83**	0.97**	1		
12	0.29	0.33*	-0.06	0.08	-0.06	-0.22	-0.2	0.08	-0.52**	-0.87**	-0.82**	1	
13	0.26	0.32*	-0.07	-0.07	-0.12	-0.31*	-0.31*	0.1	-0.33*	-0.81**	-0.72**	0.77**	1
14	-0.32*	-0.27	0.001	0.13	0.07	-0.14	-0.05	0.15	-0.13	-0.17	-0.17	0.18	0.001

Note: * and ** are significant at p=0.05 and p=0.01, respectively
(1: Plant height, 2: Number of secondary stems, 3: Leaf area index, 4: Number of flowers, 5: Flower yield, 6: Shoot yield, 7: Biological yield, 8: Flower harvest index, 9: Chlorophyll a, 10: Chlorophyll b, 11: Total chlorophyll, 12: Chlorophyll a/b ratio, 13: Carotenoid, 14: Proline.

CONCLUSION

This study demonstrated that the combined application of phosphorus, potassium, and humic acid fertilizers significantly enhanced the vegetative growth, yield, and physiological characteristics of chicory plants. Increased leaf area index, flower number, flower yield, shoot yield, and biomass indicated improved plant growth. Furthermore, elevated chlorophyll content and reduced free proline levels suggested enhanced photosynthetic activity and reduced stress in treated plants. However, excessive application of these fertilizers had detrimental effects on plant growth. Therefore, a balanced application of these nutrients is crucial. Based on the results, applying 8 kg/ha of Sangral (phosphorus source), 10 kg/ha of potassium sulfate (potassium source), and 0.5 kg/ha of humic acid powder is recommended for chicory cultivation in the Arak region. In conclusion, the combined application of these fertilizers can be considered an effective strategy to improve chicory yield and quality. However, optimal fertilizer rates and ratios should be determined based on specific soil and plant conditions.

Conflict of interest

The authors declare no competing interests.

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