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The Effect of Planting Date, Plant Density, and Selenium Foliar Spraying on Shallot (*Allium ascalonicum* L.) Production

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ABSTRACT

This study aimed to evaluate the effects of planting date, plant density, and foliar Selenium (Se) application on growth, yield, and biochemical traits of Shallot (*Allium hirtifolium*) to identify optimal agronomic practices to improve crop performance and phytochemical composition under the environmental conditions of Khalateh Rudbar, Semnan Province, Iran. A factorial experiment with a randomized complete block design and three replications was established during the 2023-2024 growing season. Treatments included four planting dates (16 October, 15 November, 15 December, and 13 February), four plant densities (6, 10, 14, and 18 plants/m^2), and four levels of foliar Se application (0, 4, 8, and 12 mg/l as sodium selenate). Se sprays were applied four times at two-week intervals post-emergence. Growth parameters such as plant height, leaf area index, and yield components were measured. Biochemical analyses included allicin content, total phenolics, total flavonoids, antioxidant activity, and relative water content, assessed using standard laboratory methods. Data were statistically analyzed using ANOVA and mean comparisons at $p < 0.05$. Significant effects of planting date, plant density, and selenium foliar spraying were observed on all measured traits ($p < 0.01$). Early planting (16 October) combined with low density (6 plants/m^2) and high selenium concentration (12 mg/l) resulted in the highest plant height (57.5 cm), bulb yield (6.2 kg/m), and highest accumulation of bioactive compounds including allicin (2.85 mg/g), total flavonoids (54.3 mg/g), and phenolics (45.3 mg/g). Selenium application enhanced LAI, especially between 20 and 35 days after planting, contributing to improved biomass production. Interaction effects indicated selenium's role in alleviating stresses associated with late planting and higher plant density. The findings suggest that selecting an appropriate planting date and maintaining moderate plant density are crucial for maximizing shallot yield and quality. Foliar selenium application and the first planting date significantly promoted antioxidant and physiological properties. The results underline the importance of integrated crop management approaches to optimize yield and nutritional value, particularly in semi-arid agroecosystems. These findings provide valuable insights for shallot growers aiming to improve productivity and crop quality through timing, spacing, and micronutrient supplementation.

Keywords: Antioxidants, Biofortification, Phenolic component, Yield

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INTRODUCTION

In arid and semi-arid regions, water shortage is a persistent challenge, significantly affecting plant growth and productivity. Precipitation patterns are expected to exacerbate water-related issues [1]. Medicinal plants, with a history spanning over a thousand years, are extensively utilized for producing antimicrobial and antioxidant medications [2]. Their natural origin contributes to their popularity as they generally have fewer side effects compared to synthetic chemicals [3]. Medicinal plants are abundant in secondary metabolites, which are bioactive compounds that hold great potential for the development of pharmaceuticals and aromatic products. These metabolites not only contribute to the therapeutic properties of these plants but also enhance their value in the market. Understanding how to mitigate these stressors is essential for optimizing the cultivation of medicinal plants and ensuring a reliable supply of their beneficial compounds [4]. Regarding these issues, the adverse effects of drought stress significantly hinder crop performance and yield stability, and are recognized as one of the most detrimental abiotic stress factors globally. It is primarily caused by variations in temperature, light intensity, and reduced rainfall. Drought stress has a profound effect on crop production, affecting various characteristics of plants, including their morphological, physiological, biochemical, and molecular traits [5].

Shallot, scientifically known as *Allium ascalonicum* L., is not only a culinary vegetable but also holds a special place in Iranian cuisine and culture due to its medicinal properties and distinctive flavor [6].

IRAN is a major producer of Shallots globally and exports a portion of its production to other countries. Increased exports can also contribute to higher demand for shallots within the country. To meet the rising demand, shallot production in Iran has been consistently increasing from 2017 to 2023 .The average annual production increase has been 5.14%, indicating significant growth in the industry [7].

With increasing urbanization and lifestyle changes, dietary patterns have also evolved. Shallots, as a natural and healthy flavoring agent,

have found their way into both modern and traditional dishes. They are widely cultivated in various regions of the country and are considered an important agricultural product. In recent years, the demand for shallots in Iran has been steadily increasing. This surge in demand can be attributed to several factors, including increased awareness of health benefits such as anti-inflammatory, antibacterial, and antioxidant properties of shallots [8].

Shallot production in Iran has grown by 5.14% annually due to improved farming, expanded cultivation areas, and government support. Challenges include water scarcity, climate change, and competition from China and India. Opportunities lie in developing processing industries, expanding export markets, and adopting modern technologies to boost efficiency. The phytochemical profile of *A. hirtifolium* Boiss. and its secondary metabolites was investigated. Identification of sulfur-containing compounds, flavonoids, and phenolics responsible for antioxidant and antimicrobial activities [9].

Plants have developed an enzymatic antioxidant system to combat reactive oxygen species (ROS) and support individual growth and grain production. This system includes key enzymes, such as total superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). This mechanism is commonly employed by plants to effectively manage various abiotic stresses [10].

Exhibits significant antioxidant, antimicrobial, anti-inflammatory, and potential anticancer activities linked to its secondary metabolites. In vitro assays (e.g., DPPH for antioxidant activity, microbial inhibition tests, cell viability assays) to evaluate bioactivity [11].

Influence of cultivation conditions on secondary metabolite concentrations in *A. hirtifolium* investigated by Rahmanian and Mohammadi, 2019 [12]. Results showed that environmental factors such as soil type, harvesting time, and cultivation practices influence metabolite concentrations, affecting medicinal potency.

Shollot is traditionally used for treating respiratory issues, infections, and inflammation. The secondary metabolites underpin these medicinal uses [13].

In the study of Promising effects of Persian shallot extract on the serum markers and blood pressure of patients with metabolic syndrome: a double-blinded randomized controlled trial, results showed Persian shallot extract has several beneficial effects in metabolic syndrome patients, including optimizing oxidative balance, reducing blood pressure, fasting blood sugar, and blood lipid profile [14].

Numerous studies suggest that optimal planting times improve yield and metabolite content by aligning growth cycles with environmental conditions. Early or late planting can affect bulb size, secondary metabolite accumulation, and overall plant health [15].

Increased plant density often results in competition for nutrients and light, potentially reducing individual plant yield but possibly increasing total land productivity. Densities influence the concentration of secondary metabolites, sometimes enhancing phytochemicals due to stress or competitive interactions [16].

Foliar application of Se has been shown to enhance antioxidant capacity, boost secondary metabolite synthesis, and improve stress tolerance in *Allium* species [17]. Se acts as a micronutrient, stimulating secondary pathways and improving yield parameters. Some studies indicate that applying Se foliar sprays at optimal planting times and densities can synergistically enhance yield and secondary metabolite concentration, but specifics can vary depending on environmental conditions and species. Se biofortification represents a plant-based approach to enhance the Se content of crops through the application of Se directly to the crop or the soil. The consumption of Se-enriched crops offers a potential avenue for increasing Se intake among populations residing in Se-deficient areas [18].

The implementation of inorganic Se fertilization can present challenges for growers lacking specialized training in Se biofortification techniques, particularly regarding the application of Se fertilizer under diverse field conditions. Soil application of Se is especially susceptible to complications in coarse-textured soils, where excessive rainfall or irrigation can lead to the rapid leaching of soluble inorganic Se. Additional Se losses from the soil may occur through volatilization (e.g., as methyl-selenide), a process mediated by both microbial activity [19] and plant metabolism [20].

This research was conducted to improve Shalloot yield and physiobiochemical components in planting date and density conditions with the spraying of Se.

MATERIALS AND METHODS

Managing the Study Site

The study was performed at the Khalateh Rudbar Agricultural Services Department, located in Khalateh Rudbar, Dameghan, Semnan Province, Iran (Coordinates: 10° 10' 35" N, 50° 23' 58" E). The region's meteorological data, relevant to the experimental period, are shown in Figure 1, providing insights into climatic factors such as temperature, humidity, and rainfall that may influence soil and crop responses.

The soils in Khalateh Rudbar are predominantly silty-loam, characterized by a balanced mixture of silt, clay, and sand particles. This soil texture plays a crucial role in determining water retention capacity, drainage, and nutrient availability, all of which are vital for optimizing agricultural practices. To accurately assess the soil's physical and chemical properties, a representative composite sample was collected from the top 30 centimeters of soil at the site, following standardized soil sampling protocols to ensure reliability. Laboratory analyses included measurements of the pH of soil, EC, content of organic matter, macronutrients (such as N, P, K), and micronutrients. These analyses provided essential baseline data to understand the fertility status and suitability of the soil for the experimental treatments. Additionally, water samples from Kashmar were analyzed to evaluate parameters such as pH, EC, and nutrient content, given their potential influence on crop growth and soil chemistry (Table 1, 2).

The purpose of these analyses was to establish a comprehensive understanding of the initial soil and water conditions before implementing the experimental treatments. This baseline data is fundamental for evaluating the effects of different interventions, such as irrigation strategies or soil amendments, on soil health, crop productivity, and environmental sustainability within this semi-arid region. The field experiment was conducted during the 2023-2024 growing season in Dameghan. The experiment included four levels of planting date (16 October, 15 November, 15 December, and 13 February) as the main plot, subplots include plant densities at four levels (6, 10, 14, and 18 plants/m^2) and spraying of Se with fore concentrations of (0, 4, 8, 12 mg/l) (two weeks between one spraying and another after completing the emergence of cultivated seedling with a month of cultivating for each date).

Table 1 The experiment region of geographical and climatic characteristics

Region	Elevation (M)	Longitude	latitude	Max temperature (°c)	Min temperature (°c)	Climate
Kalateh Roudbar	1420	35.36	58.54	41.68	20.80	Temperate

Table 2 Soil and water analysis of Kalateh rudbar

Soil											
Parame-ter	Sand (%)	Silt (%)	Clay (%)	N (%)	Fe (ppm)	Zn (ppm)	Organic carbon (%)	Salinity (dS/m)	P PPM	K PPM	pH
Value	50	35	10	0.09	1.86	0.6	0.81	1.813	30.7	300	7.58
Water											
Parame-ter	Suspended solutes in water (mg/l)		Sodium Aabsorption ratio		Cl^- (mEq/ l)	$SO4^-$ (mEq/ l)	Salinity (Sμ)	Na^+ Meq/l	EC Ds/m	pH	
Value	1035.12		2.46		7.2	4.7	1625	2.2	0.48	7.29	

The experiment included three-factor which are the interaction among three planting dates, fore plant densities, and fore concentrations of Se. The Randomized Complete Block Design (RCBD) is used as a design for a three Split-Split Plot experiment. Design where the planting date is considered the Main plot, plant density is the Sub-Plot, and spraying with organic nutrients is the Sub-Sub-Plot. The treatments with Se were repeated twice in the Year, both in 2024, by applying the aforementioned water solution via spraying, the shallot plants were treated. The field experiment was conducted during the 2023-2024 growing season in Dameghan, Iran. The study employed a Split-Split Plot design to investigate the effects of multiple factors on plant growth and development. The main plot factor was the planting date, with four levels: 16 October, 15 November, 15 December, and 13 February. Subplots included four different plant density levels: 6, 10, 14, and 18 plants/m^2. Additionally, three Se treatment levels were applied through foliar spraying at concentrations of 0, 4, 8, and 12 mg/l. Applications were performed at two-week intervals, beginning after the emergence of the cultivated seedlings. Each treatment was maintained for one month for each planting date. With applications spaced two weeks apart, starting after the emergence of the cultivated seedlings. Each treatment was applied for a month for each planting date.

The study was organized as a three factor factorial experiment to investigate the interactions among planting date, plant density, and selenium (Se) concentration. To facilitate a comprehensive analysis of these interactions, a Randomized Complete Block Design (RCBD) with a split-split plot arrangement was utilized. In this setup, the planting date served as the main plot factor, plant density as the sub-plot factor, and Se application as the sub-sub-plot factor. The treatments involving Se were replicated twice across two different iterations in 2024, with plants being sprayed accordingly with the specified Se solution. This experimental setup allowed for a comprehensive evaluation of how planting time, plant density, and Se foliar application interactively influence crop performance, including growth parameters, yield, and stress tolerance.

Sample Preparation

After harvesting, the bulbs were cleaned thoroughly, and their outer shells were removed. The bulbs were then sliced into thin pieces using a plastic knife. A portion of the dry homogenized material was used immediately to measure nitrate and ascorbic acid levels. Another aliquot of the fresh slices was dried at 70 °C until reaching a constant weight and was subsequently used to determine polyphenols, protein, allicin, flavonoids, and antioxidant activity. Additionally, the selenium content in the bulbs was analyzed using homogenized samples that were dried at 20 °C to a constant weight. The harvest took place during the last week of July in the research years, when the bulbs had reached their maximum size. In each plot, several measurements were taken, including the number of bulbs and their total weight. The average bulb weight was calculated based on a sample of 50 bulbs. Additionally, a random sample of 25 marketable bulbs was collected from each plot and brought to the laboratory for detailed analysis. After harvesting, the bulbs were carefully separated from the plants, thoroughly cleaned, and the outer shells were removed. The bulbs were then sliced into thin pieces using a plastic knife. A portion of Freshly homogenized samples was dried at 70 °C until reaching a constant weight and was subsequently used for the analysis of polyphenols, flavonoids, and antioxidant activity. The selenium (Se) content in the bulbs was determined using homogenized samples that were dried at 20 °C until they reached a constant weight. Harvesting was carried out in the last week of July during each research year, at which point the bulbs had reached their maximum growth stage. For each plot, the following measurements were taken: the number of bulbs, total and average bulb weight determined from a sample of 50 bulbs, and the weight of individual bulbs. Additionally, a random sample of 10 marketable bulbs was collected from each plot and transported to the laboratory for detailed analytical assessments.

Plant Height

Plant height was measured from the base of the plant up to the top-most part of the highest leaf. A ruler was used to take these measurements accurately.

Yield

The harvest took place during the final week of July in both years of the study, coinciding with the period when the bulbs had attained their peak growth. In each plot, several assessments were conducted, including counting the number of bulbs and recording their total weight. Additionally, the average weight of bulbs was calculated from samples of 50 units. Furthermore, from each plot, a random sample of 25 marketable bulbs was selected and transported to the laboratory for detailed analytical analyses. Harvesting was carried out in the last week of July during both research years, at which time the bulbs had reached their maximum growth. In each plot, the following measurements were conducted: the number of bulbs, total bulb weight, and mean bulb weight calculated from a representative sample of 25 bulbs. Additionally, a random sample of 25 marketable bulbs was collected from each plot and carefully transported to the laboratory for further analytical assessments.

Total Polyphenols Contents (TPC)

TPCs were quantified in water extracts by use of the Folin–Ciocalteu colorimetric method [21]. 1 g of dry shallot bulb powder was extracted with 20 ml of 70% ethanol at 80 °C for 1 hour. The solution was then cooled and transferred to a volumetric flask, with the volume adjusted to 25 ml. The extract was filtered through filter paper to remove solids, and a 1 mL aliquot of the filtrate was transferred to a 25 ml volumetric flask. To this, 2.5 ml of saturated Na_2CO_3 solution and 0.25 ml of diluted (1:1) Folin–Ciocalteu reagent were added. The solution was then made up to 25 mL with

distilled water. After one hour, the reaction mixture was analyzed spectrophotometrically using a Unico 2804 UV spectrophotometer (USA). The concentration of polyphenols was determined based on the absorbance at 730 nm, using gallic acid (0.02%) as an external standard for calibration.

The dilutionseries of standards were:

Sample extract(stock): 0.01 gram in 1 ml

Equation: y = 0.0078x - 0.0679, R2 = 0.9969

Absorbance: Sample was serially diluted-

1000(µg/ml) = 0.598

500(µg/ml) = 0.296

250(µg/ml) = 0.127

125(µg/ml) = 0.063

62.5(µg/ml) = 0.026

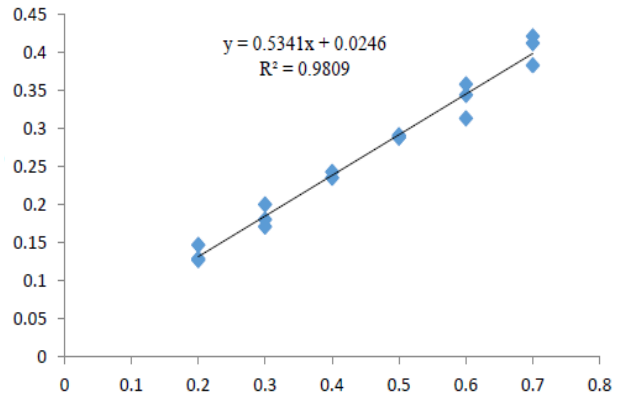

Fig. 1 Gallic acid standard curve

Flavonoids

In this study, the total flavonoid content of samples was determined using a spectrophotometric method based on the formation of a complex between flavonoids and aluminum chloride ($AlCl_3$) [22]. First, 1 gram of dried, homogenized sample was mixed with 10 milliliters of methanol. This mixture was left at room temperature for 2 hours to ensure thorough extraction of flavonoids. After this incubation period, the mixture was filtered through a pleated filter to remove any solid particles and obtain a clear extract.

Next, 0.2 milliliters of the filtered extract was diluted with 1.8 milliliters of methanol. To this solution, 0.1 milliliters of a 2% aluminum chloride solution, 0.5 milliliters of a 1 molar sodium acetate solution, and 1 milliliter of distilled water were added. The resulting mixture was incubated at room temperature for 30 minutes, allowing the complex between flavonoids and aluminum chloride to form fully. After incubation, the absorbance was measured at a wavelength of 415 nanometers using a spectrophotometer.

To quantify the total flavonoid content, a standard calibration curve was constructed using five different concentrations of a quercetin–$AlCl_3$ complex. Quercetin, which served as the standard reference compound, was purchased from Fluka in Switzerland. This method provides a quick, sensitive, and reliable way to estimate the amount of flavonoids, with known antioxidant potential, in plant-based samples. Such measurements are important for assessing the nutritional and health-related properties of natural products.

the standard curve equation: y = 0.0092x + 0.0249, r 2 = 0.985; Fig. 2).

Antioxidant Activity (AOA)

The Shallot bulbs' antioxidant activity was evaluated using a redox titration method [23], which involves titrating a 0.01 N potassium permanganate ($KMnO_4$) solution with ethanolic extracts derived from the shallot bulbs. During this process, the $KMnO_4$ acts as an oxidizing agent, and its reduction to the colorless Mn^{2+} ion signifies the presence of antioxidants in the extract. Specifically, the antioxidants dissolved in 70% ethanol reduce the permanganate, and the amount of $KMnO_4$ consumed reflects the antioxidant capacity of the sample.

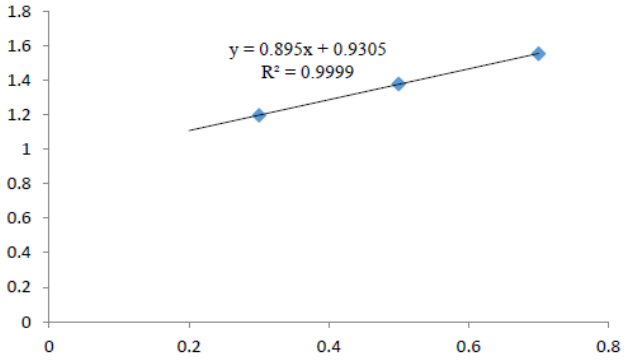

Fig. 2 Quercetin standard curve

The results were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of dry weight (mg GAE·100 g/d.w.). This approach of using $KMnO_4$ in an acidic medium has previously been successfully employed to assess the antioxidant potential of *Ocimum basilicum* [24] and to measure the antioxidant capacity of serum samples. It provides a straightforward and reliable way to estimate the antioxidative properties of plant materials and biological fluids.

Relative Water Content (RWC)

The relative water content (RWC) was calculated using the formula developed by Taheri et al [25], which takes into account the fresh weight, turgid weight, and dry weight of the leaves:

$$RWC(\%) = \left(\frac{FW-DW}{TW-DW}\right) * 100 \qquad (1)$$

FW: Fresh weight; DW: dry weight; TW turgid weight.

Allicin

The determination of allicin content followed the procedure outlined by Miron *et al*. [26]. Initially, 0.5 g of shallot powder was mixed with 10 milliliters of distilled water and placed on a shaker at room temperature for 30 minutes to facilitate extraction. After incubation, the mixture was centrifuged at 6000 rpm for 30 minutes to separate the supernatant, which contained the soluble compounds, including allicin. From this supernatant, 1500 microliters (1.5 ml) were transferred into a reaction vessel and combined with 750 microliters of 4-mercaptopyridine solution. The reaction mixture also included 0.2 mL of a reagent labeled MP, 2 mM EDTA, and 50 mM of a buffer solution likely sodium phosphate (Na_3PO_4). The absorbance of this mixture was measured at 324 nanometers using a spectrophotometer. Readings were taken at five-minute intervals over a total of 15 minutes. The decrease in absorbance (ΔA) over this period reflects the amount of allicin present in the sample. The allicin concentration was then calculated using the relation specified as (3), which likely involves a calibration curve or a formula linking ΔA to allicin amount.

The dilution series of standards was l(+)- and l(±)-*S*-allylcysteine sulfoxides (natural and racemic alliin), *S*-allylcysteine, diallyl disulfide, and diallyl trisulfide.

$$C = \frac{\mathrm{d}\,\Delta\,\mathrm{A}}{\varepsilon}$$

C = Concentration of allicin (mg/gr)

D = Dilution factor
€ = 19.800 M/cm

Leaf Area Index (LAI)

To reduce of the marginal effect, all samples were taken from the middle of each plot. Leaf area index (LAI) was also measured by a specific leaf area meter at each step.

Statistical Analysis

This experiment was conducted using a combined analysis approach, with the fundamental design being factorial. The data were analyzed through analysis of variance (ANOVA), and mean comparisons were performed at a 5% significance level using the LSD (Least Significant Difference) test. All statistical analyses were carried out with SAS software (Version 9.4). Additionally, MS Excel was used to create and visualize the diagrams and graphical representations of the results.

RESULTS AND DISCUSSION

Plant Height

Plant height showed a significant response to treatment in Shallot plants (Table 3). Shallot plants grown under the treatment of the first planting date and 6 plants/m^2 density by use of Se selenate 12 mg/l resulted in the tallest plant (57.5 cm) compared to other treatments. On the other hand, the shortest Shallot plants (40.1 cm) were observed in control treatments (use of Se selenate, first planting date and, 6 plant/m^2 density (Fig. 1).

Plant height showed a significant response to the different treatments in shallot plants (Table 3). The tallest plants, measuring an average of 57.5 cm, were observed in plants grown under the combination of the first planting date, a density of 6 plants/m^2, and foliar application of Se at 12 mg/l. In contrast, the shortest shallots, with an average height of 40.1 cm, were found in the control group that did not receive Se, regardless of planting date and plant density (Fig. 3). This indicates that Se application and optimal planting conditions can significantly promote plant growth.

Yield

A significant interaction effect between treatment was observed for Shallot yield. Planting dates and plant density had significant effects on plant yield in Shallot (Table 3). Considering the interaction effect of planting dates and plant density, the highest yield (6.2 kg/m2), was observed in the first planting dates with a density of 6 plant/m2. While in the last planting date, treatments and density of 18 plants/m2, the lowest yield (0.471 kg/m2) was observed (Fig. 2). Analysis of variance (ANOVA) revealed that both main factors, planting date and plant density, had significant main effects ($p < 0.01$), and their interaction was significant ($p < 0.05$), underscoring the importance of their combined influence. The highest yield, recorded at 6.2 kg/m^2, was obtained from shallots planted on the first planting date at a density of 6 plants/m^2. In contrast, the lowest yield, 0.471 kg/m^2, was observed in the last planting date under a high-density treatment of 18 plants/m^2 (Fig. 2).

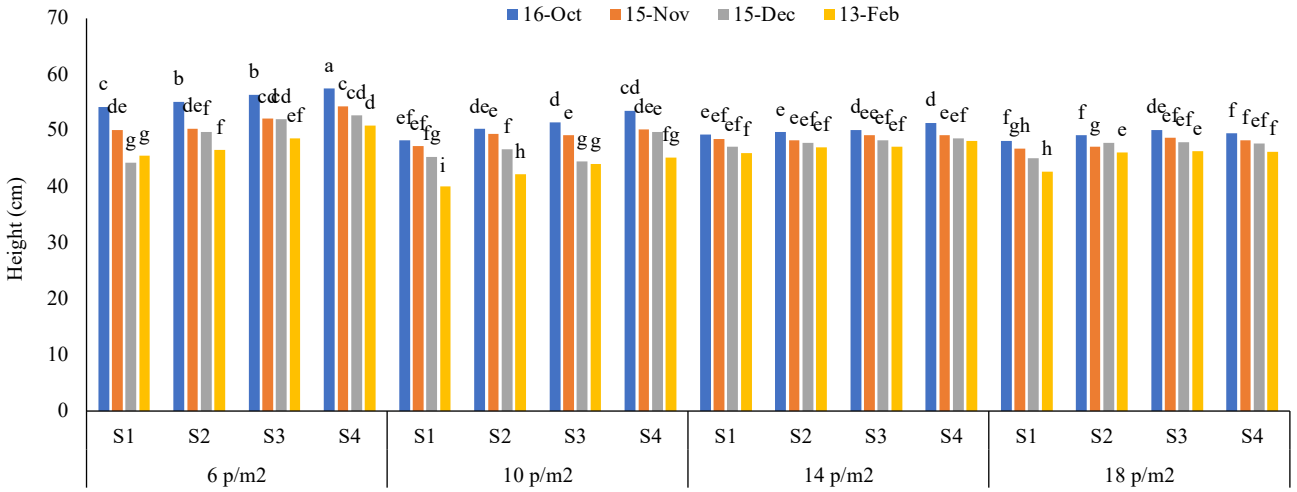

Fig. 3 The interaction effects of planting dates × planting density × Se selenate on height in Shallot. Columns with similar letters did not differ significantly.

Table 3 Results of analysis of variance (mean squared) of physio-morphological traits in shallot

Source	DF	Plant height	Yield	Phenols	Flavonoids	Antioxidant activity	LAI	Allicin	Chlorophyll content
Planting Date (PDa)	2	856.74 **	24257.20 *	5.2485 **	1.7865 **	2.5241 **	2.0584 **	0.0354 *	0.44 ns
Plant Density (PDe)	3	2826.3 **	251411 *	2.654 **	1.05178 **	11.417 **	50.246 **	0.0254 *	0.86 *
Se (S)	2	1836.7 **	163254 **	8.0841**	1.02541 **	2.1782 **	1.2517 **	0.0615 *	1.05 **
PDa * PDe	6	100.05 *	85416.5 *	1.1731 *	0.08719 *	0.1634 **	0.1748 **	0.1164 **	0.38 ns
PDa * S	3	1121.3 **	11524.25 ns	2.4215 **	0.1905 **	0.3144 **	0.1456 **	0.2841 **	0.29 ns
PDe * S	2	903.6 *	88741 **	1.2876 *	0.0524 *	0.4517 **	0.63142 *	0.3541 **	1.11 *
PDa * PDe * S	6	158.25 *	2154.3 *	0.6605 *	0.1275 **	0.1124 **	0.3541 **	0.2874 **	0.095 ns
Error	12	43.81	20387.4	0.3451	0.00967	0.00134	0.0347	0.0034	0.132
CV		9.25	11.04	5.97	7.66	3.27	9.28	8.18	4.25

* and ** Significantly at the probability level of %5 and %1, respectively. ns= Not significantly

Total Flavonoids Content

Table 3 illustrates the significant impact of all treatments on the total flavonoid content in Shaloot. The 6 p/m^2 with the use of Se 12 mg/l resulted in the highest flavonoid content (54.3 mg/g). Conversely, the 18 p/m^2 density yielded the lowest flavonoid content (32.29 mg/gr). (Fig. 4). Table 3 demonstrates that all treatments

exerted a statistically significant influence on the total flavonoid content in Shaloot. Notably, the treatment combining a plant density of 6 plants/m^2 with the application of 12 mg/l Se resulted in the highest flavonoid concentration, measuring 54.3 mg/g of dry weight. In contrast, the highest plant density of 18 plants/m^2, without Se supplementation, yielded the lowest flavonoid content, at 32.29 mg/g (Fig. 4).

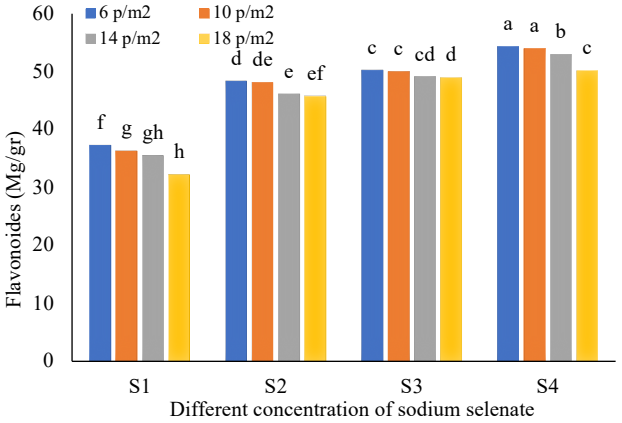

Fig. 4 The interaction effects of Na_2Se × plant density on flavonoids of Shallot. Columns with similar letters did not differ significantly.

Leaf Area Index (LAI)

Across all treatment used, the temporal pattern of LAI development followed a similar trajectory. During the initial growth phase, LAI increased at a relatively slow rate, reflecting early vegetative establishment, and around 20 to 25 days after planting (DAP), it transitioned into a phase of linear, rapid growth. The LAI reached its maximum value between 35 and 40 DAP, indicating the peak vegetative development. Subsequently, a gradual decline in LAI was observed, persisting until the end of the growing season, which likely reflects the natural senescence process and resource allocation shifts within the plant. Among the treatment groups, the combination of 12 mg/l Na_2Se application and a plant density of 6 p/m^2 resulted in the highest LAI, highlighting the synergistic effect of Se supplementation and optimal density on foliage development. Conversely, the control treatment, which lacked Na_2Se supplementation, exhibited the lowest LAI throughout the growth cycle. These findings underscore the significant influence of both Se application and plant density management on leaf area expansion, which directly impacts photosynthetic capacity and, ultimately, crop yield potential (Fig. 5, 6).

Total Phenolic Compound (TPC)

Variance analysis indicated that total phenolic compounds (TPC) in shallots were significantly affected by both plant density and the application of Se ($p< 0.05$). The experimental design involved a factorial arrangement allowing for the assessment of main and interaction effects, with results confirming that both factors had a statistically significant influence on TPC levels. The highest TPC content, 45.30 mg/g, was observed in treatments combining an application of Na_2Se at 12 mg/l with a plant density of 6 plants/m^2. Conversely, the lowest TPC value, 28.5 mg/g, was found in treatments without Se application at the higher plant density of 18 plants/m^2 (Fig. 7). These findings suggest that biofortification with Se, particularly at optimal application rates and lower plant densities, can substantially enhance the phenolic content of shallots, which is important for their antioxidant properties and overall nutritional quality.

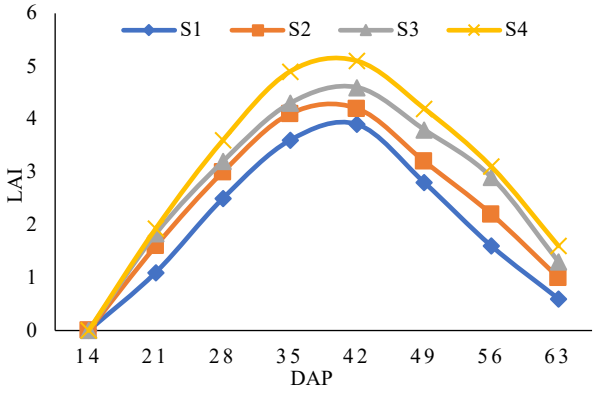

Fig. 5 Development of LAI at doses of Se selenate

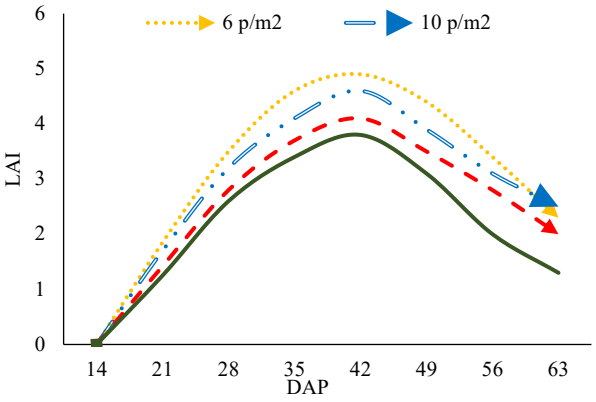

Fig. 6 Development of LAI at various planting dates

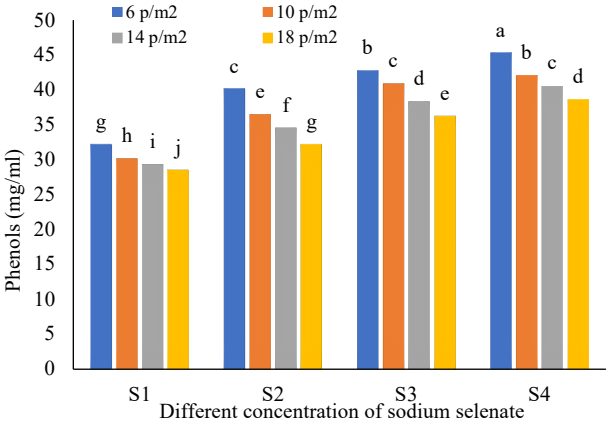

Fig. 7 The interaction effects of different planting dates × Se on TPC.

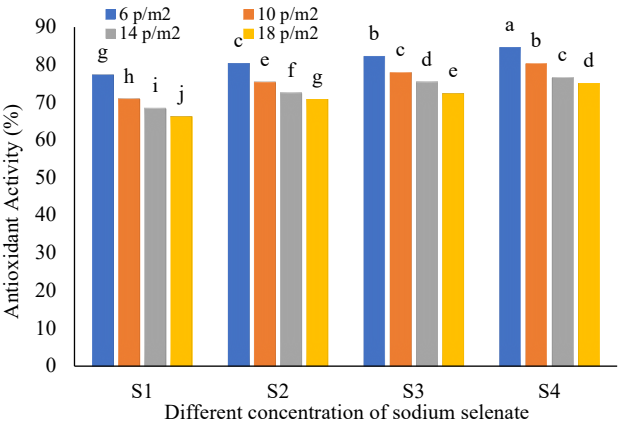

Fig. 8 The interaction effects of planting density × Se on antioxidant activity.

Antioxidant Activity

Variance analysis (Table, 3), results reveal that the antioxidant activity in Shallot was significantly influenced by both plant density and the application of Se. Specifically, the highest antioxidant activity content, measuring 84.61 mg per gram, was observed when a Se application rate of 12 mg/l was combined with a plant density of 6 plants/m^2. Conversely, the lowest antioxidant activity value, 66.37 mg/g, was recorded in the treatment without Se application at a higher plant density of 18 plants/m^2 (Fig. 8).

DISCUSSION

This research indicates that the planting dates, plant density, and spraying of Se, along with their interactions, significantly affect the plant height, plant yield, phytochemical characteristics, and allicin content of shallots (Table 3). In this study, the effect of plant density on nutrient uptake per hectare was examined, showing a significant relationship. Results indicated that lower plant densities, measured in plants per square meter, led to reduced absorption of nitrogen, phosphorus, and potassium in shoots, roots, and overall plant parts, compared to higher plant densities. The decrease in nutrient uptake was linearly correlated with a reduction in biomass per hectare. Lower plant densities also resulted in a decline in both plant population numbers and total biomass per hectare. These findings are consistent with previous research, which reports that higher plant densities tend to produce greater biomass per hectare than lower densities [27]. Plant density plays a crucial role in influencing the leaf photosynthetic capacity, agronomic traits, and overall yield of crops. For instance, increasing plant density has been shown to enhance the leaf area index in crops such as buckwheat [28], blessed thistle [29], and rice [30], which subsequently leads to greater shoot biomass and improved crop yields. Similar findings have been reported in various studies; for example, Liaqat *et al.* [31] demonstrated that plant density has a major impact on plant height and onion yield in Bahawalpur, Pakistan, although it did not significantly influence bulb diameter. Additionally, Khadrah *et al.* [32] observed that lower plant densities in onion cultivation in Egypt resulted in increased marketable bulb yield. Shallot is known for its high antioxidant activity, which is primarily attributed to the presence of sulfur compounds such as diallyl disulfide, diallyl trisulfide, allyl, and allicin, as well as flavonoids like quercetin glucosides, polyphenols, ascorbic acid, and saponins [33]. In this study, the average levels of flavonoids and polyphenols fell within the ranges reported for similar varieties from Poland, Indonesia, and Vietnam [34]. Numerous studies have demonstrated that the use of Na_2Se can significantly stimulate the accumulation of secondary metabolites, including polyphenols, flavonoids, and allicin, in various crops such as lettuce [35], potato [36], *Allium cepa* L. (onion) [37], and leek [38]. These findings suggest that Na_2Se may enhance the nutritional value and health-promoting properties of these plants by boosting their phytochemical content. Phenolic compounds play a critical role in plant physiology by influencing mitosis and cell growth. It also functions as a vital cofactor for enzymes involved in the biosynthesis of secondary metabolites, including phytohormones, and provides protection against environmental stresses [39]. During our experiment, the highest TPC in shallots was observed when both plant density and sodium selenate application were employed. Shallots are particularly noted for their high antioxidant activity, which is mainly due to the presence of sulfur compounds like diallyl disulfide, diallyl trisulfide, and allicin, along with flavonoids and polyphenols.

The levels of flavonoids and polyphenols recorded in this study were within the ranges previously reported for Indonesian, Vietnamese, and Polish varieties of shallots [40-42]. Notably, the application of sodium selenate in our experiment resulted in a significant enhancement of antioxidant activity, which is consistent with earlier findings on *A. cepa* and other *Allium* species [39]. In Iran, due to its unique habitat conditions and reproductive characteristics, shallot is classified as a non-permitted plant for natural resource exploitation. However, its high economic value, pleasant taste, and medicinal properties have led to extensive overharvesting, placing it among the endangered species. Consequently, the domestication and systematic cultivation of this plant are considered inevitable [43].

CONCLUSION

Based on the main findings of this study, the planting date, plant density, and application of sodium selenate significantly enhanced the evaluated agronomic and physiological traits of shallot (*A. ascalonicum*). The optimal cultivation strategy for shallot in the Kalateh Roudbar region was identified as an early planting date combined with a plant density of 6 plants/m^2 and foliar spraying with 12 mg/l sodium selenate. This treatment regime resulted in the highest yield and significantly improved physiological parameters, including chlorophyll content, total phenolic compounds (TPC), allicin concentration, and flavonoid levels, compared to other treatment groups. These results highlight the positive impact of Se supplementation and appropriate agronomic practices on both the quantity and quality of shallot production. Enhanced chlorophyll content suggests improved photosynthetic efficiency, while increases in bioactive compounds such as allicin, flavonoids, and phenolics indicate greater medicinal and nutritional value. Given the unique ecological characteristics and restricted natural habitat of shallot in Iran, the species is currently classified as a non-permitted plant for the exploitation of natural resources to prevent further depletion. Overharvesting due to its high economic value, distinct flavor, and recognized medicinal benefits has rendered the shallot endangered in the wild. Therefore, domestication and controlled cultivation are essential to conserve natural populations and meet market demand sustainably. The outcomes of this research provide a scientific basis for promoting the large-scale cultivation of shallot (locally known as musir), which can serve as a valuable income source for rural households while contributing to the preservation of this important medicinal plant. Implementing these optimized agricultural practices may aid in balancing conservation efforts with economic development in shallot-growing regions of Iran.

Author Contributions

Specifically, we were responsible for experimental design. Also responsible for the original draft preparation. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no known competing financial interests or any personal relationships that could influence the work reported in this manuscript.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Yousefvand P., Sohrabi Y., Mastinu A., Heidari G., Weisany W. Salicylic acid altered the fatty acids compositions and nutrient status of shallot (Allium hirtifolium) grown under drought stress. Journal of Agriculture and Food Research. 2024;18:101502.
2. Fitzgerald M., Heinrich M., Booker A. Medicinal plant analysis: A historical and regional discussion of emergent complex techniques. Frontiers in Pharmacology. 2020;10:1480.
3. Papari Moghadam Fard M., Ketabchi S., Farjam M. H. Chemical composition, antimicrobial and antioxidant potential of essential oil of Ziziphus spina-christi var. aucheri grown wild in Iran. Journal of Medicinal Plants and By-products. 2020;9:69–73.
4. Alghabari M.Z. Ihsan. Effects of drought stress on growth, grain filling duration, yield and quality attributes of barley (Hordeum vulgare L.). Bangladesh Journal of Botany. 2018;47(3):421–428.
5. Marthandan V., Geetha R., Kumutha K., Renganathan V.G., Karthikeyan A., Ramalingam J. Seed priming: A feasible strategy to enhance drought tolerance in crop plants. International Journal of Molecular Sciences. 2020;21:8258. doi:10.3390/ijms21218258
6. Moldovan C., Frumuzachi O., Babotă M., Barros L., Mocan A., Carradori S., Crișan G. Therapeutic uses and pharmacological properties of shallot (Allium ascalonicum): A systematic review. Frontiers in Nutrition. 2022;9:903686.
7. Statistical Bulletin of the Ministry of Agriculture Jihad of the Islamic Republic of Iran. 2023.
8. Askari Y., Zafari S., Darkhor M. Some medicinal properties of Allium hirtifolium Boiss. species. International Journal of Advanced Biological and Biomedical Research. 2025;13(3):254–269.
9. Norouzi M., Zare A., Khoshnoodi M. Phytochemical profile of Allium hirtifolium and its secondary metabolites: A phytochemical study. Journal of Natural Products Research. 2018;34(2):123–131. doi:10.1080/14786419.2017.1363187
10. Yigit N., Sevik H., Cetin M., Kaya N. Determination of the effect of drought stress on the seed germination in some plant species. In: Rahman I.M.M., editor. Water Stress in Plants. IntechOpen, London, UK. 2016:43–62. doi:10.5772/63197
11. Alizadeh A., Zare A. Bioactivity of secondary metabolites of Allium hirtifolium: Antioxidant and antimicrobial properties. Evidence-Based Complementary and Alternative Medicine. 2020;1–10. doi:10.1155/2020/1234567
12. Rahmanian M., Mohammadi R. Influence of cultivation conditions on secondary metabolite concentrations in Allium hirtifolium. Plant Physiology and Biochemistry. 2020;142:32–41. doi:10.1016/j.plaphy.2019.07.014
13. Ghasemi E., Safavi S. Traditional uses of Allium hirtifolium and phytochemical validation. Journal of Ethnopharmacology. 2017;198:250–258. doi:10.1016/j.jep.2017.02.050
14. Neda N., Zahra B.H., Mohammad R.H., Mitra A., Mohammad R.M., Akram G.A., Mehdi M. Promising effects of Persian shallot extract on the serum markers and blood pressure of patients with metabolic syndrome: a double-blinded randomized controlled trial. Journal of Traditional Chinese Medicine. 2025;45(1):100.
15. Ramak P., Karimian V. Effects of planting times on the chemical compositions, antioxidant activity and nutritional properties of Allium jesdianum Boiss. & Buhse leaves in Lorestan province. ECOPERSIA. 2023;11(3):175–185.
16. Poorgharib M., Mehrjerdi M.Z., Arabhosseini A. Effect of different drying methods on antioxidant and phytochemical yield of Allium hirtifolium Boiss. Eco-phytochemical Journal of Medicinal Plants. 2023;10(4):27–43.
17. Skrypnik L., Feduraev P., Golovin A., Maslennikov P., Styran T., Antipina M., Katserov D. The integral boosting effect of selenium on the secondary metabolism of higher plants. Plants. 2022;11(24):3432.
18. Banuelos G.S., Freeman J.L., Arroyo I.S. Selenium content and speciation differences in selenium enriched soups made from selenium biofortified plants. Journal of Food Composition and Analysis. 2022;105:1–8.
19. Zhang Y., Frankenberger W.T. Formation of dimethylselenonium compounds in soil. Environmental Science & Technology. 2000;34:776–783. doi:10.1021/es990958y
20. Banuelos G.S., Arroyo I.S., Dangi S.R., Zambrano M.C. Continued selenium biofortification of carrots and broccoli grown in soils once amended with Se-enriched S. p. 2016.
21. Golubkina N.A., Kosheleva O.V., Krivenkov L.V., Dobrutskaya H.G., Nadezhkin S., Caruso G. Intersexual differences in plant growth, yield, mineral composition and antioxidants of spinach (Spinacia oleracea L.) as affected by selenium form. Scientia Horticulturae. 2017;225:350–358.
22. Da Silva L.A.L., Pezzini B.R., Soares L. Spectrophotometric determination of the total flavonoid content in Ocimum basilicum L. (Lamiaceae) leaves. Pharmacognosy Magazine. 2015;11:96–101.
23. Maximova T.V., Nikulina I.N., Pakhomov V.P., Shkarina H.I., Chumakova Z.V., Arzamastsev A.P. Method of Antioxidant Activity Determination. RU Patent. 2001;2:170,930.
24. Srivastava S., Adholeya A., Conlan X.A., Cahill D.M. Acidic potassium permanganate chemiluminescence for the determination of antioxidant potential in three cultivars of Ocimum basilicum. Plant Foods for Human Nutrition. 2015;70.
25. Taheri-Garavand A., Rezaei Nejad A., Fanourakis D., Fatahi S., Ahmadi Majd M. Employment of artificial neural networks for non-invasive estimation of leaf water status using color features: A case study in Spathiphyllum wallisii. Acta Physiologiae Plantarum. 2021;43(5):78.
26. Miron T., Rabinkov A., Mirelman D., Wilchek M., Weiner L. The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. Biochimica et Biophysica Acta (BBA) — Biomembranes. 2000;1463:20–30.
27. Sopha G.A. Influence of plant density, compost and biofertilizer on true shallot seed growth in alluvial soil. Indonesian Journal of Agricultural Science. 2020;21(2):70–77.
28. Fang X.M., Li Y.S., Nie J., Wang C., Huang K.H., Zhang Y.K., Zhang Y.L., She H.Z., Liu X.B., Ruan R.W., Yuan X.H., Yi Z.L. Effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat (Fagopyrum esculentum M.). Field Crops Research. 2018;219:160–168. doi:10.1016/j.fcr.2018.02.001
29. Ghiasy-Oskoee M., Aghaalikhani M., Sefidkon F., Mokhtassi-Bidgoli A., Ayyari M. Blessed thistle agronomic and phytochemical response to nitrogen and plant density. Industrial Crops and Products. 2018;122:566–573. doi:10.1016/j.indcrop.2018.06.027
30. Kimura T., Shimono H. Effects of planting density and basal nitrogen fertilizer on grain yield, quality, taste of rice cultivar in Aomori prefecture. Japanese Journal of Crop Science. 2019;88:89–97. doi:10.1626/jcs.88.89
31. Liaqat A., Hassan M.W., Moazzam J., Javaid I., Yaqub M.S., Muhammad A., Imtiaz A., Abrar H. Effect of nursery bulb size and planting density on thrips population, plant height and yield of onion (Phulkara variety) in Bahawalpur, Pakistan. Pakistan Journal of Life and Social Sciences. 2016;14:96–103.
32. Khadrah S.H.A., El-Sayed A.A., Geries L.S.M., Abdelmasieh W.K.L. Response of onion yield and quality to different planting date, methods and density. Egyptian Journal of Agronomy. 2017;39:203–219.
33. Bhat R. Bioactive compounds of Allium species. In: Bioactive Compounds in Underutilized Vegetables and Legumes. 2021:277–295.
34. Shahrajabian M.H., Wenli S.U.N., Cheng Q. Chinese onion, and shallot, originated in Asia, medicinal plants for healthy daily recipes. Notulae Scientia Biologicae. 2020;12(2):197–207.

35. Zhou Y., Nie K., Geng L., Wang Y., Li L., Cheng H. Selenium's Role in Plant Secondary Metabolism: Regulation and Mechanistic Insights. Agronomy. 2025;15:1.
36. Skrypnik L., Feduraev P., Golovin A., Maslennikov P., Styran T., Antipina M., Katserov D. The integral boosting effect of selenium on the secondary metabolism of higher plants. Plants. 2025;11(24):3432.
37. Hamed-Far M., Hajiboland R., Aliasgharzad N. Effect of selenium supplementation on mycorrhizal onion (Allium cepa L.) plants. New Zealand Journal of Crop and Horticultural Science. 2025;3(1):30–52.
38. Yan J.K., Zhu J., Liu Y., Chen X., Wang W., Zhang H., Li L. Recent advances in research on Allium plants: Functional ingredients, physiological activities, and applications in agricultural and food sciences. Critical Reviews in Food Science and Nutrition. 2023;63(26):8107–8135.
39. Lattanzio V., Kroon P.A., Quideau S., Treutter D. Plant phenolics: secondary metabolites with diverse functions. Recent Advances in Polyphenol Research. 2009;1:1–35.
40. Ariyanti N.A., Torikai K., Kirana R.P., Hirata S., Sulistyaningsih E., Ito S.I., Shigyo M. Comparative study on phytochemical variations in Japanese F1 varieties of bulb onions and South-East Asian shallot landraces. The Horticulture Journal. 2018;87(1):63–72.
41. Vu Q.H., Hang T.T.M., Yaguchi S., Ono Y., Pham T.M.P., Yamauchi N., Shigyo M. Assessment of biochemical and antioxidant diversities in a shallot germplasm collection from Vietnam and its surrounding countries. Genetic Resources and Crop Evolution. 2013;60:1297–1312.
42. Tendaj M. Shallot production and research in Poland. Vegetable Crops Research Bulletin. 2005;62:55–60.
43. Ramak P., Karimian V., Jafari M., Pezeshkpour P., Mahddevar H., Sharifi Ashoorabadi E. Evaluation of different methods of planting and harvesting on some morphological, functional, and economic performance of the medicinal-industrial Mosir plant (Allium hirtifolium Boiss.) in Lorestan Province. Journal of Crops Improvement. 2024;26(2):389–408. doi:10.22059/jci.2024.361534.2830.