Effect salicylic acid and silicon on physiological and phytochemical indices of Scrophularia striata L. under water stress

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Article

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Effect salicylic acid and silicon on physiological and phytochemical indices of *Scrophularia striata* L. under water stress

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Abstract

Water stress, a main factor, exerts influence on the physiological and phytochemical responses of plants. Therefore, to scrutinize the physiological and biochemical reactions of *Scrophularia striata* under drought stress, a factorial experiment was conducted employing a completely randomized design with three replications plants were subjected to varying concentrations of salicylic acid (0 and 100 PPM), silicon (0 and 1 g/L), and drought stress at two levels (50 and 100% of field capacity) within greenhouse conditions. Data analysis results revealed that drought stress leads to an increase in Ca^{+2} and Na^{+} levels in Ilam ecotype under the FC50% treatment. Additionally, Mg^{+2} levels in Ilam ecotype, under the control × SA treatment, exhibit an augmentation in comparison to the stress treatment. The application of silicon resulted in an elevation of potassium in Abadanan ecotype under the FC50% treatment. Simultaneous utilization of SA and Si would demonstrate an increased K^{+}/Na^{+} ratio in Ilam ecotype within the control × SA × Si treatment. Nitrate levels exhibit an increase in Ilam ecotype under the FC50% × SA × Si treatment. The analysis of amino acid compounds in the tested samples showed that drought stress increased Tryptophan in Ilam ecotype in the FC50% treatment, using SA increased Tryptophan, Phenylalanine and using Si increased Argenine and Valine. 45volatile compounds were identified for the first time in the stem of *S. striata* by technique (GC-MS). In general, the results the simultaneous use of these two compounds can play an effective role in modulating drought stress in *S. striata*.

Keywords: Drought stress, GC-MAS, Salicylic acid, *Scrophularia striata* L, Silicon.

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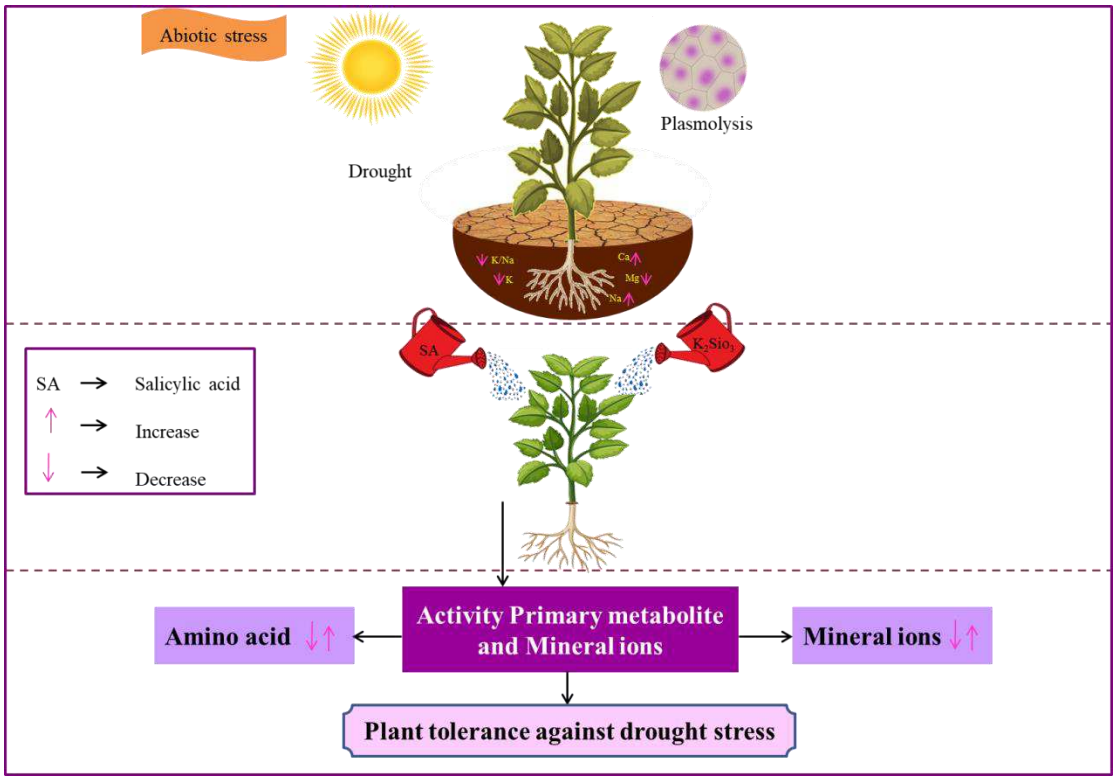
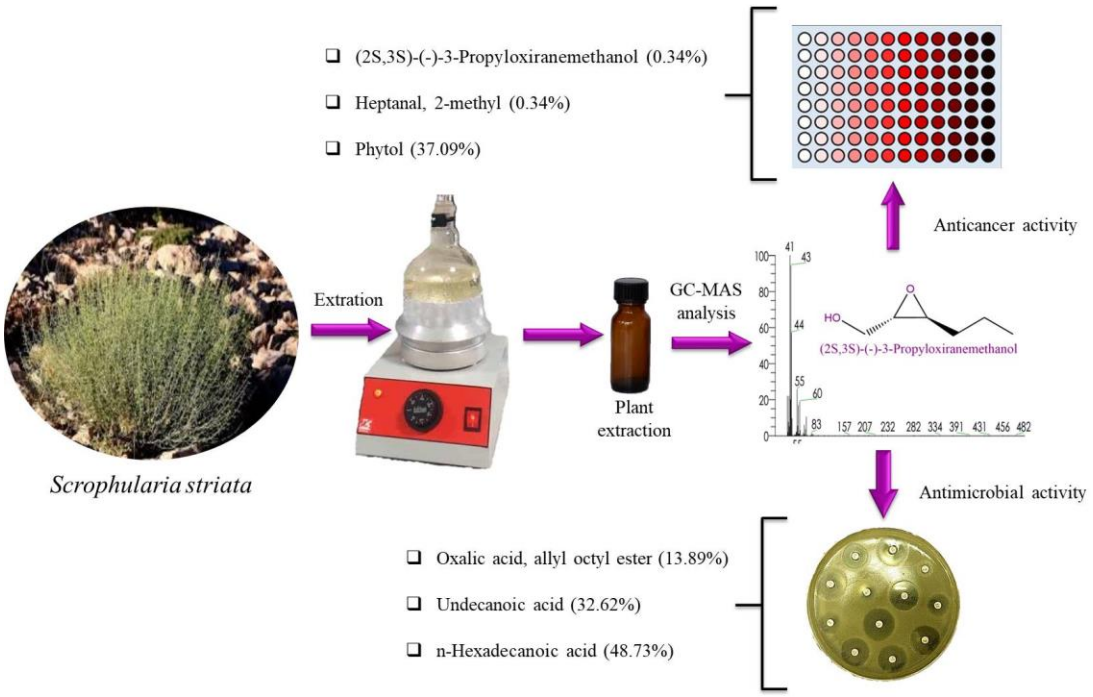


Graphic abstract of Si- and SA-treated *S. striata* under drought stress shows that the content of some amino acid and nutrient elements as well as ROS production increased after the application of drought stress. These biochemical changes have finally moderated the drought stress.



Graphic abstract GC -MAS of bioactive compounds from *S. striata*.

Introduction

Plants thrive in dynamic environments, making them susceptible to various stressors that significantly impact their growth and yield. These stressors encompass factors such as water stress or flooding, extreme temperatures, varying light intensities, salinity, and nutrient deficiencies. These environmental challenges disrupt the physiological and biochemical efficiency of plants, as highlighted by[1]. Drought stress is one of the most basic abiotic stresses that causes a significant reduction in crop yield by disrupting metabolic processes such as photosynthesis, translocation, ion absorption and nutrient metabolism[2]. Amino acids are simple nitrogen-containing organic compounds that are soluble to varying degrees in water and originate from the metabolism of sugar and nitrogen and are used in the structure of proteins. When the plant is exposed to environmental stress, amino acids play an important role in the plant by changing the osmotic regulation inside the cell[3]. Free amino acids are the most important metabolites in organisms that act as the basic components of proteins and, in addition, lead to the synthesis of several secondary metabolites through metabolic pathways; metabolites that have different functions in homeostasis and plant response to different biotic and abiotic stresses[4]. Drought reduces the activity of root surface enzymes involved in nutrient assimilation[5]. In addition to preventing the absorption of nutrients by the roots, drought stress limits the supply of nutrients to the shoot and leads to a reduction in the growth and yield of agricultural crops[6]. Among approximately 250,000 plant species around the world, only 17% have been scientifically investigated for medicinal potential. The chemical and biological diversity of plants represents a potentially non-renewable and unlimited source for use in the development of new pharmaceuticals[7]. The genus *S. striata* Boiss of the *Scrophulariaceae* family is a herbaceous, perennial, wild plant with a plant height between 30 and 50 cm. It grows widely in several regions of the world, especially Iran, Turkey and Azerbaijan. This plant grows in pastures, hillsides and rough areas in Iran. Due to its medicinal properties, different parts of this plant have been used to treat various diseases such as scrofula, scabies, tumor, exoma, psoriasis, rheumatism and chronic infectious diseases as Iranian folk remedies. It is claimed that this plant is capable of treating various diseases such as conjunctivitis, colds Gastritis, hemorrhoids, wounds and burn infections. All parts of the plant have been used in traditional medicine, and the extract of its shoot is used to treat second and third degree burns[8], [9]. SA is a phytohormone that has been increasingly known as an enhancer of abiotic stress tolerance in plants and modulates protein metabolism, nutrient uptake and growth under drought stress by signal transduction[10]. Si is the second most frequent element is in the earth's crust. Although Si is not considered as an essential element in plants, its accumulation can play a role in improving growth, especially in plants under drought stress. Using Si for improving the growth of crops such as *rice, sorghum, corn*, and *soybeans* is reported[11]. Considering the economic importance and the increasing need of society to consume medicinal plants, increasing the area under cultivation of these products is more than necessary. On the other hand, our climate does not allow increasing their cultivation under drought, so increasing studies have been conducted in this field, which have attempted to increase the area under cultivation in arid and semi-arid areas using special mechanisms and creating special conditions. Therefore, the present study investigated the simultaneous use of salicylic acid and silicon on physiological indicators and phytochemical compounds and the effect of these changes on the inhibition of free radicals in *S. striata* L. under drought stress.

Results

Elemental concentration

Variance analysis results indicate that drought stress has a significant effect on the accumulation of Ca^{+2}, Na^{+}, K^{+}, Mg^{+2}, K^{+}/Na^{+} and NO_3 at the probability level of $P \leq 0.01$. But no significant difference was observed between the two ecotypes in terms of NO_3 accumulation. The combined impact of ecotype, stress, salicylic acid (SA), and silicon (Si) demonstrated a

statistically significant influence on the concentrations of Ca^{+2}, Na^{+}, K^{+}, Mg^{+2}, and K^{+}/Na^{+}at a probability level of $P \leq 0.01$, as indicated in (Table 2). Post hoc comparisons using LSD revealed that the lowest levels of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} were observed in Abdanan ecotype under the control * Si treatment, Ilam ecotype under the control * Si treatment, Abdanan ecotype under FC50% * Si treatment, and Ilam ecotype under the control * Si treatment, respectively. The lowest concentration of Na^{+} was noted in Abdanan ecotype under FC50% * SA * Si treatment and Abdanan ecotype under the control * Si treatment. Conversely, the highest concentrations of Na^{+} and Ca^{2+} were observed in Ilam ecotype under FC50% treatment, while the highest levels of Mg^{2+} and K^{+} were noted in Abdanan ecotype under FC50% * Si treatment and Ilam ecotype under the control * SA treatment, respectively. Notably, no statistically significant effect was observed in Abdanan ecotype under FC50% * Si treatment and Ilam ecotype under FC50%* SA * Si treatment (refer to Figure 2).

Identification of chemical compounds

A total of forty-eight distinct compounds were identified in the shoot of *S. striata* across various treatments using GC-MAS (refer to Figure. 3,4; Table 3). These compounds included oxalic acid allyl octyl ester (ranging from 0.6% to 38.59%), undecanoic acid (13.58% to 29.03%), 9-octadecenal (5.7% to 40.82%), phytol (3.58% to 38.94%), oxalic acid allyl hexadecyl ester (0.6% to 3.81%), n-hexadecanoic acid (2.64% to 48.73%), 2-decen-1-ol (1.5% to 11.2%), and (2S,3S)-(-)-3-propyloxiranemethanol (ranging from 0.34% to 64.04%) (Table). Water deficit stress exerted a significant influence on the oxalic acid allyl octyl ester content in the *S. striata* extract. Notably, Ilam ecotype demonstrated the highest concentration (38.15%) of the specified compound when subjected to FC50% × SA × Si treatment, while the lowest concentration (0.19%) was observed in Ilam ecotype under control treatment. These findings highlight a significant surge (99%) in the content of oxalic acid allyl octyl ester in Ilam ecotype under FC50% × SA × Si treatment as compared to control treatment. The application of silicone ether exerted a noteworthy influence on the levels of 9-octadecenal. Moreover, Ilam ecotype exhibited the highest content (56.48%) of this compound under FC50% × Si treatment, whereas the lowest content (5.8%) was detected in control treatment. This indicated a substantial increase (89.91%) in 9-octadecenal content in Ilam ecotype under FC50% × Si treatment as compared to control treatment. Additionally, the presence of salicylic acid had a distinct impact on the levels of (2S,3S)-(-)-3-propyloxiranemethanol, with the highest concentration (64.04%) and the lowest (0.34%) observed in Ilam ecotype under FC50% × SA and control × Si treatments, respectively. The results indicated a notable rise (99.4%) in Ilam ecotype under FC50% × SA treatment as compared to control treatment. In Abdanan ecotype, the highest concentration of 2-decen-1-ol (11.2%) was observed under FC50% × Si treatment, whereas the lowest concentration (1.5%) was noted in Ilam ecotype under SA × Si treatment. Additionally, FC50% × Si treatment showed a significant increase (86.61%) as compared to control treatment. Drought-induced stress resulted in a decrease in the levels of phytol and n-hexadecanoic acid. Ilam ecotype exhibited the greatest phytol content (38.94%) when subjected to control × SA × Si treatment, while the highest n-hexanoic acid content (48.73%) was recorded in Ilam ecotype under control × Si treatment. The lowest phytol content (3.58%) was noted in Abdanan ecotype under FC50% × SA treatment, and the lowest content of n-hexanoic acid was witnessed in Abdanan ecotype under FC50% × Si treatment. Furthermore, control treatment exhibited an increase (90.9%) compared to the drought stress treatment in this regard. It is noteworthy that drought stress did not impact the content of oxalic acid, allyl hexadecyl ester, and undecanoic acid, resulting in a reduction in these components in the stress treatment compared to the control.

Composition and concentration of free amino acids

Figure. 5 illustrates the HPLC analysis of free amino acids in the stems of S. striata subjected to water deficit stress. The amino acid content analysis results indicated that aspartic acid (ASP) and glutamic acid (Glu) levels remained unaffected by drought stress. The highest content of ASP and Glu, at 89.5%, was seen in Ilam ecotype under the control × SA × Si treatment, while the lowest levels, at 33.6% and 40.2%, were recorded in the Abdanan ecotype. Notably, Ilam ecotype in the control × SA × Si treatment exhibited a significant increase in ASP (62.5%) and Glu (55.09%) content compared to Abdanan ecotype in the control × Si treatment. Salicylic acid (SA) and silicon (Si) demonstrated a significant impact on serine (Ser) content, particularly in Ilam ecotype under the control × Si treatment and Abdanan ecotype under FC50% × SA treatment. The highest Ser content, at 78.5%, was observed in Abdanan ecotype under the control × Si treatment, whereas the lowest was noted in Abdanan ecotype under FC50% × SA treatment. Furthermore, it was evident that Ilam ecotype in the control × Si treatment experienced a notable increase (44.9%) compared to the stress treatment. The combined application of Si and drought stress significantly elevated arginine (Arg) content, with the highest percentages (45.2% and 45.7%) recorded in Ilam and Abdanan ecotypes under FC50% × Si treatment, and the lowest percentage (26.6%) observed in Ilam ecotype under the control treatment.The findings revealed that the Ilam and Abdanan ecotypes under FC50% × Si treatment exhibited an increased percentage of 41.54% compared to the control treatment. The analysis indicated that the highest alanine (Ala) content, registering at 158.6%, was observed in Ilam ecotype under the control treatment, while the lowest percentage (105.5%) was recorded in Abdanan ecotype under the SA × Si treatment. It was observed that Ilam ecotype under the control treatment (33.49%) possessed the highest Ala content among the treated plants. Regarding tyrosine (Tyr), the highest content, at 28.7%, was identified in Ilam ecotype under the control treatment, whereas the lowest percentage (15.5%) was noted in Ilam ecotype under the control × Si treatment. The application of SA and Si resulted in a reduction in glycine (Gly) content under drought stress conditions, with Ilam ecotype in the control treatment exhibiting the highest Gly percentage (46.3%) and the lowest (23.6%) recorded in Abdanan ecotype under FC50% × SA × Si treatment. The comparative analysis revealed that the control treatment led to an increase in glycine (Gly) percentage at 49.03% compared to the stress treatment. Drought stress exhibited a significant impact on the percentages of valine (Val) and methionine (Met). The highest levels of Val (83.7%) and Met (80.6%) were observed in Abdanan ecotype under FC50% × Si treatment and Abdanan ecotype under FC50% treatment. Conversely, the lowest Val percentage (57%) was noted in Ilam ecotype under FC50% × Si treatment, and the lowest Met percentage (61.6%) was observed in Ilam ecotype under FC50% × SA × Si treatment. Regarding leucine (Leu) and isoleucine (Ile), the highest percentages (33.5% and 31.2%) were identified in Ilam ecotype under control × Si treatment and Ilam ecotype under the control treatment, while the lowest percentages (16.6% and 13.2%) were observed in both Ilam and Abdanan ecotypes under SA × Si treatment. The control treatment significantly increased Leu and Ile percentages by 50.45% and 57.7%, respectively, compared to drought stress. SA in conjunction with drought stress had a significant effect on tyrosine (Tyr) content, with the highest Tyr percentage (32.7%) noted in Ilam ecotype under FC50% × SA treatment and the lowest in both Ilam ecotype and the control treatment. The comparative results indicated that the control treatment (48.63%) increased the Tyr percentage compared to the stress treatment. For phenylalanine (Phe) and histidine (His), the highest percentages (48.8% and 170.4%) were observed in Ilam ecotype under FC50% × Si treatment, while the lowest percentages (22.2% and 120.2%) were recorded in Abdanan ecotype under control × SA × Si treatment. The control ecotype exhibited reduced Phe and His content (54.1% and 40.61%) compared to drought stress treatment. Regarding tryptophan (Trp), the highest percentage (30.3%) was obtained from Ilam ecotype under FC50% treatment, while the lowest (18%) was recorded in Abdanan ecotype

under the control treatment. The results indicated that Ilam ecotype under FC50% treatment increased Trp content by 40.6% as compared to the control treatment (refer to Figure. 6, 7).

Discussion

A decrease in osmotic potential is a recognized response mechanism employed by various plants to cope with drought stress. Osmotic regulation relies on two categories of osmolytes: organic solutes and inorganic ions, each having distinct mechanisms[12]. The findings of this study indicate that water deficit stress led to an increase in Na^{+} and Ca^{+2} levels, accompanied by a reduction in Mg^{+2} and K^{+}. Elevated Na^{+} and decreased K^{+} absorption disrupts ion exchange in numerous species under drought stress, potentially suppressing growth by diminishing osmotic capacity, maintaining turgor pressure, and inhibiting metabolic processes[12]. The rise in Ca^{+2} content may be attributed to reduced cell membrane permeability and an increase in H^{+}-ATPase activity in plants experiencing drought stress[6]. Enhanced Ca^{+2} levels contribute to improved water retention, enhanced plasma membrane hydration, cell wall cohesion, and prevention of potassium entry into stomatal guard cells during drought stress[13]. Magnesium (Mg^{+2}) is an essential component of chlorophyll structure, and its deficiency can diminish photosynthetic activity, disrupting the transfer of photosynthetic products from source to sink, ultimately resulting in growth reduction[14]. In line with the current study's results, previous research has reported growth reduction in *Glycyrrhiza uralensis* L. under drought stress conditions[12]. In this investigation, applying salicylic acid resulted in an increase in Mg^{+2} in Ilam ecotype under control * SA treatment. Salicylic acid has been shown to enhance a plant's capacity to endure water deficit stress by promoting mineral uptake[15]. The impact of SA on plant growth and metabolism is contingent on factors such as plant ecotype, stress severity, and SA concentration[16]. In concordance with our study, [17]. documented an augmentation of Mg^{+2} content in plants subjected to drought stress when treated with SA. Silicon (Si) application led to an increase in K^{+} in Abdanan ecotype under FC50% × Si treatment. Typically absorbed through plant roots in solution form, Si has the potential to augment ion-exposed sites by enhancing root length and surface area, thereby enhancing water and nutrient absorption during drought conditions[18]. Regarding the findings of the present research, similar findings were reported in the literature, where Si increased K^{+} levels in *Mangifera indica* under drought stress[19]. Simultaneous application of SA and Si increased NO_3 levels in Ilam ecotype under FC50% × SA × Si treatment and Abdanan ecotype under FC50% × Si treatment, although no significant difference was observed. Drought-induced reductions in soil nutrient concentrations, influenced by microbial activity, were mitigated by SA and Si application, stimulating and enhancing nutrient availability[13]. Water stress, impacting enzyme activity involved in nutrient uptake at the root surface Finally, it reduces the absorption of nutrients[20]. The K^{+}/Na^{+} ratio in Ilam ecotype under control × SA × Si treatment increasing It is worth mentioning that applying salicylic acid (SA) and silicon (Si) did not exert an influence on the K^{+}/Na^{+} ratio of plants experiencing stress. Prior research suggests that, during drought stress, the osmotic equilibrium is contingent upon factors such as the plant ecotype and the duration of the stress period[12]. A decrease in the content of Na^{+} was seen regarding Abdanan ecotype under FC50% **SA* *Si treatment and Ilam ecotype under the control*Si treatment, although no significant difference was noted. In agreement with our study, the reduction in sodium absorption in Glycyrrhiza glabra may be attributed to the deposition of silica in the cell walls of leaves and stems under drought stress conditions[12]. Amino acids, serving various functions including synthesis, enzyme activity, compatibility as solutes, and maintaining redox homeostasis, play a fundamental role in responding to drought stress[21]. Moreover, findings of our research revealed a significant tryptophan (Trp) increase in Ilam ecotype under FC50% treatment when subjected to water deficit stress. Trp has the potential to be converted into serotonin (Sero), a compound with diverse functions in the plant cell wall that contributes to

cell wall reinforcement. Additionally, Trp can be metabolized into the growth regulator melatonin, aiding plants in coping with drought stress [22]. In line with our present findings, Flahi et al. (2018) observed an increase in tryptophan (Trp) in *S. striata* when subjected to drought stress. Amino acids such as alanine (Ala), arginine (Arg), aspartic acid (Asp), glycine (Gly), glutamic acid (Glu), histidine (His), leucine (Leu), isoleucine (Ileu), methionine (Met), and serine (Ser) exhibited an increase in the control treatment. Concomitant with the decline in the uptake of nutrient compounds like NO_3 by the roots and their transfer to the shoot, amino acid synthesis also decreased[23]. Water deficit stress, through the reduction of the plant's transpiration rate, diminishes the transfer of nitrogen from the root surface to the shoot. This limitation in nitrogen uptake from the plant surface, as nitrogen is a constituent of amino acids, likely contributes to the reduction in amino acid content[14]. Contrary to many studies where the majority of free amino acids show an increase [24]. our study observed both increases and decreases in amino acids under stress. This discrepancy might be attributed to the fact that not all amino acids increase at low stress levels, and higher stress intensity is required to elevate their concentrations. However, the application of salicylic acid (SA) and silicon (Si) increased the content of aromatic amino acids (phenylalanine and tyrosine), aliphatic amino acids (valine), and arginine. The accumulation of phenylalanine, tyrosine, and tryptophan, which play a pivotal role in secondary metabolite biosynthesis and act as antioxidants, is likely a fundamental mechanism in responding to drought stress[21], [22]. In accordance with the outcomes of our current investigation, prior studies have noted an elevation in the content of phenylalanine (Phe) and tyrosine (Tyr) in S. striata under drought stress. Glutamate has been recognized as a safeguarding and signaling osmolyte, playing a role as a molecule associated with drought stress in higher plants. The reduction in glutamate content might be attributed to the development of compatible metabolites such as arginine [25]. Silicon (Si) enhances the plant's capacity to uptake ammonium and sulfur from the soil, leading to the production of arginine and valine[26]. In alignment with our study, previous reports have indicated that salicylic acid (SA) application increased tyrosine levels in soybean and beans[27]. Branched-chain amino acids (BCAAs), including valine, play a crucial role in tolerating short-term periods of drought stress, potentially serving as an alternative source for the tricarboxylic acid cycle (TCA) by delaying drought stress, it maintains homeostasis[4]. In agreement with our findings, [24] reported that silicon application increased arginine and valine in plants exposed to drought stress. This study identified the major chemical compounds in the stem extract based on their abundance, highlighting that stress significantly impacts the extract and its constituents [28]. The biological impacts of plant extracts and essential oils can vary significantly based on their chemical compositions, influenced by factors such as geographical conditions, species diversity, and plant genotype[28]. In line with the findings of this study, [29] noted an increase in the chemical compounds of *Satureja hortensis* L. under drought stress. In the present research, the levels of phytol, oxalic acid allyl hexadecyl ester, and undecanoic acid were observed to decrease due to drought stress. These outcomes may be attributed to the stress-induced modulation of enzymatic activity involved in the biosynthesis of these compounds[28]. The investigation into the components of phytochemical compounds revealed distinct responses to drought stress among different ecotypes. Considering the absence of previous research on the impact of salicylic acid and silicon on medicinal herbs under drought stress, it appears that the significant components of phytochemical compounds are influenced by both different ecotypes and the stress conditions.

Conclusion

Plants employ various strategies to counteract the effects of drought stress, including augmenting osmotic regulators and secondary metabolites, In the current investigation, drought stress resulted in an elevation of Na^+ and Ca^{+2} levels, as well as an increase in tryptophan, while concurrently leading to a reduction in K^+ levels and the K^+/Na^+ ratio. Conversely, the

application of SA and Si reduce the detrimental impact of drought stress by amplifying the presence of organic, ionic, and chemical compounds. The utilization of SA and Si may conceivably mitigate drought stress in *S. striata* albeit with a dual effect that includes both positive and negative aspects.

Materials and methods

Plant material and Cultivation

The seeds of two ecotypes of *S. striata* were collected in January 2020 from the north and south of Ilam Province (refer to Figure.1; Table 1). The botanical characteristics of the studied samples were confirmed based on (Flora Iranica). In addition, a number of plant samples were collected and stored in the Ilam University Gene Bank under the code IUGB02076 for later use by other researchers after obtaining the necessary cooperation and permission from the above gene bank in accordance with the existing guidelines and regulations [30]. Prior to germination, the seeds underwent a thorough cleansing process involving a one-hour immersion in water, followed by a brief 30-second exposure to a 10% sodium hypochlorite solution. Subsequently, the seeds were subjected to multiple rinses with distilled water. To overcome dormancy, a treatment with 400 ppm gibberellic acid was administered for a duration of 48 hours, facilitating the subsequent germination proces.s[30], [31]

Plant growth condition

The seeds were positioned within a designated germinator model (KBWF 240, Germany) at a controlled environment of 25°C and a relative humidity of 90%. Following a 48-hour duration, the germinated seeds were relocated to petri dishes containing moist filter paper within the same germinator. Post-germination, the seedlings were transplanted into small paper pots filled with a mixture of soil, organic fertilizer, and clay possessing specific physicochemical attributes, including electrical conductivity (EC) of 1.3 micro m/cm, pH of 7.4, humidity of 28%, and an N: P: K ratio of 0.01: 9.4: 240%. Subsequently, these seedlings were transferred to a greenhouse, where meticulous care was provided for a period of 30 days, after which they were further transplanted into pots with dimensions of 12 cm depth and 18 cm diameter.

experimental design

To explore and analyze the reaction or behavior of plants to drought stress treated with silicon and salicylic acid, a factorial experiment was performed based on a completely randomized design with three replications in the research greenhouse of the Faculty of Agriculture of Ilam University during 2020-2021. In this experiment, SA and Si with molecular formulas $C_7H_6O_3$ and K_2SiO_3 were purchased from Merck (Germany). Four months after planting, when the plants reached the stage of four to five leaves, they were subjected to foliar spraying with salicylic acid (SA) and silicon (Si) at two different doses. This application was carried out in the early morning to ensure complete coverage of the plant foliage and, then, repeated eight times over a four-week period. Two days subsequent to the foliar treatment, drought stress was induced at two distinct levels for a duration of 50 days. Sampling occurred six months after the initial planting, spanning from morning to noon. Furthermore, after a span of 198 days, the plants were harvested from the soil surface in the morning[31]. The determination of field capacity employed the methodology outlined by [32]–[34]through the utilization of Equation (1).

FC% = (FSW-DSW/DSW)*100
[FC: moisture Soil, WSW: Wet soil weight, WSD: Dry soil weight]

Extraction of crude extract

The shoot of the plant was dried at room temperature without light and shade, and powdered using a mill. Then, it was subjected to sequential extraction using methanol solvent (80:20)[35].

Gas chromatography-mass spectrometry (GC-MS)

GC Mass analysis was performed on a Thermo Finnigan capillary GC that directly coupled to a mass spectrometer (model TRACE GC 2000, TRACE MS plus) equipped with a HP 5MS non-polar fused silica capillary column (30 m, 0.250 mm, 0.25 µm film). The oven temperature program was 40◦C (2 min), then 4◦C/min to 150 (2 min) and finally to 280◦C/min. Injector temperature was set at 260◦C. the diluted samples of 1 µl have injected in the splitless mode[36].

Osmotic regulating compounds

Chromatographic separation of amino acids

The extraction of free amino acids from the stem (0.5 g FW) was conducted following the procedure outlined by Falahi et al. (2018). A Unicam-Crystal-200 High-Performance Liquid Chromatography (HPLC) system from England, featuring an MD-1510 diode-array detector set at 263 nm (λmax), was employed. Data acquisition and processing were carried out applying Borwin-PDA Version 1.50 software developed by JMBS Developments in Grenoble, France. Samples, introduced by a 20-µL loop through a 7125 valve (Rheodyne, Cotati, CA), were directed onto a Purospher RP-18 column (250 × 4 mm, 5 µm internal diameter). The column, maintained at 25°C, operated with 1.0 mL/min flow rate, utilizing eluent A being 50mM acetate buffer (pH 4.2) and eluent B being acetonitrile. Peak identification and quantification were performed by comparing the retention time of amino acid standards with those present in the extracted sample, following the methodology outlined by[37].

Elemental analysis

The leaf samples were dried in the shade and then ground. 1 g of milled powder was ashed in a furnace (LAC, Czech Republic) at 550°C for 6 hours. The ash was dissolved in 5 ml of hydrochloride and diluted with 50 ml of distilled water. The concentration of Na^+, K^+, Mg^+ and Ca^+ was measured using an atomic absorption spectrophotometer (NOUAA400P, Analytic Jena)[28].

Nitrate measurement

0.1 g of ground leaf powder was rubbed with 2 ml of 0.02% acetic acid. Then, it was centrifuged and the extract was used to measure nitrate. Three types of reagents were used for the measurement: type 1 (saturated solution of sulfamic acid), type 2 (0.5% salicylic acid in 96% sulfuric acid) and type 3 (0.07% NaOH). 15 µl of the extract was mixed with 10 µl of reagent 1 and 200 µl of reagent 2. The samples were left at room temperature for 10 minutes. Then, 2 ml of reagent 3 was also added and left again for 20 minutes at room temperature. Finally, the absorbance was read by a spectrophotometer (SPEECORD 50 n, Germany) at 420 nm. K_2NO_3 (0-10 mmol) was used to prepare standard solutions. The amount of this substance was calculated in micrograms per gram of dry weight[38].

Statistical analysis

The present study was conducted as a factorial experiment based on a CRD. Three replications were considered for each treatment. Data analysis was done using SAS 9.4. Mean comparison of the treatments was done using least significant difference (LSD) at the level of $P < 0.05$, and GraphPad 9 was used to draw the graphs.

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Author contributions

The project was conceived and supervised by A.F, H. S. S. and F. SH. developed F. SH and A.F. and H. S. S wrote the manuscript. All authors critically read and approved the final manuscript. This study was financially supported by e research center No. 1764/32 of the esteemed Vice Chancellor for Research and Technology of Ilam University, to which the authors are most grateful.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Abbreviation

SA, salicylic acid., Si, silicon., Glu, glutamic., ASP, aspartic., Arg, argenine., Ala, alnine., Gly, glycine., Val, valine., Met, methionine., Tyr, tyrosine., Leu, leucine., Iso, isoleucine., His, histidine., Phe, phenylalanine., Trp, tryptophan., Ser, serine., Thr, threonine., BCAAs, Branched-chain amino acids, TCA, tricarboxylic acid cycle.

Table 1. Geographical location of the collection of ecotypes in this study

No	Location	latitude	Longitude	Altitude
1	Ilam	47°25′	32°59′	88 m
2	Abdanan	23°21′30″	46°51′19″	1427 m

Table2. Analysis of variance on Mineral Nutrient Content in *S. striata* treated with SA or Si under drought stress

S.OV	df	µg g^{-1}DW					
		Ca^{+2}	Na^{+}	K^{+}	Mg^{+2}	K^{+}/Na^{+}	NO^{-}_{3}
var	1	71.95**	230.2**	1549.2**	25.3**	1.9**	0.0002^{ns}
stress	1	528.47**	613.6**	44597.1**	96.1**	5.9**	0.0137**
SA	1	162.84**	696.54**	16889.2**	106**	6.2**	0.001*
Si	1	92.37**	64.84**	1680**	41.7**	4.14**	0.0002^{ns}
var*stress	1	92.87**	244.39**	4769.2**	25.2**	0.012^{ns}	0.0004^{ns}
var*SA	1	94.27**	236.96**	133.1^{ns}	56.5**	18.9**	0.0007^{ns}
var*Si	1	71.95**	141.28**	270.2**	78.2**	16.3**	0.0008^{ns}
stress*SA	1	378.8**	852.35**	70697.4**	34.91**	13.96**	0.0018**
stress*Si	1	36.38**	37.47**	2830.8**	15.64**	4.32**	0.0013**
SA*Si	1	61.4**	52.64**	3259.4**	0.143^{ns}	10.78**	0.0002^{ns}
var*stress*SA	1	8.25**	89.29**	385.9*	58.8**	7.19**	0.0018**
var*stress*Si	1	80.93**	247.92**	895.9**	3.11**	6.97**	0.0011*
var*SA*Si	1	0.66^{ns}	2.13*	31145.7**	53.5**	50.62**	0.0045^{ns}
stress*SA*Si	1	130.51**	292**	24466.7**	11.6**	12.84**	0.0001**
var*stress*SA*Si	1	139.2**	410.4**	1648.5**	116.6**	15.7**	0.0004^{ns}
Error	32	0.55	0.38	59.7	0.22	0.24	0.0001
CV%	-	3.31	2.83	4.6	1	6.43	9.83

ns: non-significant, * and **: significant at 0.05 and 0.01 probability level, respectively. df: degree of freedom.

Tabale 3. GC-MS of bioactive compounds present in the methanolic extracts of Stem derived from in grown plants of *S. striata*

S. No	RT	Compound name and Structure	Formulae	Mwt (g/mol)	Area%	Biological activity
1	7.14	2-Pentanone, 4-hydroxy-4-methyl	$C_6H_{12}O_2$	116.1583	0.84%	Antibacterial. (Ismailet al., 2013)
2	9.77	1,6-Heptadien-4-ol	$C_7H_{12}O$	112.17	0.59%	Antifungal. (Todeschini et al., 2018)
3	12.66	1,5-Hexadiene, 2-methyl	C_7H_{12}	96.1702	0.27%	Antibacteia. (Li et al., 2013)
4	22.35	Hydroperoxide, heptyl	$C_7H_{16}O_2$	132.2	0.66%	Antibacteia. (Li et al., 2013)
5	22.36	2-Decenal, (Z)	$C_{10}H_{18}O$	154.25	0.52%	Pesticides. (Ntalli et al., 2016)
6	23.48	Phenol, 2-(1,1-dimethylethyl)	$C_{10}H_{14}O$	150.2176	5.93%	Antifungal. (Devi et al., 2021)
7	23.82	Phenol, 2-methyl-5-(1-methylethyl)	$C_{10}H_{14}O$	150.2176	27.74%	Antimicrobia. (Magi et al., 2015)
8	30.48	Propanoic acid, 2-methyl-, anhydride	$C_8H_{14}O_3$	158.1950	1.07%	No activity reported
9	32.27	Oxalic acid, allyl octyl ester	$C_{13}H_{22}O_4$	242.31	13.89%	antimicrobial
10	33.92	Butanal, 3-methyl	$C_5H_{10}O$	86.1323	1.02%	Antimicrobial. (Krusong et al., 2020)
11	34.05	17-Pentatriacontene	$C_{35}H_{70}$	490.9	18.83%	Antibacterial. (Albratty et al., 2023)
12	34.95	Isobutyl nitrite	$C_4H_9NO_2$	103.12	0.21%	No activity reported
13	35.8	2-Nonen-1-ol, (E)-	$C_9H_{18}O$	142.24	0.73%	flavouring ingredients. (Ijagem et al., 2020)
14	35.79	Dodecanoic acid, 2-penten-1-yl ester	$C_{17}H_{32}O_2$	268.4	0.7%	antibacterial and insecticidal. (Mishra and Sree., 2009)
15	35.87	2-Undecanone, 6,10-dimethy	$C_{13}H_{26}O$	198.3449	1.31%	Anti inflammation. (Ahuchaogu et al., 2018)
16	35.88	Pentanal, 2,4-dimethyl	$C_7H_{14}O$	114.19	2.11%	Antimicrobial. (Sharma et al., 2015)
17	36.04	(2S,3S)-(-)-3-Propyloxiranemethanol	$C_6H_{12}O_2$	116.16	0.34%	Anti-cancer. (Gopu et al., 2021)
18	36.8	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228.3709	7.53%	Antibacteria and Antifungal. (Agoramoorthy et al., 2007)
19	36.09	5-Octen-1-ol, (Z)	$C_8H_{16}O$	128.2120	0.42%	No activity reported
20	36.30	Oxirane, dodecyl	$C_{14}H_{28}O$	212.3715	0.23%	Antifungal and antibacterial. (Gajera et al., 2020)
21	36.31	2-Decen-1-ol	$C_{10}H_{20}O$	156.26	1.5%	Antimicrobial. (Banni et al., 2023)
22	36.69	Heptanal, 2-methyl	$C_8H_{16}O$	128.2120	0.34%	Anticancer. (Maqsood et al., 2022)
23	36.76	Oxalic acid, allyl tridecyl ester	$C_{18}H_{32}O_4$	312.4	3.11%	Antimicrobial. (Ruthisha et al., 2017)
24	36.77	Oxirane, [(tetradecyloxy)methyl]	$C_{16}H_{32}O_2$	256.42	3.55%	antibacterial, insect antifeedant and antioxidant activities, also natural mediators for allergic asthma and they also act as a biogenetic. (Kalaivani et al., 2021)
25	36.80	Octanoic acid, methyl ester	$C_9H_{18}O_2$	158.2380	4.29%	Antimicrobial. (Brophy et al., 2008)

S. No	RT	Compound name and Structure	Formulae	Mwt (g/mol)	Area%	Biological activity
26	36.94	Hydroperoxide, hexyl	$C_6H_{14}O_2$	118.17	1.69%	Antibacteia. (Li et al., 2013)
27	36.99	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4- methyl	$C_{24}H_{45}N_2O_3^+$	409.6	0.7	No activity reported
29	37.18	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	48.73%	Anti-inflammatory, Antioxidant, Pesticide, Nematicide, Inhibitor. (Gopu et al., 021
28	37.2	Undecanoic acid	$C_{11}H_{22}O_2$	186.29	32.62%	Antifungal agent, treatment of ringworm. (El-Naggar et al., 2023)
30	37.21	2,3-Epoxy hexano	$C_6H_{12}O$	100.15900	27.8%	Antibacterial, Antioxidant. (Sudaryadi et al., 2022)
31	38.47	Oxalic acid, allyl hexyl ester	$C_{11}H_{18}O_4$	214.26	0.6%	Antimicrobial. (Ruthisha et al., 2017)
32	38.48	Oxalic acid, allyl hexa decyl ester	$C_{21}H_{38}O_4$	354.5	0.69%	Antimicrobial. (Ruthisha et al., 2017)
33	38.55	Z-1,9-Hexadecadiene	$C_{16}H_{30}$	222.41	0.18%	No activity reported. (Devi et al., 2015)
34	38.62	2-Cyclopentene-1-undecanoic acid	$C_{16}H_{28}O_2$	252.39	0.87%	antifungal and antioxidant. (Margret Kanimozhi and Rose., 2023)
35	38.63	9,12,15-Octadecatriena	$C_{18}H_{30}O_2$	278.4	2.12%	Antibacterial. (McGaw et al., 2002)
36	38.75	Octane, 2-methyl	C_9H_{20}	128.25	20.35%	No activity reported
37	38.76	Phytol	$C_{20}H_{40}O$	296.5	37.09%	Antimicrobial,Anti inflammatory,Anticanc er, Diuretic, and antimalarial. (Yarazar et al., 2022)
38	38.98	5,10-Dioxatricyclo[7.1.0.0(4,6)] decane	$C_8H_{12}O_2$	140.18	30.77%	No activity reported. (Yasmeen et al., 2017)
39	38.97	2-(Prop-2-enoyloxy)pentadecane	$C_{18}H_{34}O2$	282.5	1.61%	No activity reported
40	39	10-Undecyn-1-ol	$C_{11}H_{20}O$	168.28	21.22%	Antifungal. (Neoh et al., 2008)
41	39.04	1,6-Anhydro-3,4-dideoxy-β-D-manno-hexapyranose	$C_6H_{10}O_5$	162.14	1.43%	No activity reported. (Amudha et al., 2014)
42	39.16	1,2,4-Trioxolane, 3,5-dipropyl	$C_{14}H_{12}O_3$	228.24	11.92%	No activity reported
43	39.18	2-Dodecenoic acid	$C_{12}H_{22}O_2$	198.30	1.64%	Antimicrobial. (Marques et al., 2015)
44	39.22	Cyclopentane undecanoic acid	$C_{16}H_{30}O_2$	254.41	2.72%	Antimicrobial. (Chenniappan et al., 2020)
45	42.41	Oxalic acid, allyl heptyl ester	$C_{12}H_{20}O_4$	228.28	0.54%	Antimicrobial. (Ruthisha et al., 2017)

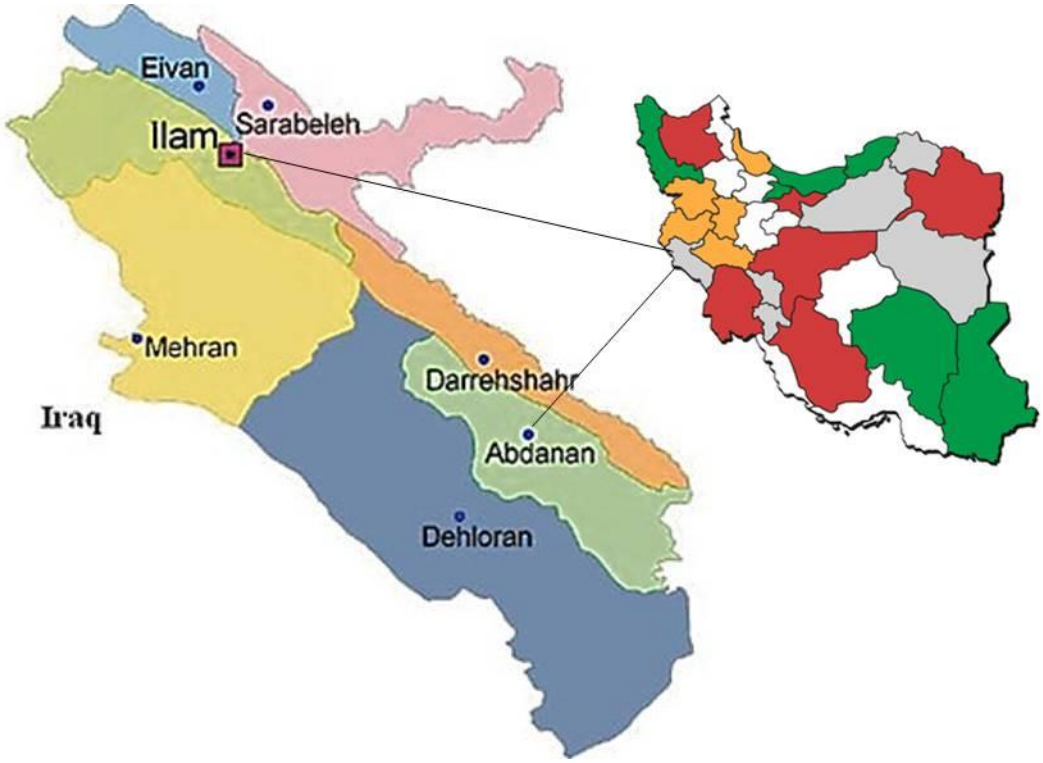

Fig. 1 Seed *S. striata* collection site from Ilam province and Abdanan district

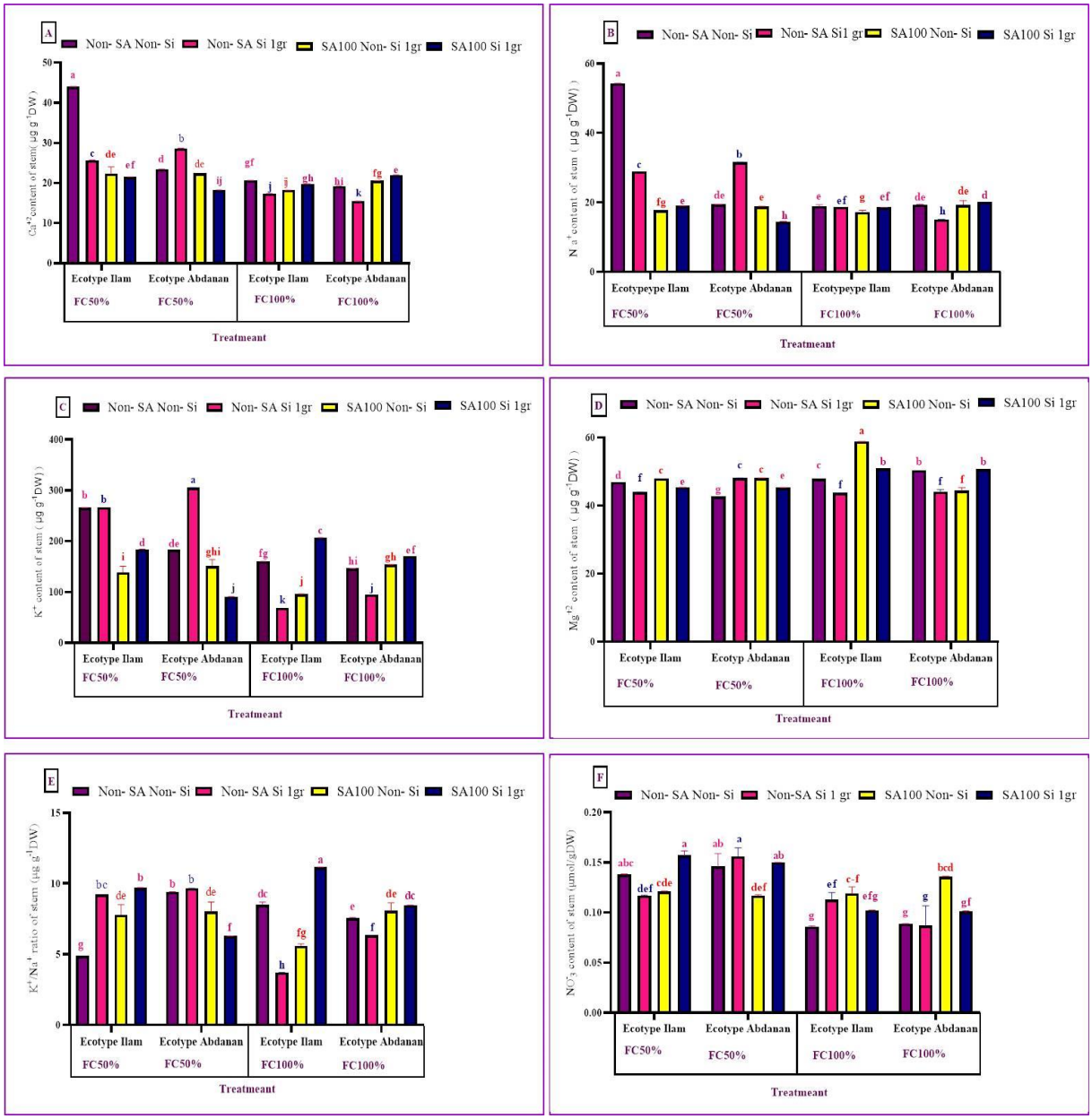

Fig. 2 Effects of salicylic acid (0 and 100 ppm) and silicone (1 g/liter) on the content of Ca^{+2} (A), Na^{+} (B), K^{+} (C), Mg^{+2} (D), K^{+}/Na^{+} (E) in aerial parts of *S. striata* under drought stress. Data show the mean of three replicates ± SD (standard deviation). P≤0.05 is shown by various letters.

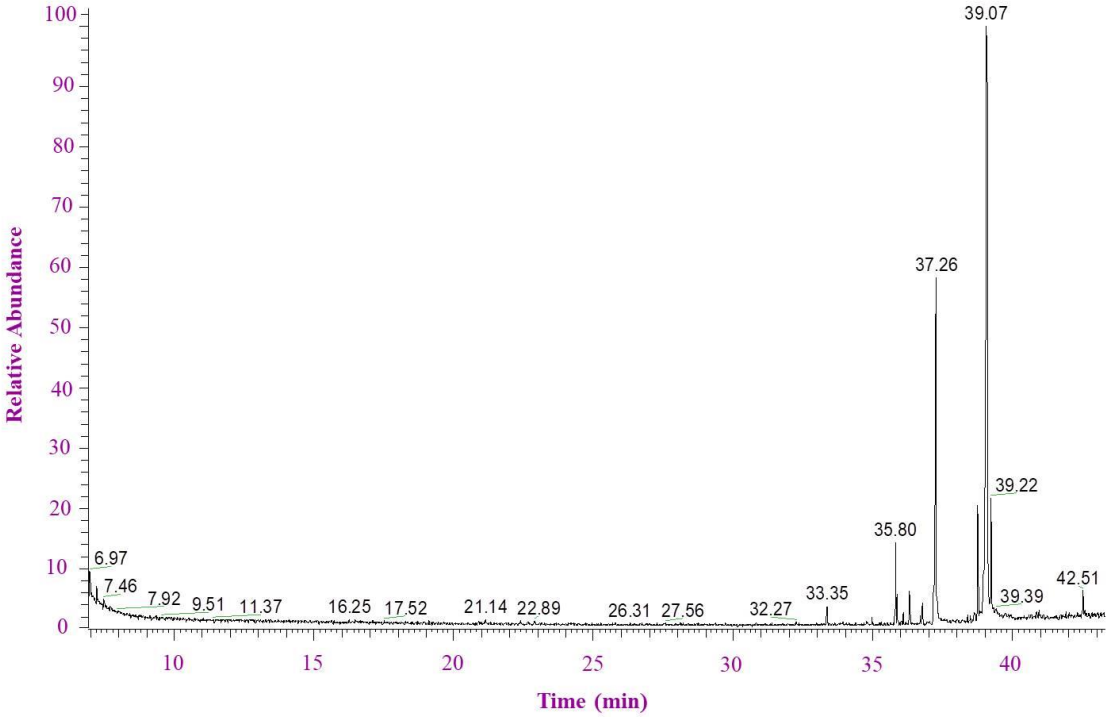

Fig. 3 GC-MS chromatogram of methanolic extract of *S.striata*

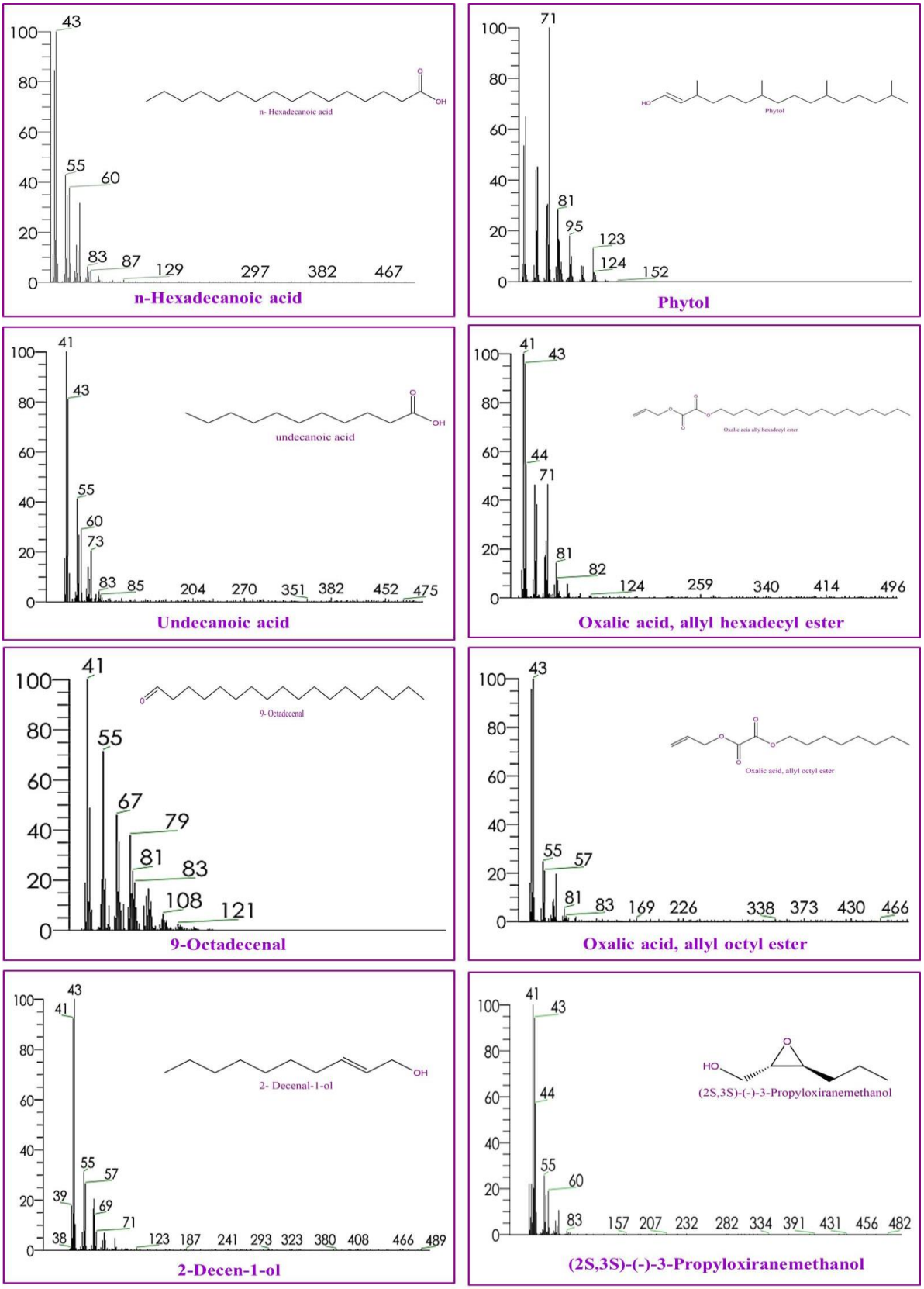

Fig. 4 Chemical structures of the major components of essential oils of *S.striata* grown in Iran climatic

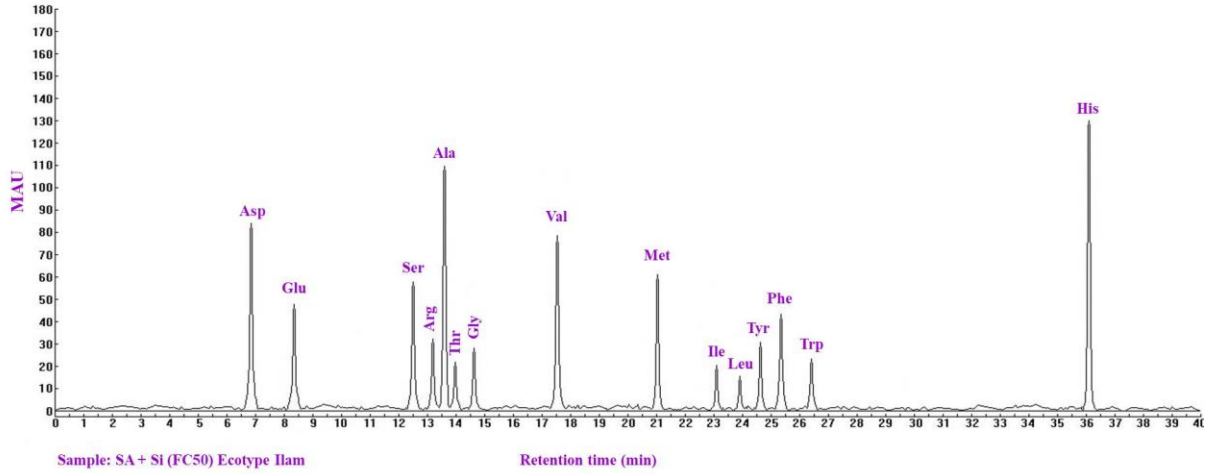

Fig. 5 Representation of amino acids chromatogram of *S. striata* Stem

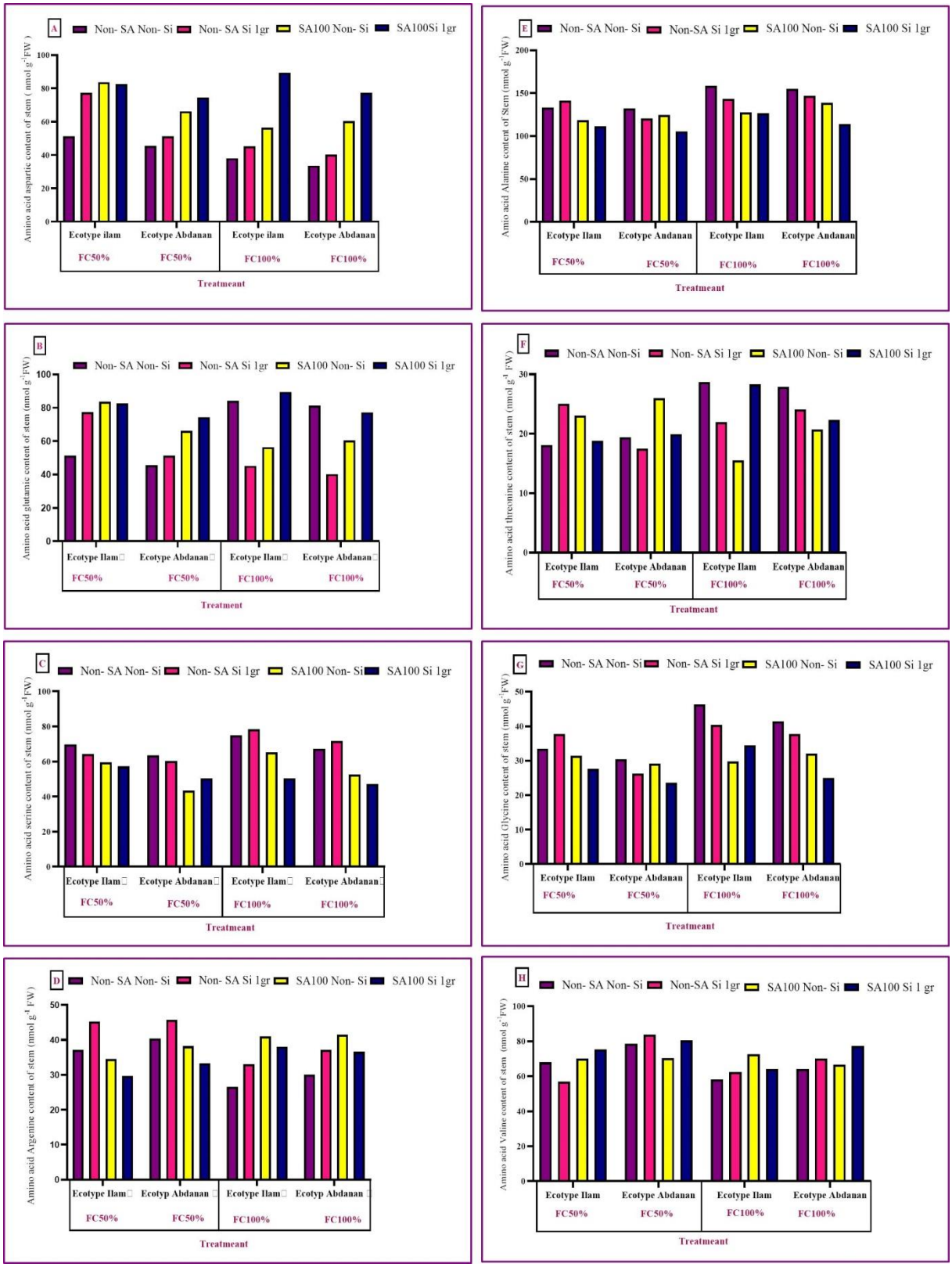

Fig. 6 Comparison of the mean content of aspartic (A), glutamic (B), serine (C), argenine (D), alanine (E), threonine (F), glycine (G), valine (H) in salicylic acid (0 and 100 ppm), and silicon (1 g/liter) treated *S.striata* under drought stress.

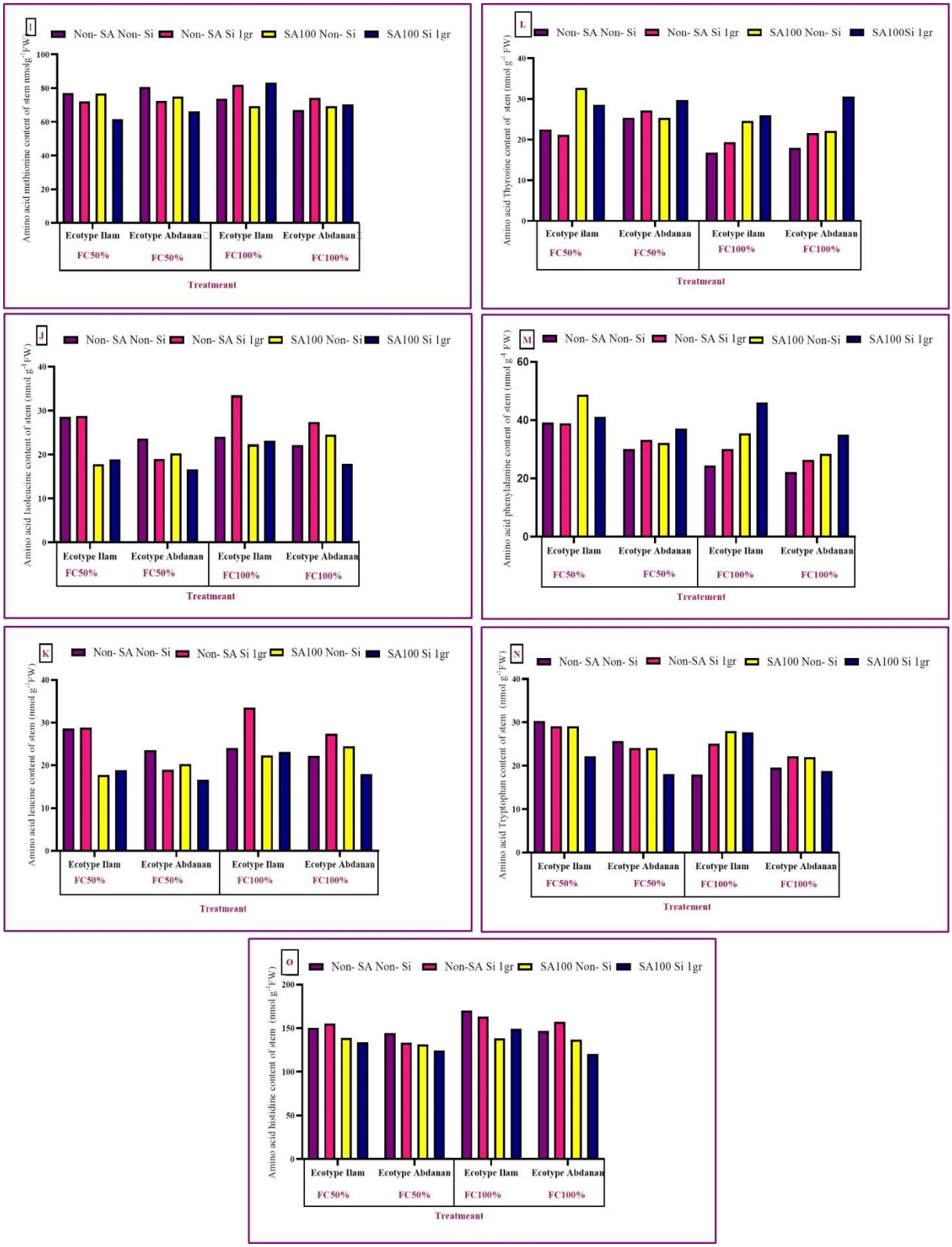

Fig. 7 Comparison of the mean content of methionine (I), Isoleucine (J), Leucine (K), thyrosine (L), phenyalanine (M), tryptophane (N), histidine (O) in salicylic acid (0 and 100 ppm), and silicon (1 g/liter) treated *S.striata* under drought stress.

Supplementary Files

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- Tryptophan.docx
- Valine.docx
- aspartic.docx
- ca.docx
- leusine.docx
- methionine.docx
- phenylalanine.docx
- threonine.docx