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# Variation in yield and biochemical factors of German chamomile (*Matricaria recutita* L.) under foliar application of osmolytes and drought stress conditions

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**ABSTRACT**

**Introduction:** Chamomile is one of the oldest and most valuable medicinal plants from the Asteraceae family. In addition to pharmaceutical uses, its essential oil is extensively used in perfumery, cosmetics, food industry and aromatherapy. This experiment was conducted with the aim of investigating the effect of osmolytes foliar application on biochemical characteristics, and the yield of German chamomile under drought stress conditions.
**Methods:** The experiment was conducted as a split plot with randomized complete block design with three replications during the 2016-2017 growing season. Three levels of irrigation, 50 (control), 100 (mild stress) and 150 mm (severe stress) evaporation from evaporation pan class A, and spraying treatments, NS (no-spraying), W (distilled water), MeJA (methyl jasmonate), SA (salicylic acid), HA (humic acid), GB (glycine betaine) and GABA (γ-aminobutyric acid) were considered as the main plots and sub-plots, respectively.
**Results:** Analyzed data indicated that proline, total soluble sugars (TSS), and essential oil yield were enhanced with the increase of drought intensity and the maximum amount was registered under severe stress, while the severe drought caused a substantial reduction in protein concentration of leaves and dried flower yield. Proline concentration of leaves significantly increased with exogenously applied spraying treatments under severe drought. All spraying treatments except GB under severe stress, caused higher TSS concentration than those subjected to mild stress. SA, HA, and GABA treated plants had significantly higher protein concentration compared to NS treatment. Plants that treated with GABA had the highest dried flower and essential oil yield.
**Conclusion:** The present study suggests that osmolytes foliar application can ameliorate the detrimental effects of drought on chamomile plant through alteration in yield and biochemical variables.

*Implication for health policy/practice/research/medical education:*
Foliar application of osmolytes can effectively reduce the damages of drought stress in German chamomile through its protective effect on growth.

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## Introduction

Drought stress affects a variety of physiological and biochemical processes in plants and leads to reduced growth and final crop yield. Many of the morphoanatomical, physiological, and biochemical changes that occur in plants make them consistent with drought conditions. Osmoregulation is one of the important physiological adaptations that occur due to the reduction of cellular water potential stabilizing the physiological processes necessary for plant growth (1).

The organic solutes, commonly referred to as compatible osmolytes or compatible solutes, not only participate in osmoregulation but they may also maintain the structure of different biomolecules and membranes (2), or scavenge free radicals and protect DNA from the destructive effects of ROS (3). Stressed plants can accumulate some important compounds such as proline, which play an important role in cell osmotic adjustment and protect cell

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components during dehydration (4), salicylic acid (SA), which may play as a constitutive defense compound (5), free amino acids (6) and phytohormones (7).

An alternative method for increasing plant drought tolerance is the exogenous use of organic solutes (e.g. glycine betaine [GB], GABA [γ-aminobutyric acid], methyl jasmonate [MeJA], SA, humic acid [HA]) which is currently being considered for high performance, feasibility, cost and labor-effectiveness (1).

GB, a quaternary ammonium compound, is considered as a strong osmoprotectant against drought (8) due to its small size, solubility in water, and not interfering with other metabolites within the cell. Furthermore, when exogenously applied as a foliar spray, GB can easily penetrate through the leaf epidermis and move to other organs to effectively contribute to enhanced stress tolerance (9). The major function of GB in the plant cell under osmotic stress is to stabilize the structure of enzymes, complex proteins, and membranes (10).

Plant species contain a non-protein four-carbon amino acid called GABA. In response to drought stress, plants accumulate GABA rapidly (11). GABA acts as an osmolyte, antioxidant, or reactive oxygen scavenger that help the plant to tolerate stresses (12). Exogenous GABA application may lead to antioxidant response in the plants (13).

SA is a phenolic compound with a broad distribution in plants that plays an important role during the plant response to drought stress. In particular, the foliar application of SA is effective in providing resistance to the plants against drought stress. Furthermore, SA may function in crosstalk with other phytohormones, ROS and glutathione (GSH) in events involved in the transcription of sets of defense genes (14).

HA can play a role as a growth regulator, regulating hormonal levels, improving plant growth, and increasing stress tolerance (15). Humic substances are well known as plant growth stimulants by enhancing absorbance and transport of nutrients, reducing uptake of toxic elements, increasing membrane permeability, respiration, photosynthesis, and phosphate uptake and acting as growth hormones (16). HA foliar application due to increased rubisco activity, photosynthesis and carbohydrate increases tolerance to stress conditions (17)

MeJA is considered as a plant hormone. The major function of MeJA and its various metabolites is regulating the plant responses to drought stress as well as plant growth and development. When MeJA is exogenously applied, promote petiole abscission, activation of ethylene synthesis, inhibition of root growth, chlorophyll production, and pollen germination (14).

German Chamomile is an annual plant belonging to the Asteraceae family. Chamomile distribution is in the west, northwest and south of Iran, and its use in Iranian folklore medicine has a long history. Over 120 compounds have been identified in German chamomile essential oil, while, chamazulene, bisabolol oxides A and B, farnesene and α bisabolol oxide are the most important ones (18). Chamomile has anti-inflammatory, anti-allergic anti-spasmodic and anti-bacterial features (19).

Abiotic environmental stresses such as drought have the greatest impact on medicinal plants (20). Farhoudi et al (19) reported that moderate drought stress increased essential oil content and essential oil yield of chamomile, however, severe drought stress reduced the growth, photosynthesis and essential oil yield of this plant. The findings of Pirzad et al (21) showed that drought stress reduced chamomile leaf chlorophyll content, but proline content, soluble sugar and relative water content of leaves were not affected by drought stress.

Considering the drought stress as one of the most important limiting factors of yield in many crops (22), the present study was conducted to determine whether and how osmolytes (growth regulators) application alleviates the bad effects of drought stress on the yield and biochemical characteristics of German chamomile.

## Materials and Methods

### Experimental site

The experiment was conducted between 15 March to 25 June 2016 at Medicinal Plants Research Center of Shahed University, Tehran, Iran (latitude: 51°, 08´, longitude: 35°, 34´, altitude: 1190 m above the sea). This site is characterized by a semi-arid climate with the annual average temperature of 17.6°C. Rainfall distribution and average temperature during crop growing season at the experimental site are presented in Figure 1. Soil physicochemical specifications were measured at 0-60 cm depth before conducting the experiment (Table 1). The experiment was based on split plot with randomized complete block design with three replications to investigate the changes of yield and biochemical characteristics of German chamomile under drought stress in response to exogenous application of osmolytes.

### Plant material

Seeds of German chamomile (*Matricaria recutita* L.) cultivar Bodegold were provided from Kohsar-e Arak Company, Arak, Iran. Sowing was done manually on March 15, 2016 on a well-prepared seedbed. Seeds were planted by hand in 1.5×1.5 m plots having a density of 75 plants $m^{-2}$. Each plot consisted of five rows with 10 cm intra-row spaces and 30 cm inter-row space. Before planting, the seeds were mixed ten times with wind sands. The weeds were controlled manually during the growing season. Irrigation took place immediately after sowing, then once every 6-day interval according to agronomic practices in the district. Irrigation was carried out manually sprinkled until the plant establishment. The plants were thinned before the rosette stage and usually

**Table 1.** Some physicochemical properties of the soil of the experimental site

| Fe (mg/kg$^{-1}$) | K (mg/kg$^{-1}$) | P (mg/kg$^{-1}$) | Electrical conductivity (dS/m) | pH | N | Organic matter % | Sand % | Silt % | Clay % | Soil texture |
|---|---|---|---|---|---|---|---|---|---|---|
| 4.7 | 234.2 | 10.2 | 4.2 | 7.8 | 0.04 | 0.7 | 74 | 6 | 20 | Sandy loam |

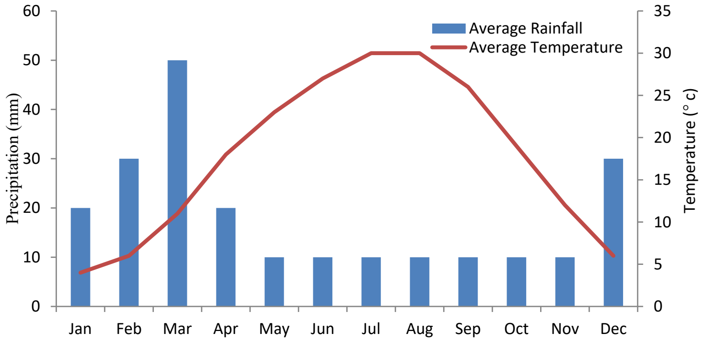

**Figure 1.** Rainfall distribution and average temperature during the experiment period in 2016.

four-leaf to create the desired density. At 80% flowering stage (80 days after planting), measurements were carried out on plants in all experimental units.

## Treatments

Experimental factors were arranged in irrigation regimes as main plots irrigation after 50, 100, 150 mm evaporation from evaporation Pan class A as control, mild and severe stress, respectively (23) and foliar application of osmolytes as subplots [(NS: no spraying, W: distilled water, MeJA: foliar application of 75 µm MeJA solution (24), SA: foliar application of 2 mM SA solution (25), HA: foliar application of 2.5 L/1000 humic acid solution (26), GB: foliar application of 5 mM GB solution (27), and GABA: foliar application of 50 mM GABA solution (28)]. The drought treatments were imposed after the end of spring rainfall (50 days after planting). Evaporation rate from class A pan was measured daily and irrigation of each treatment was performed after reaching the evaporation rate to the desired value. In order to determine the irrigation time in each treatment, 48 hours after irrigation, daily and continuous sampling was carried out on the soil in order to determine the percentage of soil moisture content (23). Plumbing irrigation systems were used to accurately apply irrigation regimes. The volume of irrigation water consumed in each plot was calculated by the equation 1:

$$V_{irrig} = ( F_c - \theta_m ) \times \rho_b \times D_{root} \times A \qquad (1)$$

Where:
V= irrigation volume (lit)
$F_{cv}$= gravimetric soil moisture content at field capacity
$\theta_m$ = gravimetric soil moisture content before irrigation
$\rho_b$ = the bulk density (kg/m$^{-3}$) of the experimental soil
$D_{root}$ = root development depth (m)
A= area of each plot (m$^2$)

Plants were treated with a foliar application of the freshly prepared aqueous solution of the GABA, GB, SA, MeJA, and HA. The first applications of GB, SA, MeJA, and HA were at 45 days after sowing (vegetative stage) and the second applications at 60 days after sowing (flowering stage) using a hand-held sprayer to completely cover the plant foliage. Plants were treated with GABA only at flowering stage. Control plants were sprayed with distilled water.

## Biochemical analysis

Fully expanded leaf material was gathered at the 80% of flowering stage. Leaf samples were placed in liquid nitrogen and stored at -80°C until variables were measured.

## Determination of proline concentration

Proline concentrations of leaf tissues were determined by Bates et al method (29). The last developed leaves were used to determine proline concentration. 0.5 g of the leaf samples were homogenized in 10 mL of 3% aqueous sulfosalicylic acid and passed through the Whatman No. 2 filter paper. Two milliliters of the filtrate were mixed with 2 mL acid-ninhydrin and 2 mL glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 hour at 100°C. The reaction mixture was extracted with 4 mL toluene and the chromophore-containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm.

## Total soluble sugars

The concentration of total soluble sugars (TSS) was determined by Omokolu et al method (30). Briefly, 0.1 g of the fresh leaf was crushed in a mortar and 5 mL of 80% hot ethanol was added to it. Extraction was repeated 4 times with ethanol and the extract was placed at 70°C to evaporate the alcohol. To remove chlorophyll, the extract mixed with chloroform (1:5). After vortexing, it was left for 5 minutes. The upper transparent section was centrifuged for 10 minutes at 10,000 rpm for measurement of soluble sugars content by anthrone method according to McCready et al method (31). TSS concentration was determined using a LAMBDA 850 UV/Vis spectrophotometer at 620 nm. The concentration of TSS was calculated by the standard glucose curve and expressed in mg/g.dw$^{-1}$.

### Protein assay

The concentration of leaf soluble proteins was measured using the Bradford method (32). The leaves of chamomile were homogenized in ice-cold extraction buffer (50 mM potassium phosphate, pH 7.4, 1 mM EDTA). To measure protein concentration, 20 µl of extraction was diluted with 80 µL of extraction buffer. Protein solution containing 10 to 100 µg protein in a volume up to 0.1 mL was pipetted into test tubes. Five microliters of Bradford Reagent was added to the test tubes and the contents were mixed for 2 minutes by vortexing. The absorbance was measured 15 minutes after addition of Bradford Reagent at 595 nm. Protein concentration in the samples is obtained according to the spectrophotometer number and standard curve of bovine albumin serum (BSA) protein and expressed in mg/g.fw$^{-1}$.

### Extraction of essential oil

The essential oil content of chamomile dried flowers (30 g) was extracted by hydrodistillation method for 4 hours, using a Clevenger apparatus in 500 mL round-bottom flask with 300 mL distilled water according to the method described by Baghalian et al (33).

### Dried flower yield

After each harvest, the chamomile flowers were weighed accurately with a precision of 0.001 g. Then the samples were dried at room temperature (20–25°C) and the yield of each experimental unit was calculated (21).

### Statistical analysis

Analysis of variance of all data was done by the general linear model (GLM) procedure of SAS (SAS Institute, Cary, NC). Treatment means were separated using the least significant difference (LSD) test at the $P \leq 0.05$ level of probability.

## Results

Protein, and total soluble sugar concentrations were significantly ($P<0.01$) affected by osmolytes application (Table 2). Irrigation regimes affected protein, total soluble sugar, and proline concentrations ($P<0.01$). There was a significant interaction effect ($P<0.01$) of irrigation regimes and foliar application of osmolytes throughout the experiment on the proline, and total soluble sugar concentrations (Table 2). Exogenous application of osmolytes didn't have any significant effect on proline concentration (Table 2). The irrigation regimes appeared to influence ($P<0.01$) the dried flower yield (Table 2). According to Table 2, the interaction between irrigation and spraying was significant ($P<0.01$) on dried flower and essential oil yield. Also, spraying treatments significantly ($P<0.01$) affected the essential oil yield of chamomile.

### Proline concentration

Exogenous GABA application increased leaf proline concentration in mild stress compared with severe stress. GABA treated plants under severe stress had 22.2% and 19.27% lower proline concentration than NS and water sprayed plants. Stressed plants (mild and sever stressed plants) showed significant increase of leaf proline in combination with SA, HA, GB, and MeJA the same as NS and water sprayed plants (Table 3). The highest amount of proline was related to the HA and severe stress combined treatment (42.95 µm/gfw$^{-1}$), and the lowest amount belonged to the GB and control combined treatment (25.11 µm/gfw$^{-1}$) (Table 3).

### Total soluble sugars

In severe stress condition, the TSS content decreased considerably by osmolytes application compared to NS treatment (Table 3). Spraying with MeJA and HA under control treatment increased TSS content in comparison to NS under same drought level. But, plants that treated with

**Table 2.** Analysis of variance for proline, TSS, protein and the yield of German chamomile (*Matricaria recutita* L.) subjected to three irrigation regimes and foliar application of osmolytes

| SOV | *df* | Proline | TSS | Protein | Dried flower yield | Essential oil yield |
|---|---|---|---|---|---|---|
| Replication | 2 | 5.79$^{**}$ | 632.3$^{**}$ | 16.21$^{**}$ | 12945.01 $^{**}$ | 1.15** |
| Irrigation regimes (I) | 2 | 898.01$^{**}$ | 1309.8$^{**}$ | 103.26$^{**}$ | 49525.8 $^{**}$ | 0.38 $^{ns}$ |
| Error a | 4 | 5.89 | 29.67 | 11.27 | 1746.6 | 0.45 |
| Spraying (S) | 6 | 7.18$^{ns}$ | 1126.06$^{**}$ | 5.27 $^{**}$ | 1215.4 $^{ns}$ | 0.66 $^{**}$ |
| I × S | 12 | 46.49$^{**}$ | 433.8$^{**}$ | 0.66 $^{ns}$ | 2821.6 $^{**}$ | 0.56 $^{**}$ |
| Error b | 36 | 11.00 | 59.71 | 1.57 | 814.4 | 0.18 |
| CV | | 9.8 | 18.02 | 17.91 | 13.58 | 22.9 |

* and ** significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; *ns* not significant.

SOV, Source of variation; CV, coefficient of variation; Error a, Main plot error; Error b, Sub plot error; TSS, total soluble sugars.

**Table 3.** Mean free proline content (μmol/g FW$^{-1}$) TSS (mg/g DW$^{-1}$), dried flower yield (kg/ha) and essential oil yield (kg/ha) in chamomile plant under different irrigation regimes and spraying treatments

| Irrigation regimes (I) | Spraying (S) | Proline | TSS | Dried flower yield | Essential oil yield |
|---|---|---|---|---|---|
| 50 mm | NS | 26.66 $^{b}$ | 40.82 $^{b}$ | 262.6 $^{ab}$ | 1.53 $^{b}$ |
| | W | 26.49 $^{b}$ | 15.52 $^{d}$ | 281.6 $^{ab}$ | 1.73 $^{ab}$ |
| | MeJA | 25.52 $^{b}$ | 64.18 $^{a}$ | 256.7 $^{ab}$ | 1.68 $^{ab}$ |
| | SA | 29.4 $^{ab}$ | 22.8 $^{c}$ | 262.4 $^{ab}$ | 1.61 $^{ab}$ |
| | HA | 25.21$^{b}$ | 61.57 $^{a}$ | 241.1 $^{b}$ | 1.45 $^{b}$ |
| | GB | 25.11 $^{b}$ | 37.11 $^{b}$ | 264.6 $^{ab}$ | 2.06 $^{ab}$ |
| | GABA | 32.35 $^{a}$ | 21.72 $^{c}$ | 290.5 $^{a}$ | 2.24 $^{a}$ |
| 100 mm | NS | 29.61 $^{c}$ | 26.82 $^{c}$ | 183.3 $^{ab}$ | 1.73 $^{abc}$ |
| | W | 32.02 $^{bc}$ | 29.68$^{bc}$ | 213.3 $^{a}$ | 2.13 $^{ab}$ |
| | MeJA | 33.22 $^{bc}$ | 39.27 $^{abc}$ | 212.7 $^{a}$ | 2.18 $^{a}$ |
| | SA | 31.21 $^{bc}$ | 37.58 $^{abc}$ | 163.5 $^{b}$ | 1.47 $^{c}$ |
| | HA | 34.87$^{abc}$ | 50.09 $^{ab}$ | 207.6 $^{a}$ | 1.97 $^{abc}$ |
| | GB | 35.86 $^{ab}$ | 57.64 $^{a}$ | 173.7 $^{ab}$ | 1.57 $^{c}$ |
| | GABA | 40.71 $^{a}$ | 31.7 $^{bc}$ | 165.7 $^{b}$ | 1.67 $^{c}$ |
| 150 mm | NS | 42.23 $^{a}$ | 75.41 $^{a}$ | 141 $^{bc}$ | 1.24 $^{b}$ |
| | W | 40.32 $^{a}$ | 38.99 $^{c}$ | 107.2 $^{c}$ | 1.15 $^{b}$ |
| | MeJA | 40.56 $^{a}$ | 58.67 $^{b}$ | 203.6 $^{ab}$ | 2.09 $^{ab}$ |
| | SA | 42.43 $^{a}$ | 41.57 $^{c}$ | 182.5 $^{ab}$ | 2.04 $^{ab}$ |
| | HA | 42.95 $^{a}$ | 57.24 $^{b}$ | 183.8 $^{ab}$ | 2.15 $^{ab}$ |
| | GB | 41.25 $^{a}$ | 47.25 $^{c}$ | 191.6 $^{ab}$ | 2.37 $^{a}$ |
| | GABA | 32.55 $^{b}$ | 44.53 $^{c}$ | 222 $^{a}$ | 3.07 $^{a}$ |

Means with different letters in each column are significantly different for each sample ($P<0.05$); FW = fresh weight; DW= dry weight, 50 mm, 100 mm, and 150 mm: 50, 100, and 150 mm of evaporation from class A pan respectively.

NS: No-spraying, W: Distilled water, MeJA: Methyl jasmonate, SA: Salicylic acid, HA: Humic acid, GB: Glycine betaine, GABA: γ-amino butyric acid.

SA, GB, and GABA and subjected to control treatment, showed lower TSS content compared to NS treatment. All osmolytes in combination with control and mild stress treatments had higher TSS content compared to W treatment under same stress levels (Table 3).

### Soluble protein concentration

The treatment of plants with W, MeJA, SA, HA, GB, and GABA increased by 10, 14, 21, 26, 13 and 26%, in terms of soluble proteins respectively, compared to untreated control plants. Among all spraying treatments, GABA, HA, and SA had the greatest effect on the protein content, respectively. The soluble protein concentration of plants treated with GABA, HA, and SA was significantly higher than untreated plants (Figure 2A). Severe stress caused the substantial decline in protein content of chamomile than control treatment (50 mm). Protein concentration gradually declined with the increase of drought stress and the minimum amount of protein content (4.94 mg/gfw$^{-1}$) in chamomile leaves was achieved under severe stress. Severe stress had 47% lower protein content as compared to control (Figure 2B).

### Dried flower yield

The mean comparison for the interaction between the irrigation regimes and spraying showed that the highest value for dried flower yield was 290.5 kg/h, obtained under normal irrigation conditions (control) in plants that treated with GABA (Table 3). Furthermore, the lowest dried flower yield was 107.2 kg/h, which was obtained under severe stress conditions when plants sprayed with W (Table 3).

### Essential oil yield

The mean comparison of the interaction between irrigation and spraying treatments revealed that the chamomile plant had the most and least essential oil yield with averages of 3.07 (kg/h) and 1.15 (kg/h) under severe stress condition and spraying with GABA and W, respectively (Table 3). In all sprayed plants, the essential oil yield was significantly higher than untreated plants and those that received W under severe stress conditions (Table 3).

## Discussion

In the current study, foliar application of all osmolytes significantly improved proline concentration under mild and severe drought stress while the response of proline to GABA application was positive only at mild stress. Jeshni et al (34) found that the proline content of chamomile was enhanced by severe water deficit conditions. The reason for the proline concentration increase under water shortage conditions is protein degradation (35). Increase in proline due to exogenous application of SA under abiotic stress conditions in other plant species has also been reported (36). Anosheh et al (37) revealed that exogenous application of SA increased the levels of free

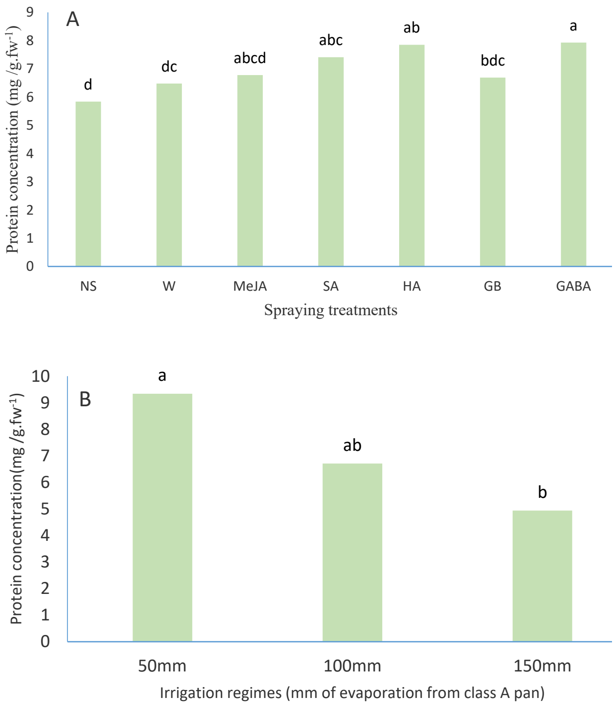

**Figure 2.** The soluble protein concentration of chamomile leaves affected by foliar spray of MeJA, SA, HA, GB and GABA (A) and three irrigation regimes (B). Similar letters are not significantly different according to LSD ($P \leq 0.05$). NS: No-spraying, W: Distilled water, MeJA: Methyl jasmonate, SA: Salicylic acid, HA: Humic acid, GB: Glycine betaine, GABA: γ-amino butyric acid, 50 mm, 100 mm, and 150 mm: 50, 100, and 150 millimeter of evaporation from class A pan, respectively.

proline in wheat cultivars under drought stress. The above results are in agreement with our results. Researchers have reported that γ-glutamyl kinase and proline oxidase are effective in controlling proline levels and environmental stresses in plants (38). The increased proline accumulation after SA application resulted from induced γ-glutamyl kinase activity and inhibited proline oxidase activity (39). In the same trend, under drought stress, in the leaves of processing tomato, the content of proline increased (40). About increasing proline concentration in the plant due to the use of GB, it can be noted in plant cells that the synthesis of proline begins through glucose, GB is formed in the first cycle of 3-phosphoglycerate and serine, when proline synthesis occurs in the final stages of the synthesis of amino acids. GB application and its cellular uptake cause the synthesis of amino acids to produce proline and other amino acids instead of the synthesis of GB (8). For this reason, proline increased with GB application under stress conditions.

HA foliar application under severe stress strongly increased proline concentration. Beheshti et al (41) observed that HA application increased proline content in drought stress conditions in Lima beans. The highest level of proline was achieved at 110 mm evaporation from class A pan and using 6 L/h of HA and its lowest amount of control treatment without HA application, respectively. HA increases the growth rate and the production of biomass in plants by increasing the production of nitrogenous organic compounds, such as protein and amino acids (42). Researchers examined the effects of HA on Echium plant under stress conditions and observed that the highest amount of osmotic regulators such as proline achieved at the severe drought stress and foliar application of 1.5 to 3 lit HA (43), which strongly confirms our results. Miranshahi and Sayyari (44) reported that summer savory plants sprayed with 75 µM MeJA showed the highest proline content in mild and severe stress condition. Similarly, Anjum et al (45) reported that exogenous application of MeJA, extensively increased the level of proline in drought-stressed maize plants compared with control plants and helped maintain the relative water content of leaves.

Our results suggested that GABA treated plants indicate an increasing and decreasing trend of proline content in mild and severe stress conditions, respectively. Shelp et al (46) reported that GABA could act as an osmolyte and also support the synthesis of osmolytes such as proline in water deficit conditions. The positive influence of GABA on the plant growth physiology and various biochemical responses toward abiotic stresses like drought and heat stress has been proved recently in different plant species. Shang et al (47) found that GABA exogenous application significantly increased the amount of endogenous GABA and proline in peach fruit under chilling injury, which resulted from the increased activities of glutamate decarboxylase, Δ1-pyrroline-5-carboxylate synthetase, and ornithine δ-aminotransferase and decreased proline dehydrogenase activity. Li et al (48) reported that the lower proline content of GABA-treated plants could reflect lesser heat-induced damages in creeping bentgrass. Additionally, based on the analysis of metabolic pathways, increased GABA shunt could be the supply of pyruvate and succinic semialdehyde to feed the TCA cycle instead of going to proline metabolism.

Our results demonstrated that drought stress conditions resulted in enhanced levels of TSS in leaves. Dubey and Singh (49) reported that under stress condition the soluble sugars in the plant would be increased for maintaining the osmotic potential of the cell. In addition, plants will be able to store sufficient carbohydrate to maintain basic cellular metabolic.

Results of El-Mageed et al (50) suggested that SA significantly increased the TSS concentration in squash leaves under deficit irrigation stress. These findings are consistent with our findings, which showed that the concentration of soluble sugars with SA spraying increased under both mild and severe stress. Thus, it was concluded that increasing the concentration of salicylic acid-induced soluble sugars can contribute to osmotic regulation, or a substrate to the synthesis of protein and polysaccharide in the root, and thereby for the growth of whole plants

(51). As mentioned previously, the exogenous application of GB increased the concentration of soluble sugars under mild stress. Similar to the results of this study, GB in corn plants under drought stress also increased the content of sugars. The effect of GB on increasing the binding of chloroplast membranes and other cell membranes under stress conditions, as well as increasing leaf area and photosynthetic pigments, can be reasons that induced the increase of soluble sugars content (52).

Our study showed that HA application significantly increased TSS concentration under mild stress conditions, but under severe stress, TSS concentration decreased significantly compared to control and NS treatments. Similar results to our obtained results were obtained by Bakry et al (53) and El-Bassiouny et al (54) on flax plant. The significant increase in TSS content led to the conclusion that the photosynthetic efficiency was increased in response to HA and thus led to enhance biosynthesis of carbohydrates which are utilized in the growth of chamomile plants.

In our experiment MeJA caused increase in TSS concentration under mild stress, however significant decrease in TSS compared to control, NS and W treatments under severe stress observed. Nazarli et al (55) reported that SA and MeJA treatments increased proline content and soluble sugars in both controls and stressed chamomile plants. But, the highest effect of these treatments on compatible solutes was observed in stressed plants. Increasing the amount of proline and sugars in the plants would lead to the resistance against losing water, protect turgor, reduce the membrane damage and accelerate the growth of plants in stress conditions (56).

Awate et al (57) reported that the foliar application of plant growth regulators such as SA and GABA to water-stressed *Simarouba glauca* plants results in an increase in total and reducing sugar content of leaf, stem, and root as compared to unsprayed and well-watered plants. Hare et al (58) reported that accumulation of soluble sugars may be due to osmotic regulation during desiccation tolerance, which plays an important role in plant survival in water conditions. Results of the current study demonstrated significant enhancement of soluble protein concentration as a result of osmolytes spraying on chamomile plants during drought stress. Plants that were treated with GABA, HA, SA, and MeJA had 26%, 25%, 21% and 14% higher protein than untreated plants. Kabiri et al (59) showed that soluble protein concentration increased in *Nigella sativa* plants with 10 μM SA under drought stress. It seems that SA affected the hormonal system that it may well contribute to protective reactions of plants, acceleration of reparative processes, and the effect on protein concentration (60). HA acid improved the mobility and efficiency of the nutrients and increased the amount of zinc and iron in the leaf, as a result, increased photosynthesis, carbohydrate and protein production (61). Spraying with HA in beans increased the protein concentration of the plant by increasing the rate and amount of nutrients uptake (54). GABA application significantly improved protein concentration of *Zea mays* cultivars (Zhengtian 68 and Yuecainuo 2 cultivars) by 11.98 and 5.77%, respectively, at 3 days after treatment as well as significantly improved protein content of (Yuebainuo 6 and Zhengtian 68 cultivars) by 5.07 and 23.07%, respectively, at 7 days after treatment (62).

The response of soluble proteins to the MeJA treatment was similar to that of other osmolytes. Abdelgawad et al. (63) found that MeJA increased the total protein content of *Zea mays* in comparison with the corresponding plants under water stress conditions. These results may be due to the increase in protein synthesis or to the decrease in its degradation. GB application resulted in an increase (up to 12.7%) in the soluble protein content of chamomile leaves. This might be possible that increase and accumulation of endogenous GB could have triggered protein synthesis in chamomile leaves which is also confirmed by Makela et al (64) in tomato leaflets.

Exposing chamomile plants to drought stress led to a substantial decrease in their soluble protein concentration. Reductions in protein concentrations may be one of the symptoms of oxidative stress that are observed repeatedly in plants under drought stress (65). In the two genotypes of chamomile studied by Bączek-Kwinta et al (66) different pattern of changes in the protein content of their leaves observed: during drought the increase in WT(wild-type), while the decrease in C6/2 cultivar is noticed. Presumably, WT plants synthesize various protective protein molecules in leaves whereas the metabolism of C6/2 specimens is“anthesis-oriented” most of the photosynthates are allocated to developing flowers instead of being turned into protective proteins.

Spraying with GB and SA, especially GABA, significantly increased the dried flower yield of chamomile in severe stress compared to other stress levels. The beneficial effects of GB foliar application on the yield of German chamomile have been observed under conditions of water shortage in other plants such as tomatoes (9), corn (67), cotton (68) and wheat (69). Gorham et al (68) expressed the increase in cotton yield as a result of increased plant growth. In their research, it was found that malate content increased by the use of GB, which plays an important role in the photosynthetic cycle, and the formation of assimilates for sustained vegetative and reproductive growth. Gharib (70) reported growth and yield increase due to SA application in basil plants. Research on the use of GABA is very limited on the flower yield of German chamomile. In a study with the use of different concentrations of GABA, the number of male and female flowers in Bitter gourd increased significantly and the highest number of male and female flowers per plant was obtained at concentrations of 1.5 and 2 mg/L (71).

In the present study, essential oil yield in the severe stress level showed a slight increase, which is consistent with other research results. The essential oil yield of the varieties of Proso, Bodegold and *Anthemis tinctoria* cultivars under moderate drought stress (75% field capacity), not only did not decrease but also the percentage of chamazulene increased (72). The same trend as in the present study has also been reported in thyme. So, the most essential oil yield in thyme was obtained in 70% of field capacity and by deviating from this moisture to 90% and 50% of field capacity, essential oil yield decreased (73).

In plants, GABA accumulation is rapidly increased in response to biological and environmental stresses and cause plant resistance to these stresses (74). GABA, by increasing the activity of proline-5-carboxylic acid and suppressing the activity of pyruvate dehydrogenase, results in proline accumulation in the plant. Since proline is one of the most sensitive stress tolerant osmolytes, proline accumulation during stress will maintain cellular structure and prevent cell damage (74). Combination of water stress and growth regulators such as GABA, salicylic acid, Abscisic acid, and Putrescine have induced the synthesis of secondary metabolites in the *Simarouba glauca* plant. Application of such compounds in stress conditions reduces the effects of water stress and induces the synthesis of biologically active compounds and improves the pharmaceutical potential of the plant (75). In the study, the effect of SA on basil and marigold plants was investigated, increasing the quantity and quality of basil essential oil in concentrations of 4 to 10 M was reported (70). Alavi-Samani et al (76) showed that interactions between irrigation regimes and MeJA, MeJA, species and irrigation regimes or species on the bitter gourd essential oil (*Momordica charantia*) were not significant, but the triple interaction of irrigation regimes, MeJA and species were significant. Improvement of essential oil yield with the use of MeJA is probably due to the prolongation of the growth period, increased absorption of nutrients and changes in the number of leaf gland and monoterpenes synthesis (77).

## Conclusion

This research shows the beneficial consumption of elicitors. The proline response in severe and mild stress was positive for HA and GABA application. Compared to other spraying treatments, MeJA increased TSS content in severe and no stress conditions. The highest amount of soluble proteins was obtained with the use of HA, however, the use of GB significantly decreased the level of soluble proteins. The response of essential oil and dried flower yield to exogenous osmolytes application was positive. The effect was more pronounced with GABA treatment. The present study suggests that the applied chemicals appeared to mitigate the negative effects of drought stress on chamomile yield and the biochemical characteristics. This improvement would result from the protecting effect of the provided chemicals on the growth and metabolism of chamomile plants under drought condition.

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## Authors' contributions

FMS conceived and designed the experiments, MAD revised the manuscript, MHF and AP analyzed the data and FMS wrote the paper. All authors read and confirmed the publication of the article.

## Conflict of interest

Authors declared that they have no conflict of interest.

## Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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