

## Phytochemical and Morphological Attributes of Borage (*Borago officinalis*) Affected by Salicylic Acid as an Enhancer

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### Abstract

The objective of this study was to determine the response of borage (*Borago officinalis*) phytochemical and morphological attributes in relation to application of different levels of salicylic acid. Borage planted in pots and salicylic acid was sprayed on the shoots at concentrations of 0, 0.5, 1.0 and 1.5 mM with 6 replications in a completely randomized block design. Morphological attributes such as shoot height, shoot dry matter, flower dry matter, flower weight and shoot wet weight were measured. In the other hand, phytochemical attributes such as ion leakage, chlorophyll *a*, *b*, carotenoid, and antioxidant activity were also investigated under induced stress by different concentrations of salicylic acid. Results showed that some morphological traits such as shoot dry matter (31.23%), flower dry matter (20.43%), flower weight (38.54 g), shoot wet weight (109.43 g), possessed the highest values under treatment of 1.5 mM salicylic acid. Also, some phytochemical traits in plants treated with 1.5 mM salicylic acid reached the highest values during the growth stage with the exception of ion leakage (0.54%). These measured traits with higher values consisted of chlorophyll *a* (4.16 mg.l<sup>-1</sup>), chlorophyll *b* (1.65 mg.l<sup>-1</sup>), total chlorophyll (5.81 mg.l<sup>-1</sup>), carotenoid (1.18 mg.l<sup>-1</sup>), antioxidant activity (53.73%).

**Keywords:** Borage, medicinal plants, morphological, phytochemical attributes, salicylic acid

### Introduction

Borage (*Borago officinalis* L.) is a herbaceous annual plant belonging to the Boraginaceae family (Janick *et al.*, 1989). A recent study proved that the presence of biologically certain active compounds is responsible for the pharmacological activity in dried flowers of borage. Thus, it can be valuable source of inflammation removal substances and promotion of skin regeneration, being is effective in treatment of eczema and wounds (Berti *et al.*, 2012; Marzena *et al.*, 2012).

Plant growth regulators stimulate growth and secondary metabolites' biosynthesis in various aromatic plants (Shukla *et al.*, 1992). Salicylic acid is one of the plant growth regulators, which is involved in various plant physiological processes, such as growth regulation, photosynthesis, flower induction, nutrient uptake, plant water relations and mechanisms of plant resistance and tolerance to biotic and abiotic stresses (Hayat *et al.*, 2010; Popova *et al.*, 1997). Several studies have demonstrated that exogenous salicylic acid application enhances plant growth and development. Al-Shareif (2006) showed that spraying on *Carum carvi* plants with salicylic acid at different concentrations significantly increased vegetative growth parameters, including plant height, branch number and herb dry weights/plant, essential oil percentage and oil yield/plant. Fariduddin *et al.* (2003) reported that mustard plants sprayed with low concentrations of salicylic acid produced

larger amounts of dry matter and had higher photosynthetic rate in comparison with control plants. In another study, the effect of salicylic acid on silymarin; linoleic acid content and lipoxigenase activity in hairy root cultures of *Solanum marianum* treated with salicylic acid was reported. Nivedithadevi *et al.* (2010) also found all plants treated with salicylic acid to have more whole plant fresh weight, dry weight, root length and stem length. They also stated that after the leaves' pigment analysis, chlorophyll, carotenoid, anthocyanin and xanthophylls pigment contents were increased due salicylic acid treatments. In addition, Hussein *et al.* (2011) revealed that growth traits of maize plants were improved as a result of spraying salicylic acid on the plants. Salicylic acid application on corn and soybean also resulted in growth of leaf area and dry weight of plants (Khan *et al.*, 2003; Hayat *et al.*, 2005).

Based on the mentioned reports, application of salicylic acid plays important role in achieving the maximum potential of productivity in crop plants. It can also affect the growth and development of medicinal plants and the accumulation of active substances in their organs (Azhar 2011; HariPriya *et al.*, 2010). While suitable concentration of this growth regulator, in different plants, is known to raise the concentration of secondary metabolites (Selmar, 2008; Zobayed *et al.*, 2007), it also has to be taken into consideration in case of borage medicinal plant.

Despite of studies regarding the response of different plants to exogenous application of salicylic acid in various

conditions, there is no information available about borage response to salicylic acid application.

The aim of this study was to determine the phytochemical and morphological attributes of borage (*Borago officinalis*) in relation to application of different levels of salicylic acid.

## Materials and methods

This research was conducted with the objective of evaluating the effect of various concentrations of salicylic acid on the morphological and phytochemical characteristics of the *Borago officinalis* medicinal plant.

The experiment was arranged in a completely randomized design with four replications. Treatments consisted of various salicylic acid concentrations of: 0, 0.5, 1.0 and 1.5 mM. Spraying was done four times at 15-days intervals. Finally, at the end of the experiment, the attended parameters were measured and recorded.

### Morphological attributes

Plants harvested at full bloom were transferred to laboratory for some morphological attributes record, such as shoot height, shoot wet weight and flower weight. Flowers and shoots were oven dried (Model BM120, Iran) with a temperature of 40 °C for 72 hours and their dry matters were calculated.

### Phytochemical attributes

#### Ion leakage

Ion leakage percentage was calculated after the following phases: two distilled washed leaf discs were put into 10 ml of double distilled water, and after transferring from the overnight holding temperature of 25 °C, the electric conductivity of the solution was measured (Lt). Then sample tubes were put into the autoclave with the temperature of 120 °C for one hour and the recorded electric conductivity expressed as Lo. Finally, the ion leakage was calculated based on the below formula (Simon, 1977):

$$IL = \frac{Lt}{Lo} * 100$$

#### Chlorophyll a, b and carotenoid

Total leaf chlorophyll content (SPAD value) was measured using chlorophyll meter (SPAD 502, Minolta, Japan). A method introduced by Pora *et al.* (2002) was used to measure and calculate the amount of chlorophyll a (665.2 nm) and b (652 nm) and also carotenoids content determined using spectrophotometer (6405 UV/Vis – Jenway, England) and expressed as g per ml via following equations:

$$[Chl a] = 16.29 E 665.2 - 8.45 E 652$$

$$[Chl b] = 30.66 E 652 - 13.58 E 665.2$$

$$[Chl a+b] = 22.12 E 652 + 2.71 E 665.2$$

$$[Carotenoid] = \frac{[(1000 \times A470) - 1.63 \times Chl a - (104.96 \times Chl b)]}{221}$$

Antioxidant activity Antioxidant capacity was determined by neutralizing properties of free radical DPPH (Ebrahimzadeh *et al.*, 2010). 100 ml of the extract was centrifuged and 2900 ml DPPH solution was added.

Mixture was quickly stirred and maintained at room temperature for 15 min in dark conditions. The decrease in absorption was determined at a wavelength of 515 nm using a spectrophotometer (model uv-1800 PC, Japan). Afterwards, the antioxidant capacity of the extracts was calculated and expressed as the inhibition percentage of DPPH.

$$\%DPPHsc = (A_{cont} - A_{samp}) \times 100 / A_{cont}$$

$$A_{samp} = DPPH + \text{Absorption of the sample}$$

$$A_{cont} = \text{Absorption of DPPH}$$

$$\text{Percentage of inhibition} = \%DPPHsc$$

## Results

The ANOVA indicated that all measured attributes of borage plant were significantly affected by the different treatments of salicylic acid, with the exception of shoot dry matter.

### Morphological attributes

#### Shoot height

Plants treated with 1.5 mM SA produced the highest shoot height, so that it reached to maximum as compared to the control, showing a significant difference (Tab. 1).

#### Shoot dry matter

Considering that no significant difference was found between treatments in terms of control plants shoot dry matter, but the control plants possessed the minimum shoot dry matter and plants grew under 1.5 mM SA possessed the maximum ones (Tab. 1).

#### Flower dry matter

The highest flower dry matter was recorded at 1.5 mM SA and show significant difference with control. The plants under 0.5 and 1.0 mM SA followed an ascending trend and had no significant differences with each other, with control nor the highest level of SA (Tab. 1).

#### Flower weight

Flower weight showed a significant and ascending trend from control to 1.5 mM SA, while there was no significant difference between two intermediate levels of SA (Tab. 1).

#### Shoot wet weight

There was no significant difference between the two high levels of SA (1.0 and 1.5 mM), while these levels had significant difference with 0.5 mM SA and the control.

Tab. 1. Effect of different salicylic acid concentrations on different morphological attributes

Salicylic acid (mM)	Attributes				
	Shoot height (cm)	Shoot dry matter (%)	Flower dry matter (%)	Flower weight (g)	shoot wet weight (g)
0	47.26 c	27.49 a	15.97 b	20.34 c	64.72 c
0.5	48.60 bc	28.07 a	17.56 ab	28.83 b	80.11 b
1	52.40 ab	29.23 a	17.80 ab	32.52 b	99.51 a
1.5	54.00 a	31.23 a	20.43 a	38.54 a	109.43 a

Means with the same letters are not significantly different.

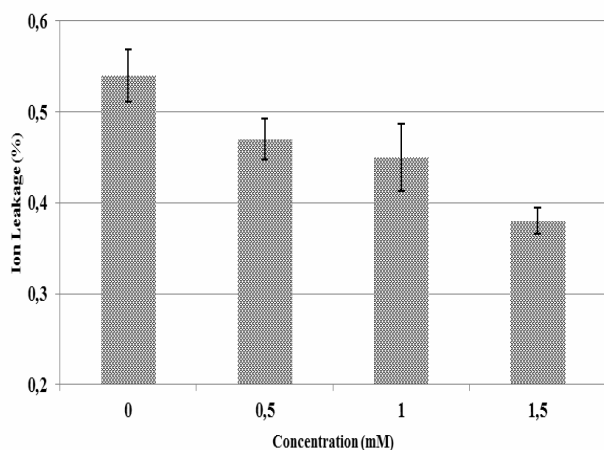


Fig. 1. Effect of different concentrations of SA on ion leakage

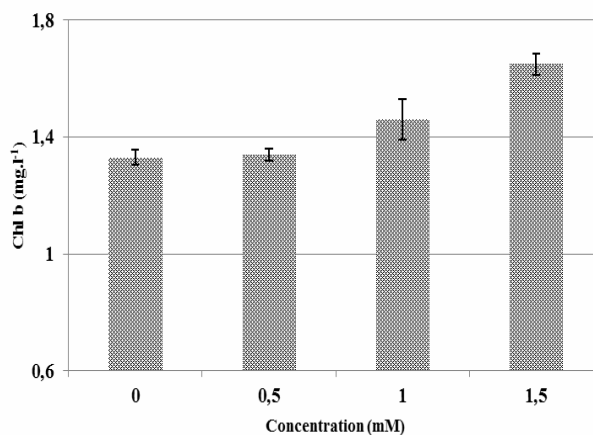
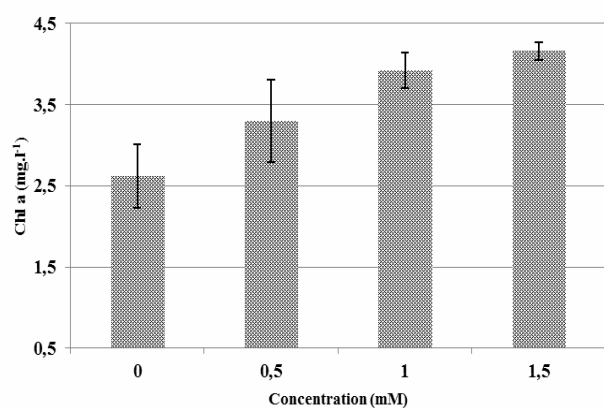
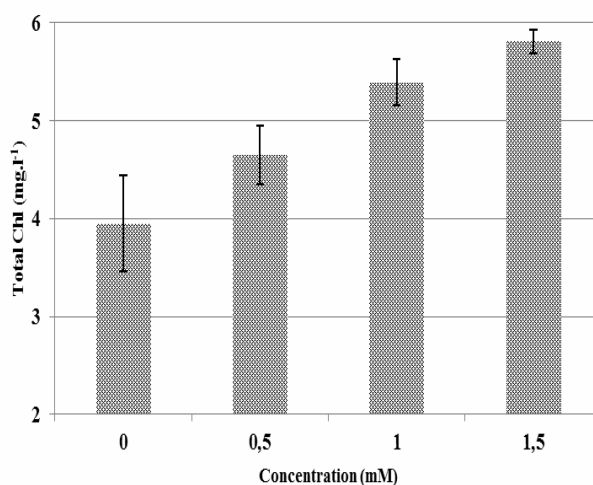
Fig. 3. Effect of different concentrations of SA on *chlorophyll b* contentFig. 1. Effect of different concentrations of SA on *chlorophyll a* content

Fig. 4. Effect of different concentrations of SA on total chlorophyll content

### Phytochemical attributes

#### Ion leakage

As it can be seen in Fig. 1, the ion leakage was significantly affected by various concentrations of SA. The greatest increase in ion leakage (0.54%) was found at control plants and a descending trend was observed toward the high concentration of SA.

#### Chlorophyll a

Although there was neither significant difference between two upper SA concentrations (1.5 and 1 mM) nor between two lower concentrations (1 and 0 mM), an increasing trend of *chlorophyll a* content was recorded. As shown in Fig. 2, the lowest *chlorophyll a* content belonged to the control, while the highest belonged to 1.5 mM SA concentration.

#### Chlorophyll b

No significant difference was observed between control and the lowest concentration (1.0 mM) of SA for *chlorophyll b* content, but a significant difference was recorded between the highest (1.5 mM), the intermediate (1.0 mM) and the lowest concentration of SA (Fig 3).

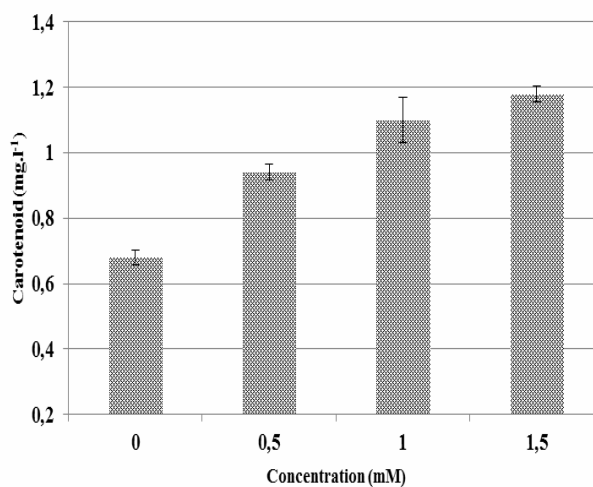


Fig. 5. Effect of different concentrations of SA on carotenoid content

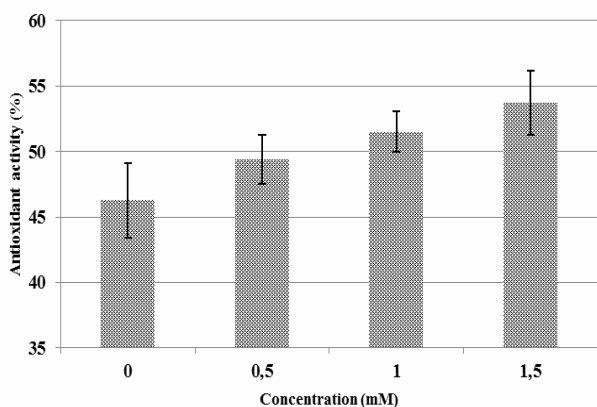


Fig. 6. Effect of different concentrations of SA on antioxidant activity

#### Total chlorophyll

Results showed that samples followed an ascending trend for total chlorophyll from control ( $3.95 \text{ mg.l}^{-1}$ ) to the highest ( $5.81 \text{ mg.l}^{-1}$ ) SA concentration (Fig 4).

#### Carotenoid

The most carotenoid amount was recorded at the highest SA level ( $1.18 \text{ mg.l}^{-1}$ ) and the lowest amount was presented at the control ( $0.68 \text{ mg.l}^{-1}$ ). All levels of SA represented significantly different amounts of carotenoid (Fig 5).

#### Antioxidant activity

There was significant difference between control and plants treated with high concentration of SA for antioxidant activity. Hence, no significant difference between control and plants treated with 0.5 mM SA was observed and the same pattern for plants treated with 1.0 mM and 1.5 mM SA. In the other hand, plants under 0.5 and 1.0 mM SA condition were not significantly different for antioxidant activity (Fig. 6).

#### Discussion and conclusions

As the results showed, some morphological and phytochemical traits in plants treated with 1.5 mM salicylic acid reached the highest values. This growth regulator causes the number of flowers by increasing protein production and isosymes bands formation (Khurama *et al.*, 1992). Salicylic acid also retards senescence and increase the flowering and flower life (Metwally *et al.*, 2003). Plants may act differently facing to stresses; one of them is stress avoid by completing growth cycle and leads to early flowering and seed formation (Hapkinz, 1999). Since salicylic acid is one of the growth regulators produced under stress conditions, it seems that spraying it on plants can control physiological actions such as increment of cell number and size (khan *et al.*, 2003; Popova *et al.*, 2003). These actions are the interaction of salicylic acid and other growth regulators such as auxin and cytokinens (Taiz and Zeiger, 1999). Several reports have been shown the positive effects of salicylic acid; in soy bean and cucumber (Gutierrez-Coronado *et al.*, 1998); odor violet (Hussain *et al.*, 2011). The inductive effects of this hormone on growth can be related to increase

of cell division in meristem zones and also its effects on other growth regulators (Shakirova *et al.*, 2003).

This growth regulator also affects the photosynthetic pigments. Suitable concentrations of salicylic acid inhibit chlorophyll degradation and increases photosynthesis. This action occurs by inhibition of chlorophyll oxidase enzyme activity (Belkhadi *et al.*, 2010). Increment of chlorophyll content improves the photosynthesis and better growth (Hapkinz, 1999). Salicylic acid effect on pigments content depending on its concentration as investigated on oregano, sweet basil and barley (Gharib, 2006; Canakci and Munzuroghlu, 2009). Singh *et al.* (2010) found that application of salicylic acid increased the nitrogen, protein and nitric reductase in cucumber. Based on their report, it is concluded that salicylic acid may increase the chlorophyll content by indirectly increment of nitrate reductase activity and nutrient uptake.

Several factors can affect the carotenoid content such as application of fertilizers or growth regulators. It seems that application of salicylic acid act as a hardening procedure and increase the cell antioxidant activity which leads to cell membrane protection and photosynthetic pigments synthesis and finally improves the growth indexes and secondary metabolites synthesis (Momeny *et al.*, 1391). Duysen and Freman (1975) reported that salicylic acid is a pigments regulator factor under stress condition which plays its role by regulating the ethylene content.

In conclusion, these results could suggest that salicylic acid protects plants against biotic and abiotic stresses and enhance their resistance to damages, so it has had a positive impact on most of the mentioned traits and can be used as one of the promising growth regulators in medicinal plants.

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