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# Influence of Salicylic and Jasmonic acid on Chlorophylls, Carotenes and Xanthophylls contents of Lemon balm (*Melissa officinalis* L.) under Salt stress conditions

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**ABSTRACT: Soil salinity is one of the most important limitations that reduce crop yield in arid and saline irrigation. Water resource solution substances known as salts. Most of the salts exist in water resources are chlorides and sodium. While salinity can improve soil structure, it can also create negatively affects on photosyntetically pigment formation and their survival. This research was carried out in order to investigate the effects of different salinity levels (0, 25, 50 and 75 mM NaCl), salicylic acid (0 and 0.7 mM) and Jasmonic acid (0 and 100 µM) on some pigment contents in Lemon balm (*Melissa officinalis* L.). The results indicated that pigment contents such as chl a, chl b and chl a+b significantly decreased and carotenes and xanthophylls increased against salinity stress. Also application of salicylic acid and Jasmonic acid significantly increased all pigment contents. Under all salinity stress conditions, 0.7 mM salicylic acid and 100 µM Jasmonic acid foliar applications could significantly increase all evaluated photosynthetically pigments. Consequently, it was concluded that synergistic interaction between salicylic acid and Jasmonic acid could reduce the harmful effects of salt stress on Lemon balm efficient pigment contents.**

**Key words**: Salinity, Salicylic acid (SA), Jasmonic acid (JA), Lemon balm (*Melissa officinalis* L.), Pigment contents

## INTRODUCTION

Lemon balm (*Melissa officinalis* L.) belongs to the family Lamiaceae, is among the most often used medicinal plants due to their beneficial health effects. This plant used to give essential oil and extract to different food and beverage products. It has also been applied as a medicinal plant for making different medication of headaches, gastrointestinal disorders, nervousness, rheumatism, skin complaints, wounds and burns, conjunctivitis and poor eyesight, menstrual irregularities, varicose veins, hemorrhoids, duodenal ulcers and etc (Bisset, 2001; Neda *et al.,* 2004; Van Wyk & Wink, 2004).

Jasmonic acid and methyl jasmonate (MeJA), totally called jasmonates, are considered as endogenous regulators that play important duties in regulating stress responses, plant growth and development (Creelman & Mullet, 1997). Concentration of endogenous jasmonates increase during stress conditions such as treatment with elicitors, wound damagings, ozone, deficit and salinity stresses (Kanna *et al.,* 2003; Pedranzani *et al.,* 2007). Jasmonic acid is known as new groups of endogenous growth substances recognized in many crops and medicinal plants genotypes. They influence a wide range of physiological and developmental functions (Parthier *et al.,* 1992). In addition, Moons *et al.,* (1997) demonstrated that jasmonates a contradictory regulate the expression of salt stress inducible proteins, related to salinity in rice. Previous researches indicated that jasmonates compounds have an important duty in signaling drought-induced antioxidant responses, including ascorbic acid (Li *et al.,* 1998; Ai *et al.,* 2008).

Sheteawi (2007) reported that jasmonic acid foliar application on soybean under salt stress conditions reduced the damaging influence of salt and gained to the higher photosynthesis and yield. In addition, Salimi *et al.,* (2012) stated that methyl jasmonate consumption on chamomile under salt stress showed the considerable increase in proline amino acid concentration that leads to cell membrane stability and salt resistance. Carotenoids are the second most abundant naturally occurring pigments on earth, with more than 750 members. carotenoid pigments are mainly C40 lipophilic isoprenoids and synthesized in all photosynthetic organisms (Bacteria, algae, and plants), as well as in some non-photosynthetic bacteria and fungi photosynthesis, photomorphogenesis, photoprotection, and development. These pigments also attending as precursors for two plant hormones and a various collection of apocarotenoids (Nisar *et al.,* 2015). Carotenoids range from colorless to yellow, orange and red, with variations reflected in many fruits, flowers, and vegetables, which contribute to their economic value as well. Several eyecatching examples include carotenes from carrots and sweet potatoes, lycopene from tomatoes and watermelon, capsanthin and capsorubin from red peppers and lutein from marigold products named apocarotenoids are necessary for different biological processes in plants.

The photosystems formation and light harvesting antenna complexes for photosynthesis and photoprotection, and regulation of growth and development (Ruiz-Sola & Rodrı´guez-Concepcio´ na, 2012; Havaux, 2014). Kaydan *et al*., (2007) showed that seed soaking pre-treatment using salicylic acid, positively, affected the osmotic potential, shoot and root dry mass, $K^+/Na^+$ ratio and photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) concentration in wheat tissues, under both salt and non-salt treatments. The xanthophylls act as screening pigments in the retina absorbing damaging blue light, but they may also help to the antioxidant defense of retinal sections (Wrona *et al.,* 2004; Krinsky & Johnson, 2005). Photosynthetic organisms and microalgae use atmospheric carbon dioxide and sunlight to make different nutritional and bioactive substances such as carotenoids, antioxidants, fatty acids and phycobiliproteins (Yen *et al.,* 2013). carotenoids, which are lipophilic pigments with an isoprenoid structure, are synthesized in photosynthetic organisms. Beginning carotenoids act as accessory pigments in light harvesting during photosynthesis. Secondary carotenoids can photoprotect the photosynthetic machinery from surplus light by inhibition reactive oxygen species (Skjånes *et al.,* 2013). carotenoids are the most main groups of whole grain phytochemicals. carotenoids act as antioxidants by quenching single oxygen and free radicals (Liu, 2004) carotenes, a component of photosynthetic reaction centre is accumulated as lipid globules in the triplet-state chlorophyll or by reacting with singlet oxygen (1O2), and also, it acts as a light filter. Besides its physiological duties, carotenes have a wide range of applications (Telfer, 2002). The more outcome of salt stress includes the accumulation of fl-carotene. These physiological responses would appear to function as mechanisms designed to protect the organism against conditions of stress, in special high-intensity irradiation (Ben-Amotz & Avron, 1989). Although other investigator's finding on *D. tertiolecta* Butcher have quoting NaCl tolerance range of 0.05-3 M (Jahnke & White, 2003). The main objective of this study was to investigate the effect of salicylic acid and Jasmonic acid foliar application on photosynthetic pigment concentration changes in leaves of Lemon balm (*Melissa officinalis* L.) against different salt stress conditions.

## MATERIALS AND METHODS

*A. Experimental design*

Due to study the effect of salicylic and Jasmonic acid on chlorophylls, carotenes and xanthophylls contents of Lemon balm (*Melissa officinalis* L.) under salt stress conditions, a green house pot culture experiments were conducted during 2013- 2014 in Islamic Azad University, Yadegar-e-Imam Khomeini (RAH) Shahre-rey Branch.

The experimental design was as factorial based on completely randomized design (CRD) with 4 replications. The salinity levels (0, 25, 50 and 75 mM NaCl), salicylic acid (0 and 0.7 mM) and Jasmonic acid (0 and 100 µM) were considered. The ratio 3:1:1 of sand, clay and manure fertilizer were used as media. Lemon balm seeds were obtained from Iranian Institute of medicinal Plant, Alborz, Iran. Seeds were disinfected in 3% (v/v) of formaldehyde for 4 minutes and washed with distilled water for 5 times to prevent fungal infection. The seeds were planted directly in 7 kg soil capacity pots. To avoid loss of nutrients plants trace elements out of the pots, plastic plate were placed under each pot and the collected leaches were put back to experimental pots.

*B. Chlorophyll content*

Total chlorophyll concentration is a unifying parameter for determine the effect of specific interventions. Concentrations of Chl a and Chl b and total chlorophyll (Total Chl = Chl a + Chl b) were identified by spectrophotometry.

According to the Lichtenthaler and Welburn (1983). They offered the following equations to determine chlorophyll a and chlorophyll b concentrations in 80% acetone extracts:

Chlorophyll a (µg/ml) = 12.21 (A663) - 2.81 (A646)
Chlorophyll b (µg/ml) = 20.13 (A646) - 5.03 (A663)

*C. Carotenes and xanthophylls content*

Carotenes and xanthophylls contents were determined by absorbance of xanthophylls fraction at 445 mµ and carotene fraction at 445 and 451 mµ. For facility, normal hexane can be used as a blank for all absorbance measurements without introducing significant error. Evaluation amounts of pigments in total extract, xanthophylls extraction and carotenes extraction as follows (Blessin, 1962):

Pigment, ppm = A × V ×1000/a × L × W

A: Absorbance, V: Volume, L: Cell pass in cm, W: Sample weight (g),

*D. Absorptivity in carotenoids*

Carotenes are recalculated as equivalent xanthophylls to adjust all values to the same wave length and reference standards (Blessin, 1962).

**Table 1: Calculations are based on the fallowing wave lengths and absorptivity.**

| Pigment | Wavelength (*mµ*) | Absorptivity (*L/g- cm*) |
|---|---|---|
| Total pigments | 445 | 231 |
| Xanthophylls | 445 | 231 |
| Carotenes | 451 | 251 |
| Carotenes | 445 | 231 |

## RESULTS

As shown in Table 2, analysis of variance generally showed a significant effect of the salinity, salicylic acid and jasmonic acid for all experimated characters. The salt stress concentration was found to have inhibitory effects on all evaluated chlorophylls content of fully mature leaves. So increase in salinity concentration to 75 mM caused a significant reduction in chl a (12.12 µg/ml), chl b (5.98 µg/ml) and chl a+b (18.10 µg/ml) and meaningful Increasing in carotenes (71.05 Absorbance/g Fw) and xanthophylls (2.11 Absorbance/g Fw) (Table 3). Therefore the different salt levels were located in different statistical groups. The simple effects of salicylic acid on examined traits were demonstrated that after its consumption during lemon balm growth stages, all evaluated traits significantly changed, so the chl a (24.45 µg/ml), chl b (13.61 µg/ml), chl a+b (38.06 µg/ml), Carotenes (46.39 Absorbance/g Fw) and Xanthophylls (1.70 Absorbance/g Fw) increased (Table 3). Simple effect of jasmonic acid on main and accessory photosynthetically pigments content revealed that all experimented traits had significantly changed (Table 2). In this case, the chl a, chl b, chl a + b, Carotenes and Xanthophylls increased 14.69%, 15.31%, 14.93%, 9.67% and 1.80% compare to control alternatively. The results confirmed that salicylic acid foliar application was more impressive on pigment contents than jasmonic acid (Table 3). The findings revealed that only double interaction effects of salinity and SA and salinity and JA on carotenes were significant. In such circumstances, the highest carotenes were obtained in 75 mM salinity and 0.7 mM SA (77.82 Absorbance/g Fw) and 75 mM JA (75.19 Absorbance/g Fw) application (Tables 4 and 5). Simultaneous spraying of SA and JA showed synergistic increasing percent effect compared to control for carotenes (40.74%) and xanthophylls (7.10%).

**Table 2: Analysis of variance for experimental traits of lemon balm.**

| S.O.V. | d.f. | Mean of Square (M.S.) | | | | |
|---|---|---|---|---|---|---|
| | | Chl a | Chl b | Chl a+b | Carotenes | Xanthophylls |
| **Salinity (S)** | 3 | 1370.3** | 13.35** | 28.46** | 11075.7** | ** 2.62** |
| **Salicylic acid (SA)** | 1 | 442.5** | 3.30** | 8.08** | 1355.68** | 0.13* |
| **Jasmonic acid (JA)** | 1 | 174.6** | 1.23** | 3.30** | 467.45** | 0.04ns |
| **S × SA** | 3 | 22.6ns | 0.07 ns | 0.13ns | 289.27** | 0.06ns |
| **S × JA** | 3 | 8.50 ns | 0.02 ns | 0.05ns | 90.28* | 0.01ns |
| **SA × JA** | 1 | 13.99 ns | 0.38 ns | 0.55ns | 55.21ns | 0.01ns |
| **S× SA × JA** | 3 | 0.48 ns | 0.003 ns | 0.001ns | 8.70ns | 0.003ns |
| **Error** | 48 | 10.17 | 0.12 | 0.19 | 30.20 | 0.02 |
| **CV (%)** | - | 14.61 | 10.44 | 7.76 | 13.13 | 9.87 |

ns, * and **: No significant and significant at 5% and 1% level of probability respectively

**Table 3: Simple effects of Salinity, Salicylic and Jasmonic acid on pigment contents of lemon balm.**

| Treatments | | Chl a (µg/ml) | Chl b (µg/ml) | Chl a+b (µg/ml) | Carotenes (Absorbance/g Fw) | Xanthophylls (Absorbance/g Fw) |
|---|---|---|---|---|---|---|
| **Salinity (mmol)** | 0 | 33.74a | 12.64 a | 54.38 a | 9.63 d | 1.19 d |
| | 25 | 23.88 b | 13.09 b | 36.97 b | 33.90 c | 1.47 c |
| | 50 | 17.59 c | 8.67 c | 26.26 c | 52.81 b | 1.85 b |
| | 75 | 12.12d | 5.98 d | 18.10 d | 71.05 a | 2.11 a |
| **Salicylic acid** | Non application | 19.21 b | 10.58 b | 29.80 b | 37.30 b | 1.61 b |
| | Application | 24.45 a | 13.61 a | 38.06 a | 46.39 a | 1.70 a |
| **Jasmonic acid** | Non application | 20.15 b | 11.12 b | 31.27 b | 39.787 b | 1.64 a |
| | Application | 23.62 a | 13.13 a | 36.76 a | 44.05 a | 1.67 a |

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%

**Table 4: Mean comparison of Salinity and Salicylic acid effects on pigment contents of lemon balm.**

| Salicylic acid | Salinity(mM) | Chl a (µg/ml) | Chl b (µg/ml) | Chl a+b (µg/ml) | Carotenes (Absorbance/g Fw) | Xanthophylls (Absorbance/g Fw) |
|---|---|---|---|---|---|---|
| **Non application** | 0 | 29.60 a | 17.82 a | 47.45 a | 11.35 f | 1.24 a |
| | 25 | 20.82 a | 11.62 a | 32.45 a | 27.80 e | 1.41 a |
| | 50 | 15.91 a | 7.74 a | 23.66 a | 45.75 c | 1.75 a |
| | 75 | 10.52 a | 5.12 a | 15.64 a | 64.29 b | 2.03 a |
| **Application** | 0 | 37.88 a | 23.45 a | 61.31 a | 7.90 f | 1.14 a |
| | 25 | 26.94 a | 14.56 a | 41.50 a | 39.99 d | 1.53 a |
| | 50 | 19.27 a | 9.60 a | 28.87 a | 59.86 b | 1.94 a |
| | 75 | 13.72 a | 6.84 a | 20.56 a | 77.82 a | 2.19 a |

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%

**Table 5: Mean comparison of Salinity and Jasmonic acid effects on pigment contents of lemon balm.**

| Jasmonic acid | Salinity (mM) | Chl a (µg/ml) | Chl b (µg/ml) | Chl a+b (µg/ml) | Carotenes (Absorbance/g Fw) | Xanthophylls (Absorbance/g Fw) |
|---|---|---|---|---|---|---|
| **Non application** | 0 | 31.18 a | 19.03 a | 50.18 a | 10.47 g | 1.21 a |
| | 25 | 21.93 a | 12.11 a | 34.03 a | 30.00 f | 1.44 a |
| | 50 | 16.80 a | 8.37 a | 25.19 a | 50.40 d | 1.82 a |
| | 75 | 11.11 a | 5.33 a | 16.44 a | 66.91 b | 2.06 a |
| **Application** | 0 | 36.29 a | 22.25 a | 58.59 a | 8.79 g | 1.17 a |
| | 25 | 25.82 a | 14.08 a | 39.90 a | 37.79 e | 1.51 a |
| | 50 | 18.61 a | 9.04 a | 27.65 a | 55.90 c | 1.88 a |
| | 75 | 13.13 a | 6.64 a | 19.76 a | 75.19 a | 2.16 a |

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%

**Table 6: Mean comparison of Salicylic acid and Jasmonic acid effecton pigment contents of Lemon balm.**

| Salicylic acid | Jasmonic acid | Chl a (µg/ml) | Chl b (µg/ml) | Chl a+b (µg/ml) | Carotenes (Absorbance/g Fw) | Xanthophylls (Absorbance/g Fw) |
|---|---|---|---|---|---|---|
| **Non application** | Non application | 17.08 a | 19.19 a | 26.27 a | 33.65 a | 1.57 a |
| | Application | 23.04 a | 12.95 a | 35.97 a | 45.54 a | 1.71 a |
| **Application** | Non application | 21.34 a | 11.96 a | 33.32 a | 40.94 a | 1.65 a |
| | Application | 26.05 a | 14.37 a | 40.43 a | 47.36 a | 1.69 a |

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range Test at 5%

## DISCUSSION

The results showed that salt stress significantly reduced chl a, chl b, chl a+b and decreased Carotenes and Xanthophylls. As the matter of fact salinity at vegetative and generative growth stages affects leaf pigment contents of Lemon balm. The changes in various photosynthetic pigments of low and middle salt adapted plants as lemon balm confirmed, there was considerable changes in chlorophylls and some carotenoids elements content in investigated plant. So it seems that in terms of the xanthophylls cycle pigments, there was change in the pool size of the xanthophylls cycle (Amira & Abdul Qados. 2011). Chlorophylls content changes was the same but its decrease slop in Chl a was more than Chl b followed by salt stress intensity after 21 days of treatment.

The chlorophyll contents are sensitive to salt exposure and a decline in chlorophylls due to salinity has been reported in various plants, such as pea (Ahmad and Jhon 2005), wheat (Ashraf *et al.,* 2002), rice (Anuradha and Rao 2003), and tomato (Al-Aghabary *et al.,* 2004). Diminish in chlorophylls content is possibly depends on deterrent effect of the accumulated ions of different salts on the biosynthesis of the different chlorophyll sections.

Salinity significantly approved carotenes and xanthophylls concentration and the salinity levels located in different statistical groups. Qingtao & Congming (2004) stated that a relative enrichment in lutein and zeaxanthin was observed in particular at the late stage of grain filling. So Carotenes and violaxanthin decreased faster than chlorophyll. This notable decrease in chlorophylls and improve in carotenes and xanthophylls found in this study as a result of the treatment with increased concentrations of sodium chloride, could be explained by the negative effect of salt on photosynthesis that leads to the reduction of plant growth, leaf growth, and chlorophyll content (Netondo *et al.,* 2004). It was mentioned by Jamil *et al.,* (2007 a, b) that increased concentrations of sodium chloride (zero, 50, 150 mM) increased the total chlorophyll content of sugar cane leaves (*Beta vulgaris* L.), and that it was a significant increase. However, there have been other findings indicating that salinity in the presence of high PPFD may stimulate significant pigment composition changes in sorghum (Sharma & Hall, 1992). The only photosynthetic pigment change found in our experiments is that salinity may induce in barley leaves a quite small increase in the total chlorophyll and other pigments (Abadia *et al.,* 1999). On the other hand, our results also suggest that there were some differences in the changes in the photosynthetic pigment composition. For example carotenes and xanthophylls increased in fully maturation leaves. SA and JA showed synergistic effects on chlorophylls as main photosynthetically pigments and carotenes and xanthophylls as antenna pigments and until stress substances. It was observed that in response to chlorophylls diminish fallowing salinity intensity; carotenes and xanthophylls increase to prevent descent of photosynthesis and bioprocess for secondary metabolite production and can explain the bioprocess. Cho *et al.,* (2008) explained the synergistic effects of sequential treatment with methyl jasmonate (MJ), salicylic acid (SA) and yeast extract (YE) on benzophenanthridine alkaloid accumulation and protein expression in *Eschscholtzia californica* too.

## CONCLUSION

Salinity affects differently early growth stages of plants. This abiotic stress has both osmotic and specific ion effects on plant growth (Dionisio-Sese & Tobita 2000). Application of SA and JA against salt-stressed plant showed significantly improved chlorophyll concentrations; however, their combined application (SA + JA) was clearly more efficient in mitigating the adverse effects of salinity on total chlorophylls and carotenoids in lemon balm. This effect of SA application on photosynthetic pigments was already demonstrated (Arfan *et al.,* 2007; Wasti *et al.,* 2012) but the present study indicates that such an effect be increased indirectly via SA and JA. Photosynthetic pigments and Na demonstrated an opposite trend. It may be concluded that SA and JA reduces the harmful effect of salt stress in lemon balm plants.

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