Effect of vermicompost fertilizer on antioxidant enzymes and chlorophyll contents of *Borago officinalis* under salinity stress

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Abstract

In order to investigate the effects of vermicompost on antioxidant enzymes and chlorophyll contents of borage (*Borago officinalis*) grown under salinity, a factorial experiment was conducted based on a completely randomized design with three replications. Treatments included vermicompost (0, 6, 12, and 18% of w/w soil) and four levels of salinity (0 (control), 4, 8, and 12 dS m^{-1} NaCl). As salinity was increased, the activity of antioxidant enzymes (catalase, superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, and malondialdehyde) increased significantly while photosynthetic pigments decreased. The use of vermicompost caused a significant increase in chlorophyll$_b$, carotenoids and malondialdehyde contents. The maximum activities of antioxidant enzymes were obtained in the treatment with vermicompost (18%) at salinity level of 12 dS m^{-1} and the highest amount of chlorophyll$_a$ and total chlorophyll were obtained in the treatment with 18% vermicompost and non-salinity stress (control). Therefore, the use of vermicompost as an organic fertilizer, in addition to increasing the activity of antioxidant enzymes and the amount of photosynthetic pigments, could be a good strategy to reduce the negative effects of high concentrations of sodium and chlorine ions in soils on borage (*Borago officinalis*).

Keywords: Ascorbate peroxidase; catalase; chlorophyll; fertilizer; salinity

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Introduction

Borage (*Borago officinalis* L., Boraginacea) has medicinal, industrial, and forage properties (Berti et al., 2010). Some studies have reported tolerance to salinity and high uptake of solutes by this plant (Naghdi and Sorooshzadeh, 2011). The leaves and flowers of this plant are used as a transpirant, sedative, and blood purifier (Mahmoodi et al., 2018). Chemotaxonomic studies on borage have shown that the seeds of this plant contain gamma-linolenic acid (GLA) which is used for heart disease, skin inflammation, diabetes, and arthritis (Karami and Sepehri, 2015). Borage is able to tolerate salinity through the accumulation of sodium and chlorine in its tissues (Sajirani et al., 2011).

Salinity is one of the most important non-biotic stresses (Hernandez, 2019). It causes the destruction of the lipid membranes of the cells. The content of malondialdehyde (MAD) produced as a result of the destruction of the lipid membranes can be considered as an indicator of oxidative stress under stress condition (Bagheri and Khosravinejad, 2016). Shirazi et al. (2005) reported that salinity stress caused an increase in

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sodium accumulation and decrease in chlorophyll and photosynthesis in leaves of salt-sensitive cultivars in wheat. Farhoudi (2014) reported that salinity stress significantly decreased chlorophyll$_a$ and chlorophyll$_b$ of wheat seedlings.

The use of chemical fertilizers in agricultural lands has caused environmental problems such as contamination of water resources, diminished quality of agricultural products, and reduced fertility of the soils (Chaulagain et al., 2017). In the production of medicinal plants, in addition to the quantity of production, it is necessary to pay attention to the quality of production, which is of particular importance (Chiyaneh, 2014). Medicinal plant active ingredients may respond positively or negatively to fertilizers, which is a need for more accurate nutritional studies (Shirzad et al., 2011).

Soil salinity is one of the main environmental stresses affecting plant growth and their yield (Yassin et al., 2019). Various studies have shown that oxidative stress resistance is highly correlated with salinity stress in plants, in other words, salt tolerant cultivars exhibit better antioxidant activity against oxidative stress (Pakar et al., 2016). Research shows that plants that have higher antioxidant capacity than other plants are better able to grow in saline environments (Alhasnawi et al., 2014).

In sustainable agriculture, application of vermicompost enhances the population and activity of soil-beneficial microorganisms such as mycorrhizal fungi and phosphate solubilizing microorganisms and also increases the nutrients required for the plant such as nitrogen, phosphorus, and potassium, improving the growth and yield of crops (Koozehgar Kaleji and Ardakani, 2017). In an experiment on borage, fertilizer treatments with two levels of 20 and 40 tons ha^{-1} of vermicompost showed that increasing the vermicompost content increased the concentration of nutrients and increased plant growth and yield (Abadi et al., 2012). In another study on the effect of different levels of vermicompost (0, 5, and 10 tons ha^{-1}) on the quantitative and qualitative characteristics of *Matricaria chemmomilla*, it was observed that the maximum height, fresh, and dry weight of the plants were obtained with 10 ton ha^{-1} vermicompost (Seyyed Hadi et al., 2013). Vermicompost in the saline environment could greatly reduce the negative effects of salinity stress on the growth of *Tamarindus indica* (Oliva et al., 2008). Due to the salinity of much of the Azarbaijan lands in Iran, the lack of fresh water resources and the importance of borage as a salinity-tolerant medicinal plant, and considering the potential of vermicompost fertilizer to improve soil physical and chemical conditions and its role in reducing the deleterious effects of salinity stress on plant growth, this study investigated the effect of vermicompost fertilizer on antioxidant enzymes activity and photosynthetic pigments in borage (*Borago officinalis*) under salinity stress.

Materials and Methods

To evaluate the effect of salinity and application of vermicompost on the activity of antioxidant enzymes and photosynthetic pigments of borage (*Borago officinalis*), a factorial experiment based on a completely randomized design with three replications was conducted at the Agricultural Research Station of Miandoab Azad University in Iran during 2018. Treatments

Table 1
Soil properties before plant sowing

Silt (%)	Clay (%)	Sand (%)	Total N (%)	K (mg kg^{-1})	P (mg kg^{-1})	OC (%)	EC (dS m^{-1})	pH
42.5	27.3	30.2	0.08	223.7	11.6	0.8	1.5	7.3

Table 2
Specifications of vermicompost

Specifications	Moisture (%)	K (mg kg^{-1})	P (mg kg^{-1})	Mn (mg kg^{-1})	Na (mg kg^{-1})	OC (%)	EC (dS m^{-1})
Vermicompost	27.4	0.9	0.7	0.83	473	20.7	5

included three levels of vermicompost fertilizer containing 0, 6, 12, and 18 (weight percentage of pot soil based on dry weight) and four levels of salinity including 0 (control), 4, 8, and 12 dS m^{-1} NaCl. Distilled water was used in non-stress salinity condition (control). The physical and chemical properties of soil and vermicompost are listed in Tables 1 and 2, respectively. The experiment was carried out under controlled thermal and optical conditions, so that day and night temperatures were adjusted to 25 and 18 °C, respectively. The amount of vermicompost was calculated for each pot and mixed with pot soil before planting. Seed planting was conducted on 25 March in 2018. Each test unit consisted of a pot 16 cm high and 9 cm in diameter and four seeds were planted in each pot which was reduced to two seedlings after ensuring that they were greening (Meloni et al., 2008, Khan et al., 2016). Salt stress was applied about eight weeks after planting (stages of six to eight leaves).

Photosynthetic pigments were measured by the amount of light absorbed by the extract obtained from the leaf by a spectrophotometer (Cary 300, USA) at a wavelength of 663 and 645 nm (Wang et al., 2012).

$Chl_a = (19.3\times A_{663} - 0.86\times A_{645})\ V/100W$
$Chl_b = (19.3\times A_{645} - 3.6\times A_{663})\ V/100W$
$Chl_T = Chlorophyll_a + Chlorophyll_b$
$Carotenoides = 100(A_{470}) - 3.27(mg\ chl_a) - 104(mg\ chl_b)/227$
V = volume of sample extracted
A = light absorption at 663, 645 and 470 nm wavelengths
W = fresh sample weight
$Chl_a = Chlorophyll_a$
$Chl_b = Chlorophyll_b$
Chl_T = Total Chlorophyll

Catalase (CAT)

Catalase activity was determined by calculating the decrease in H_2O_2 uptake at 240 nm using Chandlee and Scandalios (1984) method.

Superoxide dismutase (SOD)

Assessment of superoxide dismutase activity was performed by the method of Giannopolitis and Ries (1997) with nitro blue tetrazolium (NBT) photo-reduction inhibition assay at 560 nm.

Ascorbate peroxidase (APX)

First, 2 g fresh leaf tissue was weighed and then, four cc ice-cold extraction buffer was added and completely powdered in mortar before it was centrifuged at 16,000 rpm for 15 minutes. Then the upper phase of the centrifuged solution as a protein extract was used to measure the enzymatic activity. Finally, the activity level of ascorbate peroxidase (APX) was measured by the method of Nakano and Asada (1981).

Glutathione peroxidase (GPX)

To measure glutathione peroxidase enzyme, leaves were transferred to the laboratory, washed with distilled water, and immediately inserted in a Tris buffer of 16.0 M at pH = 7.5 before they were crushed. Then the membrane and cell wall digestion process was carried out with the same volume of buffer containing digitonin (wall digestive enzyme). Afterwards, 0.5 ml of the homogenate solution was removed for protein assay and the amount of protein was determined in the form of ml per mg. Finally, in the residue of the extract solution, the amount of glutathione enzyme was measured by Holy (1972) method.

Malondialdehyde (MDA)

In this method, 0.2 g fresh leaf tissue was weighed and crushed with 5 ml of trichloroacetic acid (TCA). The extract was centrifuged for 5 minutes using a centrifuge machine. To one ml of the supernatant obtained from the centrifuge solution, 5.4 ml of trichloroacetic acid (TCA) solution containing 5 g of thiobarbituric acid (TBA) was added. The mixture was centrifuged at 4000 rpm for 10 minutes. The absorbance intensity of this solution was read with a spectrophotometer at 532 nm; then, the amount of malondialdehyde was obtained by Stewart and Bewley (1980) method.

SAS software was used for statistical analysis of the data and mean comparisons were

made using Duncan's multiple range test at 5% level.

Table 3
Analysis of variance of biochemical traits on *Borago officinalis*

S.V	df	GPX	CAT	SOD	APX	MDA
Salinity stress	3	5.28**	37.54**	392.91**	30.67**	6.80**
Vermicompost	3	11.42**	20.61**	407.68**	54.13**	7.12**
Salinity stress× Vermicompost	9	2.37*	9.41**	246.04**	9.24*	5.07^{ns}
Error	45	0.92	2.46	78.27	4.21	0.75
C.V		9.31	10.25	8.63	7.49	10.71

*, **, and ns, significant at the 5% and 1% probability levels and non-significant, respectively.

Table 4
Mean comparison of combined treatment of salinity and vermicompost fertilizer on *Borago officinalis* properties

Salinity stress (dS m^{-1} NaCl)	Vermicompost (weight percentage)	Chlorophyll$_a$ (mg g^{-1} FW)	Total Chlorophyll (Mg g^{-1} FW)	APX (U mg^{-1} Protein)	GPX (U mg^{-1} Protein)	CAT (U mg^{-1} Protein)	SOD (U mg^{-1} Protein)
0	0	7.83 d	11.57 ef	0.0058 i	0.0019 ijk	0.0014 fgh	0.0899 i
	6	10.54 bc	15.63 cd	0.0069 hi	0.0023 hijk	0.0026 efg	0.1031 hi
	12	12.38 ab	18.57 b	0.0089 gh	0.0029 hi	0.0050 cdef	0.1376 ghi
	18	14.67 a	21.83 a	0.0106 fgh	0.0036 ghi	0.0068 bcde	0.1647 fghi
4	0	8.21 cd	12.17 ef	0.0228 efg	0.0078 gh	0.0042 defg	0.3534 fgh
	6	10.73 bc	16.19 c	0.0453 def	0.0155 egh	0.0056 cde	0.7001 efg
	12	12.45 ab	18.65 b	0.0733 cde	0.0254 deg	0.0075 bcd	1.1346 de
	18	12.55 ab	18.82 b	0.0873 cd	0.0300 cdef	0.0081 bcd	1.3493 cde
8	0	5.92 e	8.70 g	0.0683 cde	0.0235 deg	0.0045 cdef	1.0553 def
	6	8.31 cd	12.30 ef	0.0927 bcde	0.0320 cde	0.0064 bcd	1.4326 cde
	12	9.07 c	13.22 de	0.1013 bcd	0.0359 bcde	0.0085 bcd	1.5651 cd
	18	11.82 b	17.74 bc	0.1093 bcd	0.0376 bcde	0.0097 bc	1.6892 bcd
12	0	2.65 g	3.97 i	0.1327 bcd	0.0457 bcd	0.0068 bcd	2.0512 bc
	6	3.32 fg	4.95 hi	0.1582 bc	0.0545 bc	0.0086 bcd	2.4457 bc
	12	4.45 ef	6.68 gh	0.1883 b	0.0650 b	0.0102 bc	2.9112 b
	18	5.73 e	8.60 g	0.3280 a	0.1190 a	0.0202 a	5.0678 a

Means with similar letters in each column are not significantly different.

Results

Effect of salinity and vermicompost fertilizer on antioxidant enzymes activity

Results of analysis of variance showed that simple effects of salinity and vermicompost stress on antioxidant enzymes activity were significant; also, the interaction effects of treatments on catalase, superoxide dismutase, ascorbate peroxidase, and glutathione peroxidase were significant but for malondialdehyde, the interaction of these two factors was not significant (Table 3).

Interaction of salinity and vermicompost on the activity of superoxide dismutase, catalase, glutathione peroxidase, and ascorbate peroxidase showed that the highest superoxide dismutase enzyme (5.0678 U mg^{-1} protein), catalase (0.0202 U mg^{-1} protein), glutathione peroxidase (0.1190 U mg^{-1} protein), and ascorbate peroxidase (0.3280 U mg^{-1} protein) were obtained in the treatment with 12 dS m^{-1} NaCl and vermicompost (18%) (Table 4). Increasing salinity caused a significant increase in malondialdehyde so that the percentage of increase of malondialdehyde in salinity treatment of 12 dS m^{-1} compared to non-salinity treatment was 94.87% (Table 6). Also, the increase in vermicompost fertilizer caused a significant increase in the malondialdehyde content so that the percentage of increase of malondialdehyde in

vermicompost (18%) compared to the control (non vermicompost application) was 88.46% (Table 7).

salinity treatment of 12 dS m^{-1} (Table 6). Interaction effect of salinity and vermicompost treatments showed the highest amount of

Table 5
Analysis of variance of photosynthetic pigments of *Borago officinalis*

S.V	df	$Chlorophyll_a$	$Chlorophyll_b$	Total Chlorophyll	Carotenoid
Salinity stress	3	5.49^{**}	3.65^{**}	9.05^{**}	1.08^{**}
Vermicompost	3	2.77^{**}	1.72^{**}	4.24^{**}	0.39^{**}
Salinity stress× Vermicompost	9	0.69^{*}	0.4^{ns}	5.79^{**}	0.12^{ns}
Error	45	0.32	0.29	0.76	0.08
C.V		6.26	7.89	3.01	8.36

*, **, and ns: significant at the 5% and 1% probability levels and non-significant, respectively

Table 6
Mean comparison of physiological and biochemical traits of *Borago officinalis* under salinity stress

Salinity stress (dS m^{-1} NaCl)	MDA (μ mol g^{-1} FW)	$Chlorophyll_b$ (mg g^{-1} FW)	carotenoid (mg g^{-1} FW)
0	0.0039 d	5.54 a	1.59 a
4	0.0053 c	5.47 ab	1.25 ab
8	0.0061 b	4.31 b	1.03 b
12	0.0076 a	2.02 c	0.27 c

Means with similar letters in each column are not significantly different.

Table 7
Mean comparison of physiological and biochemical traits on *Borago officinalis* under Vermicompost fertilizer

Vermicompost (weight percentage)	MDA (μ mol g^{-1} FW)	$Chlorophyll_b$ (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
0	0.0026 d	2.95 c	1.34 d
6	0.0031 c	4.04 b	1.72 c
12	0.0042 b	4.7 ab	2.16 b
18	0.0049 a	5.56 a	2.35 a

Means with similar letters in each column are not significantly different.

Effect of salinity and vermicompost fertilizer on chlorophyll contents

Analysis of variance showed that the effect of salinity and vermicompost on photosynthetic pigments was significant at 1% probability level and the interaction of these two factors was significant for total chlorophyll ($p \leq 0.01$) and $chlorophyll_a$ ($p \leq 0.05$) while it was not significant for $chlorophyll_b$ and carotenoids (Table 5). With increase in salinity levels, $chlorophyll_b$ and carotenoids decreased significantly. The highest $chlorophyll_b$ and carotenoids contents were 5.02 and 1.59 mg g^{-1} FW in non-salinity stress treatment, respectively and the lowest mean $chlorophyll_b$ and carotenoids were obtained as 0.49 and 0.27 mg g^{-1} FW, respectively, in the $chlorophyll_a$ and total chlorophyll as 14.67 and 21.83 mg g^{-1} FW obtained from non-salinity stress and vermicompost (18%), respectively. The lowest amount of $chlorophyll_a$ and total chlorophyll obtained as 2.65 and 3.97 mg g^{-1} FW in the treatment with 12 dS m^{-1} NaCl and non vermicompost application (Table 4).

Application of vermicompost fertilizer compared to the control (no vermicompost application) significantly increased the levels of $chlorophyll_b$ and carotenoids. The highest amounts of $chlorophyll_b$ and carotenoids were obtained as 7.49 and 2.35 mg g^{-1} FW from vermicompost (18%), respectively and the lowest amounts of $chlorophyll_b$ and carotenoids were obtained as 4.79 and 1.34 mg g^{-1} FW in the non

vermicompost fertilizer treatment, respectively (Table 7).

Discussion

Iran is located in arid and semi-arid regions of the world, so soil salinity and irrigation water salinity are major problems facing the country's agriculture (Bagheri and Khosravinejad, 2016). Water and soil salinity cause morphological, physiological, and biochemical changes in plants (Sabagh et al., 2019).

In the present study, the level of superoxide dismutase enzyme in borage treated with vermicompost fertilizer under salinity stress condition was significantly higher than the control. Plants increase superoxide dismutase enzyme to reduce the effects of oxidative stress in saline conditions (Yaghubi et al., 2014).

Catalase is one of the most important antioxidant enzymes in plants (Hameed et al., 2008). Salinity stress in many plants increased the activity of catalase enzyme (Kahrizi et al., 2012). Catalase is also considered as one of the iron-containing proteins and acts in plant and animal cells when the amount of hydrogen peroxide in the environment is high (Sairam et al., 2005). Lokhande et al. (2011) reported that catalase protects cells from the effects of hydrogen peroxide. Catalase enzymatically helps plant survival by removing active oxygen species and preventing cell wall degradation (Jiang and Zhang, 2001). Ascorbate peroxidase has several essential roles in plant physiological processes such as growth, development and metabolism and also acts as a reducing agent for many free radicals specifically hydrogen peroxide. As a result, ascorbate peroxidase can minimize the damage caused by oxidative stress (Mittler, 2002; Gholinejad et al., 2014). Glutathione peroxidase enzyme degrades hydrogen peroxide by using phenolic substances as electron donor (Appel and Hirt, 2004). Due to increased activity of this enzyme under drought and salinity conditions and its role in reducing glutathione, this enzyme is one of the most important enzymes in the plant that increases the resistance of the plant to oxidative stress. Behrouzi et al. (2015) reported that increased activity of glutathione reductase enzyme increased salinity tolerance.

The results of the present study showed that the amount of malondialdehyde activity increased with increasing salinity. One of the oxidative damages caused by salinity stress is membrane lipid peroxidation which results in the production of malondialdehyde and can be considered as an indicator of the effect of salinity stress (Borzouei et al., 2012). The results of this study also indicated an increasing trend of malondialdehyde production with increasing salinity stress. This is consistent with the results of Alinia and Kazemeini (2017) on the effect of salinity on sorghum. Antioxidant enzymes are one of the fastest antioxidants against active oxygen attack. In the present study, an increase in antioxidant enzymes activity was found, which is consistent with the results of Yaghubi et al. (2014) on the effect of salinity on chickpea. Antioxidant enzymes such as catalase, peroxidase, ascorbate peroxidase, superoxide dismutase, and glutathione reductase cause inactivation of reactive oxygen species (Eyidogan and Oz, 2007). Bahrampour et al. (2019) reported that with increasing drought stress, peroxidase activity increased in the Calendula officinalis, which was in agreement with the results obtained in this study. Many studies also showed that the activity of this enzyme increases in leaves of susceptible and tolerant cultivars to salinity stress (Ashrafi et al., 2015). Increased salinity of soil in *Ocimum basilicum* decreased chlorophyll$_a$, chlorophyll$_b$, total chlorophyll, carotenoids, fresh and dry weight of plant, and leaf area (Bernstein et al., 2010). Niakan and Habibi (2016) reported that increasing drought stress in Cucurbita maxima decreased the amount of chlorophyll$_a$, chlorophyll$_b$, total chlorophyll, carotenoids, and fresh weight of plant, which is in agreement with the results obtained in this study.

Photosynthetic pigments in this study reduced under salinity stress, which could be due to the accumulation of ions in chloroplast. Salinity stress increases the production of active oxygen radicals and the decrease in the extent of chlorophyll content indicates oxidative damages (Taibi et al., 2016). This decrease may be due to the inhibition of different stages of chlorophyll biosynthesis (Zhao et al., 2019). Also the decrease

in chlorophyll concentration in plants under stress may be due to an increase in chlorophyll degradation activity by the chlorophyllase enzyme (Goldani, 2012). Carotenoids as biological antioxidants play an important role in protecting plant tissue. Lack of carotenoids may cause severe photo-oxidative damage in plant tissues (Havaux, 2013). Gomes et al. (2017) reported that salinity had a significant effect on the amount of carotenoids and the amount of carotenoids decreased under salinity stress, but the decrease was less compared to chlorophyll. In *Salvia officinalis*, effects of different salinity concentrations (25, 50, 75, and 100 mM salt) showed that increasing salinity level up to 100 mM significantly reduced plant growth by 65% (Taarit et al., 2009). Also, salinity stress in *Matricaria chamomilla* significantly reduced total chlorophyll and significantly increased proline content (Akcin and Yalcin, 2016). The effect of biofertilizers on chlorophyll content of *Ocimum basilicum* was reported to be higher than in nitrogen fertilizer treatment but no significant difference was observed between this treatment and nitroxine fertilizer treatment (Weisany et al., 2012).

The present study showed that vermicompost application increased the studied traits in *Borago officinalis*. Increased levels of photosynthetic pigments in vermicompost fertilizer treatments can be due to improved soil structure, increased soil moisture retention capacity, and nutrient supply. Also increasing soil vermicompost increased chlorophyll$_a$, chlorophyll$_b$, total chlorophyll, and carotenoids and the highest increase was observed in the fourth level of vermicompost (18%). Application of vermicompost in normal irrigation and moderate and severe stress conditions on morphophysiological and grain yield of Brassica napus was evaluated positively (Rashtbari and Alikhani, 2012). In some plants such as *Helianthus annuus*, vermicompost reduced the deleterious effects of salinity and increased physiological traits (Ahmad and Jabeen, 2009). Mena et al. (2015) reported that at low salinity levels, all levels of vermicompost, and at high salinity level, high levels of vermicompost reduced the damage effects of salinity in *Phaseolus vulgaris*. Overall, it was found that the use of vermicompost fertilizer increased the amount of photosynthetic pigments by increasing the amount of nitrogen in the plant, which resulted in the ability to absorb sunlight, produce photosynthetic materials, and ultimately increase plant growth and yield. The results of the present study also showed an increase in chlorophyll$_a$, chlorophyll$_b$, total chlorophyll and carotenoids under vermicompost fertilizer treatments.

Conclusion

Based on the results obtained in this study, it can be stated that salinity stress has different negative effects on *Borago officinalis* physiological and biochemical processes. Thus, increasing salinity levels significantly increased the activity of superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, catalase, and malondialdehyde but significantly decreased the amount of photosynthetic pigments. Antioxidant enzymes and photosynthetic pigments such as chlorophyll$_a$, chlorophyll b, total chlorophyll, and carotenoids increased in *Borago officinalis* with increasing use of vermicompost fertilizer. The interaction between salinity and vermicompost showed that the maximum antioxidant activity was obtained by vermicompost (18%) at a salinity level of 12 dS m^{-1}. Therefore, the use of vermicompost as an organic fertilizer, in addition to increasing *Borago officinalis* growth, can be a good strategy to reduce the negative effects of high concentrations of sodium and chlorine in the soil. It also appears that application of vermicompost (18%) by improving soil physical and chemical conditions can increase salinity tolerance threshold in the studied traits.

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