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Priming of *Silybum marianum* (L.) Gaertn seeds with H_2O_2 and magnetic field ameliorates seawater stress

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ABSTRACT

Silybum marianum (L.) Gaertn is an important medicinal plant and has been used as a traditional medicine for diseases of the liver and biliary tract. The effects of seed priming by H_2O_2 (Haloprimig) and magnetic field (Magnetopriming, MF) on the impacts of seawater concentration were tested using *S. marianum* at the vegetative stage. These plant species accumulate flavonoids especially slimarine that is used in liver treatment. Some soaked *S. marianum* seeds were subjected to 0.18 T MF for different time durations (0, 10, 20 and 30 min) and other seeds were soaked in different concentrations of H_2O_2 (0, 80,160 and 240μM) for 8h. H_2O_2 priming increased growth and development under water irrigation more than under sea water stress. Moreover, our results uncovered statistical evidence that the priming seeds with H_2O_2 and MF increased the tolerance of *S. marianum* to salinity. In summary, we provide clear evidence that seawater stress caused a highly significant reduction in the growth parameters and stimulation in proline and phenolic compounds. It was concluded that, application of H_2O_2 and MF of *S. marianum* could scavenge or alleviate the harmful effects of salinity stress at early seedling stage and alleviate the oxidative damage leading to improvements in physiological attributes for the plant growth under sea water stress.

1. Introduction

Milk thistle (*Silybum marianum*, *S. marianum*) is one of the chemotypes specific medicinal plants whose seeds' effective substance is used in the production of medicines for liver diseases (Davazdah and Majnoon, 2008). There are medical confirmations for anticarcinogenic and hepatoprotective activities of *S. marianum*. *S. marianum* is used vigorously in cirrhosis, prostate, skin and breast cancer, cervical cells and kidney ailments (Noreen, 2017).

Seed priming is a pre-sowing treatment in different ways so as to cause early germination and obtain better seed vigor (Evenari, 1984). Priming improves seed viability, synchronizes and accelerates germination and sprouting, increases stress resistance and antioxidant activity, and improves plant productivity and growth (McDonald, 2000). Specifically under stress conditions, it induces the germination changes and which is to maintain the germination rate and uniformity in the seedling emergence (Ashraf and Fooland, 2005). Salinity is one of the most important factors that limit plant growth and productivity (Mahajan and Tuteja, 2005). Saline water was used to be considered unusable for irrigation but research efforts during the past two decades have brought into practice some large irrigation schemes which depend on saline water (Hamdy et al., 1993). The effects of salinity and drought stress on seed germination characteristics of *S. marianum* showed that radicle and plumule length decreased by increasing of salinity and drought stress (Yazdani et al., 2010).

Using high vigor plant seeds is important in the dry parts of the world like Egypt that faces natural stresses as a result of the decrease in water availability for germination and growth. Seed priming with H_2O_2 having the capacity to enhance the multi-resistance to heat, drought, chilling and salt stress (Uchida et al., 2002). H_2O_2 is one of the main chemicals which are induced to elevate in plants by biotic and abiotic stresses. Environmental stresses are known to induce H_2O_2 and other toxic oxygen species production in cellular compartments and result in acceleration of leaf senescence through lipid peroxidation and other oxidative damage. It also changes the redox status of surrounding cells where it initiates an antioxidative response by acting as a signal of oxidative stress (Sairam and Srivastava, 2000). Some authors suggested that H_2O_2 plays a dual role in plants: at low concentration, it acts as a messenger involved in signaling and in triggering tolerance against various abiotic stresses, but at high concentrations H_2O_2 causes oxidative stress which leads to a loss

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of protein function, membrane integrity, and to programmed cell death (Asada, 1996). Hemalatha et al. (2017) reported that seed priming with H_2O_2 could be recommended for mitigating the effect of salt stress even under higher salt concentrations. However, the obtained results of Zlatica et al. (2018) revealed that the effects of H_2O_2 priming depended on soybean line and treatment, some lines responded favorably to immersion, while in others priming had an inhibitory effect, causing a significant decrease in germination.

Magnetic treatment of seeds became very popular in the agricultural sector. Pre-treatment of seedling with magnetic field is gaining more application with significant advantages such as magnetic treatment improves first stages of growth in higher plants and increases stress enzyme (Nyakane et al., 2019). Pre-sowing exposure of seeds of different crops to static magnetic field (SMF) called 'magnetopriming', is a non-destructive dry seed priming treatment that has been reported to increase percentage of germination, rate of germination and seedling vigor of many crops. There are few reports on the metabolic changes occurring during germination in the seed in response to magnetopriming under non-stressed environment (Shine et al., 2011; Bhardwaj et al., 2012). The effect of magnetic biostimulation of seeds using stationary MF was presented by Thomas et al. (2013); Kataria et al. (2015) and Kataria et al. (2017). Electric and/or magnetic treatments are assumed to enhance seed vigor by influencing the biochemical processes that involve free radicals and by stimulating the activity of proteins and enzymes (Karimi et al., 2017).

Our study here aims to improve plant production under sea water by using halopriming and magnetopriming. Thus the experiment studied the responses of *S. marianum* to H_2O_2 with different concentrations and MF with different duration time under tap water and 10% sea water irrigation in terms of growth and physiological attributes compared with their controls (water and sea water).

2. Materials and methods

2.1. Seeds collection

The seeds of *S. marianum* seeds were collected in the fall seasons from different habitats in the Mediterranean region of Egypt. The study area lies between Alexandria and Elhammam (Fig. 1) which belongs to the semi-arid climate with mild winter and warm summer, the annual rain fall is 150 mm mostly in the winter season (UNESCO, 1977).

2.2. Preparation of seeds and growth experiments

The seeds of *S. marianum* were sterilized and uniform sized and shaped seeds were divided into two groups, the first group were primed with different concentrations of H_2O_2 (0, 80, 160, 240μM) for 8h as reported by Wahid et al. (2007). While the second group was soaked in distilled water for 8h then, exposed to MF 0.18T (Magnetic susceptibility device, Teslameter LEYBOLD DIDACTIC GMBH QKD2955777, Fig. 2) with different durations (0, 10, 20, 30 min). Priming seeds were grown in plastic pots (15 cm in diameter, 20 cm in length) filled with 1kg soil (1:2 clay to sand soil), 10 seeds were planted separately each treatment had six replicates. The pots were arranged randomly under greenhouse condition in the Faculty of Education, Alexandria University. Pots were irrigated with different concentration of seawater (0, 10, 20, 30 and 50% and then 15%) from the running surface of Mediterranean Sea (Electrical conductivity 50 Ms/cm). The pots were kept at 60% water holding capacity for the soil type When the plants became well established (after 50 days) (Fig. 3), they were carefully freed from the soil by gentle motion in tap water, then the plants were washed with distilled water. Irrigated pots with more than 10 % sea water were excluded because the germination was very low and the seedlings were dead after a few days from germination.

2.3. Growth parameters and photosynthetic pigments

Fifteen individuals were selected from each treatment for determination of shoot, root lengths and the biomass. The length of shoot/root ratio was calculated for each treatment. Leaf area (A) was estimated using the following equation (Cain and Castro, 1959): $A = 0.667xLxW$. Where L is the leaf length, W is the leaf width, and 0.667 is a correction factor used to convert the rectangular product of leaf length and width into the area of the leaf. Photosynthetic pigments chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids (carot) were determined by N, N-dimethylformamide (DMF) method according to Inskeep and Bloom (1985). Chl a/b, Carot/total % and Carot/chl (a+b) % ratios were calculated, for the fresh sample.

2.4. Metabolic compounds

Aqueous extract was prepared following Migahid and El-Khazan (2002) protocol for measurements of soluble protein, amino acids and proline by the methods described in Bradford (1976),Ya and Tunekazu (1966) and Bates et al. (1973) respectively. The extract of the total

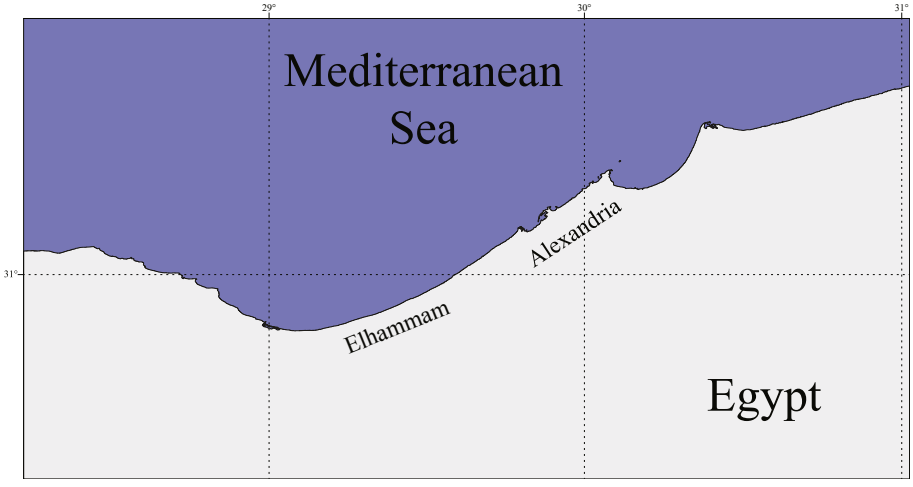

Fig. 1. The study area at Mediterranean coastal region of Egypt which lies between Alexandria and Elhammam (denoted by thick black arrow).

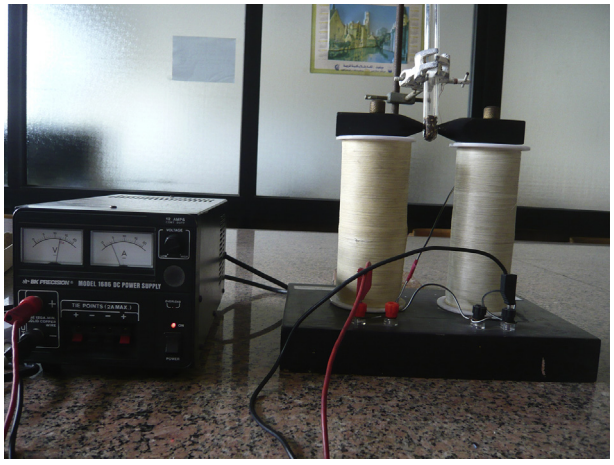

Fig. 2. Magnetic susceptibility device (0.18 T).

phenolic and flavonoid compounds were prepared from fresh samples (0.5 g) were refluxed with 5 ml absolute methanol at 50 °C for 2 h, then the extract was filtered by Whatman No. 4 filter paper and the filtrates were completed up to 5 ml with absolute methanol. Measurements of phenolic and flavonoid content were carried out spectrophotometrically according to Kaur and Kapoor (2002) and Kanatt et al. (2007) at 650 nm and 510 nm respectively.

2.5. Statistical analysis

Analysis of variance (ANOVA) was applied to assess the significant variations of the plant in response to different treatments (salinity of seawater, H_2O_2 concentrations, and MF durations). Statistical evaluation concerning all parameters was performed by using Minitab software (Minitab 12 for windows). Significant results were presented at two significant levels (highly significant at $p \leq 0.01$ and significant at $p < 0.05$). While Tukey's HSD test was applied within treatments in metabolic compounds at 0.05% level.

3. Results

3.1. Growth parameters

3.1.1. Plant biomass

Table 1 shows a highly significant decrease in the total fresh weights for *S. marianum* after sea water treatment. The reduction in fresh weight was more obvious for shoot than root. Both shoot and root's fresh weights of *S. marianum* increased significantly in response to H_2O_2 pretreatment as compared with the control. The largest increase of the shoot fresh weight (19%) after irrigation with tap water and (42%) after irrigation with 10 % sea water were attained at 160 μM H_2O_2. The opposite trend appeared in the MF treatments of the shoot fresh weight especially after tap water and 10% seawater irrigation for which there was no statistically significant reduction at 30 min MF (20.1% and 15.2% respectively) compared to control.

The shoot and root dry weights of *S. marianum* were decreased significantly in response to the seawater treatment, the reduction in the shoot was more obvious than that in the root (Table 1). The response of shoot dry weights to H_2O_2 and MF priming were a non-significant increase, whereas the highest increase of shoot dry weight after tap water and seawater irrigation were attained at 160 μM H_2O_2 (22.2% and 16.6% respectively). However, there was no obvious change in the root dry weight in response to H_2O_2. The highest increase in shoot dry weight which produced from the priming of *S. marianum* seeds with MF after irrigation with tap water was attained at 10 min MF (11.1%), and that treated with sea water irrigation was attained at 20 min MF (33.3%) as

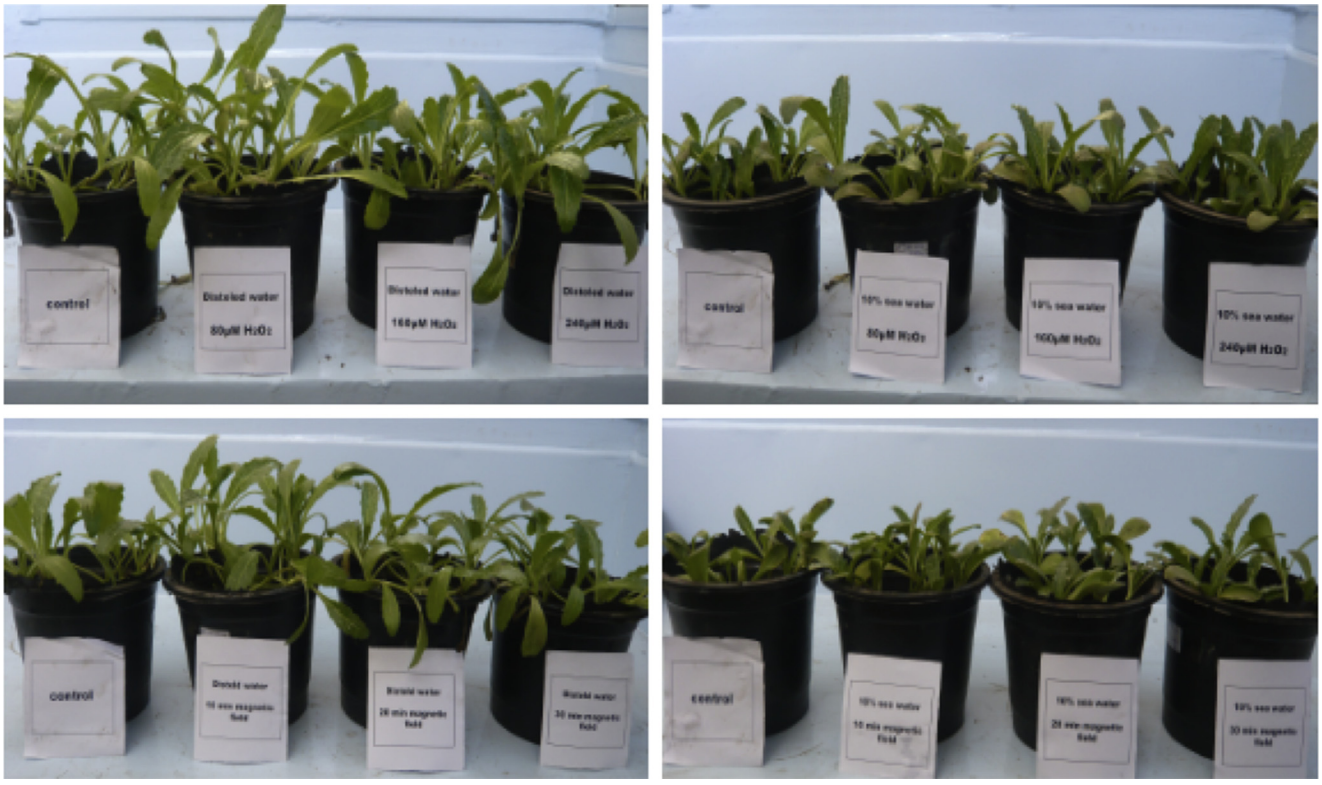

Fig. 3. Potted plants of *S. marianum* in green house of 50-days old priming seeds with H_2O_2 (0, 80, 160 and 240 μM) after irrigation with tap water and 10% sea water, while the priming with magnetic field (0, 10, 20 and 30 min duration) after both irrigation with tap water and 10 % sea water arranged respectively.

Table 1
Variations of fresh and dry weights of shoots and roots (gm) of 50 days old *Silybum marianum* priming with H_2O_2 and MF in response to salt stress. 0%: irrigation with tap water 10%: irrigation with 10% sea water.

Treatment		*Silybum marianum*											
		Fresh weight						Dry weight					
		Shoot		Root		Total		Shoot		Root		Biomass	
		0%	10%	0%	10%	0%	10%	0%	10%	0%	10%	0%	10%
Control (H_2O_2& magnet)		1.79	0.99	0.10	0.07	1.88	1.06	0.09	0.06	0.04	0.03	0.12	0.09
80 μM H_2O_2		2.08	1.04	0.05	0.06	2.13	1.10	0.10	0.05	0.01	0.02	0.11	0.07
160 μM H_2O_2		2.15	1.32	0.09	0.07	2.24	1.39	0.11	0.07	0.04	0.03	0.14	0.10
240 μM H_2O_2		1.94	1.07	0.05	0.07	1.99	1.13	0.09	0.06	0.01	0.03	0.11	0.09
F-values	Sea water conc.	66.61 **		0.14		67.13 **		35.53 **		0.08		11.79*	
	H_2O_2 treatment	16.86 **	4.16 *	3.82 *	1.00	18.67**	3.67	3.67	2.67	2.19	1.00	3.00	2.33
10 min magnet		1.76	0.97	0.13	0.04	1.89	1.01	0.10	0.06	0.05	0.01	0.15	0.07
20 min magnet		1.49	0.98	0.07	0.04	1.56	1.01	0.09	0.08	0.03	0.01	0.13	0.09
30 min magnet		1.43	0.84	0.09	0.04	1.51	0.88	0.09	0.05	0.03	0.01	0.12	0.07
F-values	Sea water conc.	46.63 **		11.76*		43.93**		19.64 **		10.57*		40.33 **	
	magnetic treatment	0.63	0.26	0.86	1.00	6.33	3.67	1.00	6.33	3.67	9.00 *	8.00 *	5.33

*Significant at ($p < 0.05$), ** highly significant at ($p \leq 0.01$).

compared with control.

3.1.2. Shoot and root lengths

There is a highly significant decrease in shoot and root lengths for *S. marianum* due to seawater treatment (Table 2). The pretreated *S. marianum* seeds with H_2O_2 exhibited a highly significant increase of shoot and root lengths. The highest increase in shoot length which irrigated with tap water and 10% sea water was attained at 80 μM H_2O_2 (16.5%). The highest increase compared to control in the root length after irrigation with tap water was obtained at 160 μM H_2O_2 (16.18%), whereas the highest increase at 10% seawater (40.5%) was recorded at 240 μM H_2O_2. It is well noted that the pretreated *S. marianum* seeds with MF exhibited longer shoot and root lengths than the control (Table 2). The highest increase of shoot length which irrigated with tap water was attained at 30 min MF (4.2%), while after seawater it was 20% at 20 min MF. On the other hand, the highest root length after tap water irrigation attained at 20 min MF (23.7%), while at 10 % sea water the highest root length (35%) was at 10 min. The shoot/root length ratio showed significant increase in the individuals under tap water and highly significant decrease in those irrigated with 10 % sea water in the pretreated with H_2O_2, while it was reduced with non-significant in case of MF priming.

3.1.3. Leaf area

The leaf area of plant decreased with highly significant p-values in response to seawater treatment (Table 3). However, the leaf area was increased with highly significant in response to H_2O_2 priming under irrigation with tap water and seawater. The highest value (0.57%) after irrigation with tap water was attained at 80 μM H_2O_2, while after seawater irrigation it was attained 70.25% at 160 μM H_2O_2 compared to control. There was a significant increase of the leaf area in which pretreated seeds with MF. The highest increase (1.6%) in the leaf area after tap water irrigation was recorded at 30 min MF, whereas it was recorded 6.9% after seawater irrigation at 10 min MF.

3.2. Photosynthetic pigments

The content of pigments in the plant at different treatments was recorded in the Table 3. The present study showed no great variation in the content of the total pigment in response to seawater and pretreated seeds treatments. The carotenoid of *S. marianum* increased significantly in response to seawater and pretreated seeds, while chlorophyll a (Chl a) showed a slight increased as compared to control in response to H_2O_2 priming. The highest increase in chl a content was at 240 μM H_2O_2 after both tap water and seawater irrigation (2.9% and 6.8% respectively). Priming with 160 μM H_2O_2 indicated that chlorophyll b (chl b) showed the highest increase after irrigation with tap water (1.9%). Meanwhile, the highest increase after 10% sea water irrigation was recorded at 240 μM H_2O_2 (3.4%). In case of carotenoid the highest increase was recorded at 160 μM H_2O_2 and 240 μM H_2O_2 treatments (1.4%) after tap water irrigation, while 80 μM H_2O_2 and 240 μM H_2O_2 obtained the highest value (5%) after 10% seawater irrigation.

Moreover, the chl a content in *S. marianum* increased significantly in

Table 2
Variations of the shoot and root length of *S. marianum* (cm ± st.dv.) priming with H_2O_2 and MF in response to salt stress. 0%: irrigation with tap water 10%: irrigation with10% sea water.

Treatment		*Silybum marianum*					
		length of shoot		length of root		Shoot/root	
		0%	10%	0%	10%	0%	10%
Control (H_2O_2 & magnet)		8.06 ± 1.38	3.61 ± 1.38	6.18 ± 1.76	5.13 ± 1.97	1.30	0.70
80 μM H_2O_2		9.39 ± 1.23	4.21 ± 0.75	6.82 ± 2.66	6.29 ± 2.61	1.38	0.67
160 μM H_2O_2		8.10 ± 0.80	3.91 ± 0.95	7.18 ± 2.39	6.75 ± 2.34	1.13	0.58
240 μM H_2O_2		8.64 ± 1.26	4.01 ± 0.88	5.94 ± 1.86	7.21 ± 2.02	1.45	0.56
F-values	Sea water conc.	745.54**		0.12		83.32**	
	H_20_2 treatment	5.34**	0.41	7.24**	5.55**	3.31*	4.31**
10 min magnet		8.11 ± 1.42	4.08 ± 0.68	7.38 ± 1.65	6.93 ± 2.41	1.10	0.59
20 min magnet		8.18 ± 1.35	4.33 ± 1.27	7.65 ± 2.59	5.77 ± 1.58	1.07	0.75
30 min magnet		8.40 ± 1.38	4.07 ± 0.84	7.52 ± 2.32	5.88 ± 1.47	1.12	0.69
F-values	Sea water conc.	432.90**		20.93**		61.05**	
	magnetic treatment	0.42	3.11*	0.28	0.57	0.57	0.39

*Significant at ($p < 0.05$), ** highly significant at ($p \leq 0.01$).

Table 3
Variations of leaf area and pigments (Chl a, Chl b and Carot.) of *S. marianum* priming with H_2O_2 and MF in response to salt stress. 0%: irrigation with tap water 10%: irrigation with10% sea water.

Treatment		*Silybum marianum*							
		Chl a		Chl b		Carot.		LEAF AREA	
		0%	10%	0%	0%	10%	0%	0%	10%
Control (H_2O_2& magnet)		2.05 ± 0.05	2.05 ± 0.17	2.05 ± 0.02	2.06 ± 0.03	0.18 ± 0.01	0.18 ± 0.02	17.89 ± 0.02	11.70 ± 0.01
80 μM H_2O_2		2.08 ± 0.07	2.09 ± 0.05	2.06 ± 0.03	2.07 ± 0.03	0.18 ± 0.02	0.19 ± 0.01	28.09 ± 0.02	17.60 ± 0.04
160 μM H_2O_2		2.10 ± 0.01	1.99 ± 0.16	2.09 ± 0.03	2.06 ± 0.05	0.21 ± 0.01	0.17 ± 0.04	21.44 ± 0.04	19.92 ± 0.04
240 μM H_2O_2		2.11 ± 0.06	2.19 ± 0.16	2.05 ± 0.04	2.13 ± 0.02	0.21 ± 0.03	0.19 ± 0.08	21.46 ± 0.03	15.68 ± 0.02
F-values	Sea water conc.	0.03		1.71		0.19		9.74*	
	H_2O_2 treatment	1.01	1.08	1.96	2.61	2.17	0.17	60.56**	23.72**
10 min magnet		2.17 ± 0.03	2.09 ± 0.14	2.11 ± 0.01	2.08 ± 0.01	0.25 ± 0.01	0.20 ± 0.04	17.08 ± 0.00	12.51 ± 0.02
20 min magnet		2.08 ± 0.06	2.19 ± 0.05	2.07 ± 0.02	2.11 ± 0.03	0.23 ± 0.01	0.21 ± 0.01	18.03 ± 0.02	11.65 ± 0.02
30 min magnet		2.16 ± 0.05	2.17 ± 0.07	2.09 ± 0.02	2.08 ± 0.03	0.24 ± 0.02	0.23 ± 0.01	18.18 ± 0.01	11.59 ± 0.01
F-values	Sea water conc.	0.00		0.12		5.63*		1119.53**	
	magnetic treatment	2.19*	2.13	2.16	3.18	8.55**	2.27	119.13**	6.28*

*Significant at ($p < 0.05$), ** highly significant at ($p \leq 0.01$).

response to MF priming under irrigation with tap water. The highest increase (5.8%) compared with control was recorded at 10 min MF, while at 20 min MF, chl a content attained the highest increase (6.8%) after seawater irrigation. The same trend was exhibited in chl b. Results revealed a slight increase in chl b due to the priming with MF. The highest increase in chl b (2.9%) was attained at 10 min MF after tap water irrigation, whereas after seawater treatment was recorded at 20 min MF (2.4%). The carotenoid content increased with highly significant in response to MF priming under tap water. The highest value was recorded at 10 min MF in irrigated with tap water (38.8%), while after 10% sea water was attained at 30 min MF (27.7%).

3.3. Metabolic compounds

3.3.1. Proteins, amino acids, proline

Fig. 4 and Table 4 show that the soluble protein of *S. marianum* showed a highly significant decrease in response to seawater after H_2O_2 primings. However, this decrease was insignificant after priming with MF. On the other hand, the soluble protein was significantly increased after priming with H_2O_2 in individuals irrigated with tap water while the increase of soluble protein after irrigation with 10% sea water was non-significant. Whereas the highest increases of soluble protein (30.5%, 4.16%) were recorded at 80 μM H_2O_2 and 160 μM H_2O_2 after tap water and 10% sea water irrigation respectively compared with the control. The increase in soluble protein in response to MF was non-significant in both plants irrigated with tap water and 10% sea water. The maximum increase in soluble protein content of *S. marianum* was recorded at the priming with 10 min MF after irrigation with tap water (22.03%), after seawater irrigation it attained 35.4% at 30 min MF.

The content of free amino acid of *S. marianum* was decreased in response to seawater and pretreated seeds with H_2O_2 and MF (Fig. 4). However, this decrease was insignificant after H_2O_2 treatment and highly significant after MF (Table 4). The content of free amino acids of *S. marianum* individuals increased non-significantly with H_2O_2 priming after both irrigation with tap water and 10% seawater treatment. The highest content in this species was recorded at 80 μM H_2O_2 after tap water irrigation and at 160 μM H_2O_2 after 10% sea water irrigation (50.7% and 16.4% respectively). On the other hand, the amino acids content exhibited highly significant increase in response to priming with MF after irrigation with sea water, the highest amino acids content after tap water irrigation was attained at 30 min MF (23.9% of control), while the highest amino acid content after irrigation with 10 % sea water was attained at 20 min MF (5.9%).

The content of free proline in the studied species exhibited highly

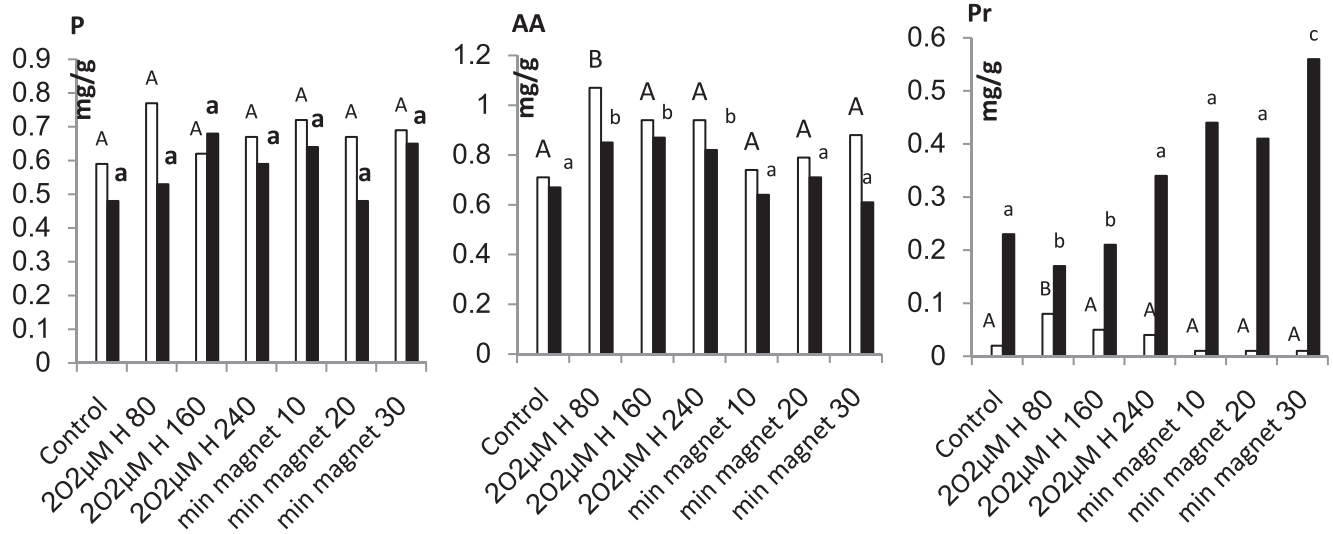

Fig. 4. Variation of proteins (P), amino acids (AA) and proline (Pr) (mg/g f. w.) in priming seeds *of Silybum marianum* with H_2O_2 and magnetic field (0.18 T). Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test. 0% sea water □ (capital letters) and 10% seawater ■ (small letters) were separately grouped.

Table 4
Statistical analysis of nitrogenous compounds (mg g^{-1}f.wt. ± st. dv.) in *S. marianum* 50-day old pretreated with H_2O_2 and MF (0.18T) and grew under salinity stress.

Treatment		Protein		Amino acids		Proline		Phenolic		Flavenoids	
		0%	10%	0%	10%	0%	10%	0%	10%	0%	10%
F-value	Sea water conc.	14.93**		3.50		58.38**		6.20		0.07	
	H_20_2 treatment	7.26*	0.81	0.81	1.86	1.09	3.33	6.98*	142.05**	1.88	24.05**
F-value	Sea water conc.	4.07		12.07**		200.89**		2.84		0.04	
	magnetic treatment	0.26	0.86	1.74	8.53**	0.60	6.95*	6.81*	17.56**	40.08**	120.57**

*Significant at ($p < 0.05$), ** highly significant at ($p \leq 0.01$).

significant accumulation in response to 10% sea water in priming with H_2O_2 and different durations of MF (Fig. 4 and Table 4). The highest accumulation of free proline was recorded at 240 μM H_2O_2 and 80 μM H_2O_2 (750%, 524% respectively), while the highest accumulation of free proline after MF was recorded at 30 min and 20 min (5500%, 913.5% respectively) compared with control. Soluble proline increased non-significantly in response to H_2O_2 priming after tap water and 10% seawater irrigation. The highest proline content after tap water irrigation was attained at 80 μM H_2O_2 (300%), while after irrigation of sea water the highest proline was attained at 240 μM H_2O_2 (47.8%). Significant increase of proline in pretreated seeds with MF was recorded at 10% seawater irrigation. The highest proline content at seawater irrigation was attained at 30 min MF (143%). However, in tap water irrigation there was a non-significant reduction (50%) at all duration of MF compared with control.

3.3.2. *Phenolic and flavonoids compounds*

The total phenolic content in *S. marianum* after H_2O_2 and MF priming showed significant increases in response to sea water treatment (Table 4 and Fig. 5). The highest content of phenolic compound was recorded at 160 μM H_2O_2 (23.6%) in plant individuals irrigated with tap water, while the highest phenolic compound content was recorded at 240 μM H_2O_2 (62.4%) in plant individuals irrigated with 10% sea water. The highest phenolic compound in the treated seeds with MF was recorded at 30 min MF (60.58%) in those individuals irrigated with tap water. After irrigation with 10% sea water these values were recorded at 20 min MF (117.7%).

The present study showed that the total flavonoids content of *S. marianum* increased with highly significant in the pretreated seeds with H_2O_2 and MF in response to seawater treatment (Table 4 and Fig. 5). The maximum increase of total flavonoid content after 10% of sea water irrigation was attained at 240 μM H_2O_2 (63.2%). In the case of MF treatment, the highest flavonoid content in this species after irrigation of tap water was attained at 10 min MF (39.2%). While the highest flavonoid content increase was more than double time after 10% of sea water irrigation was obtained at 20 min MF when compared with the control.

4. Discussion

It is well documented that salinity reduces the germination as well as seedling growth in crop plants and seed priming ameliorates salinity during early seedling growth (Afzal et al., 2006). The present study evaluates the priming effects *for S. marianum* seeds with different concentrations of H_2O_2 and different durations of MF which grown under irrigation with tap water and 10% sea water. In the present study, the salinity of seawater caused a highly significant reduction in the growth parameters of *S. marianum* compared with control. The fresh and dry weights of root and shoot of *S. marianum* decreased progressively due to salinity as compared to control. These results are in agreement with those reported by Memon et al. (2010) and Hessamoddin et al. (2015) that showed a negative effect of salinity on plant growth of several plant species.

In our study there was a highly significant reduction in shoot and root lengths of *S. marianum* due to seawater stress under different priming techniques. The rate of root growth inhibition is more prominent compared to shoot inhibition. The reductions in the shoot and root length with more reduction in root growth than shoot growth due to salt stress are similar to those El-Katony et al. (2019) and Devi et al. (2008). High salinity may inhibit root and shoot elongation due to slowing down the water uptake by the plant (Werner and Finkelstein, 1995), which may be another reason for this decrease. Kaymakanova and Stoeva (2008) reported that salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio. The shoot/root ratio in the present study of *S. marianum* showed highly significant decrease due to seawater treatment. Under prevailing experimental conditions, salinity significantly reduced leaf area of *S. marianum* these observations were

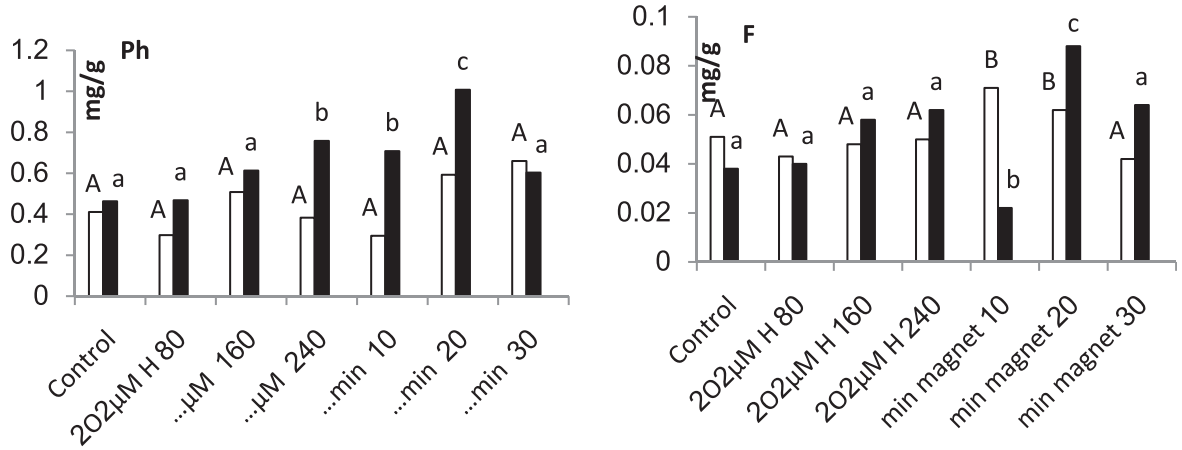

Fig. 5. Variation of total Phenolic compounds (Ph) and flavonoid (F) with mg/g f. w. in priming seeds *of Silybum marianum* with H_2O_2 and magnetic field (0.18 T). Means with identical letters within graphs do not differ significantly at the 0.05% level of probability based on Tukey's HSD test. 0% sea water □ (capital letters) and 10% seawater ■ (small letters) were separately grouped.

previously recorded in different plant species (Zhao et al., 2007 on *Avena sativa* and Yilmaz and Kina, 2008 on *Fragaria xananss*). This notable decrease in leaf area, 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 leaf growth and chlorophyll content (Netondo et al., 2004). Such reduction in leaf area of salt-stressed wheat plants may be related to the inhibition of cell division and/or cell expression (Heckenberger et al., 1998).

In the present study, these seed priming agents were also found very effective in alleviating the deleterious effects of salinity on seed germination and seedling growth H_2O_2 is a strong oxidizing agent that injures cells and damages photosynthesis at high concentration when produced internally or applied externally (Sairam et al., 2002). However, it acts as stress signal in low concentrations (Desikan et al., 2004). The present results indicate that priming seeds with H_2O_2 revealed the increase in the root and shoot fresh and dry weight, organs length and leaf area of *S. marianum* as compared with the control especially under the irrigation with 10% sea water. This result indicated that the role H_2O_2 in deleterious effects of salt stress on the plant growth may be due to the activation of antioxidants (Wahid et al., 2007). The capacity of the antioxidant defense system is often increased under stress conditions (Taibi et al., 2016).

MF is an inescapable environmental factor for plants on the earth. However, its impact on plant growth is not well understood. The intensity of MF used in this study was 0.18 T based on Majd et al. (2009), they found that suitable MF-priming 0.18 T could speed up seedling development and increase biomass. The present study exhibited that the growth parameters allow us to conclude that the magnetic treatment improves the growth of the studied species under irrigation with tap water as well as seawater treatments. Whereas the shoot and root weights, length and leaf area in the studied species were enhanced in response to MF treatment. Magneto-priming could be promising and effective tool for alleviation salinity stress on germination of barley crops (Hozayn et al., 2018). The results of Hessamoddin et al. (2015) on *S. marianum* indicated that different strengths of the magnetic field and different time durations are important factors which can influence the plant growth. Karimi et al. (2017) found that magnetic priming for 6 hours was suggested for enhancing germination and growth of sweet corn under salt stress. Podlesny et al. (2005) showed a positive effect of magnetic stimulation of seeds on the increase of pea hypocotyl and root length.

Results of the present study revealed that seed priming with MF of the studied species increase in leaf area (not-significant). Yinan et al. (2005) reported that cucumber seedlings with MF-priming grew much better than the untreated, and above ground biomass and leaf area were significantly increased. The ratio between shoot/root lengths exhibited no significant change due to priming with MF for studied species. On the other hand, Ananta and Shantha (2008) noted that there is a significant increase in germination, seedling vigor and shoot/root growth in maize and chick pea seeds exposed to static MF. Flórez et al. (2007) reported faster germination of maize seeds when exposed to MF of 125 or 250 mT for varying periods of time.

The photosynthetic pigments are some of the most important internal factors, which in certain cases can limit the photosynthesis rate. The response of plant pigments (chlorophyll a, chlorophyll b and carotenoids) to seawater stress in the present study exhibited a slight increase in pretreated *S. marianum* with H_2O_2. Under prevailing experimental conditions, the ratio of chlorophyll a/b in stressed plant of *S. marianum* did not significantly change. Increase in chlorophyll content with sea water irrigation agrees with results reported by Misra et al. (1997). They indicated that stressing rice seedlings of *Oryza sativa* with sodium chloride increased significantly the chlorophyll content of seedlings (15 days old). Also, it was mentioned by Jamil et al. (2007a and b) that increased concentrations of sodium chloride increased the total chlorophyll content of *Beta vulgaris* leaves, and that was a significant increase. The MF priming enhanced total pigments production in the studied species. Dhawi and Al-Khayri (2008) found that low intensity of MF or short period of exposure, increased chlorophyll content, whereas high-intensity MF and long exposure to MF reduced the concentration. A similar pattern of response, MF can cause an inconsistency in the function of antioxidant enzymes in *Nicotiana tabacum* (Sahebjamei et al., 2007).

In the present study, the soluble protein significantly decreased in response to seawater in *S. marianum* especially for individuals treated with H_2O_2. Similar observations have been reported by Chen et al. (2007) who found that exposing *Vigna unguiculata* plants at the age of 14 days–75 mM of NaCl reduced soluble protein content in the plant. These results were confirmed by Cheruth et al. (2008) with their study on *Catharnathus roseus* seedling and Khosravinejad et al. (2009) on *Hordeum vulgare*. There is a marked increase in soluble protein for the studied species in response to H_2O_2 as compared with the control. H_2O_2 induced small heat shock proteins (HSP26) in tomato and rice (Chevallier, 2001). Thus, H_2O_2 may play an important role in signal transduction for abiotic stress tolerance, although it is toxic at high concentrations.

Shine et al. (2017) exhibited that MF treatment influences the physiological and biochemical process in the seeds and thereby contributes to better vigor and improved crop stand. In the present study, there is an obvious increase in soluble protein as a response to MF but this increase was non-significant under both irrigations with tap water and 10% sea water. The effect of MF on protein synthesis has been studied in some experiments. It has been observed that the protein values were slightly higher in MF-exposed seedlings, in comparison with the controls (Piacentini et al., 2001). Novitsky et al. (2001) demonstrated that low-intensity MF application increased protein in onion plant. In fact, the content of free amino acids in the studied species decreased in response to seawater under H_2O_2 and MF priming but the reduction under H_2O_2 was non-significant. Also, the reduction and/or the increase in the content of free amino acid in response H_2O_2 priming were insignificant for the studied species. Under the prevailing experimental condition, the amino acids content increased significantly in response to MF in *S. marianum*.

Proline accumulation is a widespread response of plants to environmental stresses (Anjum, 2008), which is shown to be involved in defense of plants against salinity and osmotic stress (Karimi et al., 2017). The present study revealed that soluble proline in the studied species accumulated with highly significant in response to 10% sea water stress under pretreated with H_2O_2 and MF durations. It was suggested that proline accumulation may be caused by increased proteolysis or by decreased protein synthesis. The higher concentration of proline under salt stress is favorable to plants as proline participate to osmotic potential of leaf and thus to osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition. Proline accumulation may also help in nonenzymic free radical detoxifications (Khan et al., 2002). Results showed that seeds pretreated with H_2O_2 enhanced free proline content; similar results were reported by He et al. (2009), they showed that seed priming with H_2O_2 significantly enhanced free proline content. The slight increase in proline, an osmoprotectant in H_2O_2 (0.2 mM) stressed senescing rice leaves may be attributed to the free radical scavenging function of proline as reported elsewhere (Matysik et al., 2002). The MF priming seeds of *S. marianum* enhanced free proline content as compared with control, similar observations have been reported by Dhawi and Al-Khayri (2008), who found that at the lowest intensity, 10 mT, proline concentration increased in response to longer exposure durations reaching a maximum at 240 min.

Plants produce a large variety of secondary products that contain a phenol group; abiotic stresses may also modulate the level of secondary metabolites such as phenolics (Dixon and Paiva, 1995). In the present study, the content of the total phenolic compounds increased in studied species in response to the salinity of sea water but this increase was non-significant which could be a defense mechanism and a biochemical adaptation to environmental stress (Dixon and Paiva, 1995). These compounds are thought to protect the plant against salt-induced

oxidative stress (Oh et al., 2009). Phenolics are electron donors and thus could mitigate the effect of oxidative stress as an excellent substrate for antioxidant enzymes such as peroxidases (Posmyk et al., 2009). Reports on the effect of salinity on phenolic contents are limited. Total polyphenol content increased in *Mentha pulegium* leaves (Oueslati et al., 2010) and varied during fruit ripening in pepper (Navarro et al., 2006) exposed to salt stress. Bourgou et al. (2012) found that salinity influenced significantly the total phenol contents of *Nigella sativa* organs, the content increased in the shoots while it decreased in the roots. In this study, the content of the total phenolic compounds in *S. marianum* did not significantly change in response to H_2O_2 and MF. Results of the present study demonstrated that the non-significant increase in total flavonoids in *S. marianum* in response to seawater. The result expressed by Ali and Abbas (2003), they recorded that flavonoids content increase significantly in response to salt stress in *Hordeum vulgare*. But flavonoids content showed a significant increase in response to the treatment with H_2O_2 and MF.

In conclusion, our study demonstrated that sea water stress caused a highly significant reduction in the growth parameters and stimulation in proline and phenolic compounds. In fact, the present study showed that the irrigation with tap water exhibited longer shoots and well-developed leaves after H_2O_2 and MF pretreatment than seawater. Moreover, the plant individuals which produced from pretreated seeds with H_2O_2 and MF were characterized by longer shoot and root length than non-treated seeds. Priming seeds of *S. marianum* with H_2O_2 and MF in different duration may alleviate the oxidative damage, leading to improvements in physiological attributes for the plant growth under sea water stress but with different effects. This opens an unusual perspective on plant responses that should be tested under combination of H_2O_2 and MF.

Declarations

Author contribution statement

MM Migahid: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

RM Elghobashy: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

LM Bidak, AW Amin: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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The authors declare no conflict of interest.

Additional information

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