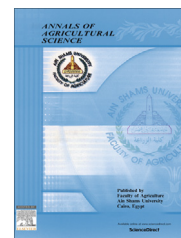




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Beneficial effects of exogenous selenium, glycine betaine and seaweed extract on salt stressed cowpea plant



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Abstract Pot experiments in sandy soil were carried out to investigate foliar applications of [selenium (5 & 10 μ M Se), glycine betaine (5 & 10 mM GB) and seaweed extract (2 & 4% SWD)] on cowpea plant, under salt stress (0 & 50 mM NaCl). Growth, yield parameters and biochemical constituents were determined. The obtained results indicated that all foliar treatments under both salinity concentrations achieved an increment in most of the growth, yield parameters and biochemical constituents. As for the effect of single treatment on growth and yield parameters, 5 μ M Se without salinity revealed higher significant values than all treatments followed by 10 μ M Se under 50 mM NaCl. Meanwhile, 4% SWE under 50 mM NaCl showed the highest values of photosynthetic pigments, proline and Phenylalanine ammonia lyase (PAL). On the other hand, 10 mM GB 50 mM under NaCl recorded the highest value of total soluble sugars (TSS), while a significant increase in protein concentrations was observed by 5 μ M Se without salinity.

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Introduction

Salinity is a standout among the most critical abiotic elements restricting yield efficiency (Munnas, 1993). Salinity has negative effect on crop generation in numerous parts of the world, particularly in watered fields of dry and semiarid locales (Schleiff, 2008). Saltiness influences numerous morphological, physiological and biochemical procedures, including seed germination, plant development, water uptake and nutrient uptake (Willenborg et al., 2004). The plant capacity to adapt salt anxiety incorporates changes at leaf level, connected with

morphological, physiological and biochemical characteristic whereby numerous plants conform to high saltiness and low soil water availability (Cicek and Cakirlar, 2008). There is solid confirmation that salt influences photosynthetic chemicals, chlorophyll and carotenoids (Stepien and Klobus, 2006).

Salinity stress leads to accumulation of reactive oxygen species (ROS) (O_2 , superoxide radicals; $OH\cdot$, hydroxyl radical; H_2O_2 , hydrogen peroxide and $1O_2$, singlet oxygen), causing an oxidative stress. ROS are mainly generated in chloroplast and mitochondria due to electron transport processes. In typical conditions, ROS are created as byproducts in metabolic pathways, however there is a harmony between ROS and the antioxidant system in the cell. At the point when plants suffer from biotic or abiotic stress for example, saltiness, ROS are

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excessively produced which unbalance the cellular redox in favor of oxidized forms, in this manner creating oxidative stress that can harm DNA, inactivate enzymes and cause lipid peroxidation (Gill and Tuteja, 2010).

Cowpea (*Vigna unguiculata* L.) has a moderate resilience to salinity, with a more noteworthy resistance than corn yet not as much as wheat, grain, sugar beet, or cotton (Hall and Frate, 1996). Fortunately, a few assortments of cowpea are adjusted to adapt to a few abiotic anxieties, for example, drought, salinity and elevated amounts of temperature and radiation (Silveira et al., 2003) which, alone or in mix, can impel oxidative harm in the plant (Foyer and Noctor, 2000).

Some plant species developed in Selenium-enriched media have shown demonstrated upgraded imperiousness to certain abiotic stresses, e.g. salinity (Kong et al., 2005; Djanaguiraman et al., 2005 and Hawrylak-Nowak, 2009).

Osmoregulators (e.g., glycinebetaine; GB) is amassed in plants as a versatile instrument to ecological stress, such as salinity (Thomas et al., 1992). In plants that combine GB, it is collected in leaves because of water shortage and salt anxiety (Rhodes and Hanson, 1993). Notwithstanding its part as osmoprotectant, GB has been accounted for to balance out photosynthetic responses, the structure of outward proteins of the PSII complex and ATP combination (Mamedov et al., 1991) as well as activation of enzymes (Gorham, 1995).

Moreover, marine bioactive substances separated from marine algae are utilized as a part of agricultural and horticultural crops, and many beneficial effects, in the terms of enhancement of yield and quality have been reported (Blunden, 1991 & Crouch and Van Staden, 1994). Seaweed has been accounted to animate the growth and yield of *Zyziplus mauritiana* Lamk (Rama Rao, 1991), enhance antioxidant properties (Verkleij, 1992), create resilience to environment stress (Zhang and Schmidt, 2000 & Zhang et al., 2003) and increase nutrient uptake from soil (Verkleij, 1992 & Turan and Köse, 2004).

The current investigation mainly focuses on studying the response of cowpea to foliar applications of selenium, glycinebetaine and seaweed extract under salt stress. Also growth, yield parameters and biochemical constituents were determined.

Materials and methods

Pot experiment

Pots experiment were performed in greenhouse of the Agric. Botany Department, Fac. Agric., Ain Shams Univ, Cairo, Egypt, during the growing two successive growing seasons which started from May to July 2014 and 2015. Six seeds of cowpea (*V. unguiculata* (L.) cv. Kareem 7) were sterilized with 10% NaClO for 1 min, washed with distilled water and sown in 1st May on 5.5 kg of air-dried sand soil filled in circular earthen pots (40 cm height and 30 cm diameter). Thinning was conducted two weeks after sowing leaving 3 plants/pot. The plants were watered every second to the third days. Recommended fertilization program was followed as designed by the Egyptian Ministry of Agriculture. Each pot received 2.2 g calcium superphosphate (15.5% P₂O₅), 1.1 g potassium

sulfate (48% K₂O) and 4.0 g ammonium sulfate in two portions.

Treatments were factorial combinations of two salinity levels [0 & 50 mM NaCl] and foliar applications of selenium (5 and 10 µM I⁻¹ Na₂SeO₄), glycine betaine (5 and 10 mM I⁻¹ GB), seaweed extract (2 & 4% SWE) as commercial seaweed extract (Algreen 600) was used and control (sprayed with distilled water)]. Tween 20 at 0.1% was used as a wetting agent. Pots were arranged in randomized complete design with nine replicates, and each replicate has three plants.

Se, GB and SWE solutions were sprayed three times on plants. The first application started two weeks after sowing date followed by second and third applications every 2 weeks-intervals. The plants leaves were thoroughly wetted until solution drops. Saline treatment was started with the first application.

Growth parameters

The following parameters were measured: fresh and dry weights of the fully expanded third leaf from the top of plants (g), area of the third leaf as measured by an Image J software (mm²), pod length (cm), average pod dry weight (g), number of pods/plant, number of seeds/pod and weight of 100 seed (g). The parameters were measured after 50 days from cultivation. Six plants were taken from each treatment to calculate the mean of each measurement.

Biochemical analyses

Each biochemical analysis was determined in 0.5 g fresh weight of the fully expanded 3rd leaf from the top of the plants. Determination of chlorophyll a & b, carotenoids, proline, total soluble sugars, total protein, superoxide dismutase activity, peroxidase activity and phenylalanine ammonia lyase activity were achieved at 50 days after sowing from the first season.

Chlorophyll a & b and carotenoids

Chlorophyll (Chl a & b) and carotenoids (Car), were extracted and estimated as per the strategy portrayed by Moran (1982). Formula and extinction coefficients were used as described by Lichtenthaler and Wellburn (1983) and expressed as mg/g fresh weight.

Proline concentration

Proline concentration was determined using a ninhydrin colorimetric method of Troll and Lindsley (1955) as modified by Peters et al. (1997). The proline concentration was calculated from the standard curve of L-proline and expressed as µg proline/g fresh weight.

Total soluble sugars

Total soluble sugars (TSS) were extracted as per the strategy portrayed by AOAC (2005) and measured according to the method recorded by Dubois et al. (1956) and expressed as mg/g fresh weight.

Total protein

Protein concentration was determined according to the method of Bradford (1976). The protein concentration was calculated using the standard curve of bovine serum albumin (BSA) and expressed as mg protein/g fresh weight.

Superoxide dismutase (SOD) assay

The activity of superoxide dismutase (SOD) (EC 1.15.1.1) was quantified by the method of Beyer and Fridovich (1987), and expressed as unit mg^{-1} protein.

Peroxidase (POD) assay

The activity of peroxidase (EC1.11.1.7) was quantified by the method of Hammerschmidt et al. (1982) and expressed as unit mg^{-1} protein.

Phenylalanine ammonia lyase (PAL) assay

Phenylalanine ammonia lyase (PAL) (EC 4.3.1.5) activity was quantified by the method of Lister et al. (1996) and expressed as unit mg^{-1} protein.

Statistical analysis

Plant growth parameters and chemical analysis were statistically analyzed using One-way ANOVA and Post hoc-LSD tests (the least significant difference) by using SAS (2003) at 0.05 level of probability.

Results*Effect of foliar application on growth parameters*

Results illustrated in Table 1 represent the effect of different treatments under salt stress on fresh weight, dry weight and leaf area of 3th leaf. It is evident that 50 mM NaCl reduced significantly growth parameters as compared to the control (non-saline) and other treatments. Under the NaCl treatments, all foliar applications showed significant increments in all growth parameters against 50 mM NaCl except the plants treated with SWE with saline. Also the plants treated with SWE under control have significant value with the control.

As for dry weight, 5 & 10 μM Se under control and NaCl concentration and 10 mM GB without salinity increased significantly dry matter above the control. On the other hand, there is no significant difference in dry weight with 5 mM GB under control and NaCl concentration as well 10 mM GB under 50 mM NaCl and 2 & 4% SWE without salinity. Perversely 50 mM NaCl singly or with 2 & 4% SWE has positive effect on dry weight against the control.

It is clear that foliar application of 5 μM Se without salinity and 10 μM Se under 50 mM NaCl achieved maximum fresh and dry weights of the 3rd leaf above the rest of treatments to reach the 5% level of significance. Also, all treatments without salinity and both Se concentrations under 50 mM NaCl increased significantly leaf area as compared to the control, while the rest treatments led to decrease the leaf area.

Effect of foliar application on yield parameters

Data presented in Table 1 demonstrate the effect of different treatments under salt stress on yield parameters. Generally, 50 mM NaCl treatment recorded the lowest value of all yield parameters; the reverse was true for 5 μM Se without salinity which led to the highest value of all yield parameters. It is evident that Se treatments led to significant increment in pod length against the control except 5 μM Se under salt stress which recorded no significant difference. Meanwhile, SWE in both concentrations has no significant difference without salinity or with 50 mM NaCl. On the other side, the rest of the treatments detected significant decrements in pod length when compared to the control.

As for pod dry weight, it is clear that there is no significant differences between control (non-saline) and 5 μM Se without salinity, while both of them recorded the highest weight in the 1st and 2nd seasons, respectively. The other treatments showed significant decrease in the pod dry weight against the control.

The general inclination of the pods number per plant was to decrease with increasing salinity stress. Applications of Se in both concentrations and 2% SWE without salinity as well 10 μM Se under 50 mM NaCl led to an increment in number of pods/plant than the other treatments, and these applications recorded 3.66, 3.00, 3.00 and 3.33 pod/plant, respectively in the 1st season while the 2nd season took the same trend. Meanwhile the other treatments even showed no significant differences or decreased the number of pods/plant as compared to the control.

Concerning the effect of foliar applications on number of seeds/pod, data indicated that either 5 μM Se without salinity or 10 μM Se under 50 mM NaCl achieved the highest number of seeds/pod as compared to the other treatments and the control in both seasons.

Regarding the weight of 100 seed, it is evident that application of 5 & 10 μM Se without salinity and all foliar applications under 50 mM NaCl recorded significant increase in weight of 100 seed as compared to the rest treatments, while the highest weights (21.7 & 20.7 g/100 seed) were recorded with 5 μM Se without salinity and 10 μM Se under 50 mM NaCl (21.7 & 20.7 g) respectively in the 1st season and (22.07 & 20.61 g) respectively during the 2nd season. The same trend was observed with the seed yield/plant.

Effect of foliar application on biochemical constituents

Data illustrated in Table 2 showed the effect of different foliar applications on biochemical constituents of 3th leaf of cowpea. As for photosynthetic pigments, chlorophyll (Chl a & b) and carotenoids (Car) were affected differently by salinity, but in both of the controls their contents decrease even more with salinity. On the other hand, Chl a & b were significantly increased at all foliar treatments than the control, except 10 μM Se, 5 mM GB & 2% SWE without salinity and 5 μM Se under 50 mM NaCl. Generally, it could be observed that plants treated with 4% SWE have the highest pigment concentration as compared with the other treatments or the control. It could be also emphasized that the biosynthesis of Chl a was in parallel with that of Chl b.

In relation to carotenoids, foliar application with 4% SWE under control and NaCl concentration and 10 μM Se under

Table 1 Growth and yield parameters of cowpea as affected by foliar application with different concentrations of selenium, glycine betaine and seaweed extract under salt stress during the two seasons (2014/2015).

Treatments	Growth parameters of 3rd leaf						Yield parameters											
	F. Wt (g)		D. Wt (g)		Leaf area (mm ²)		Pod length (cm)		Pod D. Wt (g)		Number of Pods/plant		Number of Seeds/pod		Weight of 100 seed (g)		Seed yield/plant (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
<i>NaCl 0 mM</i>																		
Control	2.63	2.58	0.363	0.354	098.27	96.76	10.73	10.55	2.67	2.56	2.33	2.27	6.66	6.47	16.20	15.99	2.51	2.35
Se 5 µM	4.16	3.98	0.560	0.536	123.81	119.74	14.00	13.92	2.72	2.70	3.66	3.69	9.00	9.10	21.70	22.04	7.15	7.40
Se 10 µM	3.38	3.40	0.483	0.478	114.46	115.60	12.66	12.78	2.24	2.31	3.00	3.12	7.33	7.42	19.90	19.94	4.37	4.61
GB 5 mM	3.09	3.14	0.363	0.371	104.18	105.04	09.00	09.07	1.86	1.91	2.33	2.38	6.00	6.07	15.20	15.23	2.12	2.20
GB 10 mM	3.11	3.22	0.443	0.461	108.58	111.12	10.33	10.48	1.91	1.93	2.33	2.41	7.33	7.45	15.60	15.83	2.66	2.84
SWE 2%	2.86	2.90	0.363	0.366	104.11	106.22	11.00	10.94	2.19	2.13	3.00	3.13	6.33	6.46	14.50	14.70	2.75	2.97
SWE 4%	3.07	3.15	0.370	0.385	107.35	109.03	12.00	12.07	2.28	2.32	2.93	2.99	7.00	7.12	15.60	15.69	3.20	3.34
<i>NaCl 50 mM</i>																		
NaCl	2.02	1.96	0.290	2.88	066.42	064.97	08.16	7.99	0.46	0.51	1.33	1.24	3.00	3.02	12.46	12.41	0.49	0.46
Se 5 µM	3.29	3.23	0.470	0.468	112.00	110.88	12.00	11.89	1.92	1.84	2.33	2.31	6.33	6.29	19.13	18.95	2.82	2.75
Se 10 µM	4.04	4.12	0.510	0.499	116.75	115.76	13.33	13.12	2.45	2.43	3.33	3.41	7.96	7.93	20.70	20.61	5.48	5.57
GB 5 mM	2.87	3.01	0.390	0.392	075.61	080.03	08.33	8.52	0.98	1.00	2.00	2.11	5.00	5.18	18.00	18.20	1.80	1.99
GB 10 mM	2.91	3.09	0.400	0.398	084.47	083.99	09.00	09.16	1.26	1.29	2.33	2.38	5.66	5.64	18.70	18.58	2.46	2.49
SWE 2%	2.32	2.41	0.303	0.316	067.29	068.54	08.83	09.03	0.96	0.99	2.00	2.06	3.66	3.72	18.30	18.62	1.34	1.42
SWE 4%	2.58	2.63	0.333	0.329	068.61	070.02	08.86	09.14	1.35	1.41	2.33	2.42	4.66	4.76	19.90	20.02	2.16	2.30
LSD 5%	0.14	0.15	0.027	0.026	4.11	4.18	1.28	1.31	0.155	0.152	1.03	1.02	1.41	1.44	0.44	0.53	0.06	0.06

Table 2 Effect of foliar application with different concentrations of selenium, glycine betaine and seaweed extract concentrations under salt stress on biochemical constituents of 3th leaf of cowpea during the first season (2014).

Treatments	Photosynthetic pigments			Biochemical properties					
	Chl a mg g ⁻¹ f. Wt	Chl b	Car	Proline µg g ⁻¹ f.	TSS mg g ⁻¹ f. Wt	Protein	SOD Unit mg ⁻¹ protein	POD	PAL
<i>NaCl 0 mM</i>									
Control	0.879	0.310	0.261	305.41	0.892	29.01	5.71	7564	11884
Se 5 µM	0.986	0.403	0.326	365.57	0.995	34.51	5.39	4855	21281
Se 10 µM	0.843	0.298	0.276	355.02	1.128	31.76	4.53	5463	16221
GB 5 mM	0.891	0.310	0.282	509.94	1.051	29.01	4.67	5882	20412
GB 10 mM	1.053	0.450	0.312	519.35	1.182	28.42	5.22	5416	20834
SWE 2%	0.918	0.342	0.241	565.85	1.189	28.29	5.44	6299	18546
SWE 4%	1.110	0.520	0.366	671.27	1.588	27.36	5.47	7224	18845
<i>NaCl 50 mM</i>									
NaCl	0.822	0.264	0.212	519.35	1.851	17.06	5.73	15917	20035
Se 5 µM	0.930	0.368	0.276	567.25	1.343	24.98	9.33	25571	20616
Se 10 µM	1.014	0.424	0.379	587.56	1.445	25.09	7.06	22088	21123
GB 5 mM	0.938	0.394	0.294	786.00	2.047	26.85	6.13	20964	21291
GB 10 mM	0.979	0.388	0.314	838.71	2.186	27.07	6.44	23649	22408
SWE 2%	1.065	0.451	0.314	810.59	1.369	26.53	5.51	14544	22466
SWE 4%	1.385	0.569	0.389	869.71	1.568	28.50	6.36	12555	23232
LSD 5%	0.053	0.082	0.069	59.1	0.11	1.82	0.38	14721	945

50 mM NaCl achieved the highest carotenoids content with no significant differences between values. It could be noticed that 4% SWE under 50 mM NaCl recorded the highest values of the photosynthetic pigments, while 50 mM NaCl singly recorded the lowest values.

As for biochemical properties, it is clear that all treatments showed significant increment in proline concentration against the control except 10 µM Se without salinity. The highest significant concentrations were recorded with plants irrigated with 50 mM NaCl and sprayed with 4% SWE followed by 10 mM GB (869.71 & 838.71 µg g⁻¹ F. Wt respectively).

Concerning total soluble sugars (TSS), all treatments showed significant increment against the control except 5 µM Se without salinity. The highest significant concentrations were recorded with plants irrigated with 50 mM NaCl and sprayed with 10 mM GB followed by 5 mM GB (2.186 & 2.047 mg g⁻¹ F. Wt respectively), when compared to all treatments. The lowest concentrations were recorded within plants irrigated with control and 5 µM Se without salinity (0.892 & 0.995 mg g⁻¹ F. Wt, respectively).

In accordance with protein, the data showed that most treatments recorded significant decrements especially under salt stress except 4% SWE and 10 mM GB or no significant differences under salt absence except 5 & 10 µM Se which showed the highest significant increments against the control (34.51 & 31.76 mg g⁻¹ F. Wt, respectively).

As for antioxidant enzymes, 50 mM NaCl singly or with 2% SWE, SOD activity increased significantly in the leaves as compared to the control but under non-saline the activity was decreased or showed no significant with the control. The highest rate activity of SOD was shown by 5 µM Se followed by 10 µM Se under 50 mM NaCl as compared with the other treatments. POD activity increased remarkably in plants under salt stress. The highest rate of POD activity was recorded with

5 µM Se and 10 mM GB under 50 mM NaCl (25571 & 23649 Unit mg⁻¹ protein) respectively. PAL activity significantly increased with all treatments when compared to the control. 4 & 2% SWE and 10 mM GB under salinity recorded the highest activity rate (23232.1, 22466.3 and 22408.2 Unit mg⁻¹ protein) respectively.

Discussion

Salinity has direct effect on osmotic potential in natural and agricultural habitats. Additionally, saltiness affects various reactions going from growth inhibition and synthesis of some compounds to increase the osmotic capability of the cell and hence permits metabolic procedures to proceed by improving some antioxidant enzyme activities (Greenway and Munns, 1980).

The present study showed that salinity reduced significantly growth and yield parameters of *V. unguiculata* below the control. In this respect, Ghoulam et al. (2002) expressed that saltiness treatment results in a progressive decrease in development among the four cowpea cultivars. Shoot fresh and dry weights of cowpea at all phases of improvement were decreased continuously with expanding NaCl concentrations (Abdelgawad and Zinab, 2014; Manaf and Zayed, 2015). Also, Safarnejad et al. (2007) demonstrated that expanding saltiness stress caused a decrease, in both shoot and root yield of *Nigella sativa*. In vegetative plants, salt anxiety causes lessened cell turgor and discouraged rates of root and leaf stretching (Werner and Finkelstein, 1995; Fricke et al., 2006), recommending that ecological saltiness acts essentially on water uptake.

The data indicated that salinity (50 mM NaCl) reduced the concentration of photosynthetic pigments (Chl a & b and Car.) and total protein in cowpea leaves. Numerous studies affirm the inhibitory impact of saltiness on biochemical procedures,

of which photosynthesis is the most critical. The impact on photosynthesis can be gauged from the impact on the photosynthetic pigments. The results of specific studies (Misra et al., 2006; Murillo-Amador et al., 2007; Taffouo et al., 2010) demonstrate that saltiness diminishes the content of photosynthetic pigments in treated plants. Heidari (2012) mentioned that chlorophyll content in leaves was also affected by salinity and this effect depends on the levels of salinity. By increasing salinity levels from 0 to 6 ds/m, chlorophyll a, b and carotenes content in two basil genotypes decreased. Also, the results of certain studies (Chen et al., 2007; Kapoor and Srivastava, 2010) demonstrate a decrease, or increase, in protein content in plants treated with different salt concentrations.

Proline increased in response to NaCl stress. In this respect, Heidari (2012) reported that by increasing salinity levels from 0 to 6 ds/m, proline accumulation in leaves of basil plants increased.

Concentrations of total soluble sugars increased at salinity stress. The results are in agreement with the previous work of Nemati et al. (2011), who reported that total soluble carbohydrates under saline conditions showed significant increment than control in both genotypes of rice. Also some studies reported that plants sugar content rose (Munns and Weir, 1981) or remained constant (Morgan, 1992) under salinity.

Regarding the activities of the antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD) and Phenylalanine ammonia lyase (PAL), an increment was detected under 50 mM NaCl against the control. In this respect Lee et al. (2001); Mittova et al. (2002, 2003) showed an expansion in these enzymes under salt stress in plants and a correlation of these catalyst levels and salt toleration exists.

Results showed that the advantageous impact of Se in plants subjected to strain conditions has in most cases been attributed to increased antioxidant activity. Research works led by Xue et al. (2001) and thereafter by Djanaguiraman et al. (2005) showed the effect of Se application in the form of selenate on senescence in lettuce and soybean, confirming that the decline in antioxidant enzyme activity was milder in plants treated with this element, which offsets oxidative damage by boosting growth in plants treated with Se. Moreover, some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses such as salinity (Kong et al., 2005; Djanaguiraman et al., 2005; Hawrylak-Nowak, 2009). Selenium as foliar application enhanced growth parameters, yield parameters and biochemical constituents of cowpea especially 5 μM Se without salinity or 10 μM Se under 50 mM NaCl. So, it seems that the high concentration of 10 μM Se under salt stress offsets the lower concentration without salinity. The data are in agreement with Kong et al. (2005), who reported that at low concentrations (1–5 μM) Se had a tendency to encourage the development, the activities of SOD and POD enzymes, in addition the piling up of water-soluble sugar in leaves of sorrel seedlings. However, at higher concentrations (10–30 μM), Se applied decreased beneficial effects on plant development and catalyst activities. Results detected that SOD and POD activities of salt-stressed seedlings enhanced when presented to concentrations ranging 1–5 μM Se. At concentrations between 10 and 30 μM , there were adverse effects on both enzymes compared with those at 5 μM Se. Hawrylak-Nowak (2009) mentioned that Se treatments at 5 & 10 μM significantly improved the growth rate and increased the photosynthetic pigments and

proline contents when subjected to salt stress in cucumber leaves. Several articles have shown that Se not only able to encourage growth and development of plants but also augment resistance and antioxidant capacity of plants subjected to various stresses (Peng et al., 2002; Djanaguiraman et al., 2005).

Data in Table 2 show that GB at both levels increased total soluble sugar concentration and antioxidative enzymes (POD & PAL) under salt stress. In this respect, Gorham (1995) showed that GB establishes the structures and activities of enzymes and protein complexes and preserves the integrity of membranes against the harmful effects of excessive salt, cold, heat and freezing. Wyn Jones (1984) and Agboma et al. (1997) suggested that the protective role of glycine betaine on Sorghum growth can be identified with its part in osmotic change where it goes about as a non-toxic cytoplasmic osmolyte. Moreover, Bohnert and Jensen (1996) revealed that GB preferentially excludes inorganic ions such as sodium from the hydration sphere of proteins and thus protects enzymes from denaturation. On the other hand, Heuer (2003) pointed that compatible solute such as GB is known to play a role in the process of osmotic adjustment in many crops accumulated under environmental stress and the main role is probably, due to insulating plant cells against the ravages of salt by preserving the osmotic balance, by stabilizing the structure of key protein such as Rubisco, by protecting the photosynthetic apparatus and by functioning as oxygen free radical.

In the present experiment, a decrease in chlorophyll a, b, and carotenoids was observed under saline conditions. The maximum reduction was recorded with 50 mM NaCl. But this reduction was very much reduced in SWE (4%) supplemented plants alone or with 50 mM NaCl. The results are in agreement with the previous work of Whapham et al. (1993), and they observed that application of seaweed (*Ascophyllum nodosum*) enhanced chlorophyll of cucumber seedlings and tomato plants. Also, seaweed extract spray enhanced the leaf chlorophyll in plants (Blunden et al., 1996). Moreover, Thirumaran et al. (2009) demonstrated that seaweed extract enhanced photosynthetic pigments such as chlorophyll a, b and carotenoids on *Cyamopsis tetragonoloba*. Also seaweed extracts as biostimulants showed the highest peroxidase enzyme activity (POD), which was observed at higher concentration.

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