Al-Azhar Bull. Sci. Vol. 19, No. 2 (Dec.): pp. 91-108

EFFECT OF SALICYLIC ACID ON PHOTOSYNTHESIS AND SOME ANTIOXIDANT ENZYMES OF STRESSED *SCENEDESMUS OBLIQUUS* CULTURES

DESOUKY, S. A

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut, 71524, Egypt. E- mail: Desouky_alazhar@yahoo.com..

Abstract

This study followed the effect of salicylic acid on growth criteria, photosynthetic pigments, photosynthesis, respiration, proline and the activities of some antioxidant enzymes of stressed *Scenedesmus obliquus* cultured for 7 days.

The growth criteria (cell number and dry weight) and total pigments of non-stressed and stressed *Scenedesmus obliquus* cultures were significantly increased, when the algal cultures were subjected to 200 mM NaCl and treated with 100and 200 ppm of salicylic acid. Also, Photosynthesis (oxygen evolution), respiration (dark oxygen uptake), proline, amino acids and catalase enzyme of non- salinized *and* salinized *Scenedesmus obliquus* cultured were significantly increased, when the algal cultures were subjected to 200 mM NaCl and treated with 100and 200 ppm of salicylic acid. However, both respiration (oxygen uptake) , lipid peroxidation as termed (malondialdhyde) and peroxidase were significantly decreased, when the algal cultures were subjected to 200 mM NaCl and treated with 400 ppm of salicylic. These values were compared with that of the control cultures.

Key word: *Scenedesmus obliquus*, Photosynthesis, respiration, Lipid peroxidation (malondialdhyde) , catalase and peroxidase.

Introduction

Salinity is considered the main problem in many countries. Thus, salinity is a limiting environmental factor for plant production, and is becoming more prevalent as the intensity of agriculture increases. Salinity and drought stress can reduce plant growth and alter ionic relations by ionic and osmotic effects (Tarakcioglu and Inal, 2002), and oxidative stress (Borsani *et al.*, 2001). The response of fresh water algae to salinity differs from one type to anther. Consequently, the growth of *Chlorella vulgaris, Scenedesmus obliquus, Ankistrodesmus falactus, Ulothrix implexa, Ulotheix subflaccida* and *Enteromorpha bublosa* algae were significantly decreased under higher levels of salinity (Whittington and Biosson,1994 and Desouky; 1995).

Generally, salinity was inhibited to the biosynthesis of photosynthetic pigments in higher plants and some fresh water algae (Czerans, 1978 and Desouky, 1995).

Photosynthesis, a key metabolic pathway in plants, is a target for salt stress. Thus, photosynthesis is considered the main vital process in the higher plants and algae. The photosynthesis process plays an important role in the building and production of many raw materials. The photosynthesis was inhibited under unfavorable conditions. In this respect, some workers found that, oxygen evolution was gradually inhibited as increasing NaCl concentration in the medium (Desouky, 1995 and Koyro, 2005).

Respiration in plants and green algae have been intensively studied by many investigators (Lloyed, 1974; Kremer, 1981 and Desouky, 1995).In this context, some workers found that the respiration rate in some plant species, were higher when subjected to moderate and higher salinization treatments (Lambers1979, 1980).

The contents of free amino acids were also found to be affected by salinity and/or water deficit. In this respect some other investigators working with some plant types found that the contents of amino acids were considerably decreased with higher salinization treatments (Desouky, 1995 and Desouky,2003 a&b).

Proline in particular, is broadly involved in the process of osmoregulation. The accumulation of proline under salt stress is a common phenomenon (Cusido *et al*. 1987).In The typical first response of all plants and green algae to salt stress is osmotic adjustment. Accumulation of compatible solutes in the cytoplasm is considered a mechanism contributing to salt tolerance (Jalell *et al*, 2007a). To control salt stress, plants increase the osmotic potential of their cells by synthesizing and accumulating compatible osmolytes such as proline and glycine betaine (GB), which participates in osmotic adjustment (Jalell *et al*, 2007 b).

Small amounts of salicylic acid are known to be present in plants. Salicylic acid (SA) plays an important role in abiotic stress tolerance, and considerable interests have focused on SA due to its ability to induce a protective effect on plants under stress. Salicylic acid (SA) is considered as a hormone-like substance, which plays an important role in the regulation of plant growth and development, glycolysis process, (Klessig and Malamy, 1994). Ion uptake and transport, photosynthetic rate, stomatal conductance and transpiration could also be affected by SA application (Khan *et al.*, 2003).Thus, the role of the SA in defense mechanisms under both biotic and abiotic stresses suggests that it also alleviates the salt stress in plants (Tissa *et al.*, 2000). Also, exogenous added to SA could regulate the activities of antioxidant enzymes and increase plant tolerance to the abiotic stress (Hu *et al.*, 2002).

In this respect, salicylic acid has qualified as a plant growth regulators due to its physiological and biological roles in plants (Raskin, 1992). SA has been suggested as signal transducer or messenger under stress conditions (Klessig and Malamy, 1994). However, the role of exogenously applied SA under salt stress on the photosynthetic pigments, membrane permeability and mineral uptake of plant are not still clear or lacks information. Extensive studies have been undertaken to elucidate the molecular biology of SA-induced systemic acquired resistance (Raskin, 1992;Levin *et al.*, 1994; Mur *et al.,* 1996). However, the physiological and biochemical basis for this phenomenon and the mechanism of signal regulation of plant resistance induced by SA are not still well known (Senaratna *et al*., 2000; Shakirova *et al.*, 2003).

Under oxidative stress conditions such as salinity, drought and low or high temperature, plants produce active oxygen species, which are harmful to plant growth due to their detrimental effects on the subcellular components and metabolism of the plant, leading to the oxidative destruction of cells. It is generally accepted that O_2^- might be converted to hydrogen peroxide (H_2O_2) and then metabolized to water by ascorbate peroxidase and glutathione reductase in plants (Shim *et al.,* 2003). Active oxygen species cause deterioration of membrane lipids, leading to increased leakage of solutes from membranes (Mishra and Choudhuri, 1999). The maintenance of membrane integrity for enhanced stress tolerance is worthwhile. This is why the role of SA on membrane leakage, lipid peroxidation, H_2O_2 production and mineral uptake of plants under saline conditions is of great importance.

When plants are subjected to saline conditions, such biochemical changes occur as production of reactive oxygen species (ROS) like superoxide, hydrogen peroxide and hydroxyl radicals (Dat *et al.,* 2000). In order to avoid the harmful effects of these reactive oxygen species (ROS), plants evolved an effective scavenging system composed of non-enzymatic antioxidants (carotenoids, ascorbate, tocopherol), and enzymatic antioxidants, such as catalase (CAT) and ascorbate peroxidase (APX). The results of most studies suggest that the resistance to salt stress is usually correlated with a more efficient antioxidative system (Gossett *et al.,* 1996 and Gueta-Dahan *et al.,* 1997). The enzymatic action of ascorbate peroxidase (APX) reduces H_2O_2 using the ascorbate as an electron donor. Catalase (CAT) is also implicated in removal of H_2O_2. Salinity induced production of reactive oxygen species (ROS) disturbs the cellular redox system in favor of oxidized forms, and thereby creating an oxidative stress that can damage DNA, inactivate enzymes and cause lipid peroxidation (Smirnoff, 1993).

This study aim to elucidate the role of some external addition of salicylic acid to alleviate the counteract of the adverse effect of salinity stress on growth parameter, photosynthesis, respiration, proline and some antioxidant enzymes system of *Scenedesmus obliquus* cultures.

Materials And Methods

Tested alga:

Scenedesmus obliquus was collected from the River Nile and was used as a test organism. Beijerinck's nutritive medium was used as a nutritive medium for enrichment and growth of the tested alga, (Stein, 1966).

Treatments:

Scenedesmus obliquus cultures were subjected to level of 200 mM NaCl and 100, 200 and 400 ppm of salicylic acid for 7 days. The control cultures (00) NaCl and (00) salicylic acid was left without any treatments.

Analytical Methods:

1-Determination of cell number:

Haemocytometer, (0.1mm depth) having improved Naubuer ruling was used. One drop of the algal suspension was pipetted on the slide, Covered and left for two minutes for alga setting. The mean counts of three replicates were taken into consideration and the results measured as cells ml^{-1} alga1 suspension.

2-Determination of dry weight:

A definite volume (100 ml) of algae suspension was filtered through weighed glass fiber filter. The cells after being precipitated on the filter were washed twice with distilled water and dried over night in an oven at 105 ^{o}C. The data were expressed as µg ml^{-1} algal suspension.

3-Determination of photosynthetic pigments:

The pigment fractions (µg $m1^{-1}$ algal suspension) chlorophyll a, chlorophyll b and carotenoids were calculated using the equations mentioned by Metzner *et al*., (1965).

Chlorophyll a = $10.3E_{663}$-$0.918E_{664}$ = (µg $m1^{-1}$ algal suspension)

Chlorophyll b = $19.7E_{664}$- $3.87E_{663}$ = (µg $m1^{-1}$ algal suspension)

Carotenoids = 4.2E (0.0264 chlorophyll a + 0.426 chlorophyll b) = (µg $m1^{-1}$ algal suspension)

4 -Oxygen evolution:

Oxygen evolution was measured by using the oxygen electrode model 97.08 as mentioned by Lessler *et al*., (1956). The data of oxygen evolution were calculated as μ mole O_2 ml^{-1} algal suspension hr^{-1}.

5-Oxygen uptake:

The dark respiration was determined using oxygen uptake in the dark as an indicator. The system mentioned above was used for the determination of dark respiration. At the end of oxygen evolution measurements, all the lights were switched off and the flasks were wrapped tightly in aluminum foil for complete darkness. The results of oxygen uptake were calculated as μ mole O_2 ml^{-1} algal suspension.

6- Proline:

It was determined according to Bates *et al,* (1973) methods. The results of proline contents were calculated (μg mg^{-1}dry weight).

7-Other free amino acids:

Free amino acids were extracted from fresh water algal suspension and calorimetrically determined using the method of Moore and Stein (1948). The free amino acid contents are calculated as μg mg^{-1} dry weight).

8- Determination of Lipid peroxidation as Malondialdehyde (MDA):

It was measured in termed Malondialdehyde (MDA). The level of lipid peroxidation in the plant tissue was quantified by determination of malondialdehyde (MDA) spectrophotometrically as described by Zaho *et al*., (1994), a breakdown product of lipid peroxidation. MDA content was determined with thiobarbituric acid reaction. A known volume (200 mls.) of the algal suspension was centerfiugated at 10,000*g* for 10 min., the cells were homogenized in 5 mls. of 0.1% from TCA (w/v). The homogenate was spun at 10,000*g* for 5 min. Four mils buffer solutions using 20% (w/v) trichloroacetic (TCA) acid containing 0.5% (w/v) thiobarbituric acid added to 3 mils aliquot of the supernatant. The mixture was crushed with sand and put in water path at 95°C for 15 min and cooled immediately, and the absorption of the supernatant was read at 532 nm. The value of Malondialdehyde (MDA) was calculated from the absorption at 600 nm. The concentration of Malondialdehyde (MDA) was calculated using the extinction coefficient of 155 $mmol^{-1}$ l^{-1} cm^{-1}.

9-Peroxidase activity: It was determined spectrophotometrically according to the method Adam *et al.,* (1995). The assay medium contained 1.5 ml of (100 mM K-

phosphate buffer) at pH 5.5, 100 µ l^{-1} of 1 mM guiaicol and 10 µ l^{-1} of enzyme extract and 190 µL of water. The reaction was started by addition of 300 ml of 1.3 mM H_2O_2. The increase in absorbance was recorded at 470 nm. The result of peroxidase activity was calculated as (µg mg^{-1} dry weight).

10-Catalase activity (EC 1.11.1.6):

It was determined Spectrophotometrically by measuring the rate of H_2O_2 conversion to O_2, this according method (Beers and Sizer, 1952; Aebi, 1984 Dietiz 2003). The Bioassay medium contained 2.9 ml of bioassay media (K-phosphate buffer at pH 7.5) containing 3 mM H_2O_2, 200 μl^{-1} of sample tissue extract .The decrease in absorbance at 240 nm was monitored. The result of catatase activity was calculated as (µg mg^{-1} dry weight).

11- Statistical method:

Four replicates were used in this study and the data were statistically analyzed to calculate the Least Significant Difference (L.S.D) according to Snedecor and Cochran (1980).

Results

The results of this study revealed the effect of exogenously additives of salicylic acid on growth criteria, the activities of some metabolic and antioxidant enzymes of sensitive *Scenedesmus obliquus* cultures for 7days.

The growth criteria (cell number and dry weight) of non salinized *Scenedesmus obliquus* cultures were significantly increased, when the algal cells were treated with 100 and 200 ppm SA. Under higher level of 200 mM NaCl and 400 ppm of SA, the growth parameters (cell number and dry weight) were significantly decreased. Similarly, when *Scenedesmus obliquus* cultures were subjected to 200 mM NaCl only, the growth parameters were significantly declined (Table, 1).

On the other hand, *Scenedesmus obliquus* cultures subjected to 200 mM NaCl and treated with different levels of (100and 200 ppm) of SA, the growth criteria were significantly increased. However, the growth parameters were significantly declined, when the algal cells subjected to 200 mM NaCl and treated with 400 ppm of SA. All of these results are compared with that of the control cultures (Table, 1).

The photosynthetic pigments of non salinized *Scenedesmus obliquus* cultures were significantly increased when the *Scenedesmus* cells treated with various levels of (100, 200 and 400 ppm) of SA only. Similarly, when *Scenedesmus obliquus*

cultures subjected to 200 mM NaCl, the photosynthetic pigments were significantly declined. Also, the photosynthetic pigments of salinized *Scenedesmus obliquus* cultures were significantly increased, than that of the control cultures, when the algal cells subjected to 200 mM NaCl and treated with levels (100 and 200 ppm) of SA. All these results were compared with that of the control cultures (Table, 2).

The photosynthesis rate (Oxygen evolution) of non-salinized *Scenedesmus obliquus* cultures were significantly increased up to the level 200 ppm of SA. Under relatively higher level of 400 ppm SA, these contents were significantly decreased.

On other hand, Oxygen evolution of salinized *Scenedesmus obliquus* cultures was increased , when the algal cell subjected to 200 mM NaCl and treated with the levels (100 and 200 ppm) of SA.All of these results are compared with that of the control cultures (Table,3).

The respiration rate of non- salinized *Scenedesmus obliquus* cultures was significantly decreased.

On the other side, the respiration rate of salinized *Scenedesmus obliquus* cultures were significantly decreased, when *Scenedesmus cells* were subjected to a level of 200 mM of NaCl and treated with various levels (100, 200 and 400ppm) of SA. All of these results are compared with that of the control cultures (Table, 3).

Proline contents of non-salinized *Scenedesmus obliquus* cultures were significantly increased. Thus the maximum values of proline contents were 370 % compared to that of the control cultures, when the algal cells were treated with 400 ppm of SA only. Also, the minimum values reached to 236 %, when the algal cultures were treated with 200 ppm of SA.

Also, the proline contents were significantly increased, when *Scenedesmus obliquus* cultures were subjected to 200 mM NaCl, when compared with that of the control cultures .However, when *Scenedesmus obliquus* cultures are subjected to 200 mM NaCl and treated with various levels (100, 200 and 400µ M) of SA the proline contents were significantly decreased, in quantities more than that of the control cultures. All of these results are compared with that of the control cultures (Table, 4).

Amino acid contents non-salinized *Scenedesmus obliquus* cultures were significantly increased up to the level 200 ppm of SA only. However, under high level (400 ppm) of SA, the values of amino acid contents were significantly

decreased. Therefore, the maximum values of free amino acids reached 354 %, when the algal cell treated to with 200 ppm of SA only.

On the other hand, amino acid contents of salinized *Scensdesmus obliquus* cultured were significantly increased, when the algal cultures were subjected to 200 mM NaCl and treated to different levels (100, 200 ppm) of SA. Thus, the maximum values of amino acids reached 317 %. All of these results are compared with that of the control cultures (Table, 4).

Lipid peroxidation is calculated as malondialdehyde (MDA) and peroxidase of non- salinized *Scenedesmus obliquus* cultures was significantly decreased. However, both lipid peroxidation as malondialdehyde (MDA) and peroxidase were significantly increased, when the algal cells subjected to 200 mMNaCl and treated with different levels (100,200 and 400 ppm) of SA (Fig., 1).

On the other hand, catalase activity was significantly increased when the algal cultures subbjrcted to 200 mM NaCl of treated with various levels of SA (Fig.1).

Discussion

This study elucidated the interactive effects between NaCl and salicylic acid on the growth parameters, photosynthetic pigments, photosynthesis, respiration, some metabolic and antioxidant enzymes activities of sensitive *Scenedesmus obliquus* cultured for 7 days.

The growth criteria (cell number and dry weight) of non salinized *Scenedesmus obliquus* cultures were significantly increased, than that of the control cultures, when the algal cells were treated with 100 and 200 ppm of SA. While, under higher relatively level 400 ppm of SA, the growth parameters were significantly decreased. These results matches with those of , Aydin Gunes *et al*, (2007) who were working with *Zea mays* L plants reported that, the growth and dried plant yield significantly increased, when treatmented with various levels of SA (0, 0.1, 0.5 and 1.0 mM). Also, SA treatments alleviated the deleterious effects of salinity on plant growth.

On the other hand, *Scenedesmus obliquus* cultures subjected to 200 mM NaCl and treated with different levels of (100and 200 ppm) of SA, the growth criteria were significantly increased. In this context, Coronado *et al.* (1998) reported that aqueous solutions of SA as a spray to shoots of soybean significantly increased the growth of shoots and roots in either greenhouse or field conditions. Recently,Some

other workers reported that SA treated wheat plants showed higher dry mass compared to those of untreated seedlings under water stress(Shakirova *et al*. (2003) and Singh and Usha (2003). Also, increases in dry matter of salt stressed plants in response to SA may be related to the induction of antioxidant response and protective role of membranes that increase the tolerance of plant to damage (Shakirova *et al.* (2003).

However, the growth parameters were significantly decreased, when the algal cells were subjected to 200 mM NaCl and treated with 400 ppm of SA. All of these results were compared with the control cultures. These results are in agreement with the results obtained by Janda *et al.* (1999) who found that pretreatment of maize seedlings with 0.5 mM SA cause a decrease in growth criteria and photosynthesis. Also, salinity caused decrease in maize plant growth significantly.

The photosynthetic pigments of non- salinized *Scenedesmus obliquus* cultures were significantly increased, when the algal cells treated with various levels (100, 200 and 400 ppm) of SA only. However, when *Scenedesmus obliquus* cultures were subjected to 200 mM NaCl, the photosynthetic pigments were significantly decreased. Also, the photosynthetic pigments of salinized *Scenedesmus obliquus* cultures were significantly increased, when compared with the control cultures, when the algal cells were subjected to 200 mM NaCl and treated with levels (100 and 200 ppm) of SA.

The photosynthesis rate (Oxygen evolution) of non-salinized *Scenedesmus obliquus* cultures were significantly increased in presence of 200 ppm of SA. Under relatively higher level of 400 ppm from SA, these contents were significantly decreased. Also, Pancheva *et al.* (1996) reported that, the decrease in photosynthesis rate in barley might due to the effect of SA on the enzyme activity of Rubisco.

On other hand, Oxygen evolution of salinized *Scenedesmus obliquus* cultures was increased , when the algal cell subjected to 200 mM NaCl and treated with the levels (100 and 200 ppm) of SA. These results are in agreement with the results obtained by; Czerpak *et al.* (2002) who found that SA at lower level of 100 µM in *Chlorella* cultures a significantly increased cell number and protein content. The same authors found that the exogenously added of SA at a level of 100 µM stimulates the biosynthesis of chlorophyll" a" and carotenoids. Shakirova *et al.* (2003) concluded that the increase in growth of the treated wheat with 50 µM SA may be due to the effect of SA in cell division of merited cells causing an increase in the growth and productivity of plant.

The respiration rate of non- salinized *Scenedesmus obliquus* cultures was significantly lowered.

The respiration rate of salinized *Scenedesmus obliquus* cultures were significantly lowered , when the algal cells were subjected to 200 mM of NaCl and treated with levels (100, 200 and 400ppm) of SA.

Proline contents of non-salinized *Scenedesmus obliquus* cultures were significantly increased. Thus the maximum values of proline contents were, when the algal cells were treated with 400 ppm of SA only.

The accumulation of proline in plant cell indicates that the plant cell suffered from increased stress. So, proline contents were significantly increased, when *Scenedesmus obliquus* cultures subjected onl to 200 mM NaCl used only. However, when *Scenedesmus obliquus* cultures subjected to 200 mM NaCl and treated with various levels (100, 200 and 400μ M) of SA proline contents were significantly decreased , in amounts more than that of the control cultures.

In this context, osmotic stress induced a strong accumulation of proline (Gibon *et al.,* 2000). Proline accumulation could be used as an evaluation of tolerance or sensitivity of plants to stresses (Tal *et al.,* 1979 and Alia and Mohanty, 1991).In this respect, Singh *et al.* (1972) found a relationship between the magnitude of water stress on plants and proline accumulation as an index of osmotic resistance in barley. They also added that the higher the potential for proline accumulation the greater resistance of plants to drought.

Amino acid contents of non-salinized *Scenedesmus obliquus* cultures were significantly increased in a level of 200 ppm of SA only. However, under high level (400 ppm) of SA, the values of amino acid contents were significantly decreased.

On the other hand, amino acid contents of salinized *Scensdesmus obliquus* cultured were significantly increased, when the algal cultures were subjected to 200 mM NaCl and treated with different levels (100, 200 ppm) of SA. Also, these results are in agreement with many recent investigations (Tammam, 2003; Khodary, 2004)

Lipid peroxidation is calculated as malondialdehyde (MDA) and peroxidase of non- salinized *Scenedesmus obliquus* cultures was significantly lowered. However, both lipid peroxidation and peroxidase were significantly increased, when the algal

cells subjected to 200 mMNaCl and treated with different levels of (100,200 and 400 ppm) of SA .

On the other hand, catalase activity was significantly increased when the algal cultures subjected to 200 mM NaCl of treated in presence of various levels of SA.

The accumulation of H_2O_2 due to salt stress has been reported by many investigators (Hernandz *et al.* 1995; Fradzilla *et al.* 1997 and Dietz, 2003). Singha and Choudhuri (1990) proposed that H_2O_2 could be play an important role in the mechanism of salt injury. The main function of catalase is to convert H_2O_2 into water and oxygen. Although H_2O_2 is toxic at high concentrations, at low concentration it may play a role in signal transdation processes in both plant and animal organisms (Schreck *et al.* 1991 and Prasad *et al.* 1994). SA specifically inhibited the catalase activity *in vitro* and induced an increase in H_2O_2 concentrations *in vivo* in tobacco plants (Chen *et al*. 1993 and Chen *et al* 1997). The addition of H_2O_2 may help to induce other protective mechanisms (Prasad *et al*.1994, Rao, *et al.,* 1997 and Siegrist , *et al.*,2000).

Table (1) Cell number (cell ml^{-1} algal suspension), dry weight (µg ml^{-1} algal suspension) of *Scenedesmus obliquus* cultures subject to 200mM NaCl and treated with various levels (100, 200and 400 ppm) of SA for 7 days.

Treatments NaCl mM : SA ppm	Cell number	% Control	Dry weight	% Control
00: 00	401.00 x10 ^{4}	100.00	501.00	100.00
00 :100	481.00 x10^4	120.0	651.00	129.90
00:200	551.00 x 10 ^{4}	137.40	719.00	143.40
00 : 400	385.80 x10^4	96.20	601.00	119.90
200 : 00	330.00 x10^4	82.40	421.50	84.10
200 : 100	431.50 x10^4	107.60	551.30	110.00
200 : 200	502.30 x10^4	125.30	580.30	115.80
200:400	199.50 x10^4	49.80	360.30	71.90
L.S.D at 0.05 %	3.170		2.70	

L.S.D significantly at $p \leq 0.05$, n =4

Table (2) :Photosynthetic pigments: Chlorophyll a, chlorophyll b, carotenoids and total pigments (µg ml $^{-1}$ algal suspension) of *Scenedesmus obliquus* cultures subject to 200mM NaCl and treated with various levels (100, 200and 400 ppm) of SA for 7 days.

Treatments NaCl mM : SA ppm	Chloro. a	% Control	Chloro.b	% Control	Carto.	% Control	Total pigments	% Control
00: 00	5.08	100.00	3.05	100.00	2.42	100.00	10.55	100.00
00 :100	6.27	123.42	4.23	138.69	3.25	134.30	13.75	130.33
00:200	7.31	143.89	5.22	171.14	4.24	175.20	16.77	158.96
00 : 400	5.60	110.23	3.90	127.87	3.13	128.93	12.63	119.72
200 : 00	4.10	80.71	2.12	69.51	2.00	82.64	8.22	77.91
200 : 100	5.22	102.76	4.50	147.54	2.81	116.12	12.53	118.77
200 : 200	6.81	134.06	4.82	158.33	3.19	131.82	14.82	140.47
200: 400	3.20	62.99	2.12	69.51	1.82	75.21	7.14	67.68
L.S.D at 0.05 %	0.02		0.023		0.035		0.052	

L.S.D significantly at $p \leq 0.05$, n =4

Table (3) :Oxygen evolution and O_2-uptake (µg ml $^{-1}$ algal suspension hr.$^{-1}$) of *Scenedesmus obliquus* cultures subject to 200mM NaCl and treated with various levels (100, 200and 400 ppm) of SA for 7 days.

Treatments NaCl mM : SA ppm	Oxygen evolution	% Control	Oxygen uptake	% Control
00 : 00	2.313	100.00	1.603	100.00
00 :100	3.215	139.00	1.315	82.00
200: 00	4.210	182.00	1.220	76.10
00 : 400	1.898	82.10	1.403	87.52
200 : 00	1.498	64.80	2.525	157.50
200 : 100	4.210	182.0	1.410	88.00
200 : 200	4.808	207.90	1.468	91.60
200:400	1.995	86.30	1.018	63.50
L.S.D at 0.05 %	0.027		0.28	

L.S.D significantly at $p \leq 0.05$, n =4

Table (4) :Proline contents and amino acids (µg mg $^{-1}$ dry weight) of *Scenedesmus obliquus* cultures subject to 200mM NaCl and treated with various levels (100, 200and 400 ppm) of SA for 7 days.

Treatments NaCl mM : SA ppm	Proline	% Control	Other free amino acids	% Control
00: 00	1.22	100.00	2.00	100.00
00 :100	3.81	313.60	4.07	203.5
00:200	2.87	236.50	6.90	345.0
00 : 400	4.50	370.60	1.92	95.90
200 : 00	5.21	428.64	1.03	51.70
200 : 100	3.23	265.70	5.81	290.50
200 : 200	3.41	280.70	6.34	317.00
200:400	5.22	429.60	1.07	53.44
L.S.D at 0.05 %	0.023		0.040	

L.S.D significantly at $p \leq 0.05$, n =4

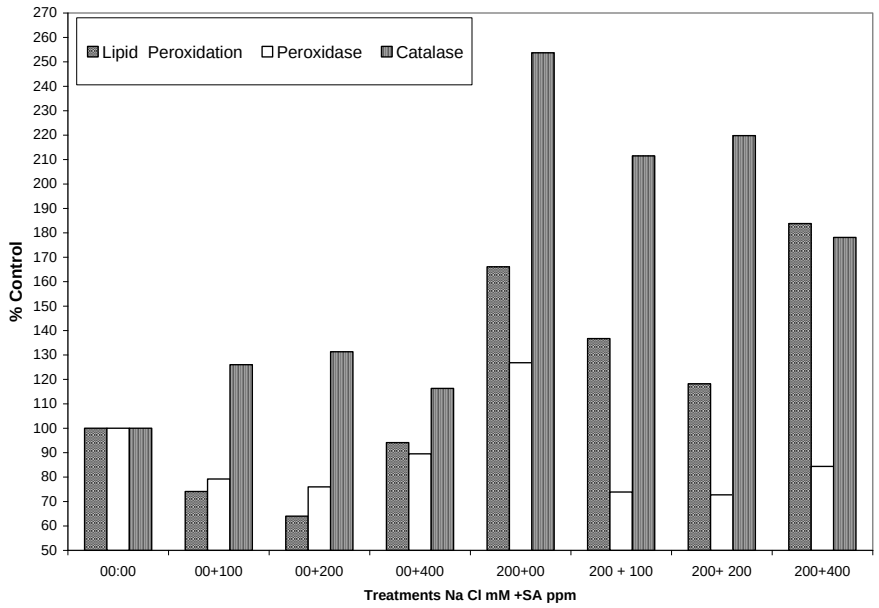

Fig.(1): Lipid peroxidatio as a Malonalidhyde (MDA)) , peroxidase and catalase activities (µ nmole $^{-1}$ µg dry weight) of *Scenedesmus obliquus* cultures subject to 200mM NaCl and treated with various levels (100, 200and 400 ppm) of SA for 7 days.

References

1. ADAM, A.L.; BESTWICH, C.L.; BARAN,B AND MANSFIED,J.W. (1995) : Enzymes regulating the accumulating of active oxygen species during the hypersensitive reaction of bean to *Pseudomonas syringae* pv. Physiol.Plant.197: 240-249

2. AEBI, M. (1984) : Catalase invitro. Meth.Enzym. 105: 121-126

3. ALIA, P. S. AND MOHANTY, P. (1991): Proline enhances primary photochemical activities in isolated thylakoid membranes of *Brassica juncea* by arresting photoinhibitory damage. Biochem. Biophys. Res. Commun. 181: 1238-1244.

4. AYDIN GUNES' , ALI INA, MEHMET ALPASLAN, FIGEN ERASLAN ESRA GUNERI BAGCIAND NURAY CICEK ,(2007) : Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity.J.Plant Physiol. Vol. 164, Issue 6, 728-736

5. BATES, L.S.; WALDREN, R.P. AND TEARE, I.D. (1973): Rapid determination of free proline for water stress studies. Short communication. Plant Soil 39: 205-207.

6. BEERS, R., AND SIZER,I. (1952): A spectrophotometric method for measuring to breaking of hydrogen peroxide by catalase , J.Bio.Chem.195: 133-144.

7. BORSANI O., V. VALPUESTA AND M.A. BOTELLA, (2001) :Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings, *Plant Physiol.* 126 (2001), pp. 1024–1030.

8. CHEN Z., LYER S., COPLAN A., KLESSIG D.F. AND FAN B. (1997): Differential accumulation of salicylic acid and salicylic acid-sensitive catalase in different rice tissues. Plant Physiol. 114: 193-201.

9. CHEN Z.; SILVA H. AND KLESSIG D.F. (1993): Active oxygen species in induction of plant systematic acquired resistance by salicylic acid. Science 262: 1883-1886.

10. CORONADO,M.A.G. ;C.T. LOPEZ AND A.L. SAAVEDRA, (1998) : Effects of salicylic acid on the growth of roots and shoots in soybean, *Plant Physiol Biochem* 8 , pp. 563–565.

11. CUSIDO, R.M.;PALAZON, J, ALTABELLA, T AND MORALES, C.(1987) : Effect of salinity on soluble protein, free amino acids and nicotine contents in *Nicotine restica* L . Plant and Soil.102: 55-60.

12. CZERANS,K. (1978) : the use of selected algal tested in determining the influence of potassium and sodium on the eutrophication of waters . Acta . Hydrobiol.,20 : 323 – 344.

13. CZERPAK,R.,BAJGUZ A., GROMEK,M., KOZŁOWSKA, G. AND NOWAK,I. (2002):Activity of salicylic acid on the growth and biochemistry of *Chlorella vulgaris* Beijerinck. Acta Physiologia Plantarum

14. DAT J., S. VANDENABEELE, E. VRANOVA, M. VAN MONTAGU, D. INZE AND F. VAN BREUSEGEM, (2000) : Dual action of the active oxygen species during plant stress responses, *Cell Mol. Life Sci.* 57 ,pp. 779–795.

15. DESOUKY, S.A, (2003 a): Effect of exogenously additives of fumaric acid upon growth, photosynthesis, respiration, proteins, free amino acids, DNA and RNA of stressed *Chlorella vulgaris* Beijer cultures. Al-Azhar Bull.Sci. Vol. 14 (1): 75-88.

16. DESOUKY, S.A, (2003 b): Alleviation the toxicity effect of lead acetate by riboflavin on growth parameters, photosynthesis, respiration, carbohydrates, proteins, free amino acids and proline of *Chlorella vulgaris* Beijer cultures. Al-Azhar Bull.Sci. Proceeding of the 5th. Int.Sci.Conf, 25-27 March 2003.PP: 277-279

17. DESOUKY, S.A. (1995): Effect of some organic additives on salinized *Chlorella vulgaris.*Ph. D. Thesis. Fac. Sci .Assiut Univ., Assiut,Egypt PP :1-187.

18. DIETZ, K .J. (2003): Plant Peroxidases. Ann. Rev. Plant Biol. 54:93-107.

19. FRADIZILLA M.N., FINCH R. AND BURDON R.H. (1997): Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J.Exp. Bot. 48: 325-331.

20. GIBON, Y., SULPICE, R., AND LARHER, F. (2000): Proline accumulation in canal leaf discus subjected to osmotic stress is related to the loss of chlorophylls and to the decrease of mitochondrial activity. Pysiol.Plant. 110: 469-476

21. GOSSETT, D.R., S.W. BANKS, E.P. MILLHOLLON AND M. CRAN LUCAS, (1996) : Antioxidant response to NaCl stress in a control and a NaCl-tolerant cotton cell line grown in the presence of paraquat, buthionine sulfoximine and exogenous glutathione, *Plant Physiol.* 112 (1996), pp. 803–809.

22. GUETA-DAHAN, Y., Z. YANIV, B.A. ZILINSKAS AND G. BEN-HAYYIM, (1997): Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in *Citrus, Planta*. 203: 460–469.

23. HERNANDEZ, J.A., OLMOS, E.CORPAS, F.J, SEVILLA, F. AND RIO, L.A. (1995): Salt-induced oxidative stress in chloroplasts of pea plant. Plant Sci.105: 151-167.

24. HU ,Y.L., Y.L. LIU, Q. CHEN AND A.H. BIAN, (2002) : Thermotolerance related to antioxidation induced by salicylic acid and heat hardening in tall fescue seedlings, *J. Plant Physiol. Mol. Biol.* 28 :89–95.

25. JALEEL, C.A., MANIVANNAN, P, A. KISHOREKUMAR, B. SANKAR AND R. PANNEERSELVAM, (2007 b): Calcium chloride effects on salinity-induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*, *C. R. Biologies* 330 : 674–683.

26. JALELL, C.A., GOPI, R., B. ,SANKAR, P. MANIVANNAN, A. KISHOREKUMAR, R. SRIDHARAN AND R. PANNEERSELVAM, (2007a): Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress, *S. Afr. J. Bot.* 73: . 190–195.

27. JANDA TS, TARI I AND PALDIE (1999): Hydroponic treatment with salicylic acid decreases the effect of chilling in maize (*Zea mays*) plants. Planta 208:175-180.

28. KHAN ,W., B. PRITHIVIRAJ AND D.L. SMITH, (2003) : Photosynthetic responses of corn and soybean to foliar application of salicylates, *J. Plant Physiol.* 160, pp. 485–492

29. KHODARY, S.E.A. (2004) : Effect of salicylic acid on on the growth , photosynthesis and carbohydrate metabolism in salt streets maize plants. Inter. J.Agri . Bio.6, 1:5-8.

30. KLESSIG ,D.F. AND J. MALAMY, (1994) : The salicylic acid signal in plants, *Plant Mol. Biol.* 26 (1994), pp. 1439–1458.

31. KOYRO, H.W. (2005) : Effect of salinity on growth , photosynthesis, water relations and solute composition of potential cash crop halophyte Plantogo coronpus (L). Enviro. Bot. 124 : 325-333

32. KREMER,B.P. (1981) : Aspects of carbon metabolism in marine micro algae oceanoger. Mar.Boil.Annu.Rev.19:41-94

33. LAMBERS,H. (1979) : Efficiency of root respiration in relation to growth, morphology and soil composition. Physiol.Plant.46:194-202.

34. LAMBERS,H. (1980): The physiological significance of cyanide-resistent respiration. Plant, Cell and Environ.3: 337-346.

35. LESSLER, M. A.; MALLOY, E. AND SCHWAB, C. M. (1956): Measurement of oxidative activity in biological systems by automated oxygen electrode. Fed. Proc. 24: 336-345.

36. LEVIN,,A. R. TENEKEN, R.A. DIXON AND C.J. LAMB, (1994) : H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response, *Cell* 79 (1994), pp. 583–593.

37. LLOYD, D. (1974): Dark respiration .In: Algal physiology and biochemistry, Stewart, W.D.P.ed.Univ.of California Press.PP.505-529

38. METZENER,H.,RAU,H. UND SENGER, H.(1965): untersuchungen Zur Synchronisierbarkeit einzelener Pigment-Mangel Mutanten Von *Chlorella.*Planta 65 : 186-194.

39. MISHRA A. AND CHOUDHURI M.A. (1999): Effect of salicylic acid on heavy-metal induced membrane degradation mediated by lipoxygenase in rice. Biol. Plant 42: 404-415.

40. MOORE,S. AND STEIN , W. (1948) : Photomertric ninhydrine method for use in the Chromatography of amino acids. From the laborites of Rockefeler intitute for Medical Research, New York.

41. MUR, L.A.J., G. NAYLOR, S.A.J. WARNER, J.M. SUGARS AND J. DRAPER, (1996) : Salicylic acid potentiates defence gene expression in leaf tissues exhibiting acquired resistance to pathogen attack, *Plant J* 9 (1996), pp. 559–571.

42. PANCHEVA T.V., L.P. POPOVA AND A.N. UZUNOVA, (1996): Effects of salicylic acid on growth and photosynthesis in barley plants, *J Plant Physiol* 149, pp. 57–63.

43. PRASAD T.K., ANDERSON M.D., MARTIN B.A., STEWARD C.R. (1994): Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. The Plant Cell 6: 65-74.

44. RAO M.V., PALIYATH G, ORMROD D.P., MURR D.P. AND WATKINS C.B. (1997): Influence of salicylic acid on H_2O_2 produces oxidative stress, and H_2O_2-metabolizing enzymes. Plant Physiol. 115: 137-149.

45. RASKIN, I. (1992): Role of salicylic acid in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43: 439-463.

46. SCHRECK R., RIEBER P. AND BAURERLE P.A. (1991): Reactive oxygen intermediate, as apparently widely used messengers in the activation of the NF-KB transcription factor and HIV-1. EMBO J. 10: 2247-2258.

47. SENARATNA, D. TOUCHELL, E. BUNN AND K. DIXON, (2000) :Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants, *Plant Growth Regul* 30 , pp. 57–161.

48. SHAKIROVA, F.M., FATHABUTDINOVA,A.R.,BENZRUCKOVA, M., FATKHUTDINOOA, R.A. AND FATKHUTDINOOA, D.(2003) : Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity . Plant Sci. 164 :317-322

49. SHIM I.S., Y. MOMOSE, A. YAMAMOTO, D.W. KIM AND K. USUI, (2003): Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants, *Plant Growth Regul* 39 pp. 285–292.

50. SIEGRIST J, OROBER M AND BUCHENAUER H (2000): B-Aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. Physiol. Mol. Plant Path. 56:95-106.

51. SINGH B. AND K. USHA, (2003): Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress, *Plant Growth Regul* 39 (2003), pp. 137–141.

52. SINGH, T.N., ASPINALL, D.AND PALEG, L.C. (1972) : Proline accumulation and varietal adaptability to sought barley; A Potential metabolic measure of drought resistance .Nature (London). New Biol.236 : 188-190

53. SINGHA, S. AND CHOUDHURI, M.A. (1990): Effect of salinity (NaCl) on H_2O_2 mechanism in *Vigna* and orysa seedling. Biochem.Physiol. Pflanz.186: 69-74.

54. SMIRNOFF, N., (1993) : The role of active oxygen in the response of plants to water deficit and desiccation, *New Phytol.* 125 (1993), pp. 27–58.

55. SNEDECOR,G.A AND COCHRAN,W.G. (1980) :Statistical Methods,11th Ed.,The Iowa State Univ.Press, Ames , Iowa, U.S.A , PP :172-334.

56. STEIN,J. R. (1966): Growth and mating of *Gonium pectoral* (Volvolcales) in defined media. J. Phycol. 2: 23 – 28.

57. TAL , M.KATZ, A.,HEIKEN, H. AND DEHAN, K. (1979) : Salt tolerance in wild relatives of the cultivated tomato: Proline accumulation in *Lycopericum esculentum* mill, L. *Pervianum* mill and *Solanum pennell* Corp treated with NaCl and polyethylene glycol. New Phyto. 82: 349-355

58. TAMMAM, A. A.(2003) : Response of *Vicia faba* plants to the interactive effect of sodium chloride salinity and salicylic acid treatment. Acta Agro. Hung. 51: 239-248.

59. TARAKCIOGLU , C. AND A. INAL, (2002) : Changes induced by salinity, demarcating specific ion ratio (Na/Cl) and osmolality on ion and proline accumulation, nitrate reductase activity, and growth performance of lettuce, *J. Plant Nutr.* 25 (2002), pp. 27–41

60. TISSA,S. D. DARREN, B. ERIC AND D. KINSLEY,(2000) : Acetyl salicylic acid (aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants, *Plant Growth Reg.* 30 (2000), pp. 157–161.

61. WHITTINGTON, J.,AND BIOSSON, M.A. (1994) : Na^+ influx in *Chara* under salt stress. J.Exp.Bot.45: 657-665

62. ZAHO,S.J.;XU.C.C.AND ZOU, Q. (1994): Improvements of the method for measurement of malondialdehyde in plant tissue.Plant Physiolo.commun.30:207-210