



Original Research Article

Impact of Low Salinity with Sodium Chloride on Germination, Growth and Medicinal Compounds of *Solanum nigrum* Linn.

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ABSTRACT

Keywords

Solanum nigrum,
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anti-inflammatory,
anticancer,
anti-oxidant

The study was designed to elucidate low salinity impacts on the growth and medicinal compounds contents of *Solanum nigrum*. Seeds of *Solanum nigrum* were germinated under low NaCl concentrations which enhanced *Solanum nigrum* seeds germination especially 20 mM while up of NaCl concentration to 50 mM decreased germination. Completely germination inhibition was calculated to be about 70mM. High OE (overall effect) was a low salinity responsive effect that increased germination rate and shortened germination time. A remarkable utilization of metabolites was in radical rather than plumule growth. Efficient metabolism of chl. b occurred under 10-20mM NaCl but higher concentrations was suppressive. Also, chl. a metabolism could be achieved maximally at greater NaCl concentration (40-50mM) as it was less sensitive to salinity as that of chl. b. Carotenoids content increased with most NaCl concentrations. Reduction in mono and di-sugars resulted from general inhibition in photosynthetic pigments (chlorophyll a and b) and its conversion into insoluble polysaccharides. The medicinal important secondary metabolites, varied also by the low NaCl stress as the maximum phenolic compounds were achieved by 50 mM NaCl and increase was by 28.5% control value. However, phenolic compounds metabolism is a significant response to salinity as they have important anti oxidant role during salinity stress.

Introduction

Solanum nigrum Linn. is commonly known as Black Nightshade and it is a dicot weed in the Solanaceae family (Cooper and Johnson, 1984). *Solanum nigrum* is a popular plant in part due to its toxic content of solanine, a glycoalkaloid found in most parts of the plant, with the highest concentrations in the unripened berries (Cooper and Johnson, 1984). It is known to possess various

biological activities like antibacterial, protective effect, antidiabetic activity antifungal, anti-inflammatory, anticancer, anti-oxidant, antipyretic and cytotoxic activity. Root, whole plant and leaves are used but fruits of black colour are not used as they possess toxicity, therefore they are not used for medicinal purposes. Reddish

brown coloured fruits are used for edible purpose (Rajani, *et al.*, 2012).

The nature and the intensity of the response of an individual plant to a particular stress factor may vary depending upon age, degree of adaptation, and seasonal and even diurnal activity (Larcher, 1995). In recent decades, the study of salinity stress effects has gained increasing importance in many fields of biology.

The saline growth medium causes many adverse effects on plant growth, which are due to a low osmotic potential of soil solution (osmotic stress), specific ion effects (salt stress), nutritional imbalances, or a combination of these factors (Ashraf, 1994 and Marschner, 1995).

Much salinity resulted from NaCl cause at least three problems: (A) Osmotic pressure of external solution become more than osmotic pressure of plant cells which require regulating of cells osmotic pressure to prevent dehydration of plant cells. (B) Uptake and transform of nutrition ions such as potassium and calcium, by excess sodium would make problems. (C) High Na⁺ and Cl⁻ rates would cause direct toxic effects on enzymic and membranous systems. One of the effects of salinity stress is reducing photosynthetic activity which was caused by decreasing in both b and a chlorophylls and hence photosynthetic capacity (Francois and Maas, 1993). According to studies, chlorophyll content and photosynthetic rate in salinity stress decreased in Mustard plant compared to control plants (Afroz, *et al.*, 2005). Also, the decline in photosynthesis by salinity stress could be due to low stomatal conductance, depression in carbon uptake and metabolism, inhibition of photochemical capacity, or a combination of all these factors (Mundree, *et al.*, 2002). Carbohydrates are supplied mainly through

the process of photosynthesis and photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Ashraf and Harris, 2004 and Parida and Das, 2005).

Several salt-induced proteins have been identified in plants species and have been classified as salt stress proteins, which accumulate only due to salt stress. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over (Singh, *et al.*, 1987) and may play a role in osmotic adjustment.

Plant phenolics could be enhanced as powerful antioxidants in plant tissues under different stresses, such as salinity (Dixon and Palva, 1995). Total phenolic content increased with salinity levels in fruits like apple and strawberry (Navarro, *et al.*, 2006 and Keutgen and Pawelzik, 2008). Recently, Rezazadeh, *et al.*, (2012) working with artichoke leaves, concluded that moderate saline induced the saline tolerance pathway via increasing total phenolic and flavonoid compounds. On the other hand, salinity induces disturbance of the metabolic processes leading to an increase in phenolic compounds (Dhingra and Varghese, 1985, Ayaz, *et al.*, 2000 and Ali and Abbas, 2003).

The aim of this study is to investigate the effect of low concentrations of sodium chloride on the germination and growth parameters of the important medicinal plant, *Solanum nigrum*, as well as the role of some of its primary and secondary metabolites as a salt stress adaptation mechanism.

Materials and Methods

The seeds of *Solanum nigrum* were obtained from weed research center, Shibin EL-Koum, Egypt. Seeds were surface sterilized

with 5% Clorox for 8 minutes with continuous steering and washed thoroughly many times in distilled water. These seeds were allowed to germinate in 9 cm diameter Petri dishes, 50 seeds in each dish, on moist filter paper with water or the prepared different concentrations of NaCl (0, 10, 20, 30, 40, 50 mM). Each treatment was replicated 3 times.

The seeds were moistened every 2 days, for 24 days with the solutions or distilled water and kept at 29 ± 3 °C in the laboratory light conditions. Radical emergence was the criterion for germination. The number of germinated seeds was recorded every 2 days and the germination percentage was calculated.

At the end of experiment fresh seedlings were separated into their radicles and plumules. The plumule and radicle lengths were measured and the plumule/ radicle length ratio was calculated. Plumule and radicle were weighed as fresh and dried at 60-70 °C to a constant weight for the determination of plant radicle and plumule dry weights. Also the water content of both, plumule and radicle, was then calculated.

The determination of photosynthetic pigments content was according to (Metzner *et al.* 1965) in fresh seedling leaves samples (0.1g) which were homogenized immediately in 3 ml 85 % cold acetone water and centrifugated for 15 minutes at 3000 rpm.

These extracts were diluted to the appropriate volume (3 ml), with cold acetone and their color intensities were measured against a blank of pure 85 % aqueous acetone solution at three wavelengths, 663, 644 and 452.5 nm by using a spectrophotometer (NOVASPEC) for the determining chlorophyll a, b and

carotenoids. The following equations were applied for calculating the pigment content as mg/g fresh weight.

$$\text{Chl a} = 10.3 E_{663} - 0.918 E_{644}$$

$$\text{Chl b} = 19.7 E_{644} - 3.87 E_{663}$$

$$\text{Carotenoids} = 4.2 E_{452.5} - (0.0264 \times \text{chl a} + 0.426 \times \text{chl b})$$

The pigment fraction was expressed as µg/g leaf fresh weight.

Estimation of carbohydrates (glucose, sucrose, and starch) was estimated quantitatively using the modified Nelson's method (1944) by (Naguib, 1963). The total soluble proteins content was estimated quantitatively in the borate buffer extract using the method described by Bradford (1976).

The protein content was calculated as mg/g d.wt using a prepared calibration curve by bovine serum albumin protein. Saponin was estimated quantitatively by the method described by (Hiai *et al.*, 1975). A standard curve by cholesterol was constructed and used for the determination of saponin content (mg/g d.wt). Total phenolic content in *Solanum nigrum* seedling was estimated quantitatively using the method described by (Jindal and Singh 1975). The total phenolic compounds content was expressed as mg/g d. wt.

Obtained results were statistically analyzed using one and two ways analysis of variance (ANOVA) to determine the degree of significance for the obtained variations by the used treatments. Also, correlation coefficients were applied for investigating the significance of the relationships between the studied variables of the study plant. All of the statistical methods were according to (Bishop, 1983), while the analysis was carried out by SPSS statistical package.

Results and Discussion

Effect of NaCl on seeds germination percentage

The effect of different concentrations of NaCl, on the germination percentage of *Solanum nigrum* seeds was summarized in Fig. (1). The *Solanum nigrum* seeds germination percentage was enhanced by low NaCl treatment (20 mM) where and maximum germinate percentage reached to 72% as compared with control (62%) and giving rise to an enhancement by 10%. Application of NaCl over 20 mM treatment attenuated the enhancement of the seed germination and seed germination percentages decreased than that of the control by increasing NaCl concentration to (50 mM). The regression equation for the significant ($r = 0.779$) relationship between *S. nigrum* germination percentages and NaCl concentration revealed that about 70 mM as the highest NaCl concentration which completely inhibit the plant seeds germination.

Effect of NaCl on seed germination time:

Data recorded in (Fig.2) show that the time of starting germination of *Solanum nigrum* seeds varied by different concentrations of NaCl and the recorded variations were significant. There were recognized three germinations periods at 0-5 days, 5-10 days and at more than 10 days. In the first period the rate of germination was low and after the 10th day of germination the rate was very low while the period from 5-10 days germination rate was active under all NaCl concentrations.

The regression equations of the relationships between time and germination percentages during this active period were linear and significant under all NaCl concentrations

(Table 1). However, salinity slowed down the germination process of the plant especially the high concentration of NaCl. Low salinity may decrease time of germination in comparison with the control. The time after which 50% of seeds germination occurred (T50) due to 20mM NaCl treatment was the shortest time and was remarkably lower than that needed for control. Increasing NaCl concentrations decreased not only the rate of germination but also increased the time needed to reach the T50 of germination.

The overall effect of NaCl stress (OE) on the germination process as percentage and time could be calculated by the following equation.

$$OF = (A+B)/2$$

Where:

(Treatment of germination % - Control germination %)

$$(A) = \frac{\text{---}}{\text{Control germination \%}} \times 100$$

(Control T50 – Treatment T50)

$$(B) = \frac{\text{---}}{\text{Control T50}} \times 100$$

The overall on the germination process was enhancing under all NaCl stress treatment and it exhibited its maximum percentage (12.0) by 20 mM NaCl while it was inhibiting after 40mM and with -11.14 by 50 mM NaCl. The enhancement in germination was confirmed by the increase in the slope of the regression lines and shortening of T50 under the used NaCl concentrations.

Growth criteria

The effect of salinity stress by NaCl, on some growth criteria of *Solanum nigrum* seedling have been recorded as seedling

radicle and plumule length in Fig.(3). The results showed that seedling length was greater under NaCl concentrations from 10 to 40 mM with a maximum under 10 mM NaCl compared to control treatment. On the opposite by increasing NaCl to 50 mM the seedling length decreased remarkably.

The lengths of radicle and plumule on the seedling of *Solanum nigrum* under different concentrations of NaCl were significantly varied ($p < 0.01$). Plumule length was lower under all NaCl concentrations especially 50mM compare with control. On the opposite the length of seedling radicle was longer under all NaCl concentration with a maximum length under 50 mM NaCl in comparison with the control value. This led to significantly increased ($r=0.81$) radical/plumule ratio in seedling of *Solanum nigrum* by increasing NaCl concentration when compared with the control. The maximum value of radicle/plumule ratio was 0.459 at 50 mM NaCl concentration. This increase was due to the marked decrease in plumule length and the slight increase in radicle length.

Germination rate was active in all concentrations of NaCl in the period from 5-10 days. At the first period (0-5 days) and after the 10th day of germination the germination rate was very low

The linear regression equation of the relationship between radical/plumule ratio with NaCl concentration gave rise to 0.199 rate of increase in the radicle to plumule length by the unit increase in NaCl concentrations.

Photosynthetic pigments

The photosynthetic pigments content in the fresh leaves of *Solanum nigrum* seedling which were subjected to different

concentrations of NaCl is shown in Fig.(4). The content of chlorophyll a of *Solanum nigrum* increased as a response to the increased concentrations of NaCl, except in concentration 20 mM in comparison with the control. The maximum content of chlorophyll a was 4.057 mg/g fresh weight in plants of 50 mM NaCl concentration treatment. The minimum chlorophyll a content 2.832 mg/g fresh weight was by 20mM NaCl concentration. The maximum of chlorophyll a content increased by 27%, while the minimum content decreased by 11% of the control value.

Data of chlorophyll b content in the leaves of *Solanum nigrum* seedling under the different concentrations of NaCl (Fig.3) indicated a marked sensitivity to NaCl stress as indicated by the progressive decrease with the increase of concentrations of NaCl, except the recorded increases with the concentrations 10 and 20 mM when compared with the control. The maximum content of chlorophyll b was 2.696 mg/g fresh weight by 10 mM NaCl and was 1.9 times the control content, while the minimum chlorophyll b content 0.727 mg/g fresh weight was by 30 mM NaCl and was slightly lower than that of the control.

Data of carotenoids content in the leaves of *Solanum nigrum* treated with different concentrations of NaCl (Fig.3) exhibited an increasing trend with the increase of NaCl concentrations, except the marked decrease due to with 10 to 30 mM NaCl concentrations. The maximum content of carotenoids (3.847 mg/g fresh weight) was due to 40 mM NaCl, while the minimum of carotenoids (1.006 mg/g fresh weight) was to 10 mM NaCl. The percentage of increase for the maximum of carotenoids was by about 53% while the decrease of minimum was by about 60% of control value.

The total pigments contents in the leaves of *Solanum nigrum* were higher compare to control value by all concentrations of NaCl except 30 mM. The highest content of total pigments was 8.661 mg/g fresh weight was recorded under 40 mM NaCl and was due to the marked increase in both chlorophyll a and carotenoids. The least total pigments (5.662 mg/g fresh weight) were a result of 30mM NaCl concentration. It is also remarkable that chlorophyll a content was greater than the control of each chlorophyll b and carotenoids under all NaCl treatments. The carotenoids contents came next under all NaCl treatments except 10 mM NaCl treatment where chlorophyll b was the next.

Carbohydrates content

The carbohydrates content (Table 2) in *Solanum nigrum* significantly varied by different concentrations of NaCl. The amount of direct reducing sugars values (DRV) fluctuated around a marked decrease with increasing concentration of NaCl. However, under all NaCl treatments DRV content was lower than that under the control. The lowest amount of sugar content was due to the 30mM NaCl (24.7 mg/g d.wt).

The data of Total reducing value (TRV) referred to sucrose in seedling of *Solanum nigrum* represented in Table (2) indicated marked variations by treatment of the plant with different concentrations of NaCl. The amount of the seedling sucrose showed a great decrease with all of NaCl concentrations. The greatest inhibition of sucrose metabolism was recorded at the 10 mM NaCl (696.4 mg/g d.wt). At this NaCl treatment (10 mM) the decrease was by 13.6% then the decrease of the content of sucrose was attenuated by increasing of NaCl concentration and it reach only about 4.9% at 50 mM NaCl treatment.

The polysaccharides in seedling of *Solanum nigrum* Table (2) differed due to treatment with all concentrations of NaCl. The amount of polysaccharides showed a great increase with all concentrations of NaCl as compared with the control. The maximum content of polysaccharides was recorded at the 30mM NaCl treatment (2.6 mg/g d.wt). This maximum of polysaccharides content was about 6 times the control content. More or less than NaCl concentration to 30 mM there were increases in the polysaccharides with lower values.

The total of carbohydrates (DRV, sucrose, and polysaccharides) of *S. nigrum* seedling decreased significantly by all NaCl treatments as was show in Table (2). The minimum content of carbohydrates (730.3 mg/g d.wt) was by 10 mM NaCl but the decreasing content of carbohydrates was attenuated after 10 mM NaCl. Content of carbohydrates in *Solanum nigrum* have the following order TRV > DRV > polysaccharides.

Total protein content

The results of total soluble protein content in seedling of *Solanum nigrum* were represent in Fig (5). The mean content of total protein was increased by all concentrations of NaCl except 20 and 30 mM NaCl concentrations, when compared with control which had content of (20.7 mg/g d.wt). The maximum of total protein content was 21.2 mg/g d.wt by 10 mM NaCl, while the minimum of total protein content was 17.0 mg/g d.wt by 30 mM NaCl. The percentage of increase in total proteins by 10 mM was 2.34% while at minimum the decrease at 30 mM was by 18%.

Secondary metabolites

Saponin content in seedling of *Solanum nigrum* did not vary significantly by the

different concentrations of NaCl (Fig.6). The mean of saponin content was increased by each 10 and 20 mM NaCl with a maximum of saponin content was 13.9 mg/g d.wt by 20 mM NaCl and that was higher than the control value by about 18.2%. The other NaCl treatments decreased saponin with a minimum content 11.1 mg/g d.wt that was by 30 mM NaCl that decreased the saponin content only by 5.4% compare with control.

The phenolic compounds content in seedling of *Solanum nigrum* did not vary significantly by the different concentrations of NaCl (Fig.6) but there was an increase by all of NaCl treatment in comparison with the control except for 30 mM NaCl treatment. The maximum phenolic compounds content was 1.9 mg/g d.wt at 50 mM NaCl, while the minimum content was 1.3 mg/g d.wt at 30 mM NaCl. The percentage of increase of phenolic compounds at 50 mM NaCl was by 28.5% while the minimum was lower by 13% in comparison with control.

The study was designed to learn the effect of salinity on the growth and medicinal compounds contents of *Solanum nigrum*. Seeds of *Solanum nigrum* seedling were subjected to the effect of low concentrations of NaCl for investigating their impacts on growth of the plant seedlings and their important constituents. The seeds germination was enhanced in *Solanum nigrum* with the low NaCl treatments especially 20 mM which led to the highest percentage of germination in comparison with the control while increasing of NaCl concentrations to 50 mM decreased the seed germination when compared with control. The completely inhibition in the seed germination was calculated from the regression equation to be about 70mM. This may be an adaptive strategy of seeds to prevent germination under stressful

environment, this ensuring proper establishment of the seedlings (Gill *et al.*, 2003). Prisco and O'Leary (1970) and Gill and Singh (1985) reported that NaCl stress inhibits seed germination because it may inhibit radical emergence through hindering water absorption and through mobilization of reserves from storage organs (Sheoran and Grag, 1978 & Gomes Filho *et al.*, 1983). Sharma *et al.* (2004) reported that the decrease in the rate of seed germination under drought and salinity stress conditions may be due to the fact that the seeds seemingly developed an osmotically enforced "dormancy".

The time of starting germination of NaCl stressed *Solanum nigrum* varied by different concentration of NaCl. Germination rate was active in all concentrations of NaCl in the period from 5-10 days. At the first period (0-5 days) and after the 10th day of germination the germination rate was very low. These results agreed with those obtained by Muhammad, *et al.*, (2006) where they found that the mean time to germination of almost all Phaseolus species increased with the addition of NaCl and the germination time was greater in higher concentration as compared to low concentrations. However, this study confirmed high OE due to increased in germination rate and shortening of the time of germination *Solanum nigrum* in response to low salinity levels over that occurred under control.

The plumule and radicle growth in length and length ratio were changed by the applied different concentrations of NaCl. The length of the radicle was increased by all concentrations of NaCl compared with the control. In the contrary decreases in the length of plumule were achieved due to all concentrations of NaCl compared with the control. A remarkable utilization of metabolites in radical growth rather than

plumule growth was occurred in response to NaCl salinity stress. This caused an increase in ratio of radicle/plumule significantly with increasing NaCl concentration due to the decreased length of the plumule and increased of radicle. For this reason, radicle and plumule length provides an important guide to the response of plants to salt stress. These results are in accordance with those obtained by Jamil and Rha, (2004). Also, *Solanum melongena* seedling length and the fresh and dry weights of root and shoot increased with increasing level of salinity up to 8.5 m. mhos/cm EC, indicating that the plant tolerate quite high level of salinity at seedling stage (Basalah, 2010). The photosynthetic pigments chlorophyll a, b and carotenoids varied in *Solanum nigrum* seedling leaves. Chlorophyll a content was increased with increasing concentrations of NaCl, except 20 mM which clearly decreased it in comparison with the control. The maximum percentage of chlorophyll a was increased by 27%, while the minimum was decreased by 11%.

The content of chlorophyll b gradually declined with increasing concentrations of NaCl, with the exception of the increase in concentrations with 10 and 20 mM of NaCl concentrations with a maximum increase reached to 1.9 times with 10 mM when compared with the control. Those results showed that chl. b metabolism occurred efficiently under slight NaCl stress condition (10-20mM) and higher than this was suppressing to its metabolism. Also, chl. a metabolism could be achieved maximally at greater NaCl concentration stress (40-50mM) as its metabolism was less sensitive to salinity as that of chl. b. Tolerance of chl.a than chl. b to salinity was also recorded by Elhaak et al. (2014).

The content of carotenoids in leaves of *Solanum nigrum* seedlings generally

increased with increasing concentrations of NaCl exception of a significant decrease in the concentrations 10 and 30 mM. It is worthy to mention that the highest value of the content of the pigments in the concentration of 40 mM was due the marked increase in chlorophyll a and carotenoids. The total content of chlorophyll a was higher than chlorophyll b and carotenoids compared with the control. Similar results were also found by Afroz, *et al.* (2005).

Marked reduction was observed in Direct Reducing Values (DRV) with the increase in the concentration of NaCl content. The lowest DRV was recorded in the concentration of 30 mM NaCl. These results agree with the results of many authors Boyer and Mayer, (1980), Abdel-Aziz *et al.*, (1985), Perry *et al.*, (1987), Omar *et al.*, (1993) and Al-Hakimi and Hamada, (2001). This reduction in the soluble reducing sugars might be a result of the general inhibition in the photosynthetic pigments (chlorophyll a and b) or due to the conversion into insoluble forms as reported by Omar *et al.* (1993). The present results confirmed both processes under the used NaCl stress treatments. Total Reducing Value (TRV) content in plant seedlings of *Solanum nigrum* were also reduced by the concentrations of NaCl.

On the contrary, polysaccharides significantly increased in response to all NaCl treatments with a maximum content at 30 mM of NaCl treatment and this maximum was about 6 times the control content. This increase of polysaccharides by the used salinity treatment showed a responsive transformation into reserved carbohydrates on the expense of mono and di-sugars important of osmoregulation indicating that the plant under such low stress did not suffer from osmotic stress.

Table.1 Regression equations, time needed for 50% germination (T50) and overall effect for *S. nigrum* seeds germination under different NaCl treatments (mM)

T	Regression equation of germination %	Calculated maximum germination time	R ²	Time of 50% germination	(A)	(B)	Overall effect (OE)
0	y=10.80x-5.67	5.67	0.969	5.155	0	0	0
10	y=11.12x-6.8	6.8	0.944	5.108	6	0.9	2.5
20	y=13.07x-12.1	12.1	0.95	4.751	16.	8	12.0
30	y=13.86x-18.5	18.5	0.95	4.942	14	4	9.0
40	y=14.08x-17.37	17.37	0.943	4.785	15	7	11.0
50	y=10.50x-16.31	16.31	0.905	6.315	-0.81	-23	-11.14

Table.2 The carbohydrates (DRV, TRV and polysaccharides) in seedling of *Solanum nigrum* treated by different concentrations of NaCl (mM)

treatment	DRV	TRV	polysaccharides	Total carbohydrates
0	42.96	805.841	0.451	849.26
10	32.15	696.417	1.694	730.26
20	34.88	767.159	0.500	802.54
30	24.70	745.116	2.670	772.48
40	25.00	737.648	0.437	763.09
50	24.99	769.433	1.270	795.70

Figure.1 Variation in germination percentage % of *Solanum nigrum* seeds under the effect of different NaCl treatments

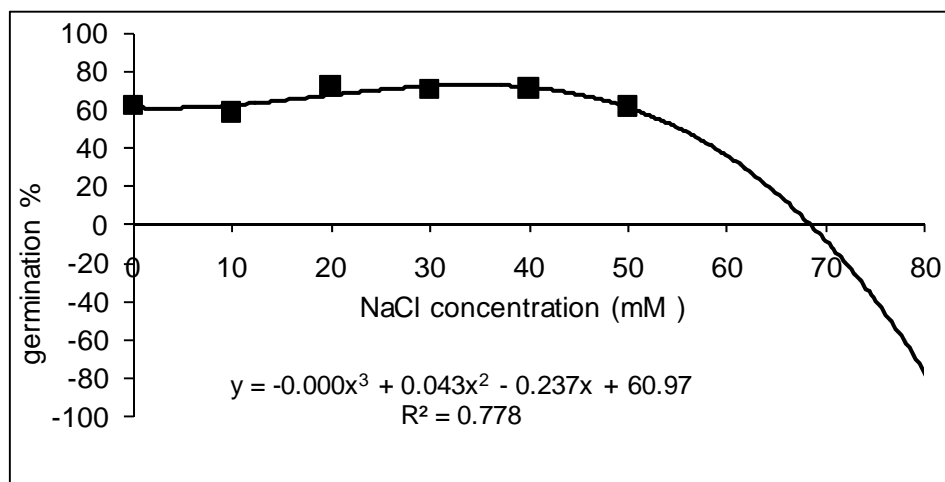


Figure.2 Time of starting germination of *S.nigrum* seeds by different concentration of NaCl

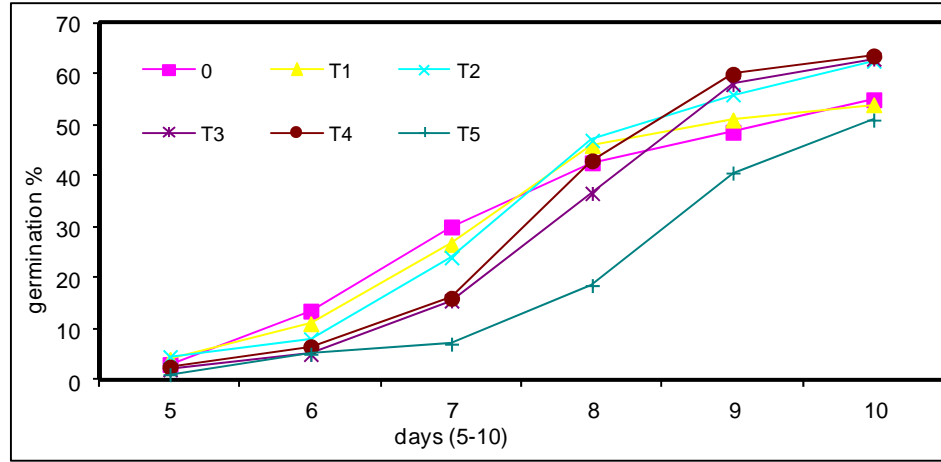


Figure.3 Length of radicle and plumule in addition two organs length ratio of seedling of *Solanum nigrum* under the different concentrations of NaCl (mM)

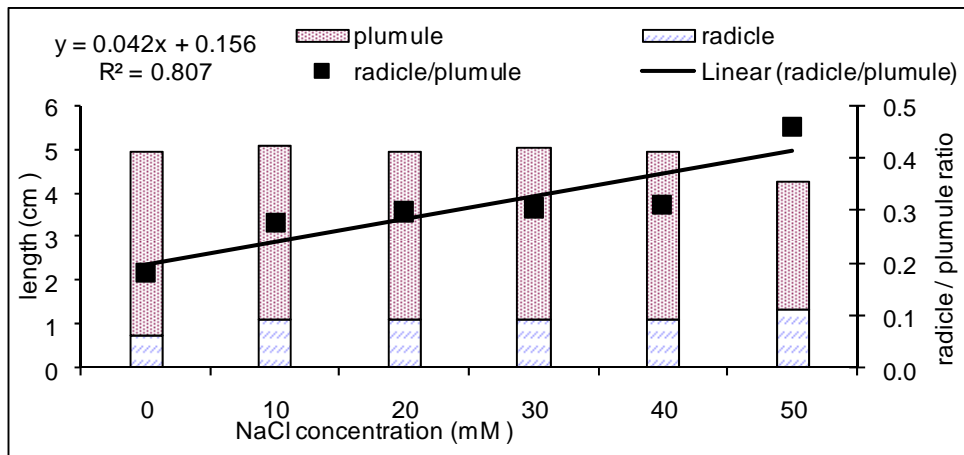


Figure.4 The photosynthetic pigments content (mg/g fresh weight) in *Solanum nigrum* seedling leaves treated with different concentrations of NaCl

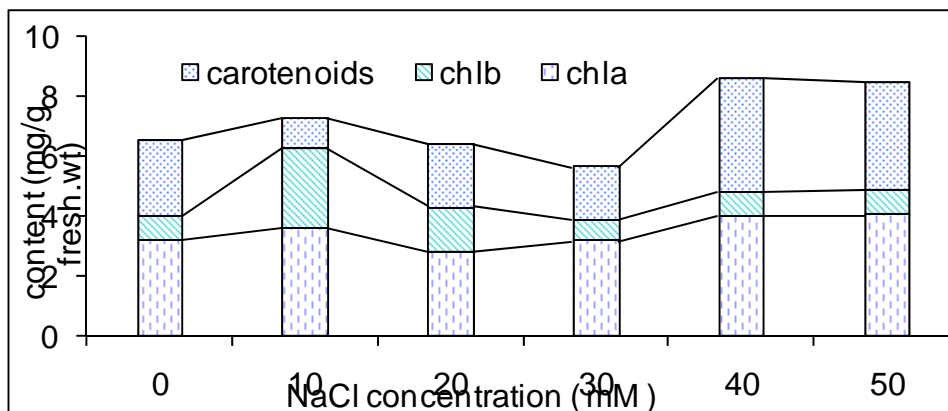


Figure.5 The total protein content (mg/g d.wt) in seedling of *Solanum nigrum* by different concentrations of NaCl (mM)

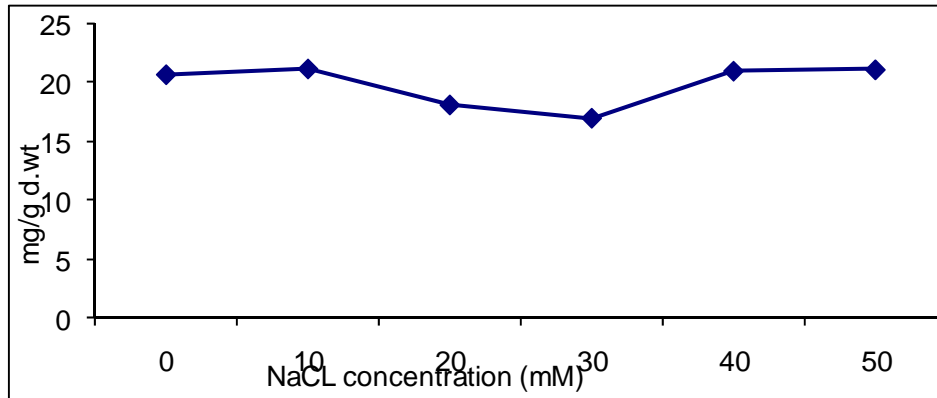
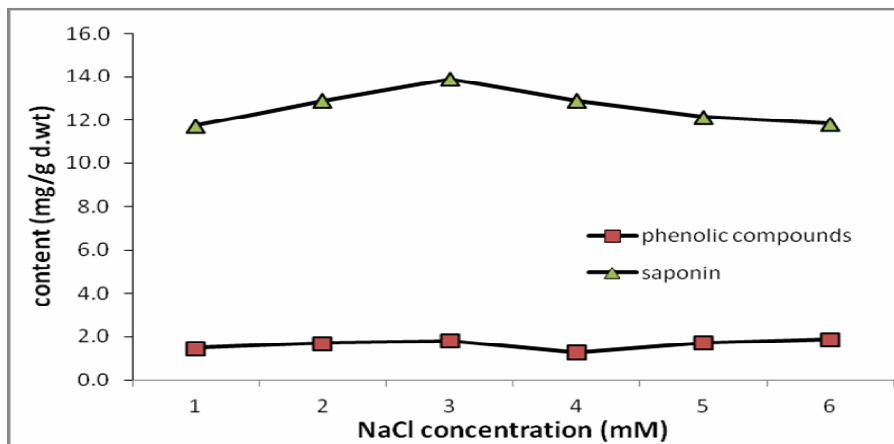


Figure.6 The mean of saponin and phenolic compounds (mg/g dry weight) in seedling of *Solanum nigrum* by different concentrations of NaCl (mM)



Rathert *et al.* (1981) found that salinity stress induced a marked accumulation of sucrose and starch in green leaves of bush beans. Accumulation of certain carbohydrate fractions or the total carbohydrate content may be induced in the stressed plants (Khattab, 1988) or markedly reduced in the other (Boyer and Mayer, 1980, and Perry *et al.*, 1987). On the other hand, Munns *et al.* (1982) reported that the sugars are accumulated under saline conditions as a result of decreased utilization.

The effect of various NaCl treatments on the protein content of *Solanum nigrum* seedlings was enhancing leading to a significant increase in average protein content with most concentrations of NaCl, compared with the control which is a confirmative of accumulation of soluble protein during NaCl stress (Khattab, 1988, Abd- El-Kader, 1991 and El-Kawas, 1999). Also, the increase in the protein content with increasing NaCl concentration may be due to over expression of some genes which catalyze the protein

synthesis under salt stress conditions. The highest protein content was a response of 10 mM NaCl and the percentage of increase was by about 2.34 % while the percentage decline was by 18 % at 30 mM.

The secondary metabolites which have medicinal importance, varied also by those studied treatments of sodium chloride. Saponin content did not significantly differ in seedlings of *Solanum nigrum* by the different concentrations of NaCl except the slightly high value of saponin content by both 10 and 20 mM of NaCl. Total phenolic compounds Content in seedling of *Solanum nigrum* that developing in different concentrations of NaCl did not vary significantly. Although there were slight increases by all NaCl treatments compared with the control. The maximum phenolic compounds were achieved by 50 mM NaCl and the percentage of increase was 28.5 compared with control. However, phenolic compounds metabolism is a significant response to salinity as they have important anti oxidant role during salinity stress. The increase in phenolic compounds with NaCl stress was found by Navarro, *et al.* (2006), Keutgen and Pawelzik, (2008) and Rezazadeh, *et al.* (2012). Also, Dhingra and Varghese (1985) and Ayaz *et al.* (2000) reported that salinity stress induced disturbance of the metabolic processes leading to an increase in phenolic compounds. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals because Phenolics possess hydroxyl and carboxyl groups, able to bind cations (Jun *et al.*, 2003).

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