

Spermidine as modulator of growth, some metabolic activities and reproductive development of *Helianthus tuberosus* plants grown in two types of soil

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Abstract: Growth, yield and some metabolic activities of *Helianthus tuberosus* plants were studied in response to the treatment with spermidine under normal and saline soil. The obtained results revealed that, under saline conditions, treatments with spermidine (50 & 100 ppm) generally enhanced most of the growth and yield characteristics (shoot length, root length, fresh and dry weights of shoots and roots/ plant, number of tubers / plant and tubers weights) of *Helianthus tuberosus* plants. Plants which planted in saline soil showed significant decreases in contents of chlorophyll (a), (b) & (a + b), carbohydrates, soluble proteins, while phenols, carotenoids and free proline contents were increased throughout the experimental period. At saline conditions, catalase activities (CAT) and peroxidase activities (POX) were increased. Treatment with spermidine caused significantly increases in CAT and POX activities in plants grown in saline or non-saline soil.

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Keywords: spermidine; saline soil; *Helianthus tuberosus*; metabolic activities

1. Introduction

Soil salinity considered the major factors that reduce plant growth in many regions in the world. Two important limitations for crop production in arid and semi-arid regions are water shortage and salinity (Sepaskhah and Yousofi-Falakdehi, 2010). In salt-affected soil, there are many salt contaminants, especially NaCl which readily dissolves in water to yield the toxic ions, sodium ion (Na⁺) and chloride ion (Cl⁻). Also, the water available in the salt-contaminated soil is restricted, inducing osmotic stress (Pagter *et al.*, 2009; Siringam *et al.*, 2011).

Jerusalem artichoke (*Helianthus tuberosus*), which is also called the sunchoke, sunroot or earth apple, is an Angiosperm plant species of Asteraceae family (Tassoni *et al.*, 2010). More importantly, as *Helianthus tuberosus* possesses prophylactic and medicinal properties for sufferers of sugar diabetes, and act as a biogenetic factor for the development of natural intestinal microflora after disbacteriosis (Rakhimov *et al.*, 2003), it has been becoming an interesting research topic and it is even labeled as a new cultivated crop (Terzic and Atlagic, 2009).

Approaches have been carried out to minimize the adverse effect of salinity by using polyamines a foliar spray (Amri and Shahsavari, 2010). Polyamines influence plant development in many ways and the response of plants to abiotic stress involves complex physiological and biochemical responses, including changes in the concentration and ratio of different metabolites (El-Bassiouny *et al.*, 2008). Although most of the known

polyamines are regulators of growth, maximum plant growth may depend on the ratio rather than the absolute levels of these substances in plants. These ratios may change dramatically in response to salinity stress, thereby leading to physiological disturbances associated with a general reduction in growth (Sood and Nagar, 2003). Also pretreating seed with optimal concentrations of polyamines can effectively improve germination as well as growth and yield of crops under both normal and stress conditions (Abd El Monem, 2007; Karimi and Rahemi, 2012). So, the aim of the present work was to evaluate the use of polyamines for improving growth and productivity as well as increasing salt tolerance of *Helianthus tuberosus* plant, and also to study the oxidative defense mechanism of *Helianthus tuberosus* plants against salt stress. Via determination of the growth characters and some essential metabolites throughout the different stages of the growth.

2. Material and Methods

2.1. Methods of planting, treatments and collection of samples

Uniform buds *Helianthus tuberosus* L. tubers were planted in two types of soils in Kafr El Shikh one represented non-saline soil (Table 1) and the other place is saline soil (Table 2) in terraces. The developed plants were irrigated whenever required. Plants grown in either saline or non-saline soil were divided into 3 groups representing the following treatments:

- Treated with distilled water (control).
- Spermidine (50 ppm spd as spermidine).

- Spermidine (100 ppm spd as spermidine).

The plants of *Helianthus tuberosus L.* were treated twice with the above mentioned treatments (as foliage spraying). The first treatment was made when the age of plants was 60 days, while the second treatment was made when the age of plants was 90

days of sowing. The plant samples were collected for analysis when the plants were 70 (Stage I) and 100 (Stage II) days old. At the end of the growth season (180 days), analyses of the tubers yielded from the different treatments as well as the control were done.

Table 1. Chemical properties of the used soil (Non-saline soil)

TSS ppm	pH	E.C. mmhos/cm	Cations meq/L				Anion meq/L			
			Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	SO ₄ ²⁻	HCO ₃ ⁻	CO ₃ ²⁻
768	7.9	1.24	2.5	0.5	2	1	4	1	1	Zero

Table 2. Chemical properties of the used soil (Saline soil)

TSS ppm	pH	E.C. mmhos/cm	Cations meq/L				Anion meq/L			
			Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	SO ₄ ²⁻	HCO ₃ ⁻	CO ₃ ²⁻
4760	8.3	7.25	4.75	1.5	5	2.5	7	5.25	1.5	zero

2.2. Measurement of growth parameters

Plant height (m), root length (cm), number of leaves per / plant, fresh and dry weights of shoots (Kg/plant), fresh and dry weights of roots (g/plant), number of tubers, weight of tuber were determined at different growth stages.

2.3. Chemical analysis

Three plant samples were taken during the growing season, at vegetative growth stage, flowering stage and yield stage.

Photosynthetic pigments were estimated using the method of **Vernon and Selley (1966)**. Contents of soluble carbohydrates were measured according to the method of **Umbriet et al. (1969)**. Contents of soluble proteins were estimated according to the methods of **Lowery et al. (1951)**. Phenolic compounds were estimated according to the methods of **Daniel and George (1972)**. Proline contents tubers were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000g at 4°C for 10 min. The supernatants were used for proline estimation according to the method of **Bates et al. (1973)**. Enzymes catalase (CAT) and peroxidase (POX) were estimated according to the methods of **Kong et al. (1999)**.

2.4. Statistical analysis

The data were subjected to the proper statistical analysis of variance of a randomized complete block design For comparison between treatments means, using t test, (PASW® Advanced Statistics 20, 2010) at 5% level was used. The values recorded in the values of the biochemical analysis are means of three replicates. Discriminant analysis is

used to classify several observations, into these known groups (**Härdle and Simar, 2007**).

3. Results and Discussion

3.1. Growth responses

The obtained results (Figs. 1-8) showed a retarded growth in salt-stressed plants. Plant height, fresh and dry weight of both shoots and roots and the number of leaves/plant were significantly decreased under saline condition. The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the plants competitors between Na⁺ and Cl⁻ and further anions and cations may result in a reduced plant growth and yield (**Keutgen and Pawelizik, 2008**). Many studies have shown that biomass partitioning between roots and shoots is strongly influenced by the most limiting resource under stress growth conditions, and resource deficiency is often ameliorated by increasing the biomass allocation to the part of the plant responsible for acquiring the most limiting resource (**Jamil et al., 2006**).

Results of the present study (Fig. 8) showed that, with a few exceptions application of spd was of stimulatory effects regarding the growth characters as well as the yield of the tested plants. This was the case in plants grown under both saline and non-saline conditions. These results are in agreement with the previous data obtained by **El-Bassiouny et al. (2008)**; **Mostafa et al. (2010)**. The use of polyamine to improve the yield and yield components was recommended by other investigators. In this regard, **Abd El-Wahed and Gamal El-Din (2005)** on chamomile indicated that the sprayed plants with polyamine showed a marked increase in the number of pods/ plant, weight of pods/ plant and seed index.

The improving of plant growth under salt stress conditions by spraying with PAs may be due to the role of PAs on enhancing cell division activity, increasing of proline accumulation (Hussein *et al.*, 2006). Polyamines are currently considered to be regulators of plant growth and development owing to their effects on cell division and differentiation (Verma and Mishra, 2005). Alsokari (2011) investigate the control of physiological stress resulted from salinity on cowpea plants using foliar spray with certain growth regulators (spermine). He found that, growth regulation treatments alleviated, with variable degrees, the harmful effects of salt stress on plant growth measurements of different criteria during different periods of growth.

Hussein *et al.* (2006) also reported that PAs improved all metabolic activities in pea plants under salinity stress conditions and make plants more tolerant to stress, thereby increase growth and yield of pea plants. In addition, Suleiman *et al.* (2002) stated that PAs associated in the regulation of cellular ionic environments, maintenance of membrane integrity and prevention of chlorophyll loss and stimulation of synthesis of proteins and nucleic acids. Furthermore, PAs were effective in improving grain yield in wheat under saline conditions (Iqbal *et al.*, 2006).

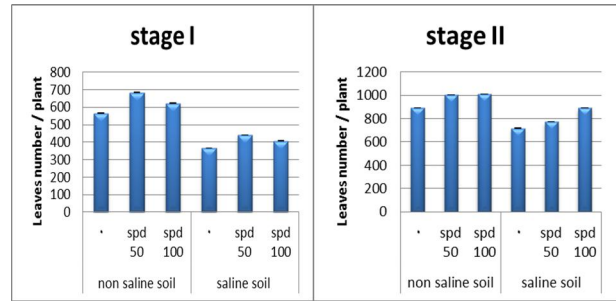


Figure 3. Effect of salinity, spermidine and their interactions on no. of leaves of *Helianthus tuberosus* plants

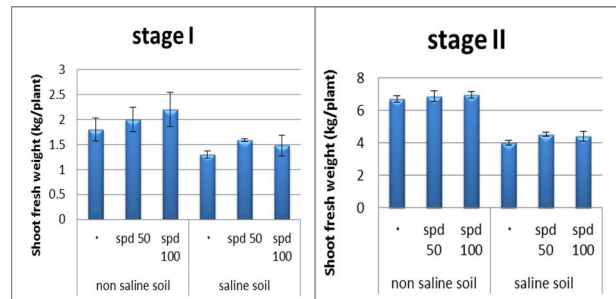


Figure 4. Effect of salinity, spermidine and their interactions on fresh weight of shoot (kg) of *Helianthus tuberosus* plants

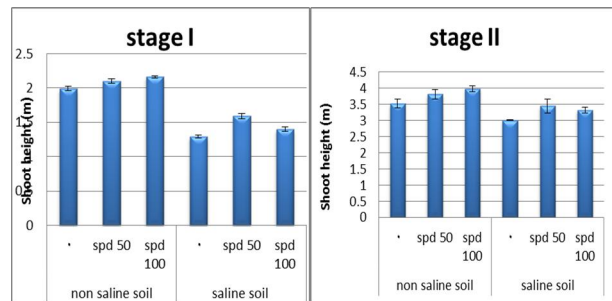


Figure 1. Effect of salinity, spermidine and their interactions on shoot height of *Helianthus tuberosus* plants

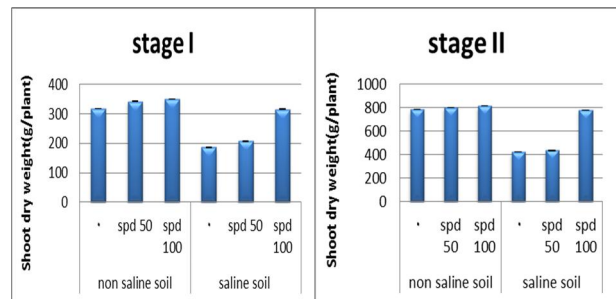


Figure 5. Effect of salinity, spermidine and their interactions on dry weight of shoot (g) of *Helianthus tuberosus* plants

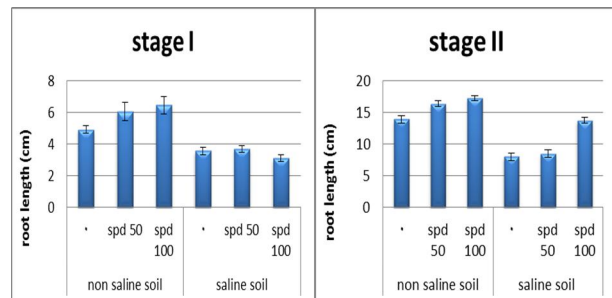


Figure 2. Effect of salinity, spermidine and their interactions on root length of *Helianthus tuberosus* plants

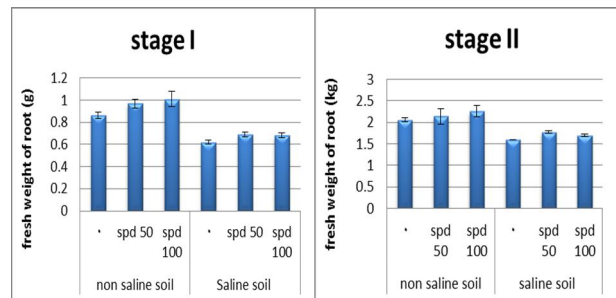


Figure 6. Effect of salinity, spermidine and their interactions on fresh weight of root (g) of *Helianthus tuberosus* plants

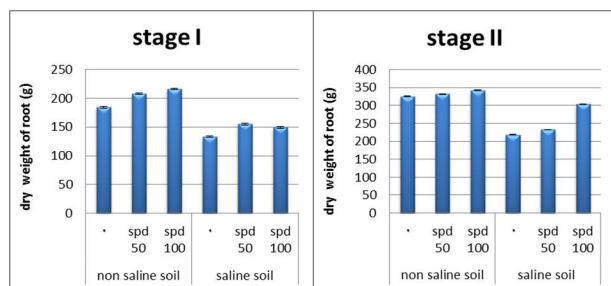


Figure 7. Effect of salinity, spermidine and their interactions on dry weight of root (g) of *Helianthus tuberosus* plants

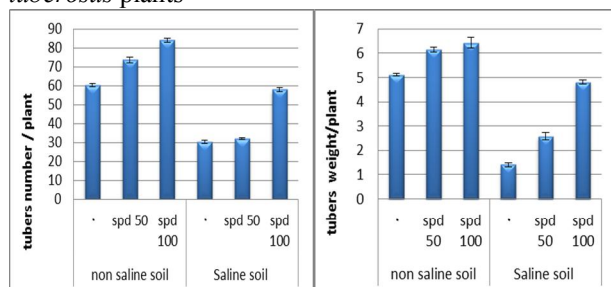


Figure 8. Effect of salinity, spermidine and their interactions on No. of tubers and tubers weight of *Helianthus tuberosus* plants

3.2. Photosynthetic pigments

Photosynthesis is essential for the normal growth and metabolism of plants. It depends on the normal availability of chlorophylls in the chloroplasts and on several enzymes which function during the light and dark phases. A normal functioning of the dark phase results in the production of monosaccharide, disaccharides and polysaccharide by the plant.

In the present investigation, results (Figs. 9-12) clearly reveal a reduction in the photosynthetic pigment levels (chlorophyll a, chlorophyll b and carotenoids) in the leaves of helianthus plants due to saline soil. This finding is supported by the study of **Almodares et al. (2008)** on sorghum, and **Sofy (2011)** on lentil. The reduction in chlorophyll contents due to salinity is probably due to the inhibitory effect of the accumulated ions (Na^+ and Cl^-) on the biosynthesis of the different chlorophyll fractions (**Mohammed, 2007**).

Results of the present work (Figs. 9, 10, 11) showed that, at different soils, contents of chlorophyll (a) and (b) as well as total chlorophyll (a + b) were, mostly, significantly increased due to the application of spd. Also, contents of carotenoids were significantly increased throughout the two stages of growth specially in plants grown under saline condition. In this regard, **El-Bassiouny and Becheta (2001)** reported that PAs mitigate stress through modulating stomatal movement, providing a link between stress condition,

PAs levels and stomatal regulation. The addition of PAs in nutritive solution reduced rice growth in the absence of NaCl and did not offer protection in the presence of salt (**Ndayiragije and Lutts, 2006**).

Chattopadhyay et al., (2002) added that PAs prevented chlorophyll loss, inhibition of photochemical reactions of photosynthesis. Also **Huiguo et al. (2006)** found that exogenous application of PAs protect PSII against water stress at both transcriptional and translational levels and allow PSII to retain a higher activity level during stress in wheat seedling resulting in the increase in chlorophyll contents.

Gupta et al. (2003) found that PAs increased the total chlorophyll contents of wheat plants. Also **El-Bassiouny and Becheta (2001)** reported that exogenous application of putrescine (1.25 mM) induced significant increase in chlorophyll, Mg contents and total soluble sugars.

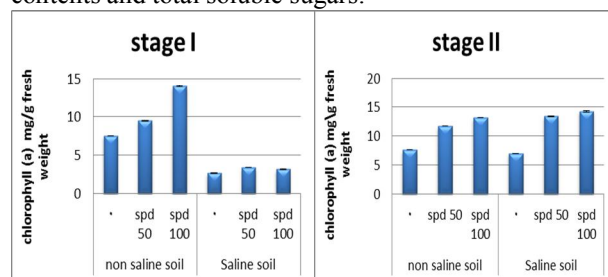


Figure 9. Effect of salinity, spermidine and their interactions on chlorophyll a content of *Helianthus tuberosus* plants

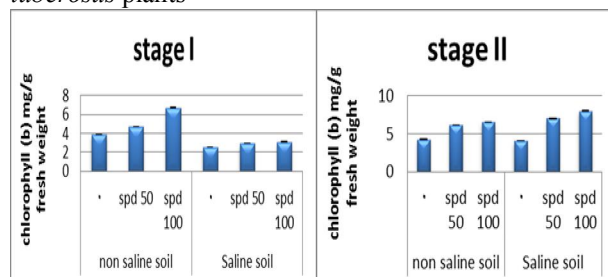


Figure 10. Effect of salinity, spermidine and their interactions on chlorophyll b content of *Helianthus tuberosus* plants

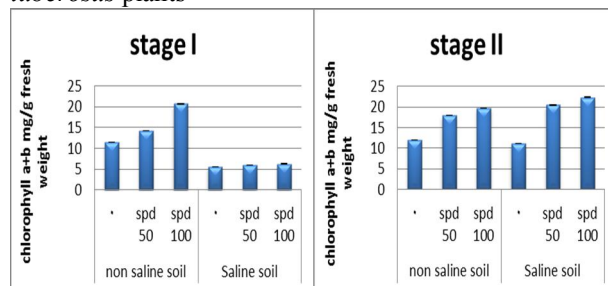


Figure 11. Effect of salinity, spermidine and their interactions on chlorophyll a + b content of *Helianthus tuberosus* plants

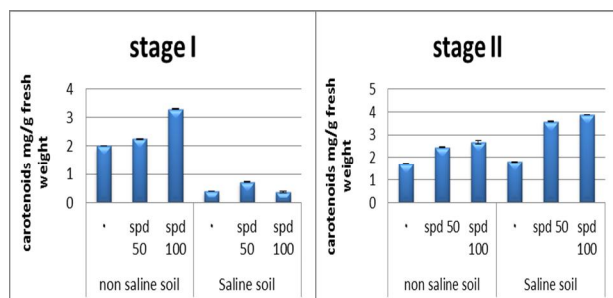


Figure 12. Effect of salinity, spermidine and their interactions on Carotenoids content of *Helianthus tuberosus* plants

3.3. Carbohydrates

In the present study, results show that total soluble carbohydrates decreased in helianthus plants grown in saline soil. **Rejeskova et al. (2007)** indicated that salinity caused a decrease in total carbohydrates in *Olea europaea* L. shoots. The decrease was probably not due only to osmotic stress but also to the presence of toxic Na^+ and Cl^- ions.

Results of the present work (Figs. 13, 14, 15) showed that contents of total carbohydrates (total soluble sugars) were, generally, significantly increased in shoots, roots and in yielded tubers in response to the treatment with spd. In this regard, several investigators proved that polyamine plays an important role in carbohydrate metabolism **El-Bassiouny et al., (2008)** found that application of PAs treatment had favourable effect on the synthesis and accumulation of carbohydrates in leaves of wheat plants. In most cases, plants sprayed with PAs at the different concentrations had higher contents of total carbohydrates in their leaves, compared to the untreated control. Moreover, **Liu et al. (2002)** found that spraying Brassica plants with PAs gave the highest value of total soluble sugar percentage. PAs alleviated the adverse effects of salt stress on total soluble sugars concentration and this is at least partially due to enhanced amylase activity and chlorophyll content, reported as a response (**Sood and Nagar, 2003**).

Data of the present investigation (Figs. 13, 14, 15) reveal that the reduction in the total carbohydrate levels induced by high salinity counteracted by low and high applied concentrations of spd. These effects were observed at all growth stages but with different degrees. Application of spd generally increased the total soluble carbohydrates in helianthus plants in saline soil. It suggested that in the presence of spd, the leaves accumulated more compatible osmolytes as soluble sugar, glucose and fructose, a sugar alcohol, sorbitol and proline (**Szepesi, 2006**).

Hussein et al. (2006) reported that increasing the sugars concentration by putrescine application may be due to the role of putrescine on improving plant growth; carbohydrate content resulted in increasing plant tolerance to salinity stress.

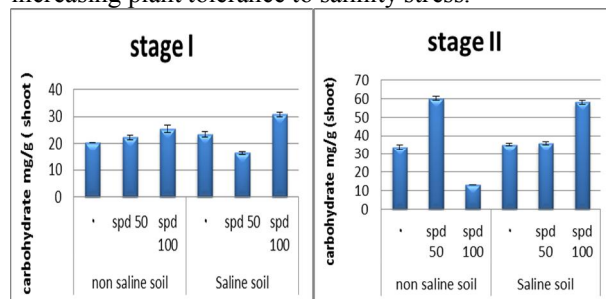


Figure 13. Effect of salinity, spermidine and their interactions on carbohydrate content (shoot) of *Helianthus tuberosus* plants

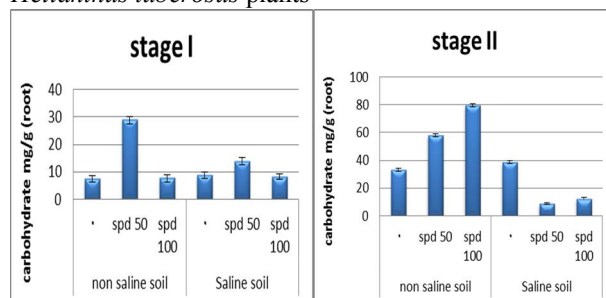


Figure 14. Effect of salinity, spermidine and their interactions on carbohydrate content (root) of *Helianthus tuberosus* plants

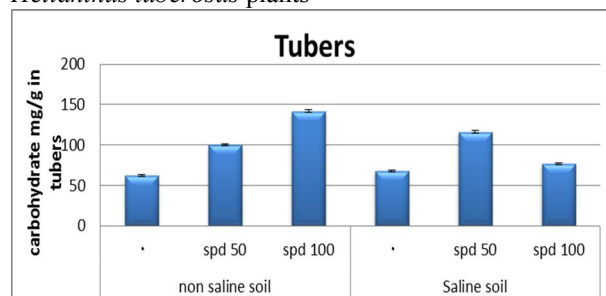


Figure 15. Effect of salinity, spermidine and their interactions on carbohydrate content (tubers) of *Helianthus tuberosus* plants

3.4. Protein

Our results (Figs. 16, 17, 18) showed that the total soluble protein decreased significantly in shoots roots and yielded with saline soil. In *phyllanthus amarus* plants, NaCl reduced the protein content (**Jaleel et al., 2008**); these results were in agreement with **Sofy (2011)** in lentil.

Results of the present study (Figs. 16, 17, 18) indicated that protein contents in shoots, roots as well as in the yielded tubers of helianthus plants were, generally, increased in response to the all used concentrations of spd. The obtained results are in

agreement with those of **Jaleel et al. (2008)** on *Phyllanthus amarus* plant, indicated that foliar application of PAs markedly increased the protein contents in the yielded seeds. **El Tayeb (2005)** found that PAs treatment significantly increased the contents of soluble protein in the shoots of barley plants. **Sood and Nagar (2003)** mentioned that spraying wheat plants with PAs gave the greatest values of crude protein present per rose.

Results of the present work (Figs. 16, 17, 18) revealed that, helianthus plants, treatment with spd, in saline soil, resulted in, mostly, significant increases in protein contents in shoots, roots and also, in the yielded tubers. These results are in harmony with those obtained by **Amira Abdul Qados (2010)** on mung bean, where they found that, contents of proteins increased as a result of PAs application with saline soil.

El-Bassiouny et al. (2008) who indicated that PAs was the most effective compound in increasing soluble carbohydrate, poly saccharides, total carbohydrates, proline, total amino acid and protein contents of wheat plants and grains under normal or stressed condition.

3.5. Total phenols

Phenols act as free radical scavengers as well as substrates for many antioxidant enzymes (Martin-Tanguy, 2001). In the recent study of phenol levels (Figs. 19, 20, 21) were significantly increased in the shoot, root as well as yield tuber of helianthus plants which grown in saline compared with those of unstressed control plant through the experimental period.

Results of the present study revealed that contents of total phenol in shoots of spd-treated plants were significantly increased throughout the stages of growth. These increases were found to be in response to the applied concentrations of spd. The potent effect of spd as regards the contents of total phenols was recorded by other investigators. In this regard, **Agastian et al. (2000)** deduced that application of various concentrations of PAs induced high significant increases in the total phenol content of cowpea shoots. This increase in total phenol contents in response to PAs treatments concurrently with increase in IAA contents in shoots of cowpea plants led to the suggestion that most of phenol compounds are diphenols and polyphenols which may inhibit IAA oxidase activity resulting in auxin accumulation, which reflected in stimulated growth and yield of the treated plants.

The exogenous application of putrescine significantly increase the accumulation of phenols in stressed cowpea shoots and roots as being compared with their reference controls (grown at 100 and 200 mM NaCl). The increase in the accumulation of the

phenolic compounds in stressed cowpea shoots and roots might be due to their increase in their biosynthesis. The increase in phenol levels have been reported in a number of plants grown under stress condition (**Farag Abeer, 2009**).

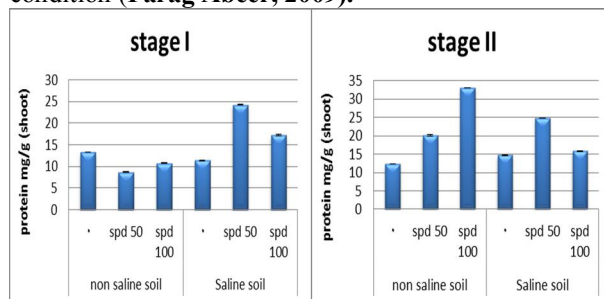


Figure 16. Effect of salinity, spermidine and their interactions on protein content (shoot) of *Helianthus tuberosus* plants

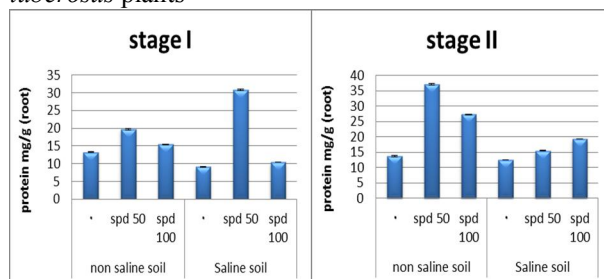


Figure 17. Effect of salinity, spermidine and their interactions on protein content (root) of *Helianthus tuberosus* plants

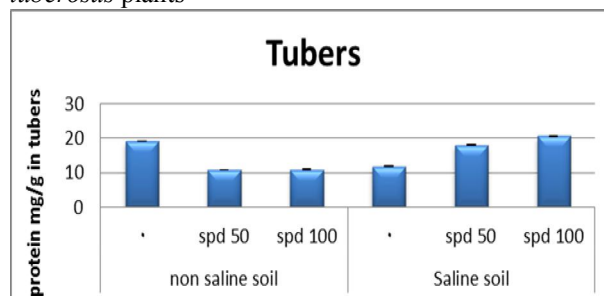


Figure 18. Effect of salinity, spermidine and their interactions on protein content (tubers) of *Helianthus tuberosus* plants

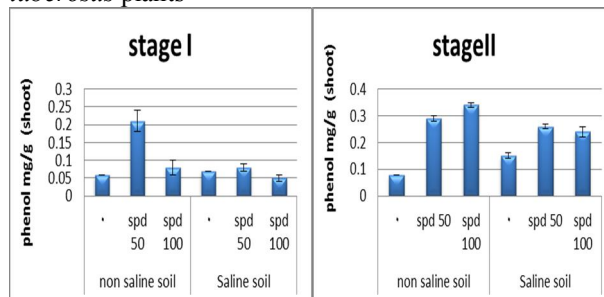


Figure 19. Effect of salinity, spermidine and their interactions on phenol content (shoot) of *Helianthus tuberosus* plants

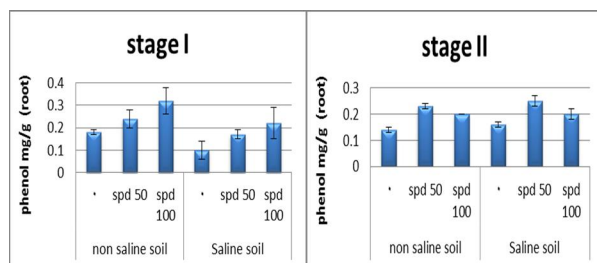


Figure 20. Effect of salinity, spermidine and their interactions on phenol content (root) of *Helianthus tuberosus* plants

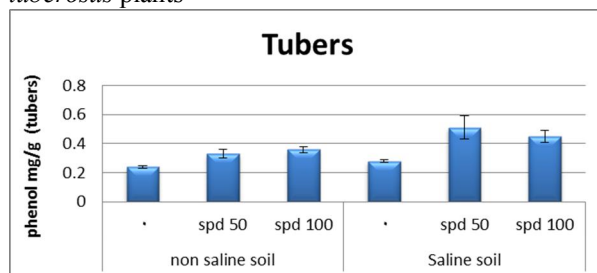


Figure 21. Effect of salinity, spermidine and their interactions on phenol content (tubers) of *Helianthus tuberosus* plants

3.6. Total proline

In organisms ranging from bacteria to higher plants, there is strong correlation between increased cellular proline levels and the capacity to survive under salt stress. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub cellular structure (membrane and proteins) scavenging free radicals and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007).

Our results (Figs. 22, 23, 24) showed that the proline contents increased significantly in plants which grown in saline soil. These results are in agreement with Lobato *et al.* (2008) in *Vigna unguiculata L.* and Sofy (2011) in lentil.

Many plant species naturally accumulate proline and glycine betaine as major organic osmolytes when subjected to different a biotic stress such as drought, salinity, extreme temperature and heavy metals. Proline is thought to play adaptive roles in mediating osmotic adjustment and protecting sub-cellular structure in stressed plants (Ashraf and Foolad, 2007).

Furthermore, Kavi Kishor *et al.* (2005) suggested that proline which is accumulated under stress conditions might serve as a sink for excess reductants providing the NAD^+ and $NADP^+$ necessary for maintenance of respiratory and photothetic processes. When proline synthesis generates $NADP^+$, its degradation produces $NADPH$ which is essential for buffering cellular redox potential in the cytosol as well as plastids.

Proline was markedly increased in salt-stressed plants. Used polyamine affects the proline level of these plants. The interaction was significant spd, in control conditions, significantly increased proline concentration in *Vigna* plants as compared with corresponding untreated plants. The data presented showed that proline concentration was greatly increased in the salt-stressed plants and this is compatible with the fact that many higher plants accumulate free proline in response to salt stress (Kavi Kishore *et al.*, 2005).

Hussein *et al.* (2006) found that salinity stress increased proline content in pea shoots, whereas foliar application of putrescine caused a highly significant increase of proline contents over the untreated plants (control) but still lower than that of the salinized ones. They reported that the increase in proline contents in pea shoots may be during the catabolic processes of polyamines.

Accumulation of some compatible solutes (proline and free amino acids) in stressed plants produced lower solute potential, which allows plant cell to maintain a higher water content than the corresponding control. These solutes play an important role in plants under stress conditions, where major functions of sugars are osmoprotection and/or osmotic adjustment as reported by Parida *et al.* (2002).

Duan *et al.* (2008) reported that exogenous spermidine substantially increased the level of proline in cucumber roots under salinity stress. Although the proline level was increased by NaCl in salinized cowpea plants, yet putrescine reduced this measure below them. Despite of putrescine induced reduction in proline level, it was still significantly greater than the control ones (0.0 mM NaCl). Similar results were obtained by Mansour *et al.* (2002) who found that PAs treatment decreased ethylene production and proline accumulation which were increased by NaCl. They postulated that PAs inhibits proline accumulation under salt stress via the change in the properties of P-5-C.

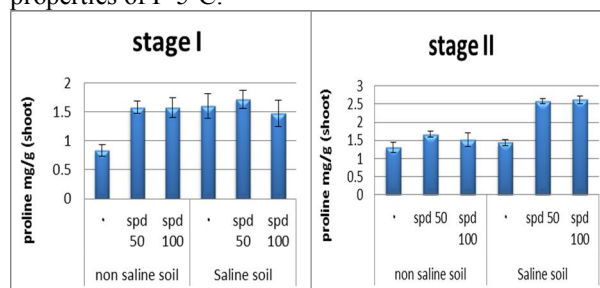


Figure 22. Effect of salinity, spermidine and their interactions on proline content (shoot) of *Helianthus tuberosus* plants

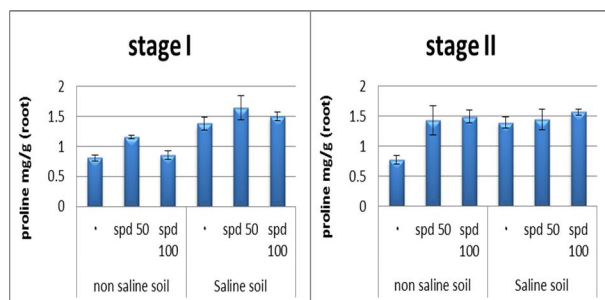


Figure 23. Effect of salinity, spermidine and their interactions on proline content (root) of *Helianthus tuberosus* plants

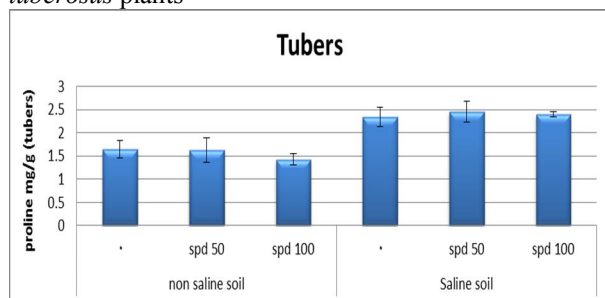


Figure 24. Effect of salinity, spermidine and their interactions on proline content (tubers) of *Helianthus tuberosus* plants

3.7. Antioxidant enzymes

Environmental stresses result in the generation of reactive oxygen species (ROS) in plants. ROS accumulate in cells and lead to the oxidation of proteins, chlorophyll, lipids, nucleic acids, carbohydrates etc. Cells have evolved intricate defence systems including enzymatic (superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), non-enzymatic systems such as ascorbic acid (ASH), phenolic compounds, alkaloids, non-protein amino acids and α -tocopherol, which can scavenge the indigenously generated ROS. Plant stress tolerance mediated by antioxidants has been shown by many workers. Antioxidant resistance mechanisms may provide a strategy to enhance plant stress tolerance. Various enzymes involved in ROS-scavenging have been manipulated, over-expressed or down-regulated to add to the present knowledge and understanding of the role of antioxidant system. ROS induce the synthesis of several plant hormones, such as ethylene, salicylic acid (SA), jasmonic acid, brassinosteroids, abscisic acid (ABA) etc. These Phytohormones are required for growth and development of plants and defend responses during environmental stresses. The present review throws light on the enzymatic and non-enzymatic antioxidants in plants to enhance stress tolerance in plants and also in particular the role of brassinosteroids and ethylene during a biotic stress tolerance in plants (Parvaiz *et al.*, 2010).

ROS are scavenged by plant antioxidant defense systems, comprising both enzymatic and non-enzymatic components (Ashraf, 2009). Environmental stresses, such as salt stress, may lead to an imbalance between antioxidant defenses and ROS levels, resulting in oxidative stress (Foyer and Noctor, 2003).

Soil salinization caused osmotic stress which stimulates the production of ROS such as anion radical (O_2^-) hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) (Azevedo Neto *et al.*, 2006) the resistance to environmental stress may therefore depend at least partially on the inhibition of ROS production by enhancing the antioxidant defense system.

In this investigation, CAT activity was significantly increased in Jerusalem plants shoots which grown in saline soil compared to unsalinized control plants. These results are in accordance with results obtained by Vardhini and Rao (2003) observed that CAT activity decreased in susceptible sorghum and maize varieties but increased in resistance varieties as compared to unstressed control.

In addition, the inhibition of CAT activity is a phenomenon that occurs in many plant species exposed to oxidative stress and is related to the accumulation of SA (Shim *et al.*, 2003). However, Cai-Hong *et al.* (2005) observed that the activity of CAT increased significantly in halophyte *suaeda salsa* L. grown under high concentration of NaCl (200 mM). They deduced that the increase in Cat activity might be related to the result of lowered H_2O_2 level.

An increase in peroxidase (POX) activity is considered as a second line of defence for scavenging the H_2O_2 under salinity (Sharma *et al.*, 2005). Data presented in this study showed that POX activity increased during salt stress and this increase was positively related to NaCl concentration. The increase in POX activity by salinity stress is established by (Cavalcanti *et al.*, 2004).

The increase in POX activity appears to be caused either by activation of existing enzymes isoforms. High reduction of plant growth (Lin and Kao, 2002) and this reduction has been attributed to POX catalysis of feruloylation of hemicelluloses and insolubilization of hydroxyproline rich glycoprotein causing cell wall stiffening (Dionisio-Sese and Tobita, 1998) resulted in the inhibition of growth elongation (Sudhaker *et al.*, 2001).

Our results showed that (Figs. 25, 26), spd increase the activities of key enzymes involved in oxidative stress such as CAT, POX as being compared with their corresponding controls (NaCl), such effect of spermidine 100 ppm was greater at NaCl. Similar results are obtained by Farag abeer (2009) observed that putrescine increase the activities of key enzymes

involved in oxidative stress such as CAT and POX, as being compared with their corresponding controls (100 and 200 mM NaCl), such effect of putrescine was greater at 100 mM NaCl. Similar results are obtained by **Tang and Newton (2005)** on *Virginia pine*. However, **Kubis (2008)** showed that exogenous application of spermidine differentially influenced enzymes of the antioxidative system under stress conditions. He observed an increase of POX activity and to a lesser degree, and reduction of SOD and CAT in spermidine treated cucumber exposed to water stress. It was proposed that the protective effect of polyamine against the damage of the superoxide depended on their conversion to conjugated forms (**Langerbartels et al., 1991**).

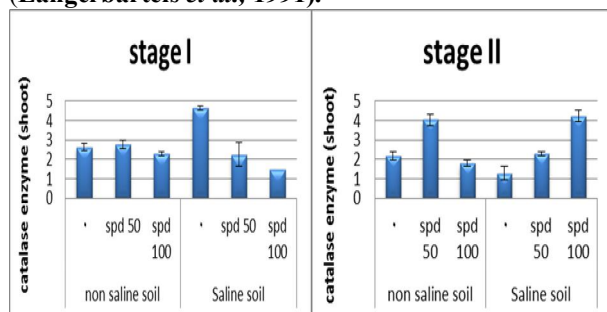


Figure 25. Effect of salinity, spermidine and their interactions on catalase enzyme (shoot) of *Helianthus tuberosus* plants

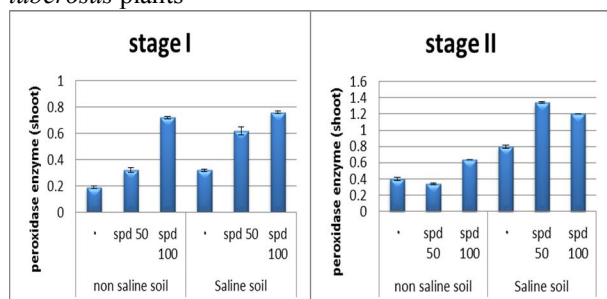


Figure 26. Effect of salinity, spermidine and their interactions on peroxidase enzyme (shoot) of *Helianthus tuberosus* plants

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