

## Study on Various Level of Salinity on Some Morphological and Chemical composition of gladiolus Plants by Foliar Spray with Glutathione and Thiamine

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**Abstract:** Two pot experiments were carried out during two successive seasons (2012/2013-2013/2014) in the greenhouse of National Research Centre, Dokki, Giza, Egypt. The main objective of this study was to investigate the effect of foliar spray with glutathione and thiamine (100 and 200 ppm) under different water salinity (1500 and 3000ppm) and their interaction on growth, flowering and chemical composition of *gladiolus L* plant. ) as well as proline. Application of saline water alone led to significant reductions in all growth and flowering parameters. The same trend was also observed concerning total carbohydrates and pigment content and mineral ions percentage). While opposite trend obtained for proline and flowering date (day) which revealed the highest significant increases under the salinity. Application of glutathione or thiamine (100 or 200 ppm for each) was found to be effective in increasing most of previous parameters both in saline and non-saline conditions (1500ppm). While applying glutathione or thiamine under 3000ppm salinity had inhibitor effect. These applications may be recommended for overcoming the harmful effect on growth, flowering and chemical composition of *gladiolus L* plant grown under different salinity levels.

**Key words:** gladiolus, salinity, glutathione, thiamine, growth, flowers, chemical, constituents.

### Introduction

Gladiolus flowers is considered a main exportable ornamental plants in Egypt, and the flower can be available the year around, the foreign markets demand Egyptian gladiolus with higher quality.

Salinity is a major factor reducing plant growth and productivity worldwide; it affects about 7% of the world's total land area<sup>1,2</sup> and is the major environmental factor limiting plant growth and productivity<sup>3</sup>. The detrimental effects of high salinity on plants can be observed at the whole plant level such as the death of plants or necrosis of plant organs and/or decreases in productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence Salinity turns fertile and productive soil into barren desert and leads to alterations and loss of biodiversity of natural flora<sup>4</sup>. Small changes in salt concentration are sufficient to suppress vegetative growth and plant development, thus inducing physiological changes with different direct and indirect effects<sup>5</sup>. Salinity stops growth and, in high concentrations, changes plant morphology and anatomy and has lethal effect on plant organism<sup>6</sup>. Cultivated plants cover the whole range of response to salinity from sensitivity to tolerance, including morphological, physiological and metabolic problems.

Ornamental plants are classified depending on their tolerance to salinity and gladiolus proved to be more tolerant<sup>7</sup>.

Glutathione (GIT) is the most important non-protein thiol present in cell animal as well as in plants and bacteria. In plants, the physiological significances of glutathione may be divided into two categories: sulfur metabolism and defense. GIT is the predominant non-protein thiol and it is an important pool of reduced sulfur<sup>8</sup> and it regulates sulfur uptake at root level<sup>9</sup>. In addition to its effects on expression of defense gene<sup>10, 11</sup>, GIT may also be involved in redox control of cell division<sup>12</sup>. GIT plays a crucial role in controlling and maintaining the intracellular redox state<sup>13</sup>. Thiamine (vitamin B1) is a necessary ingredient for the biosynthesis of co-enzyme. Thiamine pyrophosphates, so it plays an important role in carbohydrate metabolism in plant. It synthesis in leaves in plants, it is synthesized in the leaves and is transported to the roots where it controls growth. Thiamin is an important cofactor for the translocation reactions of the pentose phosphate cycle, which provides pentose phosphate for nucleotide synthesis and for the reduced NADP required for various synthetic pathways<sup>14</sup>. Pronounced increases in vegetative growth and chemical constituents of rosemary plants by foliar application of thiamine<sup>15</sup>.

The aim of the present study was to investigate the relationship between growth, flowering, and some chemical constituents decked in gladiolus as an effect of irrigation with saline water and the effect of glutathione and thymine to and their interaction understand their affect on plant balance.

## Materials and methods

Two pot experiments were carried out at the screen greenhouse of National research Centre, Dokki, Egypt, during the two successive growing seasons of 2010/ 20011 and 2011/2012 to investigate the effect of glutathione and thiamine on improving growth, flower characters and chemical constituents of gladiolus plant. Gladiolus corms were supplied by ornamental plants research, Institute of Agriculture, Giza, Egypt uniform corms where sown on the first week of December in two seasons using plastic pots ( 30 cm diameter) that were filled with loamy sand soil, the investigated soil characterized by 87% sand, 5% silt and 8% clay, pH 8.75, EC. 0.75dS/m, OM. 0.45%, CaCO<sub>3</sub> 2.2%, available nutrients were as follow P 0.74 mg/100g, K 4.1 mg/100g, Fe 5.3 ppm, Mn 4.7 ppm, Zn 0.41 ppm .The plant irrigation with saline water as (1500 and 3000ppm) sodium chloride (NaCl, Calcium chloride and sulphate magnesium (1: 1: 1) to near field capacity Plants were sprayed twice with freshly prepared solutions of glutathione and thymine each at 100 and 200ppm and combination of the different concentrations of the two factors. The spikes were cut when the basal floret became colored leaving three leaves on each plant .The new corms and cormlets were collected five weeks after the end of flowering.

Foliar application of thiamine and glutathione were carried out two times of 30 days intervals, starting at the first week of February at both seasons. All the normal culture practices of growing gladiolus corms were applied as usual manner. The experimental design was a complete randomized block with three replicates, where each replicate represented by 3 pots.

At the flowering stage sample was taken from representative three replicates randomly for each treatment, and the following parameters were determined the included the following data, plant height (cm), no. of leaves per plant, as well as fresh and dry weights of leaves (g/plant), fresh and dry weight of shoot(g/plant); flowering date(day) spike length; florets length; no. of florets/spike, No. of cormlets, and fresh and dry weight of cormlets (g/plant).

## Experimental design and statistical analysis:

The experiment was laid out to statistical analysis as factorial experiment in randomized complete block design having three replicates. The recorded data (means of the two growing seasons) were statistically analyzed using L.S.D test at 0.05 of probability<sup>16</sup>.

## Chemical analysis

### Soil testing:

Soil samples were taken before planting, air-dried and sieved through 2mm sieve. Physical & chemical characteristics were evaluated <sup>17</sup>. Soil samples were analyzed for texture with a hydrometer (, for pH and electric conductivity (EC) using water extract (1:2.5)<sup>18</sup>, for total calcium carbonate (CaCO<sub>3</sub>%): calcium meter method was

described<sup>19</sup>. Phosphorus was extracted using sodium bicarbonate<sup>20</sup>. Potassium (K<sup>+</sup>) was extracted using ammonium acetate<sup>21</sup>. Iron (Fe), manganese (Mn) and zinc (Zn) were extracted using DPTA<sup>22</sup>.

### Pant analysis

Photosynthesis pigments (chlorophyll a, chlorophyll b) and total chlorophyll (a+b) as well as carotenoids content were determined in fresh leaves as mg/g F.W<sup>23</sup>, Total carbohydrates were determined using colorimetric method was described<sup>24</sup>. Total nitrogen was determined<sup>25</sup> while phosphorus determination was carried out by calorimetrically methods<sup>26</sup>. Potassium was determined photo metrically by flame photometer method as described<sup>27</sup>. Total phenols were determined by the colorimetric method of folin-Denis as described<sup>28</sup>. Proline content (umoles g.f.w) the proline content in samples of leaf was determined<sup>29</sup>.

## Results and Discussion

### Plant Growth and Flowering

#### Characters:

Results presented in table (1,2) reveal that 3000 ppm salinity level reduced plant height, number of leaves/plant and fresh and dry weights of /shoot as well as flowering characters (number of florets/plant and fresh and dry weights of floers/plant) of gladiolus L. as compared with control treatment. Application of 200ppm, glutathione (GIT) or thymine (Th) as a foliar spray was found to be effective in increasing all the previous mentioned parameters significantly both in saline (1500 ppm) and non saline conditions. The reduction due to salinity attributed not only to inhibition of water absorption and ion toxicity but also to the nutrient disturbance under such conditions, also increasing NaCl concentration can reduce the endogenous level of IAA which may be critical to water movement through the root system of plants. Exogenous applications of a smoprotectents, plant growth regulators, fertilizers, and antioxidants have been reported to successfully mitigate the adverse effects of salinity on plant growth and metabolism<sup>30, 31, 32</sup>. The various non-enzymatic antioxidants such as carotenoids, phenols occurs ubiquitously in plants and have been reported to play a vital role in alleviating the adverse effects of salt on plant growth and metabolism in many crop plants<sup>33</sup>. exogenous application of glutathione mitigated partially or completely the adverse effects of salt stress on growth of Canola seedlings, thereby glutathione is integrated into primary metabolism and influence the functioning of signal transduction pathways by modulating cellular redox state, it may be affected nuclear gene expression which influenced by plant's external environment<sup>34</sup>. the effect of salinity (1500 and 3000ppm), glutathione (100 and 200 ppm) and ascorbic acid (100 and 200 ppm) on the growth and flowering behavior of *Tagetes erecta L.* and some chemical constituents as well as essential oil percent and components. The treatments of salinity levels (1500 or 3000ppm) reduced all growth and flowering parameters (plant height, No. of florets, fresh and dry weight of shoot and florets and No. of florets)<sup>35</sup>.

Date presented in Table (2) indicate that of flowering in both seasons was significantly decreased with increasing salinity levels stress in comparison with the control. Regarding the effect of glutathione and thiamine, it could be observed that glutathione and thiamine, promoted flowering in the two seasons in comparing with untreated plants. Concerning the interaction effects in table (2 the date indicate that the shortest time for flowering (106) days from planting) was detected in plants irrigated with thiamine of salinity and sprayed with tap water. In this respect thiamine is an important factor for the translocation reaction of the pentose Phosphate cycle, which provides pentose phosphate for nucleotide synthesis and for the role of NADP required for various synthetic pathway<sup>14</sup>Kawasaki, (1991), the author also added that, thiamine is a necessary for biosynthesis of Co-enzyme thiamine pro-phosphate, so it plays important role in carbohydrate metabolism which reflected on the increase in cornelets weights and numbers (table 3). The stimulatory effect of thiamine on flower characters was demonstrated on chrysanthemum flowers, Jasmine and gladiolus<sup>37, 38</sup>.

**Table (1): Effect of salinity levels, glutathione and thiamine as well as their interaction on plant height, number of leaves fresh weight and dry weight of leaves, fresh and dry weight of shoot of gladiolus plants.**

Characters Treatments	Plant height	No. leaves	F.W of leaves	D.Wof leaves	F.Wof shoot	D.Wof shoot
Control	35.2	5.0	7.2	1.4	28.9	4.8
1500	37.4	6.3	8.4	1.6	30.7	3.9
3000	30.5	4.2	6.2	1.0	25.4	3.1
100 GT	92.2	18.5	17.5	2.4	60.1	10.5
200 GT	95.4	19.2	18.3	2.8	65.4	11.2
100 TH	88.4	18.0	16.2	2.1	57.8	9.8
200 TH	90.2	18.7	16.5	2.3	58.9	10.4
1500+100 GT	90.6	15.7	13.4	2.0	58.2	10.0
1500+200 GT	89.9	16.2	14.8	2.5	59.3	10.9
3000+100 GT	78.3	13.7	12.1	2.0	55.6	10.2
3000+200 GT	79.8	12.8	11.3	1.8	55.8	10.3
1500+100TH	44.9	10.2	9.5	1.6	48.7	9.2
1500+200TH	46.3	10.7	9.8	1.6	48.9	9.2
3000+100 TH	42.5	8.1	7.9	0.9	44.1	7.4
3000+200TH	42.1	7.4	7.0	0.9	40.2	7.4
LSD at 5%	5.82	1.14	1.06	0.17	3.86	0.82

**GT: Glutathione****TH: Thiamine**

**Table (2): Effect of salinity levels, glutathione and thiamine as well as their interaction on flowering date, length of spike, number of florets and length of florets of gladiolus plants.**

Characters Treatments	Flowering date(days)	Length of spike	No. florets	Length of florets
Control	132	43.4	5.5	9.8
1500	117	42.8	7.4	1.0
3000	107	33.2	3.2	7.3
100 GT	127	50.3	10.5	17.2
200 GT	121	56.4	12.8	20.4
100 TH	122	48.9	9.7	15.3
200 TH	120	50.1	11.4	18.4
1500+100 GT	111	50.2	8.8	14.9
1500+200 GT	113	54.3	9.2	19.5
3000+100 GT	110	49.8	7.5	13.2
3000+200 GT	110	49.9	7.9	15.4
1500+100TH	109	45.9	4.4	9.5
1500+200TH	109	38.2	4.1	12.8
3000+100 TH	106	42.4	4.1	8.4
3000+200TH	106	42.8	4.4	8.8
LSD at 5%	11.46	3.62	0.69	1.30

**GT: Glutathione****TH: Thiamine**

**Table (3): Effect of salinity levels, glutathione and thiamine as well as their interaction on number of cormels, dry weight of cormels and diameter of gladiolus plants.**

Characters Treatments	No.cormels	D.W. cormels	Diameter of corms
Control	3.9	3.74	3.51
1500	3.7	3.70	3.5
3000	2.5	1.81	2.2
100 GT	4.6	8.32	4.9
200 GT	4.8	8.95	5.1
100 TH	4.2	7.22	4.2
200 TH	4.5	7.62	4.5
1500+100 GT	4.3	6.51	3.8
1500+200 GT	4.5	6.51	3.9
3000+100 GT	3.2	4.31	2.8
3000+200 GT	3.5	4.52	3.1
1500+100TH	3.0	3.32	3.2
1500+200TH	3.3	3.51	3.5
3000+100 TH	1.76	1.05	2.1
3000+200TH	1.89	1.22	1.98
LSD at 5%	0.32	0.44	0.30

**GT: Glutathione****TH: Thiamine**

**Table (4): Effect of salinity levels, glutathione and thiamine as well as their interaction on leaves chlorophyll a,b and carotenoids of gladiolus plants.**

Characters Treatments	Ch.a(mg/g F.W)	Ch.b(mg/g F.W)	Caroten- oids(mg/g F.W)
Control	0.384	0.201	0.211
1500	0.398	0.200	0.245
3000	0.322	0.193	0.249
100 GT	0.485	0.310	0.382
200 GT	0.498	0.323	0.394
100 TH	0.441	0.282	0.256
200 TH	0.448	0.295	0.278
1500+100 GT	0.392	0.282	0.371
1500+200 GT	0.397	0.295	0.375
3000+100 GT	0.330	0.272	0.411
3000+200 GT	0.337	0.285	0.452
1500+100TH	0.310	0.128	0.382
1500+200TH	0.323	0.135	0.392
3000+100 TH	0.278	0.111	0.456
3000+200TH	0.283	0.125	0.472

**GT: Glutathione****TH: Thiamine****Chemical Constituents:****Photosynthesis pigments:**

Data presented in table(5) reveal that chlorophyll a ; b and cartoniods decreased with increasing salinity stress in comparison with the control while, data indicated further that the gradual increase in the concentration of glutathione or thiamine. It can observed that the highest value for chl a or b and cartonoides content was found in plants application 200 GLT +1500 salinity<sup>39, 35</sup> on Khaya and *Tagetes erecta* L.

### **Total Carbohydrates**

The data in Table (5) pointed out that saline stress (3000 ppm) reduced the percentage of total carbohydrates in gladiolus L. plants. A more pronounced increase in total carbohydrates was obtained with exogenous applications of 200 ppm GLT or Th as a foliar spray in slain condition (1500ppm). The change in soluble sugars content under salt stress has been already reported for a number of species<sup>40,41</sup>. The reduction in soluble sugars may be attributed to the decline in the photosynthetic pigments or to reduction in the CO<sub>2</sub> assimilation rate, stomata conductance<sup>42</sup>. The interactive effects of glutathione or thiamine on accumulation of soluble sugars probably, attributed to the protective effects of glutathione and thiamine on the photosynthetic systems, also, plays a protective role in salinity tolerance by maintenance of the redox status<sup>43,44</sup>.

### **Proline content:**

Data in Table( 5) show that increasing salinity by irrigation with 3000 ppm led to increase in proline content in comparison with control .As for the interaction between different treatments the data in (Table 5 ) indicant that the highest accumulation of proline content was recorded in plants irrigated with salinity 3000 ppm with 200 ppm of thiamine. It has already been reported that proline and ree amino acid content increased with increased salt concentration in pearl millet<sup>45</sup>. A large number of plant species accumulate proline in response to salinity stress and this accumulation may play a role in defense against salinity stress that is why in some cases higher proline content could be correlated with a biotic stress tolerance. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported<sup>46, 47, 48</sup>.

### **Mineral Ions Percentage:**

From the results presented in table 5, it is evident that foliar application of GIT or Th (100 or 200 ppm) under non saline-conditions increased N, P and K percentage in gladiolus L. plants compared with control. While high water salinity level (3000 ppm) led to decreasing of N, P and K% to lowest ratios<sup>49,50</sup> of chamomile and sunflower plants who mentioned that glutathione and thiamine increased macro elements content. Increasing water salinity levels led to increase in Na, Cl and Ca% but decrease N and K% as well as potassium/sodium ratio. These accumulations of sodium, calcium and chlorine in plant tissues might mean that salinity is linked to its limited efficiency in keeping Na and Cl in leaf tissue below toxic levels and compensating for the lower water potentials associated with salinity by increasing tissue levels of organic solutes<sup>51</sup>.



**Table (5): Effect of salinity levels, glutathione and thiamine as well as their interaction on nitrogen, phosphorus, potassium, protein and carbohydrate of gladiolus plants.**

Characters Treatments	N%	P%	K%	Proline	Carbohy- drates Mg/g/DW
Control	0.249	0.123	3.48	15.32	14.37
1500	0.245	0.111	2.82	37.72	15.24
3000	0.211	0.023	1.75	40.21	12.11
100 GT	0.382	0.245	4.85	15.00	22.47
200 GT	0.394	0.248	5.23	15.21	26.25
100 TH	0.256	0.222	4.78	14.84	20.82
200 TH	0.278	0.245	4.79	14.92	21.11
1500+100 GT	0.371	0.208	3.85	20.45	16.82
1500+200 GT	0.375	0.210	3.92	21.23	19.11
3000+100 GT	0.211	0.142	2.41	38.25	13.15
3000+200 GT	0.252	0.152	2.64	38.45	14.22
1500+100TH	0.182	0.104	2.34	38.25	13.00
1500+200TH	0.192	0.107	2.48	38.42	13.82
3000+100 TH	0.156	0.104	2.41	41.45	10.41
3000+200TH	0.172	0.044	2.63	41.48	10.52

**GT: Glutathione****TH: Thiamine**

## References

1. Flowers TJ, Garcia A, Koyama M, Yeo AR.: Breeding for salt tolerance in crop plants-the role of molecular biology. *Acta Physiol Planta*. 1997. 19: 427-433.
2. Zhu JK. Salt and drought stress signal transduction in plants. *Ann Rev PI Biol*. 2002. 53: 247-273.
3. Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N: Ionic and osmotic effects of NaCl-induced inactivation of photo systems I and II in *Synechococcus* sp. *PI Physiol* 2000.123: 1047-1056
4. Ghassemi, F., A. Jakeman and H. Nix, 1995. Salinisation of land and water resources: Human causes. Extent.Management and Case studies. UNSW press. Sydney. Australia and CAB International. Wollingfor. UK.1-4March: 441-444.
5. Shannon, M., C. Grieve and L. Francois. In plant environmental Interaction. Ed. R.E. Wilkinson. Marcel Dekker. New York, 1994. pp: 199-244.
6. Kozłowski, T.,. Responses of woody plants to flooding and salinity-tree physiology monograph1997: 13-15.
7. Zapryanova, N. and B. Atanassova,. Effect of salt stress on growth and flowering of ornamental annual species. *Biotechnol. & Biotechnol. E.Q*, 2009.23: 177-179
8. Rennenberg, H.,. Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry*, 1982. 21: 2771-2781.
9. Lappartient, A.G. and B. Touraine. Demand driven control of root ATP sulfurylase activity and sulphateuptake in intact Canola. *Plant Physiol.*, 1996.111: 147-157.
10. Dron, M., S.D. Clouse, R.A. Dixon, M.A. Lawton and C.J. Lamp. Glutathione and fungal elicitor regulation of plant defense promoter in electroporated protoplasts. *Proc. Nati. Acad. Sci. USA.*, 1988.85: 6738-6742
11. Wingate, V.P.M., M.A. Lawton and C.J. Lamp,. Glutathione causes a massive and selective induction of plant defence genes. *Plant Physiol.*, 1988. 31: 205-211.
12. Sanchez-Fernandez, R., M. Fricker, L.B. Carben, N.S. White and N. Sheard. Cell proliferation and hair tip growth in the *Arabidopsis* root are under mechanistically different forms of redox control. *Proc. Natl.Acad. Sci. USA.*, 1997. 94: 2745-50.
13. Noctor, G. and C.H. Foyer. Ascorbate and glutathione: keeping active oxygen under control. *Plant nol.Biol.*, 1998.49: 249-279.
14. Kawasaki, T. *Modern chromatographic Analysis of Vitamins*, 2nd Ed., vol 60, New York, NY. Marcel Dekker, Inc. 1991. 319-354.
15. Yossef, A.A. and Iman, M. Talaat,. Physiological response of rosemary plants to some vitamins. *Egypt.Pharm.J*. 2003. 1:81-93.
16. Snedccor, G.W. and W. Cochran, *Statistical Methods*. 7th Edn, Jowa State Univ., Press, Jowa, USA1980.
17. Ankerman, D. and R. Large, *Soil and Plant analysis*. A&L Agricultural Laboratories, Inc., USA, 1974. 42 - 44.
18. Jackson, M.L. *Soil Chemical Analysis*. Prentice-Hall of India Private Limited, New Delhi, India, 1973: 82-86
19. Alison, L.E. and Moodle, C.D. Carborate. In: C.A. Black (ed.) "Methods of Soil analysis". Amer. Soc.Agron. Inc., Madison, Wisconsin, USA, 1965: 1379-1396.
20. Olsen, S.R., C. V. Cole, S.S. Watanabe and L.A. Dean,. Estimation of available phosphorus in soil by Extraction by sodium bicarbonate. US Dept. Agric., Circular No. 939: 1-19. Roma, Soils Bull., 1954. 48: 444.
21. Jackson, M.L. *Soil Chemical Analysis*.Printice Hall of India Private Limit. 1967. New Delhi.
22. Lindsay, W.L. and W.A. Norvell,. Development of DTPA micronutrient soil tests for zinc,iron, manganese and copper. *Soil Sci. Soc. Am. J.*, 1978. 42: 421-428.
23. Saric, M.; Kastrort R.; Cupina T.; Cuplna T. and Geric L.Chlorophyll determination.Univ. noven Sodu Parktikum is Kizlolgize Biljaka, Beogard, Haucna, Anjiga, . 1967: 215.
24. Herbert, D.P.; Phlipps, P.and Strangle R.:Determination of carbohydrates. *Method in Microbial*, 1971.58, 209-344.
25. Chapman, H.D. and Pratt P.F. *Methods of Analysis for Soils, Plants and Waters*. Univ.California Div. Agric. Sci. Berkely USA, 1961: 445.
26. King, E.J. *Microanalysis in Medical Biochemistry*, 4thed. And Achar Chill. Ltd. 1951, London.
27. Brown, J.D. and Lilland, O. Rapid determination of Ca and Na in plant material and soil extract by flame photometer. *Proc. Amer.Hort. Sci.*, 1946: 48:341-346.
28. Daniel, H.D. and C.M. George,. Peach seed dormancy in relation to indogenous inhibition and applied growth substances. *J. of American Soceity of Horticulture. Science*, 1972.97: 651-654.
29. Bates,L.S., Waldren,R.P. and Teare I.D..Rapid determination of free proline for water stress studies.*Plant Soil*. 1973. 39, 205-207.

30. Helal, M., K. Koch and K. Mengel,. Effect of salinity and potassium on the uptake of nitrogen and nitrogen metabolism in young barley plants. *Physiol. Plantarum*, 1975.35: 310-313.
31. Dunlap, J.R. and M.L. Binzel,. NaCl reduced indole3 acetic acid levels in the roots of tomato plants independent of stress induced abscisic acid. *Plant physiol.*, 1996.112: 379-384.
32. Shalata, A. and P.M. Neumann,. Exogenous ascorbic acid (vitamin C) increases resistance to salt tolerance and reduced lipid peroxidation. *J. Exp. Bot.*, 2001.364: 2207-2211
33. Hamada, A.M. Effect of exogenously added ascorbic acid, thiamin or aspirin on photosynthesis and some related activities of drought-stressed wheat plants. In: proceedings of XIth International photosynthesis Conference. Budapest, Hungary, August, 1998. 17-22.
34. Hemmat, K.,. Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline condition. *Australian J. of basic and Appl. Sci.*, 2007.1(3):323-334.
35. Rawia, A. Eid, Lobna S. Taha, Soad M.M. brahiem. Alleviation of adverse effects of salinity on growth, and chemical constituents of marigold plants by using glutathione and ascorbate. *Journal of Applied Sciences Research*, 2011.7(5): 714-721.
36. El-fawakhry, F.M. and El-Tayeb H.E. Effect of some amino acids and vitamins on chrysanthemum production. *J. Agric. Res. Alex. Univ.*, 2003. 8(4):755-766.
37. Rawia, E. Aid; Lobna, S. Taha and Soad, M.M. Ibrahim Physiological properties studies on essential oil of *Jasminum grandiflorum* L. as affected by some vitamins. *Ocean J. of Appl. Sci.* 2010.3(1): 87-96.
38. Nahed, G.A., S.T. Lobna and M.M.I. Soad,. Some studies on the effect of putrescine, ascorbic acid and thiamine on growth, flowering and some chemical constituents of gladiolus plant at Nubaria. *Ocean J. of Appl. Sci.*, 2009.2(2): 169-179.
39. Nahed G. Abdel Aziz ;Azza .M. Mazher and El-Habba .Effect of foliar spraying with ascorbic on growth and chemical constituents of khaya senegaiensis grown under salt condition. *American Eurasian Agric. & Environ. Sci.*, 2006.1(3):207-214.
40. Ashraf, M. and M. Tufail,. Variation in salinity tolerance in sunflower (*Helianthus annuus* L.). *J. Agron. Soil Sci.*, 1995.174: 351-362.
41. Amini, F. and A.A. Ehsanpour,. Soluble proteins, proline, carbohydrates and Na<sup>+</sup>/K<sup>+</sup> changes in two tomato- (*Lycopersicon esculentum* mill) cultivars under in vitro salt stress. *American J. of Biochem. And Biotechn.*, 2005.1(4): 212-216.
42. Downton, W.J.S.,. Photosynthesis in salt-stressed grapevines. *Aust. J. Plant. Physiol.*, 1977.4: 183-192.
43. Chaparzadeh, N., M.L.D. Amico, R.A. Khavari-Najad, R. Izzo and F. Navarizzo,. Antioxidative responses of *Calendula officinalis* under salinity conditions. *Plant physiol and Biochem.*, 2004.42: 695-701.
44. Ameer, K.H., S.A.A. Muhammad, U.R.A. Habib and A. Muhammed,. Interactive effect of foliarly applied ascorbic acid and salt stress on wheat at the seedling stage. *Pak. J. Bot.*, 2006. 38(5).
45. Sneha, S.; A. rishi. A. dadhich and S. chandra. Accumulation of proline and ferr amino acid in *pennisetum glaucum* (L). *Br. pak. J. biol. Sci.*, 2013.16:877-881.
46. Hare, P.D., Cress, W.A. and Staden, J. Van. Proline biosynthesis and degradation: a model system for elucidating stress-related signal transduction. *J. Exp. Bot.*, 1999.50,413-434.
47. Kavi Kishor, P.B., Sangam, S., Amrutha, R.N., Laxmi, P.S., Naidu, K.R., Rao, K.R.S.S., Rao, S., Reddy, K.J., Theriappan, P. and Sreenivasulu, N. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr. Sci.*, 2005.88, 424-438.
48. Ashraf, M. and Foolad, M.R. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. *Environ. Expt. Bot.*, 2007.59,206-216.
49. Talaat, I.M. and E.E. Aziz,. Stimulatory effect of glutathione, nicotinic acid and ascorbic acid on *matricaria*. *Egypt. J. of Appl. Sci.*, 2005. 20(21): 218-231.
50. El-Gabas, N.M.M.,. Physiological studies on the effect of ascorbic acid and micronutrients on sunflower plants grown under salinity stress. *B.Sc. (Botany). Fac. Sci.*, 2006. Al-Azhar Univ.
51. Rush, D.W. and E. Epstein,. Genotype response to salinity: Differences genotypes of tomato. *Plantphysiol.*, 1976. 57: 162-166.

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