Fast Neutrons Irradiation Induced Changes in Active Ingredients, Amino Acids and Chlorophyll Contents in *Ambrosia Maritima* (Damsisa) Influenced by Soil Water Stress.

Hanan Abdalla and Eman Selem

Botany Department, Faculty of Science, Zagazig University, Egypt
eman8_extra8@yahoo.com

Abstract: The aim of this experiment was to study the effect of different doses of fast neutrons irradiation (0.0, 10^5, 10^7 and 10^9 n/cm^2) on damsin, ambrosin, amino acids and chlorophyll contents in damsisa plant grown under different levels of soil water holding capacity 100%, 75%, 50% and 25% (W.H.C). Irradiated and un-irradiated seeds were sown in pots containing sand-loamy soil. Also, a group of irradiated and un-irradiated seeds were sown in normal soils (100%, W.H.C.) serve as a control, pots irrigated with tap water until required field capacity. It was observed that, increasing soil water stress condition decreased ambrosin, damsin, chlorophyll contents and amino acids in damsisa shoots. But, proline concentration recorded an increase along with increasing water stress treatments. While, increasing fast neutrons irradiation dose until (10^7 n/cm^2) stimulate ambrosin, damsin, chlorophyll contents and amino acids in damsisa shoots grown under different soil water stress. The obtained result showed that uses fast neutrons irradiation at dose of 10^7 n/m^2. accompanied with drought treatments would improve markedly the adverse effect of water stress on ambrosin, damsin, chlorophyll contents and amino acids in damsisa shoots.

[Hanan Abdalla and Eman Selem. **Fast Neutrons Irradiation Induced Changes in Active Ingredients, Amino Acids and Chlorophyll Contents in *Ambrosia Maritima* (Damsisa) Influenced by Soil Water Stress.** *Life Sci J* 2014;11(6):24-30]. (ISSN:1097-8135). http://www.lifesciencesite.com. 4

Key words: *Ambrosia maritima*, fast neutrons, water stress, ambrosin, damsin amino acids and chlorophylls.

1.Introduction

Damsisa or *Ambrosia maritima* L. belongs to subfamily *Tubuliflorae,* that represent a branch of family Compositae from flowering plants (Evans. 1996)**.** It is a widely distributed weed in southern parts of Egypt, Sudan, Senegal, and neighboring countries. In Egypt, it is a popular medicinal plant for the treatment of renal colic and calculi. It acts also as antispasmodic, diuretic and useful in bronchial asthma, spasms and frequent urination (Ghazanfar, 1994). Ambrosin and damsin considered as the most active ingredients in damsisa plants, (Abdelgaleil, 2010). Ambrosin was synthesized and described by Grieco *et al.* (1982), they attributed it to a group of natural products known as psuedo-guaianolides. Ambrosin used as tumor inhibitory agent (Torrance *et al.,* 1975), Moreover, Geerts *et al.* (1992) demonstrated a toxic effect of Ambrosia *maritima* to snails and nontarget organisms. Drinking decoctions of damsisa leaves were the most commonly used remedy for schistosomiasis in upper Egypt (Kloos *et al.,* 1982). It acts also as antispasmodic, diuretic and useful in bronchial asthma, spasms and frequent urination (Ghazanfar, 1994). Aqueous methanol extract of damsisa leaves had hepatoprotective and antioxidant actions in drug that induce hepatotoxicity in rats (Ahmed and Khater, 2001). Moreover, (Abdelgaleil, 2010) indicated that some sesquiterpenes isolated from *Ambrosia maritima* have marked insecticidal, herbicidal and molluscicidal activities. Recently, (Abdelgaleil, *et al.,* 2011) indicated that the sesquiterpenes isolated from *Ambrosia maritima* possessed pronounced antifungal activity against some plant pathogenic fungi.

Fast neutrons irradiation considered as a valuable tools, from which, developing varieties that economically and agriculturally important and have high productivity potential (Hashem, 2011). Moreover, γ-rays irradiation increases plant resistance to unfavorite conditions such as drought, water stresses, cold and in some condition insects or diseases (Hussein *et al.,* 2012). Moreover, irradiation has been found to produce a good mutation in the plants and consequently good yield (Jyoti, *et al*., 2008; Hussein and Atia, 2009), also many mutant varieties which are resistant to diseases, cold, drought and with high quality, have been developed (Jain *et al.,* 1998). The increase in some of amino acids in irradiated *Arachis hypogaea* and *Phaseolus vulgaris* seeds was attributed to the breakdown of proteins (Hussein. 1998). Release of free amino acids following radiation treatment was reported in wheat grains (Hashem, *et al.,* 2008); this might be due to autolysis or proteolysis. Similar results were obtained in fast neutrons irradiated wheat (*Triticum aestivum*) proteins (Selem, 2009). It was cleared that, amino acids were continually synthesized during water stress (Guerrier and Bourgeais-Chaillou, 1994;

Sundaresan and Sudhakaran, 1995: Yasser, 1998). Concerning the active ingredient in plants under water stress Hongyun Liua *et al.* (2011), stated that in *Salvia miltiorrhiza*, active constituents rosmarinic acid and salvianolic acid increased under water-stress conditions. About chlorophylls, Nikolaeva *et al.* (2010), found that, total chlorophyll content expressed per unit dry weight increased insignificantly during the first two periods of drought but decreased by 13–15% later on.

The present investigation introduces a study of the response of damsisa seeds that exposed to fast neutrons irradiation and grown in different levels of soil water holding capacity (W.H.C) and the changes that takes place in their chemical components. The main objective of this study was estimation of active ingredients (ambrosin and damsin) in flowering stage of damsisa shoots as affected by radiation treatments, drought levels and both of them, to report the changes that take place according to the treatments mentioned before and whether affects their amounts.

2. Material and Methods

Time course of the experiment

The experiment was carried out in the botanical green house of Botany Dept. (Faculty of Science, Zagazig University, Egypt) in plastic pots 5 kg soil capacity. Damsisa seeds were obtained from the Agriculture Research Center, Ministry of Agriculture, Dokki, Cairo Egypt. Damsisa seeds were then exposed to different doses of fast neutrons (0, 10^5, 10^7 and 10^9 n/cm^2), from 252 CF source (Amersham, England) Physics Department, Faculty of Science, Zagazig University. The pots (30 cm in diameter) were filled with 5 kg sand-loamy soil. Pots were divided into 4 groups, The first group consists of non irradiated control seeds (0.0 n/cm^2), the second group consists of irradiated seeds at 10^5 n/cm^2 fast neutrons, the third group consists of irradiated seeds at 10^7 n/cm^2 fast neutrons and the fourth group consists of irradiated seeds at 10^9 n/cm^2 fast neutrons. Pots of each group were subjected to the different water stress treatments as 3 pots for each water holding capacity (W.H.C), which were 100 %, 75%, 50%, and 25%. Ten seeds were germinated in each pot and left to grow for 10 dayes then plants were thinned to three plants per pot, all plastic pots were irrigated with tap water until the required field capacity. The variation in water content in the different sets was checked up and adjusted daily using a balance along the life cycle of plant groups. The experiment was terminated when the plants became five months old (during flowering stage).

Estimation of pigments

Chlorophyll pigments (Chl. a & chl. B) were determined using method described by Metzner *et al.* (1965).

Extraction and estimation of active ingredients (sesquiterpene lactones) (Mabry, 1970):

One gram of air dried leaves ground with 20 ml. chloroform; filtered and dried under vacuum. The residue was dissolved in 5 ml. ethanol (95%) + 5 ml. aqueous lead acetate (4%) then filtered and concentrated to least amount. The residue analysed directly by thin layer chromatography (TLC), using chloroform: methanol (99: 1). The sesquiterpene lactones (ambrosin & damsin) were detected as brown spots in a chamber containing iodine crystals (Harborne, 1984). The spots identified using Rf values and UV detection (Jakupovic *et al.,* 1987).

Estimation of free amino acids

Analysis was carried out at the National Center of Agricultral, Research, Giza, Egypt. Free amino acids were extracted according to the proposed method of Shad *et al.* (2002) as follow:

One grams of dry leaves were taken and soacked separately in 75% ethanol (100 ml). After 24 hours, the sample was ground and filtered. The residue was washed with a few ml of 75% ethanol and the volume was made up to 100 ml. Several amino acids were examined by using a HPLC system (HP1050) with a UV detector at 254 nm. The separation was accomplished with a ODS, C18 (5µm, 4 x 250 MM) column. The mobile phase consists of 32% (acetanol: tetrahydrofuran, 90:10 v/v), and 64% (tetrahydrofuran: water, 5/59 v/v) with 0.3 ml acetic acid and pH adjusted 5.15 with 1M NaOH. The flow rate was 1.5 ml/min, the temperature of the column was 60 ^{o}C, while the injection volume was 10 µl according to the method of Christian, (1990).

3. Results and Discussion

Ambrosin and Damsin

Active ingredients of *Ambrosia maritima* shoots during flowering stage (the plant become five months old) were shown in Table 1. It was observed that fast neutrons irradiation increase ambrosin and damsin contents in damsisa shoots produced from seeds treated by 10^7 as compared to the lowest 10^5 and highest 10^9 n/cm^2 dose of fast neutron irradiation. Likewise, fast neutrons dose10^7 n/cm^2 increased ambrosin and damsin contents in damsisa shoots as compared by normal control. Meanwhile, treatment of water stress at 75% (W.H.C), either irradiated or un-irradiated control mostly produced shoots having high ambrosin and damsin contents than the control. While, treatment of lower water stress at 25% (W.H.C), exhibited the lower contents of ambrosin and damsin contents as compared by the other levels of soil water stress.

In the same respect, the highest dose of fast neutron 10^9 n/cm^2 showed the lower contents of ambrosin and damsin contents as compared by the other doses of fast neutron irradiation. Similar findings were obtained by Hussein *et al.* (2012), on damsisa plants treated with gamma radiation and irrigated with salinity stress.

At the same respect, Razmjoo and Sabzalian (2008) indicated that increased drought significantly reduced essential oil content of chamomile. Ashraf and Harris (2004) also showed that oil content in the seed of medicinal plant, bishops weed (*Ammolei majus*). was decreased consistently with increase in external drought levels. Meanwhile. Agastian *et al.* (2000) noticed that all major processes such as photosynthesis, protein synthesis and lipid metabolism are affected, during the onset and development of drought stress within a plant, Baher *et al*., (2002), on basil. Hendawy and Khalid (2005) on *Salivia officinali*s and Khalid (2006) on *Ocimum americanum*.

Table 1: Active ingredients contents (mg/g dry wt.) as affected by fast neutrons irradiation and soil water stress in damsisa leaves at flowering stage.

W.H.C %	Ambrosin Fast neutron irradiation dose (n/cm^2)				Damsin Fast neutron irradiation dose (n/cm^2)			
	0.0	10^5	10^7	10^9	0.0	10^5	10^7	10^9
100 %	4.16 ±0.03	4.48 ±0.03	5.12 ±0.01	3.20 ±0.02	14.30 ±0.01	11.10 ±0.04	18.00 ±0.02	9.70 ±0.01
75 %	4.32 ±0.01	4.53 ±0.04	5.28 ±0.03	3.14 ±0.03	18.70 ±0.03	14.30 ±0.02	20.70 ±0.01	10.70 ±0.01
50 %	4.23 ±0.01	4.42 ±0.01	4.84 ±0.03	3.12 ±0.03	11.90 ±0.04	9.90 ±0.01	13.70 ±0.01	8.40 ±0.02
25%	3.84 ±0.03	3.96 ±0.03	4.34 ±0.01	3.10 ±0.01	9.12 ±0.03	9.14 ±0.04	11.60 ±0.03	7.80 ±0.01

All data are the means of 5 replicates ± SE.
Significant differences at $P < 0.05$

Amino acids

From data in Table 2. It was observed that the amino acid pool increased by soil water stress in shoot produced from seeds planted in soil with water stress 75 % (W.H.C) as compared to the control and the other two water stress treatments during the experiment. Meanwhile, the water stress 25% produced the lower contents of amino acids. The results obtained were similar with several reports of increase level of free amino acid pool during drought treatment in different plant species (Muthukumarasamy *et al.,* 2000 and Wang and Nil, 2000). Concerning amino acids concentration as affected by fast neutrons, it was observed that fast neutrons irradiation dose 10^7 n/cm^2 increased free amino acid contents in damsisa shoots compared to the normal control and to the lowest 10^5 and highest 10^9 n/cm^2 dose of fast neutron irradiation. Meanwhile, the highest irradiation dose 10^9 n/cm^2 mostly produced shoots having lower amino acid contents than the control set and the other two doses (10^5 and 10^7 n/cm^2) of fast neutron irradiation. The results revealed that glutamic acid (3.75 mg/g^{-1}) and arginine (3.48 mg/g^{-1}) were the prominent amino in damsisa plants. Similar results were reported by Al-Jassir (1992) on black cumin, Hussein (2010) on mungbean; Swailam (2009) on sesame and Hussein and Atia (2009) on mushroom, they observed that amino acids increased above the control values. Likewise, Hussein *et al.* (2012) observed that amino acids increased above the control values in damsisa shoots during the flowering stage in plants treated by gamma radiation and grown under salt stress. It was to mentioned that so long as levels of soil water stress increase this was accompanied by marked increase in proline concentration, as compared by its corresponding control. It is well known that proline content in the leaves of many plants get enhanced by several stresses including drought stress (Lee and Liu, 1999; Hernandez *et al.,* 2000) and fast neutrons radiation Hashem *et al.* (2008) and gamma radiation Hussein *et al.* (2012). Proline may contribute to osmotic adjustment at the cellular level, whereas many investigators recorded an accumulation of amino acids especially proline in plant exposed to drought or salinity stress (Moghaieb *et al.,* 2004; Eraslan *et al.,* 2007; Costa *et al.* 2008). Many studies suggested that proline is a protective agent of enzyme and membrane (Solomon *et al.,* 1994) and as an intracellular structure (Mudgal *et al*., 2010) or a storage compound of carbon and nitrogen for rapid recovery from stress (Jager and Meyer, 1977). Moreover, Maiti *et al.* (2000) demonstrated that proline increased in all barley genotypes with the increase in drought stress.

Also, proline is regarded as a source of energy and nitrogen for recovering tissue. So, it increased under stress levels and considered an osmotic adjuster (Costa *et al.,* 2008). It also stabilizes subcellular structures (membranes and proteins) and buffers cellular redox potential under stress (Chen and Murata, 2002). Hanafy *et al.* (2002) suggested that, the high values of total free amino acids concentration under soil stress conditions may be induced as a result of reducing the rate of incorporation of free amino acid into protein. In addition, the authors mentioned that the osmotic adjustment within the cytoplasm might be maintained by synthesis of compatible solutes such as amino acids which have deleterious effects on metabolism and growth at high concentrations.

Table 2: Amino acids concentration (mg/g dry wt.) as affected by different doses of fast neutrons irradiation and soil water stress at flowering stage of damsisa leaves during 2011/2012 season.

Soil water holding capacity (W.H.C)																
W.H.C	100%				75%				50%				25%			
Fast neutrons irradiation dose (n/cm^2)																
Amino acids (mg/g F. wt)	0.0	10^5	10^7	10^9	0.0	10^5	10^7	10^9	0.0	10^5	10^7	10^9	0.0	10^5	10^7	10^9
Proline	0.78	2.24	3.32	1.78	2.14	3.12	6.32	2.98	3.04	4.02	5.62	1.78	4.26	5.02	7.14	2.28
Alanine	0.57	0.82	1.23	0.61	0.88	0.94	1.65	0.67	0.56	0.74	0.94	0.52	0.42	0.64	0.81	0.45
Glutamic	1.62	2.12	2.84	1.43	1.96	2.40	3.14	1.62	2.65	2.63	3.75	1.42	0.00	2.74	3.84	1.22
Arginine	1.45	1.66	2.69	1.18	1.58	2.16	3.24	1.43	1.48	2.43	3.48	1.32	1.98	2.60	2.60	1.32
Threonine	0.98	1.21	2.89	1.14	1.94	1.20	3.12	1.68	0.64	2.00	2.48	1.68	0.52	1.10	1.23	1.38
Serine	0.53	1.06	1.46	0.88	0.74	1.26	1.46	0.48	0.43	1.06	2.76	0.98	0.34	1.00	1.18	0.27
Aspartic	0.70	1.32	1.72	0.86	0.78	1.73	2.14	0.68	0.95	0.72	1.32	0.48	0.44	0.52	1.22	0.40
Glycine	0.54	0.00	0.00	0.00	0.60	1.00	0.00	0.00	0.46	0.00	0.00	0.00	0.34	0.00	0.00	0.00
Cystine	0.63	0.63	0.84	0.74	0.72	0.74	0.87	0.44	0.60	0.71	0.84	0.35	0.52	0.64	0.88	0.30
Valine	0.62	0.90	1.16	0.50	0.80	0.98	1.84	0.74	0.44	0.72	1.34	0.32	0.38	0.63	0.98	0.27
Aminobutyric	0.59	0.54	0.74	0.34	0.65	0.61	0.83	0.47	0.48	0.47	0.56	0.35	0.32	0.43	0.55	0.24
Methionine	0.50	0.74	1.32	0.38	0.56	0.82	1.54	0.46	0.30	0.48	1.22	0.28	0.20	0.34	1.14	0.19
Tryrosine	0.61	0.68	0.96	0.52	0.76	0.58	0.96	0.40	0.53	0.68	1.02	0.48	0.52	0.61	0.86	0.36
Isoleucine	0.68	0.88	1.12	0.66	0.90	0.92	1.82	0.56	0.74	0.82	1.75	0.45	0.69	0.82	1.10	0.46
Leucine	0.52	0.62	0.92	0.48	0.55	0.67	1.10	0.46	0.47	0.65	0.72	0.42	0.38	0.54	0.62	0.32
Phenylalanine	0.73	0.89	1.84	0.64	0.82	1.10	1.30	0.64	0.62	1.00	1.40	0.64	0.56	0.88	1.16	0.34
Histidine	0.56	0.86	1.46	0.32	0.96	1.46	1.96	0.28	0.24	1.06	1.35	0.18	0.46	0.50	1.26	0.35
Lysine	0.86	0.90	1.12	0.55	0.98	1.10	1.28	0.66	0.72	1.14	1.32	0.68	0.62	1.04	1.25	0.56
Total (mg/g)	13.52	19.13	25.77	13.27	16.04	20.93	32.41	15.29	14.94	21.62	31.76	12.33	13.04	19.71	28.12	10.71

Chlorophyll Pigments

Table 3: Chlorophyll contents (mg/g fresh wt.) as affected by fast neutrons irradiation and soil water stress in damsisa leaves at flowering stage during 2011/2012 season.

W.H.C %	Chlorophyll a Fast neutron irradiation dose (n/cm^2)				Chlorophyll b Fast neutron irradiation dose (n/cm^2)			
	0.0	10^5	10^7	10^9	0.0	10^5	10^7	10^9
100 %	0.716 ±0.00	0.760 ±0.02	0.832 ±0.01	0.366 ±0.01	0.160 ±0.00	0.310 ±0.02	0.384 ±0.05	0.115 ±0.00
75 %	0.755 ±0.01	0.830 ±0.03	0.866 ±0.04	0.669 ±0.01	0.163 ±0.00	0.440 ±0.04	0.526 ±0.04	0.117 ±0.02
50 %	0.820 ±0.01	1.070 ±0.09	1.110 ±0.08	0.721 ±0.05	0.177 ±0.01	0.543 ±0.05	0.590 ±0.05	0.120 ±0.01
25%	1.040 ±0.02	1.310 ±0.11	1.430 ±0.10	0.835 ±0.09	0.238 ±0.05	0.550 ±0.08	0.610 ±0.09	0.126 ±0.04

All data are the means of 5 replicates ± SE.
Significant differences at $P < 0.05$

The data presented in table (3) for chlorophyll a, and chlorophyll b in leaves of damsisa plants grown at different levels of soil water stress showed a significant increase of Chl.a, and Chl.b in damsisa leaves with decreasing the soil (W.H.C.), whereas the highest values of Chl.a, and Chl.b attained in plant leaves subjecting to 25 % W.H.C., while the lowest values attained in plant leaves subjecting to 100%

W.H.C. Results obtained in the present work are in agreement with some other workers for example. Diab (1990) reported that, water deficit increased photosynthetic pigments concentration of flax plant. Also, Vora *et al.* (1994) found that, chlorophyll content increased in water stressed oat plants. Similarly Nikolaeva *et al.* (2010), concerning the effect of fast neutron irradiation, it was observed that fast neutrons irradiation increase Chl.a, and Chl.b in damsisa leaves produced from seeds treated by 10^7 as compared to the lowest 10^5 and highest 10^9 n/cm^2 dose of fast neutron irradiation. The fast neutrons dose10^7 n/cm^2 noticed to increase Chl.a, and Chl.b contents in damsisa shoots as compared by normal control. Meanwhile, all treatments of water stress either irradiated or un-irradiated produced shoots having high Chl.a, and Chl.b contents than the control set at the lower fast neutron irradiation dose 10^5 n/cm^2, however the highest dose of fast neutron 10^9 n/cm^2 showed a lower chlorophyll contents as compared with other doses.

Conclusion

It was concluded that fast neutrons irradiation can alleviate harmful effect of soil drought and this appear in an increase of most amino acids, chlorophyll pigments and some chemical components of damsisa plants that grow under drought stress condition.

References

1. Abdelgaleil, S. A. M.; Badawy, M. E. I.; Suganuma, T. and Kitahara, K. (2011). Antifungal and biochemical effects of pseudoguaianolide sesquiterpenes isolated from *Ambrosia maritima* L. African Journal of Microbiology Research Vol. 5(21), pp. 3385-3393.
2. Abdelgaleil, S.A.M. (2010). Assessment of mosquitocidal, herbicidal and molluscicidal potentials of extracts and phytochemicals isolated from three Egyptian plants. Alex. J. Agric. Res., 55: 59-73.
3. Agastian, P.; Kingsley S.J. and Vivekanandan, M. 2000, Effect of drought on photosynthesis and biochemical characteristics in mulberry genotypes, Photosynthetica, 38: 287-290.
4. Ahmed, MB. and Khater, M.R. 2001. Evaluation of the protective potential of *Ambrosia maritima* extract on acetammophen-inducecl liver damage. J. Ethnopharmacol., 75: 169-174.
5. Al-Jassir, M.S., 1992. Chemical composition and microflora of black cumin (*Nigella saliva* L.) seeds growing in *Saudi Arabia*. Food Chem., 45: 239-242.
6. Ashraf, M. and Harris. P.J.C. 2004. Potential biochemical indicators of drought tolerance in plants. Plant Sci.. 166: 3-16.
7. Baher, Z.F.; Mirza, M.; Ghorbani, M. and Rezari, M.B. 2002. The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis* L, Flav. Frag. J,. 17: 275-277.
8. Chen, T.H.H. and Murata, X. 2002. Enhancement of tolerance of abiotic stress by metabolic engineenng of betaines and other compatible solutes, Curr. Opin. Plant Biol., 5: 250-257.
9. Christian, G. (1990): HPLC tips and tricks. Great Britain at the iden press, Oxford. pp: 608.
10. Costa, R.C.L.; Lobato, A.K.S.; Neto, C.F.O.; Maia, P.S.P.; Alves, G.A.R. and Laughmghouse, H.D. 2008. Biochemical and physiological responses in two *Vigna unguiculata* (L.) Walp cultivars under water stress. J. Agron., 7: 98-101.
11. Diab, J.A. (1990): Physio-Anatomical Studies of *Linum ustatissmum* Affected by Different Treatments of Water Supply and Growth Regulating Substances. M.Sc. Thesis, Fac. Agric., Zagazig Univ., Agric. Botany Dept.
12. Eman, E. M. S. 2009. Physiological and ecological studies during the growth of wheat plant (*Triticum aestivum* L.CV) under fast neutron. Ph.D Thesis Botany Dept. Faculty of Science, Zagazig University, Egypt.
13. Eraslan, Inal F.A.; Savasturk, O. and Gunes A. 2007. Changes in antioxidative system and membrane damage of lettuce in response to drought and boron toxicity, Sci. Hortic., 114: 5-10.
14. Evans. W.C., 1996. Trease and Evans Pharmacognosy. 4th Edn., W.B. Saunders Company Ltd., London. Pages: 50.
15. Geerts, S.; Alard, F.; Belot, J. and Sidhom, M. (1992): The toxicity of *Ambrosia maritima* to snails and nontarget organisms. In: Seminar “Vector control of Schistosomiasis using native African plants”. Symoens, J.J., Geerts, S. And Triest L. (Eds.) (Brussels, 24 March 1992) pp 89-100.
16. Ghazanfar, S. (1994). CRC Handbook of Arabian Medicinal Plants. CRC Press, Boca Raton, p. 265.
17. Grieco. P.A.; Majetich, G.F. and Ohfune, Y.1982. Pseudoguaianolides. 1. Stereospecific total synthesis of the ambrosanolides dl-ambrosin and dl-damsin. J. Am. Chem. Soc., 104: 4226-4233.
18. Guerrer, G and Bourgeais-Chaillou, P. 1994. Solute contents in roots and root calli of NaCl-

tolerant and NaCl-sensitive tissues of *Lycopersicon*. Biol. Plantarum, 36: 321-328.
19. Hanafy, A.H.; Higazy, M.A.; El-Shafey, Y.H. and Moussa, S.F. 2002. Effect of drought, silicon and proline on the growth, yield and chemical composition of wheat plant. Proceeding 2nd Congress Recent Technology Agriculture, October 28-30, 2002, Cairo University, pp: 965-978.
20. Hashem, E. A. 2011. Irradiation with fast neutrons induce salinity tolerance in potato (*Solanum tuberosum* L.) callus. *Assuit Univ. J. of Botany,* 40(2): pp 61-76 (2011).
21. Hashem, E. A.; Hanafy, M. S. and Seleem, E. E. (2008): Irradiation With Fast Neutrons Induced Qualitative and Quantitative Changes In The Growth and Yield of Wheat *(Triticum aestivum*. L.) cultivar. *J. Egypt. Soc. Biotech. & Environ. Sci.* 12 (C): 1-24.
22. Harborne, J.B. (1984): Sesquiterpene Lactones, in Phytochemical Methods. 2nd ed. London. P.112.
23. Hartzendorf. T. and Rolletschek, H. 2001. Effects of NaCl-drought on amino acid and carbohydrate contents of *Phragmites australis*. Aquat. Bot., 69: 195-208.
24. Hendawy. S.F. and Khalid, K.A. 2005. Response of sage (*Salvia officinalis* L.) plants to zinc application under different drought levels. J. Appl. Sci. Res., 1: 147-155.
25. Hernandez, J.A.; Jimenez, A,; Mullineaux, P. and Sevilla, F. 2000. Tolerance of (*Pisum satiuum*) to long term salt stress is associated with induction of antioxidant defenses. Plant Cell Environ., 23: 853-862.
26. Hongyun Liua; Xiangdong Wangb; Donghui Wangc; Zhirong Zoua and Zongsuo Lianga. 2011. Effect of drought stress on growth and accumulation of active constituents in *Salvia miltiorrhiza* Bunge. *Industrial Crops and Products.* 33 (1), pp 84–88.
27. Hussein, O.S.; Hanafy, A, A.; Ghalab A.R. and El-Hefny, A.M. (2012). Some Active Ingredients, Total Protein and Amino Acids in Plants Produced from Irradiated *Ambrosia maritima* Seeds Growing under Different Soil Salinity Levels. *Amer. J. of Plant Physiol*. 7 (2): 70-83.
28. Hussain, K.; Majeed, A.; Nawaz, K.; Bhatti, K. H. and Nisar, F.K. 2009. Effect of different levels of drought on growth and lion contents of black seeds (*Nigella sativa* L.). Curr. Res. J. Biol.Sci.. 1: 135-138.
29. Hussein, O.S. 1998. Effect of certain treatments on some metabolites particualrly the toxic substances, in *Arachis hypojaea* and *Phaseolus vulgaris* seeds. Ph.D. Thesis, Botany Dep., Fac. of Sci., Ain Shams Univ., Cairo, Egypt.
30. Hussein. O.S. and Atia, L. A. 2009. Using of gamma rays in stimulation and improvement of mushroom spawns *Plerotus ostreatus* Egypt. J. Rad. Sci. Applied, 22: 275-285.
31. Hussein, O.S., 2010. Effect of ascorbic acid and or sodium chloride on irradiated mungbean seeds.Arab J. Nucl. Sci. Applied, 43: 281-288.
32. Hussein, M.M. and Mandour, M.S. (1984): Growth and chemical composition of corn plants as affected by nitrogen fertilizer and soil moisture treatments. Bull. N.R.C., Egypt. 5: 406-414.
33. Jager. H. J. and Meyer, H.R. 1977. Effect of water stress on growth and proline metabolism of *Phaseolus vulgaris* L. Oecologia, 30: 83-96.
34. Jain, S.M.; Ahloowalia, B.S. and Veilleux, R.E. 1998. Somaclonal variation and Induced Mutation in Crop Improvement, Vol. 12, KLuwer Academic Publishers. The Netherland. pp: 203-218.
35. Jakupovic, J.; Sun, H.; Geerts, S. and Bohlmann, F. (1987): New pseudoguaianolides from *Ambrosia maritima*. Planta Medica, 52, 49-51.
36. Jyoti, P. M.C.; Sandeep, K.; Subrata, P.; Jiin-Shuh, J.; Alok, C.S.; Anindita, C. and Subhas, C. S. (2008): Effects of gamma irradiation on edible seed protein, amino acids and genomic DNA during sterilization. Online. www.sciencedirect.com.
37. Khalid, K. A.. 2006. Influence of water stress on growth, essential oil and chemical composition of herbs (*Ocimum* sp.). Inter. Agrophys., 20: 289-296.
38. Kloos, H.,W.; Sidrak, A.A.; Michael, E.W.; Mohareb and Higashi, G.I. 1982. Disease concepts and treatment practices relating to *Schistosomisis haematobium* in Upper Egypt. J. Trop, Med. Hyg., 85: 99-107.
39. Lee., T.M. and Liu, C.H. 1999. Correlation of decreased calcium contents with proline accumulation in the marine green macroalga *Ulna fasciata* exposed to elevated NaCl contents in seawater. J. Exp. Bot., 50: 1855-1862.
40. Nikolaeva, M. K.; Maevskaya, S. N.; Shugaev, A. G. and Bukhov, N. G. 2010. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. *Russian Journal of Plant Physiology*, 57: (1), pp 87-95.
41. Maiti, R.K.; Moreno-Limon, S. and Wesche-Ebeling, P. 2000. Response of some crops to various abiotic stress factors and its

physiological and biochemical basis of resistances. Agric. Rev, 21: 155-167.
42. Mabry, T. J. (1970): In Phytochemical Phylogeny (ed. Harborne, J.B.), Academic Press, London, pp. 269-300.
43. Metzner, H.; Rau, H. and Senger, H. (1965): Undersuchungen zur synchronisier barkeit ein zlner-pigment-mangel mutation von *Chlorella.* Planta, 65, 186.
44. Moghaieb, R. E. A.; Saneoka, H. and Fujita, K. 2004. Effect of drought on osmotic adjustment, glycinebetaine accumulation and the betaine aldehyde dehydrogenase gene expression in two halophytic plants, *Salicornia europaea* and *Suaeda maritima*. Plant Sci, 166: 1345-1349.
45. Mudgal, V.; Madaan, N. and Mudgal, A. 2010. Biochemical mechanism of salt tolerance in plants: A review. Int. J. Bot, 6: 136-143.
46. Muthukumarasamy, M; Gupta, S.D. and Panneerselvam, R. 2000. Influence of triadimefon on the metabolism of NaCl stressed radish. Biol. Plant, 43: 67-72.
47. Razmjoo, K.P.H, and Sabzalian, M.R. 2008. Effect of drought and drought stresses on growth parameters and essential oil content of *Matricaria chamomilla*. Int. J. Agric. Biol., 10: 451-454.
48. Shad, M.A.; Ansari, T.M.; Perver, H.; Rubab, M. and Mohamood, T. (2002): Changes in sugar, amino acid and mineral contents of leaves of two mango varieties affected by quick decline disease. J. Boil. Sci. 2(10): 694- 696.
49. Solomon, A, Beer, S. Waisel, Y. Jones, G.P. and Paleg, L.G. 1994. Effect of NaCl on the carboxylation activity of rubisco from tamanx ordams in the presence and absence of proline related compatible solutes. Physiol. Plant,, 90: 198-208.
50. Sundaresan, S, and Sudhakaran, P.R. 1995, Water stress-induced alterations in the proline metabolism of drought-susceptible andbtolerant cassava (*Mamhot esculenta*) cultivars. Physiol. Plant, 94: 635-642.
51. Swailam, M.H, 2009. Quality characteristics of irradiated sasame seeds during storage. J. Rad. Sci. Applic, 22: 323-342.
52. Torrance, S. J.; Wiedhopf, R.M. and Cole, J. R. (1975): Ambrosia tumor inhibitory agent from *Hymenoclea salsola* (*Asteraceae*). *Journal of Pharmaceutical Science.* Vol. 64 (5): 887-888.
53. Vora, A.B.; Geetha, M. and Petal, J.A. (1994): A study on effects of decreasing field moisture status on growth and metabolism of *Avena sativa* L. var HFO 114. Advances in Plant Sciences. 7: 1, 72-83.
54. Wang, Y. and N. Nil, 2000. Changes in chlorophyll, ribulose bisphosphate carboxylase-oxygen ase, glycme betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *J. Hortic. Sci. Biotechnol,* 75: 623-627.
55. Xiong, L. and Zhu, J.K. 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environ*., 25: 131-139.
56. Yasser, H. A. H. 1998, Physioogical and ecological studies during the growth stages of an Egyptian medicinal plant. M.Sc. Thesis Botany Dept. Faculty of Science, Zagazig University, Egypt.

3/19/2014