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RESEARCH ARTICLE

Conservation Approach of Achillea santolina L. along the North Western Mediterranean Coastal Region of Egypt: Metabolite content

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Abstract

The main objective of the present study is to evaluate some metabolite contents such as total free amino acids (TFAA), free proline (FP), soluble proteins (SP) and total soluble sugars (TSS) of an endangered medicinal plant Achillea santolina grown in six different locations distributed along the north western Mediterranean coastal region of Egypt. Results of the present study showed that the highest concentration of FP as well as SP was recorded at Ras El-Hekma location. Similarly, the concentration of TFAA in belowground was attained at El-Hammam location, while that of the aboveground was attained at Dabaa location. Notably, conservation of this species is necessary to meet the unlimited needs on medicinal plants as well as to minimize the human impact on destroying the natural resources.
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Introduction

The north western Mediterranean coastal region represents the most abundant area in its floristic structure in Egypt. The natural plant communities registered in this belt constitute nearly half of the Egyptian vegetation (Boulos, 2009; Hegayz et al., 2010). Long term anthropogenic activities and climatic changes have resulted in natural habitat damage which in turn caused species extinction (Hegayz et al., 2010).

Achillea santolina L. is one of the most important medicinal plants grows as a common weed in the north western Mediterranean region. This plant usually grows in barley and fallow fields, olive and fig plantations and at the edges of the small canals and roads (El-Darier and Tammam, 2012). Ethnobotanical studies in the region indicated that the plant medicinally used by local inhabitants as an anthelmintic and stomachic drug. Additionally, its flower heads are used to relieve rheumatic pains, toothache and for treatment of diabetes mellitus and colic (El-Darier et al., 2005). Khafagy et al. (1965) defined santolin the bitter component of A. santolina, while Elgamal et al. (1991) determined stigmasterol, clionasterol, salvigenin, eupatorin, poriferasterol, artemisetin, 6,7,3', 4'-tetramethoxy-5-hydroxyflavone and leukodin from the shoot system.

Recent studies demonstrated the allelopatheic potential of this herb against some valuable crops including Vicia faba, Hordeum vulgare and Triticum aestivum (Hatata and El-Darier, 2009; Tammam et al., 2011). Similar to other species, this herb is endangered, therefore monitoring of such a threatened species is of great value to allow conservation and protection of the plant population from extinction danger. Virtually, insufficient information is existed about the differences in the distribution pattern of different nutrients and metabolites in A. santolina harvested from different locations. The main objective of this work is to determine metabolites during three phenophases (vegetative (VG), flowering (FL) and seeding (SD)) of A. santolina among the six different locations selected in the present study. This will provide basic biological data for establishing conservation and recovery strategies for the threatened species. To achieve the goal, total soluble sugars (TSS), soluble protein (SP), free proline (FP) and total free amino acids (TFAA) are estimated in the above and underground parts of the study plant.

MATERIALS AND METHODS:

Sampling and Phytomass Determination of Plant Materials

Ten complete individuals of medium size studied species were randomly excavated during three different phenophases (vegetative, flowering and seeding) from different sites at each of six locations (Burg El-Arab, El-Hammam, Dabaa, Ras El-Hekma, Matruh and Negilla). The samples were kept in paper bags, brought to the laboratory shortly after collection. In the laboratory, each sample was rinsed four times with tap and distilled water, air-dried and then separated into different parts [aboveground (aerial shoots + reproductive organs) and underground (buried shoots + roots)]. Thereafter, the samples were oven dried at 65^{o}C to constant weight. The oven dry weight for each part was estimated and consequently the total individual phytomass was calculated. The oven dry samples were ground using Thomas-Willy laboratory Mill, Model 4. The ground samples were stored in paper bags ready for the chemical analysis.

Chemical Analysis

1. Determination of total ash and nutrient uptake

The total ash and nutrient content of the different parts of A. santolina were determined according to Allen et al. (1984). Total ash was estimated by ignition while nutrient concentration of K, Ca, Mg, Cu, Fe, Mn, and Zn was measured using Varian Spectra A 220 Atomic Absorption Spectrophotometer and used for calculation of uptake.

2. Determination of metabolite contents

The total free amino acids (TFAA) were determined following the method described by Yemm and Cocking (1955). Free proline (FP) was evaluated following the protocol described by Bates et al. (1973). On the other hand, soluble proteins (SP) and total soluble sugars (TSS) were determined by procedures described by Hartree (1972) and Dubios et al. (1956).

All these determinations were carried out on triplicates of oven-dry samples, and expressed as percentages of the oven dry weight (mg g^{-1} oven dry wt.). The values obtained from each triplicates were averaged to represent the content in the respective organ during the growing season.

Treatment of Data

All the results were verified statistically using Two-Way analysis of variance (ANOVA) test using Costat 2.0 statistical analysis soft ware (Zar, 1984). All means were tested with least square difference (LSD) where the difference of $p \leq 0.05$ was considered as significant.

RESULTS

Variation in Metabolite Concentrations

1. Total free amino acids (TFAA)

The concentration of TFAA in the aboveground parts (Figure 1, Table1, 2) attained its highest and lowest concentration at the six locations during vegetative and seeding stage respectively. Dabaa, Ras El- Hekma and Negilla seem to have the same concentration and trend of variation. The range of variation was between 1.77 and 16.74 mg g^{-1} dry wt. at Dabaa, 1.36 and 13.53 mg g^{-1} dry wt. at Ras El-Hekma and 1.275 and 16.5 mg g^{-1} dry wt. at Negilla. Likewise, Burg El-Arab, El-Hammam and Matruh exhibited relatively the same concentration and trend of variation. The range of variation was between 2.35 and 8.19 mg g^{-1} dry wt. at Burg El-Arab, 1.3 and 6.172 mg g^{-1} dry wt. at El-Hammam, and 1.47 and 9.45 mg g^{-1} dry wt. at Matruh. The maximum concentrations of TFAA in the underground parts were achieved at Ras El-Hekma (13.43 mg g^{-1} dry wt.), Burg El-Arab (3.4 mg g^{-1} dry wt.) and El-Hammam (7.475 mg g^{-1} dry wt.) during vegetative, flowering and seeding stages respectively. On the other hand, minimum values during the three phenophases were recorded at Negilla (3.700, 1.625 and 2.157 mg g^{-1} dry wt. respectively). The trend of variation in TFAA was similar allover the six locations, as the concentrations sharply decreased from vegetative to flowering and then slightly increased from flowering to seeding stage. Negilla exhibited the lowest concentrations compared to all other locations, as the range was between 1.625 and 3.7 mg g^{-1} dry wt.

2. Soluble protein (SP)

Generally, the concentration of SP in the aboveground parts (Figure 1, Table1, 2) was higher than that in the underground ones. Negilla location exhibited the highest concentrations (155 and 136.87 mg g^{-1} dry wt.) of SP in the above and underground parts respectively during the vegetative stage. The lowest concentrations in the above (47.26) and underground parts (49.070 mg g^{-1} dry wt.) were attained during flowering stage at El-Hammam and Negilla respectively. The concentration of SP in the aboveground parts at El-Hammam and Burg El-Arab decreased from vegetative to flowering stage then slightly increased from flowering to seeding stage. The trend of variation was nearly the same at the other 4 locations. The concentration decreased in the following order: vegetative > flowering > seeding stage. With respect to the underground parts, a decrease in the concentration of SP at Burg El Arab, Dabaa and Negilla was noticed from vegetative to flowering stage, followed by an increase during seeding stage. The range of variation was from 57.07 mg g^{-1}dry wt. to 69.13 mg g^{-1} dry wt. at Burg El-Arab, 58.9 mg g^{-1} dry wt. to 76.57 at Dabaa, 49.07 mg g^{-1} dry wt. to 136.870 mg g^{-1} at Negilla. An opposite trend was noticed at El-Hammam and Matruh locations, where the concentration of SP increased from vegetative to flowering, then decreased again at seeding stage. On the other hand, Ras El-Hekma location achieved a trend of variation that was different, whereas the concentration steadily increased from vegetative to seeding stage.

3. Total soluble sugars (TSS)

The concentration of TSS was more prominent in the underground than the aboveground parts especially during flowering and seeding stage (Figure 1, Table1, 2). In the aboveground parts, the variation in the concentration of TSS was an increase from vegetative to flowering stage followed by a decrease during seeding stage. This range of variation was confined between a minimum of about 26 mg g^{-1} dry wt. at Ras El-Hekma and a maximum of about 269 mg g^{-1} dry wt. at Negilla. The concentration of soluble sugars attained nearly similar minima and maxima at Dabaa (30.8 and 108.4 mg g^{-1} dry wt.) and Ras El-Hekma (26 and 106.07 mg g^{-1} dry wt.) during the three phenophases. On the other hand, a narrow range of variation was noticed at Burg El-Arab (45.49 and 54.88 mg g^{-1} dry wt. respectively) and El-Hammam (53.5 and 65.09 mg g^{-1} dry wt. respectively). At Matruh and Negilla the concentration of TSS was greatly increased from vegetative (27.66, 28.331 mg g^{-1} dry wt. respectively) to flowering (174.6, 269 mg g^{-1} dry wt. respectively), then decreased by about half its value during seeding stage (90.577, 105.78 mg g^{-1} dry wt.). In the underground parts, the trend of variation was similar in all locations over the three phenophases, as the concentration was steadily increased from vegetative to seeding stage except for Burg El-Arab and Negilla, where the concentration decreased during seeding stage. The range of variation was between a maximum of about 303.983 mg g^{-1} dry wt. at Matruh to a minimum of about 22.85 mg g^{-1} dry wt. at Ras El-Hekma. Dabaa, Ras El-Hekma and Matruh were nearly similar in their maxima (282, 294, and 303 mg g^{-1} dry wt. respectively).

Variation in the concentrations of TFAA, SP, and TSS with the phenophase and location in the above and underground parts of A. santolina was highly significant ($P \leq 0.05$) as evaluated by the Two-Way analysis of variance (ANOVA) test.

4. Free proline (FP)

Higher values of the concentration of free proline were recorded in the underground parts compared to the aboveground ones (Figure 1, Table1, 2). The maximum and minimum concentrations in the aboveground parts were attained at Negilla during vegetative (63.056 mg g^{-1} dry wt.) and seeding (4.43 mg g^{-1} dry wt.) stage. In all studied locations, the concentration of free proline attained the same trend of variation, as it decreased from vegetative to seeding except for Matruh where the value increased from vegetative to flowering then decreased during seeding stage. The lowest range of variation (5.76 to 8.085 mg g^{-1} dry wt. respectively) in the concentrations of FP was recorded at El-Hammam location. Similarly, Dabaa, and Ras El-Hekma, attained a less difference in the concentration of (FP) between each other and over the three phenophases. The underground parts attained the maximum concentration during vegetative and a minimum during seeding stage except at Burg El-Arab and El-Hammam, where the concentration decreased from vegetative to flowering followed by an increase during seeding stage. The highest concentration (78.36 mg g^{-1} dry wt.) was attained at Ras El-Hekma location during vegetative stage, while the lowest (10.72 mg g^{-1} dry wt.) was attained at El-Hammam during flowering stage. In the aboveground parts, differences in the concentration of free proline among different locations as well as different phenophase were significant ($p \leq 0.05$) as evaluated by the Two-Way analysis of variance (ANOVA) test.

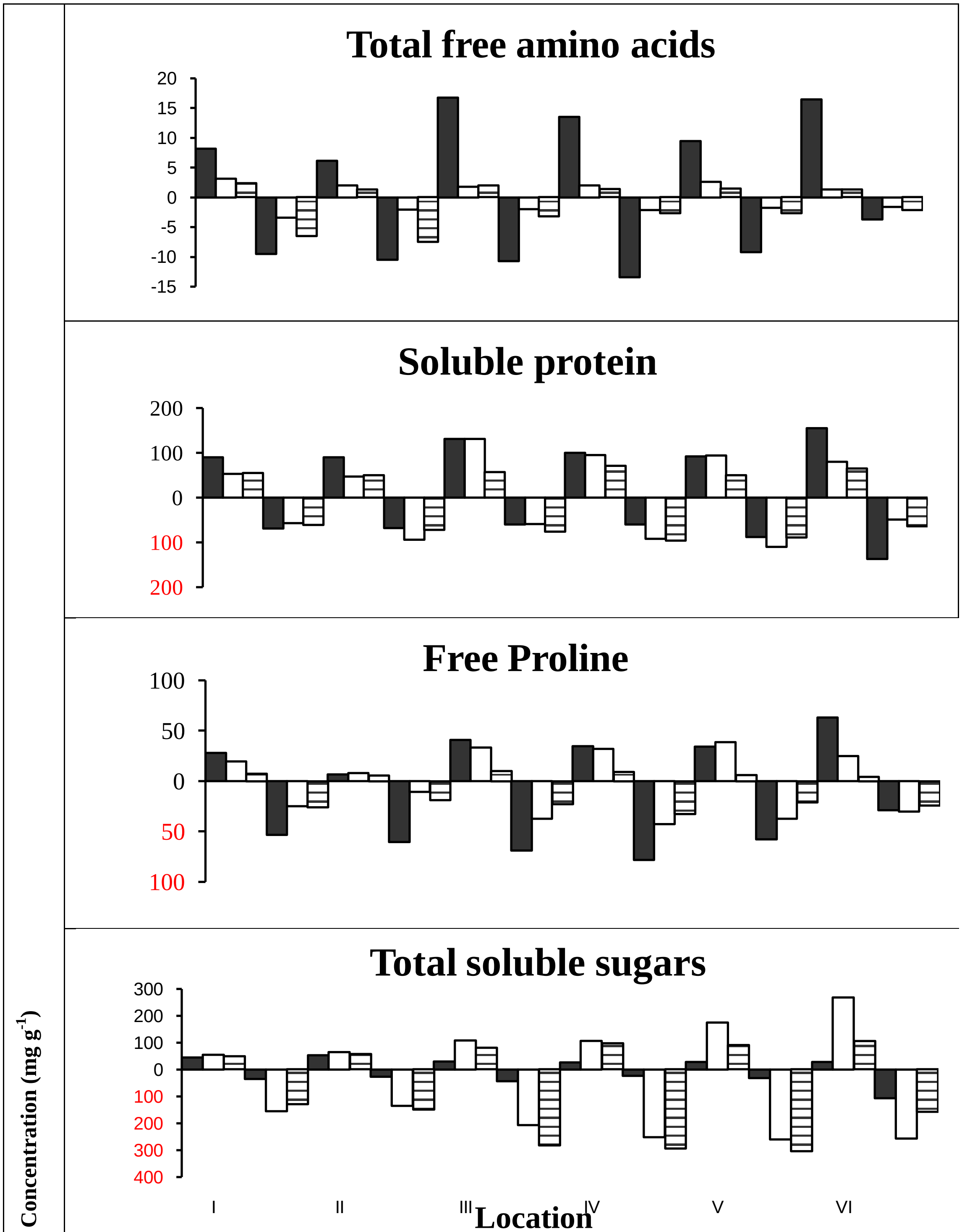

Figure 1. The mean concentration (mg g^{-1}) of total free amino acids (TFAA), soluble protein (SP), free proline (FP) and total soluble sugars (TSS) in the aboveground parts (bars above zero line) and in the underground parts (bars under zero line) of Achillea santolina during three phenophases at six locations distributed along the north western Mediterranean coastal region of Egypt. I: Burg El-Arab, II: El-Hammam, III: Dabaa, IV: Ras El-Hekma, V: Matruh, VI: Negilla. VG: vegetative, Flowering SD: Seeding

Table 1: Result of Two-way ANOVA test analyze the effects of location, phenophases and location x phenophases interaction on the concentration (mg g^{-1}) of total free amino acids (TFAA), soluble protein (SP), free proline (FP) and total soluble sugars (TSS) in the aboveground parts of Achillea santolina during three phenophases at six locations distributed along the north western Mediterranean coastal region of Egypt.

Factors	TFAA	SP	FP	TSS
Location	147.78*	141.83*	499.06*	233.80*
Phenophase	524.12*	587.34*	253.55*	107.28*
Location x phenophase interaction	202.28*	588.20*	247.19*	178.009*

*Significant at 0.05 probability level

Table 2: Result of Two-way ANOVA test analyze the effects of location, phenophases and location x phenophases interaction on the concentration (mg g^{-1}) of total free amino acids (TFAA), soluble protein (SP), free proline (FP) and total soluble sugars (TSS) in the underground parts of Achillea santolina during three phenophases at six locations distributed along the north western Mediterranean coastal region of Egypt.

Factors	TFAA	SP	FP	TSS
Location	300.30*	940.03*	0.80^{NS}	859.52*
Phenophase	352.04*	508.624*	0.64^{NS}	943.316*
Location x phenophase interaction	171.66*	123.97*	1.065^{NS}	433.75*

*Significant at 0.05 probability level

DISCUSSION

The effect of habitat conditions (e.g. land use, anthropogenic activities etc.) on the yield of A. santolina phytomass, trend of metabolite and nutrients accumulation is evident from the present results and coincide with the results obtained by El-Darier et al. (2014).

The selected locations are subjected to different styles of human activities (farming, construction, disturbance etc.) which significantly alter the relevant physiological parameters measured and exert risk on the plant existence.

Pattern of carbon allocation in plants varies significantly during the different growing stages. By the end of growing season in perennial herbs, the trend of carbohydrates accumulation is shifted to the belowground parts, suggesting a major energy reserve for the coming growing season (Lapointe, 1998). The temporal and spatial variation in the accumulation of compatible osmometabolic substances such as, soluble sugars, soluble protein and total free amino acids in the above and underground parts of A. santolina showed a significant difference among both phenophases and locations. Obviously, the maximum accumulation of metabolite in A. santolina during the three phenophases mainly achieved at location Ras El-Hekma, Matruh, and Negilla. This probably due to environmental and climatic conditions, such as seasonal precipitation, air moisture and temperature, day time and level of human activities. In addition to farming processes as well as edaphic factors including percentage of soil moisture (especially during flowering and seeding), $CaCO_3$, and K concentration. These parameters attained lowest values in these locations compared to the other three locations.

Carbohydrates play a vital role in plant cell as structural components and in cellular process. The assimilated carbohydrates act as carbon and energy source for building plant parts in addition to taking a part in organic compounds synthesis (Youssef, 1997). Based on source sink concept, there is a direct correlation between the carbohydrates transport and plant phenological stage, performance as well as growth manner (El-Darier and Youssef, 1998). Total soluble sugars accumulation mainly in the underground parts of A. santolina at location El-Hammam, Dabaa, Ras El-Hekma, and Matruh in the order of seeding > flowering > vegetative, may cause a considerable variation in the osmotic pressure of the root cell sap (Irigoyen et al., 1992). This accumulation may be due to less sugar consumption, high rate of starch breakdown into soluble sugars as well as storage of TSS in the perennating organs for the upcoming growth season (Irigoyen et al., 1992; Mohammad khani and Heidari, 2008). On the other hand, maximum values of TSS in the aboveground part all over the six locations was observed during flowering stage then decreased during seeding stage. This probably due to the high activity of plant which reaches maximum at this stage to form reproductive organs and invest much of its soluble sugar resources to produce energy necessary for this component.

Proline is one of the free amino acids that produced normally in the plant cells, but under stress conditions it tend to accumulate significantly (Lutts et al., 1999). The functional role of proline varies depending on the type of species (Raggi, 1994). The high rate of proline production in plant has several protective, adaptation and regulatory activities (Szabados and Savoure, 2010). Mohammad khani and Heidari, (2008) have reported that concentration of many metabolites like soluble sugars and proline is increased under water stress condition. In the present study, considerable variations were recorded in proline accumulation during the different phenophases among the six locations. Currently, in the aboveground part, the concentration of proline occupy the following trend, Vegetative > flowering > seeding, except location El-Hammam and Matruh which coincides with the decrease of total free amino acids in the same rhythm. The transport of proline did not coincide with the transport of soluble carbohydrates. On the other side, the level of total free amino acids, in the underground parts, showed a significant increase from flowering to seeding stage. Increased soluble protein contents at location Burg El-Arab, El-Hammam, Ras El-Hekma and Negilla signifying that accumulation of TFAA is not related to protein degradation. These findings are in accordance with Sundaresan and Sudhakaram (1995) working on Manihot esculenta. Raggi (1994) suggested that protein hydrolysis, growth as well as suppression of protein biosynthesis are the key reasons that control the variations in free amino acids concentration especially proline.

CONCLUSION

Given the interest for a continuous and regular conservation of the medicinal plant; A. santolina, study of its phytochemical properties in different representative locations is essential. Importantly, A. santolina was found to be a widespread species and adapt to a wide range of environmental conditions to keep its large geographic distributions. The measured parameters showed significant variation among different locations showing the plasticity of the species to cope with different mode of human and environmental impacts.

REFERENCES

Allen, S.; Grimshay, H.M.; Parkinson, J.A. and Quarmby, C. (1984). Chemical Analysis of Ecological Materials. Blackwell Scientific Publications Osney, Oxford, London, Edinburgh, Melbourne, pp. 565.

Bates, L.S.; Waldes, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.

Boulos, L. (2009). Flora of Egypt check list. Cairo, Egypt: Al Hadara Publishing. 410 pp.

Dubios, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A. and Smith, f. (1956). Colourimeteric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

El-Darier, S. and Youssef, R. (1998). Ecological and biochemical adaptation to drought in two fig varietis in the Western Mediterranean Desert of Egypt. Proceedings, sixth Egyptian Botanical conference, Cairo University, Giza. November 24-26, Vol. III: 455 -468.

El-Darier, S.M., Abdel-Razik, M., El-Ghamdy, M. (2005). Ecology and traditional medicine of local plants in the western Mediterranean coastal region of Egypt. International seminar on the valorization of the medicinal plants in the arid regions, Urgala University (Algeria).

El-Darier, S.M. and Tammam, A.A. (2012). Potentially phytotoxic effect of aqueous extract of Achillea santolina induced oxidative stress on Vicia faba and Hordeum vulgare. Rom. J. Biol. – Plant Biol., Vol. 57, No. 1, 3–25, Bucharest.

Salama M. El-Darier; Hoda A. Ahmed; Sania A. Kamal and Hisham A. R. Deif (2014). Conservation approach of Achillea santolina l. along the North Western Mediterranean coastal region of Egypt: Phytomass and mineral content. Journal of Global Biosciences, Vol. 3(5), 848-857.

Elgamal, M.H.; Hanna, A.G. and Duddeck, H. (1991). Constituents of Achillea santolina. Fitoterapia, 62(4): 359-368.

Hartree, E.F. (1972). A modification of the Lowery method that given a linear photometric response. Anal. Biochem., 48: 422-425.

Hatata, M.M. and El-Darier, S.M. (2009). Allelopathic effect and oxidative stress induced by aqueous extract of Achillea santolina L. shoot on Triticum aestivum L. Plant. Egypt. J. Exp. Biol. (Bot.) 5: 131 – 141.

Hegazy, A.K., Kabiel, H.F., Boulos, L. and Sharashy, O.S. (2010). Conservation approach to the demography and dynamics of protected and unprotected populations of the endmic Ebenus armitagei in the Western Mediterranean Coast of Egypt. Journal for Nature Conservation, 18: 151 – 158.

Irigoyen, J.J., Emerich, D.W., and Sánchez-Díaz, M. (1992). Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants. Physiol. Plant. 84: 55-60.

Khafagy, S.M.; El-Fatatry, L.A.; and Amer, M.S. (1965). Preliminary characterization of santolin, bitter principal of Achillea santolina L. growing in Egypt. Acta Pharm. Suec., 2(6): 403-410.

Lapointe, L. (1998). Fruit development in Trillium, dependence on stem carbohydrate reserves. Plant Physiol. 117: 183–188

Lutts, S., Majerus, V., and Kinet, J.-M. (1999). NaCl effects on proline metabolism in rice (Oryza sativa) seedlings. Physiologia Plantarum 105: 450-458.

Nayer Mohammadkhani and Reza Heidari (2008). Drought-induced Accumulation of Soluble Sugars and Proline in Two Maize Varieties. World Applied Sciences Journal 3 (3): 448-453.

Raggi, V. (1994). Changes in free amino acids and osmotic adjustment in leaves of water-stressed bean. Physiol. Plant., 91: 427-437.

Sundaresan, S. and Sudhakaran, P.R. (1995). Water stress-induced alterations in the proline metabolism of drought-susceptible and tolerant Cassava (Manihot esculenta) cultivars. Physiol. Plant., 94: 635-642.

Szabados, L. and Savoure´, A. (2010). Proline: a multifunctional amino acid. Trends in Plant Science, 15 (2): 89-97.

Tammam, A.A., El- Bakatoushi, R. and El-Darier, S.M. and (2011). The phytotoxic potential of Achillea santolina L. (Asteraceae) on Vicia faba L. and Hordeum vulgare L. The Asian international journal of life sciences, 20 (2): 443 – 464.

Yemm, E.W. and Cocking, E.C. (1955). The determination of amino acids with ninhydrin. Anal., 80: 209-213.

Youssef, R.S.A. (1997). An ecological study of the physiological and biochemical response of some desert plant species to draught and salinity stresses in the Western Mediterranean region of Egypt. Ph.D. thesis, Alexandria University, pp 132.

Zar, J.H. (1984). Biostatistical Analysis Prentice-Hall: Inc. (ed). New Jersey, 718pp.