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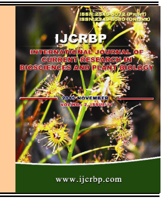

Original Research Article

Study of the Effect of Guava (*Psidium guajava*) Juice as Biological Mean on the Ability of Sunflower (*Helianthus annuus*) Plants to Withstand Deficit Irrigation

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Abstract

This study aims to evaluate the feasibility to deficit irrigation and whether significant savings in irrigation water are possible without significant reduction in the plant's characters. These aims supposed to be done by using Guava juice (*Psidium guajava*) residue as biological mean to assist sunflower (*Helianthus annuus*) plant subjected to deficit irrigation. Seeds were germinated under the natural conditions of Mecca city, 15 days later; the seedlings were divided into two groups, controls and treatment. These include: irrigation with 75 % soil water capacity SWC (C1), 50% SWC (C2), 25% SWC (C3). The second group represents the treatments, in which 5 mg of guava residue were added to represent T1, T2 and T3. Many plants characters were measured throughout the experimental period. These include: Morphological Characters (plant height, root shoot ratio and leaf area, Biochemical Characters, which include estimation of the metabolic compounds (soluble protein and amino acids), and water use efficiency. The results revealed that the main adaptation mechanisms of *Helianthus annuus* are high root length, low leaf area, high protein contents. Plant juice residues aided *Helianthus annuus* to improve many morphological and biochemical characters.

Keywords

Amino acids

Deficit irrigation

Helianthus annuus

Psidium-guajava

Soluble proteins

Water use efficiency

Introduction

Sunflower (*Helianthus annuus*, L.) is considered as one of the four important annual crops in the world for edible oil. Sunflower seeds contain 24 – 49 % of oil and the cake contains 25 – 35 % of protein which is mostly used as food for livestock because of its high biological value. Furthermore, sunflower seeds are eaten as salted, whole seeds as roasted nut meats. Moreover, oil is characterized by its high content of unsaturated fatty acids such as oleic and lenoleic which represent 90 % of total fatty acids (Elham et al., 2009). Water has become an increasingly precious natural resource as population growth throughout the region has strained supplies. The major agricultural use of water is for irrigation, which, thus, is affected by decreased supply. Another popular opinion that the cause of the total amount of available freshwater supply is decreasing because of climate change. Climate change has caused receding glaciers, reduced stream and river flow, and shrinking lakes and ponds. Many aquifers have been over-pumped and are not recharging quickly. Although the total fresh water supply is not used up, much has become polluted, salted, unsuitable or otherwise unavailable for drinking, industry and agriculture. To avoid a global water crisis, farmers will have to strive to increase productivity to meet growing demands for food, while industry and cities find ways to use water more efficiently (Chartres and Varma, 2010).

Water stress caused major reductions in height, leaf number, leaf area index, fresh and dry weight of cotton plants and some *Cucurbitaceae* members (Timpa et al., 1986) and (Akıncı and Losel, 2009, 2010) pointed that out drought-tolerant genotypes of most crop plants are those giving some yield in a particular water-limited environment. (Jones ,1993) classified as "*drought avoidance"*, the adaptations by which plants survive in regions subject to drought, in addition to drought tolerance, since this name fitted the actual situation more accurately than Levitt's term "*drought escape*". (Kramer, 1980) has reviewed strategies of drought tolerance, including (1) rapid maturation before onset of drought, or reproduction only after rain, (2) postponement of dehydration by having deep roots, (3) protection against transpiration or storing water in fleshy tissues, (4) allowing dehydration of the tissues and simply tolerating water stress by continuing to grow when dehydrated or surviving severe dehydration.

Recently, a great attention has been focused on the possibility of using natural and safety substances in order to improve plant growth. In this concern, antioxidants have synergistic effects on growth, yield and yield quality of many plant species (Al- Qubaie, 2012). This important bioprocess is characterized by microbial growth in insoluble substrate, in the presence of small amounts of free fluid, using the nutrients and residual elements present in these residues (Pinto et al., 2005). Even though this biotechnological technique has been known for centuries, it has awoken a renewed interest on the part of researchers and industries worldwide.

Objectives

This research was aimed to evaluate the response of sunflower when some biological means such as plant juice residues are added, as biological means, to assist the plant to survive under low watering regimes. Guava residue was chosen because Guava is, an important member of the Myrtaceae family, is believed to have originated in Central America. It grows throughout the tropics and sub-tropics and is one of the most widely consumed tropical fruits. The world leading producer of fresh guava production is India. Brazil is among the top six world producer countries (FAO, 2010). It shows great potential for the processing industry, mainly due to high contents of vitamins C and A.

The mineral residue of guava was higher than that of pineapple, likely due to the presence of seeds. Lousada Junior et al. (2006) found higher mineral residue values for the pineapple sub-product than those of guava, because of the presence of husks in the residue. The residue used in the study did not contain a significant amount of husks, but rather was composed mainly of pulp and stalk residue. In addition, higher concentrations of Al, Ca, Cl, Fe, K, Mg Na, S and Si were determined in the sugarcane juice samples. Furthermore, Guava fruit extract juice contained higher levels of Na ions, which would encourage particle dispersion (Zhang et al., 2006) during clarification i.e. high turbidity of clarified juice. The higher proportion of P in juices would generally result in better juice clarification, because a larger proportion of calcium phosphate precipitate would form, improving adsorption of suspended solids and sweep flocculation.

Materials and methods

Soil selection and seed germination

A sandy-clay –loam soil was used in germinating sunflower seeds following many researchers (e.g. Abdullah and Ghulam, 1991) for germinating sterilized seeds of sunflower. This soil has been chosen after 80% germination ratio was obtained. Sunflower seeds were planted in plastic pots under the natural conditions of Mecca city, Saudi Arabia, during Winter and part of Summer, (Mecca retains its hot temperature in winter, which can range from 18°C (64°F) at night to 30°C (86 °F) in the day. Summer temperatures are extremely hot, often being over 40 °C (104 °F) during the day, dropping to 30 °C (86 °F) at night. Rain usually falls in Mecca in small amounts between November and January (Mecca Climate and Weather Averages, 2014).

Preparation of juice residues

Residues of Guava (*Psidium guajava* L.), commonly used plant in many countries around the world, were collected from Juice making places. Guava residues are normally thrown as rubbish and can increase environmental pollution by collecting many insects mainly house flies. The residues were used after air drying and grinding as powder, and mixed with the irrigation water (a large tea spoon) 5g was added to water at the irrigation time (Fig. 1).

Fig. 1: Guava (*Psidium guajava*) fruit and guava residue mix.

The experimental design

Controls (No residues were added): which include plants Irrigated with 75% soil water capacity (SWC) C1, 50 SWC, C2 and with 25% SWC, C3. These 3 controls were subdivided into other sub-groups by adding Guava residue to water (5 g, each time) with the same SWC throughout the experimental period which extended for four months to represent T1, T2 and T3, matching their correspondence controls C1, C2 and C3, respectively.

Measurements

Plant height

Plant heights were measured weekly by metric ruler from end of the stem to the higher point of the stem using three random plants from each treatment.

Root to shoot ratio

Was measured after harvest following the procedure of Larcher, (1995), by taking the dry weights of roots and shoots using sensitive balance, root/shoot was calculated as :

$$\text{Root/Shoot} = \frac{\text{The dry weight of the root}}{\text{The dry weight of the shoot}}$$

Leaf area

Was measured in three plants from each treatment, following the procedure of Larcher, (1995), As follows:

Leaf Area= RLB

Where,

R= coefficient determined by a correlation of L and B of plant leaf 0.75 for sunflower leaf. L= Leaf length, B= maximum leaf breadth

Soluble proteins

Soluble proteins in the dry plant sample were taken in shoots and root system following the method of Bradford (1976). Comassie blue (Comassie Brilliant blue G250, Sigma.co) was used as detector, to give the blue color in the presence of protein. The amount of protein was measured using spectrophotometer (Spectrophotometer model, SPECTRONIC 20 GENESYS) at wavelength 595 nm. Fresh egg whites

(Bovine Serum Albumin, Sigma.co) dissolved in distilled water was in the standard curve. Soluble proteins were calculated as mg/g dry weights.

Amino acids estimation

The method of Stewart (1983) was used in amino acid estimation. 1 ml of plant extracts from three plants in the different treatments were put in test tubes and 1.9 ml of the mixture consisting of Ninhydrin, Citrate buffer pH, (5.5PH) and 60% glycerol was added to the tubes. pH of the mixture in the tubes was 6. The tubes were then placed in a water bath at 100^0C for 12 minutes. The tubes were transferred to cold water basin and left at room temperature .The intensity of the color was measured Using spectrophotometer at 570 nm wave length, using distilled water instead of the plant extracts as blank. Amino acid amounts were calculated as mg/g/dry weight and amino acid Glycine was used in the standard curve.

Water use efficiency (WUE)

Refers to the ratio of water used in plant metabolism to water lost by the plant through transpiration. Photosynthesis water-use efficiency (also called intrinsic or instantaneous water-use efficiency), which is defined as the ratio of the rate of carbon assimilation (photosynthesis) to the rate of transpiration, normally gives accurate results for WUE, Unfortunately, instrument for photosynthesis and transpiration was not available at time of measurements. Nonetheless, WUE was calculated as a ratio of biomass produced to water consumed throughout the experimental period as was suggested by Larcher (1995).

Statistical analyses

The results were analyzed statistically by using the SPSS BASE 18.0 for windows (SPSS Inc., Chicago, IL) packages. Data were tested by ANOVA and *F*-value (at $P \leq 0.05$), in order to relate different treatments to their correspondence controls. Data were presented as means and standard error.

Results

Plant height

As presented in Fig. 2, a stimulation of plant height by Guava juice residues was not clear treated plants. All plants treated with juice residue reported low plant heights than that of their correspondence control in weeks 4,5 and 6. Nonetheless, at T2 reported no significant differences between the treated plants and their correspondence control.

Root/shoot ratios

Results in Fig. 3 demonstrated the root/ shoot ratios of different treatments. T2 recorded a significant high values of root shoot ratio (0.36) compared to the correspondence control (C2) (0.28), followed by T1 (0.29). On the other hand, 25% control (C3) and 25% Guava (T3) recorded almost similar results.

Leaf area

Results presented in Fig. 4 revealed that leaf area of all treatments followed the natural rhythm of increase as the weeks progressed with the C1 recorded the highest leaf area throughout the experimental period, while T3 recorded the least leaf area during last three weeks (Fig. 4). On the other hand, at week 6, C1 still achieved the highest leaf area (40.47 cm^2), while T3 recorded the less leaf area (8.73 cm^2), which is 44% less than their correspondence control (C3), which recorded leaf area of 15.79 cm^2.

Water use efficiency (WUE)

Results shown in Fig. 5 Clarify the amount of WUE of different treatments. WUE showed its highest values in C3 (1.25 mg/l), while the least WUE was recorded in T1 (0.22 mg/l). Worth mentioning is that, C3 and T3 produced almost similar WUE (0.64 and 0.66 mg/l), respectively.

Soluble protein

Amount of soluble proteins showed different patterns in response to Guava juice residues as is presented in Fig. 6. General speaking, treated plants have high proteins especially T2 and T3 even more than T1. However, the highest amount of protein was recorded in C1 (6.64 mg/g dry weight) while the lowest amount was recorded in T1 (4.26 mg/g dry weight).

Amino acids content

Results of amino acid which is represented in Fig. 7, indicated that amount of amino acids is similar for all treatments at 75% (2.5, mg/g dry weight). Nonetheless, amino acids amount was double and triple at 50% and 25% water regimes. Guava at 50% and 25% water regimes showed almost triple the amount at 75% (7.5 mg/g dry weight) with 50% more than its correspondence control (5.00 mg/ dry weight).

Fig. 2: The effect of plant juice residues on plant height (cm) of sunflower plant (*Helianthus annuus*).

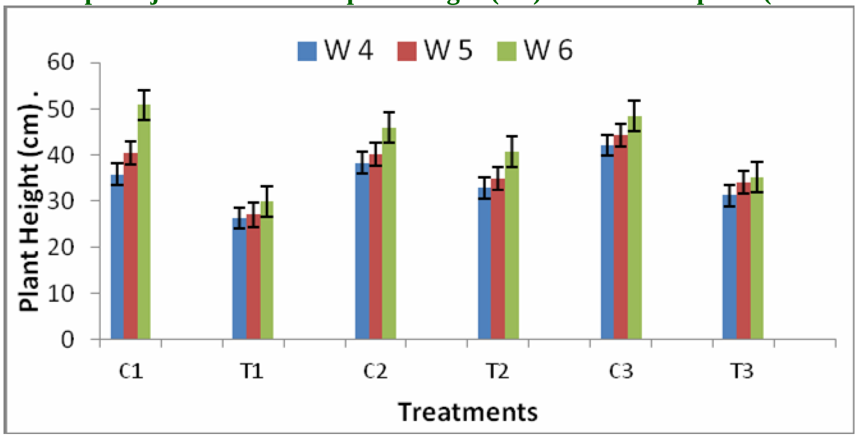


Fig. 3: The effect of plant juice residues on root/shoot ratio of sunflower plant (*Helianthus annuus*).

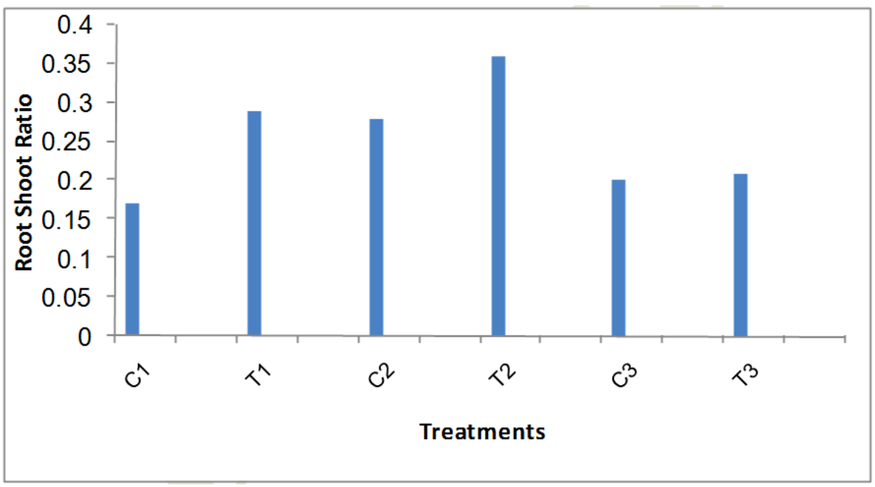


Fig. 4: The effect of plant juice residues on weekly leaf area (cm^2) of sunflower plant (*Helianthus annuus*).

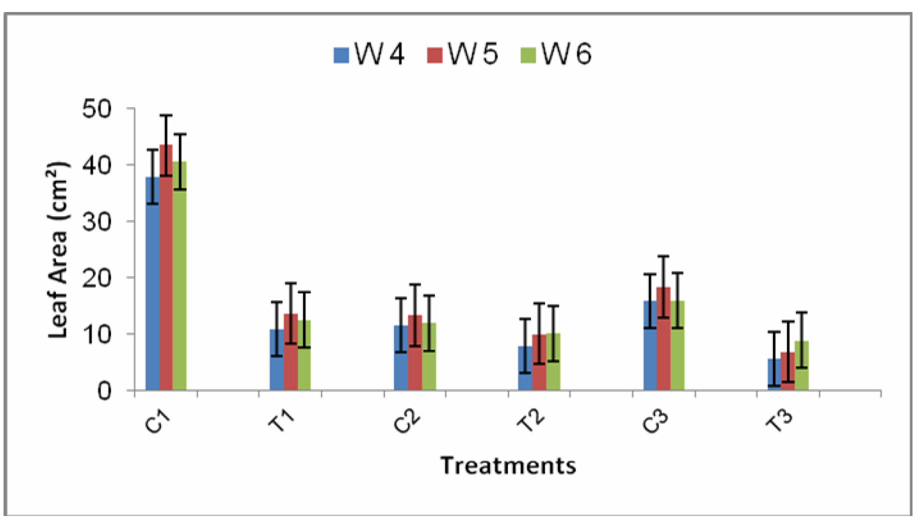


Fig. 5: Effect of treatments on mean of water use efficiency (WUE) (mg/l) in flowering stage.

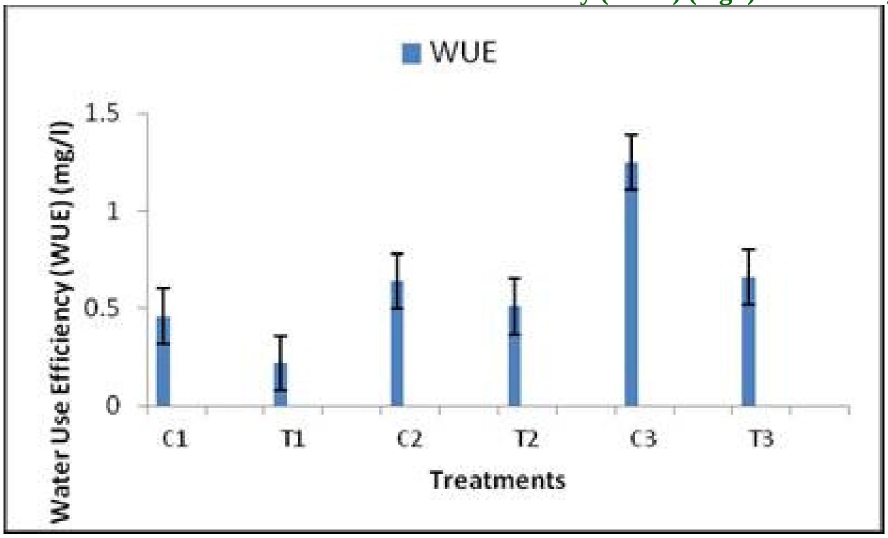


Fig. 6: Effect of treatments on protein (mg/g dry weight) in flowering stage.

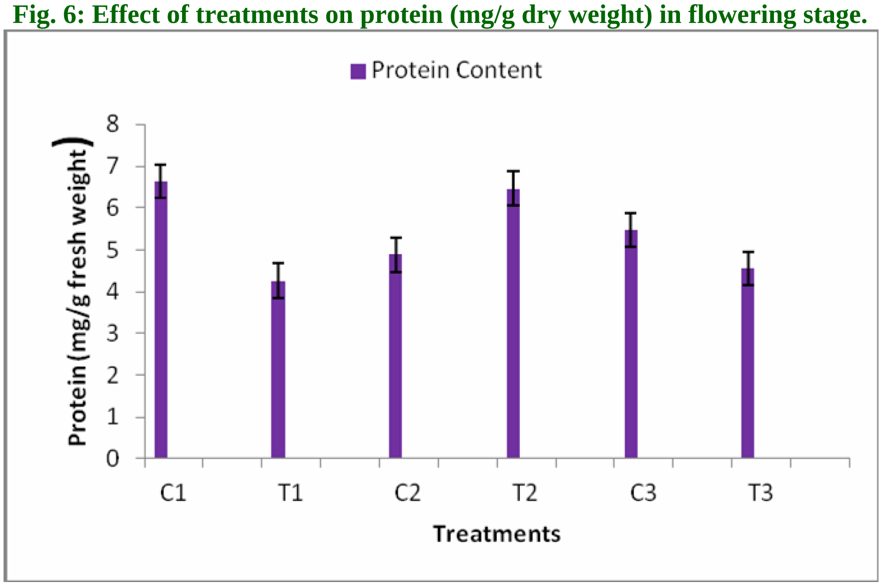
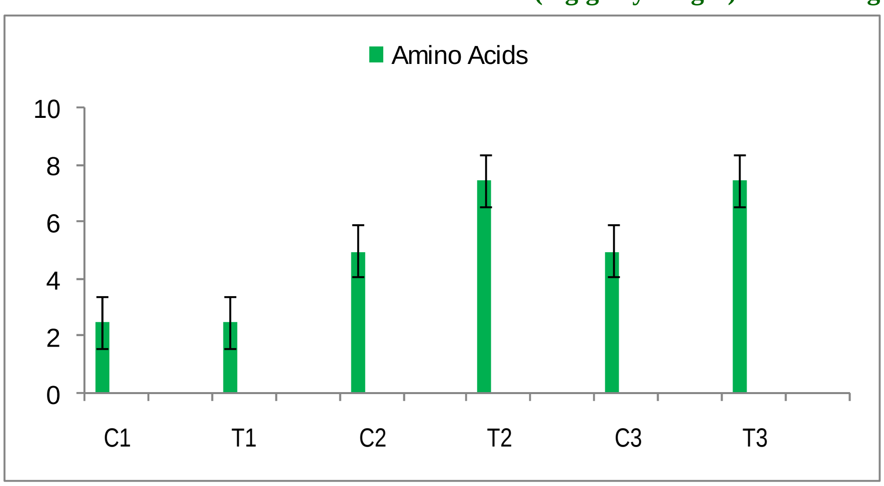


Fig. 7: Effect of Treatments on Mean of Amino acid (mg/g dry weight) in flowering stage.



Discussion

The main goal of the present study is to determine whether irrigation with Guava plant juice residues can reduce the effect of water stress of sunflower, and to find out the actual water needs of the plant in which they can get a high output with minimal irrigation water.

Plant morphological characters of Sunflower showed various responses to irrigation with plant juice residues under different water regimes. For instances, application of plant juice residue aided the stressed sunflower (T2 and T3) to increase plant height, which was obvious even at very low water regimes (T3). Water stress in general, was reported by many authors to reduce plant height of many plants (Abdullatif, 2000; AL-Kaisi, et al., 2005; Nezami et al., 2008 and Shamim, et al., 2009).

Concerning root shoot ratios, moderate water stress (50% SWC, T2), combined with Guava juice residues assisted sunflower plants to have high root shoot ratio. High root shoot ratio is known to be an adaptive mechanism against water stress in many plants, as was reported by Yardanov et al. (2003); and Mensah, (2006), who reported that, adjustments in root and shoot growth are often assumed to be a fundamental facet of a plant's phenotypic plasticity in response to its environment. An alternative view is that relatively greater root growth in response to shortages of nutrients or water could maximize a plant's probability of capturing those resources, especially if a competitor fails to respond to a comparable extent (Reich and Schoettle, 1988).

Regarding Leaf area, all plants recorded less leaf area when residues were added, compared to controls. This decrease in leaf area is considered as adaptive mechanism against water loss as assimilate saving. During leaf expansion, volumes of constituent cells can increase 10–100-fold depending upon location and function, cells such as spongy mesophyll showing the greatest increase and guard cells the least. Photoassimilate is readily available and generally sufficient but a positive turgor must be sustained for cell enlargement and leaf expansion which in turn depends on water plus inorganic resources that must all be imported (Adil et al., 2014).

Regarding water use efficiency (WUE), sunflower plants irrigated with Guava residue, have relatively high WUE values especially at T3, which recorded higher WUE than even C1. The highest Water use efficiency, which confirms the ability of the plant to resist water stress through the increase in efficiency of water use, is one of the most important characteristics of the plant for a high yield as was mentioned by Kramer and Boyer, (1995); Osakabe and Osakabe (2012) and Osakabe et al. (2013). Increases in water-use efficiency are commonly cited as a response mechanism of plants to moderate to severe soil water deficits, and has been the focus of many programs that seek to increase crop tolerance of drought. Water use efficiency (WUE), a parameter of crop quality and performance under water deficit is an important selection trait. In fact, plants have evolved various molecular mechanisms to reduce their consumption of resources and adjust their growth to adapt to adverse environmental conditions (Abdulla and Ghulam, 1991; Tahar et al., 2010; Osakabe et al., 2011; Nishiyama et al., 2013; Ha et al., 2014).

Amount of soluble proteins showed high values in plants irrigated with Guava juice residues at moderate and low water regimes (T2 and T3). Unexpectedly the lowest amount of protein was recorded in T1. Guava has high source of nitrogen, nonetheless, guava residue has lower total reducing sugar content as was reported by Bruno, et al. (2010). High proteins values were recorded at low water regime (T3). Protein accumulation may have helped sunflower to produce more biomass at low water regime. Some important studies like those of Navary et al. (1995); Shahniyar et al. (2010) and Shashi and Godara (2011), have concluded that there is a certain type of protein is linked to the sulfur group, composed under water shortage conditions and helps maintain the vitality of the plant.

Amino acids values were not affected in case of full irrigation water regime (C1 and T1). Nevertheless, amino acids amount was double and sometimes triple the value at T2 and T3. Guava residues aided sunflower plants to have the highest amino acid contents. These results were in consistence with those of Shashi and Godara (2011). Moreover, Seki et al. (2007) and Brandt et al. (2012) reported that at the biochemical level, many plants accumulate osmoprotectants such as sugars (sucrose, raffinose, trehalose), sugar alcohols (sorbitol and mannitol), amino acids (proline), and amines (glycine, betaine and polyamines). These metabolites also act as antioxidants or scavengers helping plants to avoid and/or tolerate stresses.

Conclusion

Results revealed that water deficit irrigation reduced leaves area; however, addition of residues prevents a drastic decrease in these parameters even at low water regimes. Water use efficiency was drastically reduced at 75% water regime (T1). Nevertheless, water use efficiency recorded highest values at the treatment (C3 and T3) compared to other treatments. Protein content was high at C1, but it was similar to the amount in plants irrigated with T2. Less amounts of amino acids were recorded at full irrigation (C1 and T1), while the highest values were recorded at T2 and T3.

Therefore, Guava residue can be used as safe fertilizer to benefit of sunflower under water stress. However, more researches are needed for more application in different crops.

References

Abdullah, S., Ghulam, H., 1991. Effect of irrigation intervals on yield and water use efficiency of sunflower (*Helianthus annuus* L.) in Al Ahsa, Saudi Arabia. Arid Soil Res. Rehabil. (4), 289-296.

Abdullatif, B.M., 2000. Ecophysiological Basis of Productivity in Maize (*Zea mays* L. var. Masmadu). Ph.D., Thesis, University of Malaya, Malaysia.

Adil, J., Muhammad, N., Shahid, B., Aftab, B., Rashid, M., Bilal, S., Bahaledeen, B., Mohamed, S., Hassan, T., 2014. Water stress mediated changes in morphology and physiology of *Gossypium arboretum* (var FDH-786). J. Plant Sci. 2(5), 179-186.

Akıncı, S., Losel, D.M., 2009. The soluble sugars determination in Cucurbitaceae species under water stress and recovery periods. Adv. Environ. Biol. 3(2), 175-183.

Akıncı, S., Losel, D.M. 2010. The effects of water stress and recovery periods on soluble sugars and starch content in cucumber cultivars. Fresen. Environ. Bull. 19(2), 164-171.

Al- Qubaie, A.I., 2012. Response of sunflowers cultivar Giza-102 (*Helianthus annuus* L.) plants to spraying some antioxidants. Nat. Sci. 10(11), 1-6.

AL-Kaisi, M.M., Berrada, A., Stack, M., 2005. Evaluation of and How to Solve the World's Water Problems. FT Press, USA.

Bradford, M.M., 1976. A rapid and sensitive method for quantitative of microgram quantities of protein utilizing the principle protein-dye binding. Anal. Biochem. 12, 248-254.

Brandt, B., Brodsky, D.E., Xue, S., Negi, J., Iba, K., Kangasjärvi, J., 2012. Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1 kinases and branched ABI1 PP2C phosphatase action. Proc. Natl. Acad. Sci. U.S.A. 109, 10593–10598.

Bruno, A., Araujo, S., Roberta, T., Pinto, C., 2010. Biotechnological reuse of fruit residues as a rational strategy for agro-industrial resources. J. Technol. Manag. Innov. 5(2), 104-112.

Chartres, C., Varma, S., 2010. Out of Water: From Abundance to Scarcity and How to Solve the World's Water Problems. FT Press, USA.

Elham, A.W., Norhan, E.S., Heyam, A.E., 2009. Vegetative oil blend as alternative lipid resources in diets for gilt head sea bream. Aquacul. Int. 17 (5), 421-435.

FAO, (Food and Agriculture Organization of United Nations) 2010. FAO Statistics Division [Accessed in 08/04/2010].

Ha, C.V., Leyva-Gonzalez, M. A., Osakabe, Y., Tran, U. T., Nishiyama, R., Watanabe, Y., 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. Proc. Natl. Acad. Sci. U.S.A. 111, 581–856.

Jones, H.G., 1993. Drought Tolerance and Water-use Efficiency. In: Water Deficit Plant Responses from Cell to Community (Eds.: Smith, J.A.C., Griffiths, H.). BIOS Sci. Ltd., Oxford. pp.193-203.

Kramer, P.J., 1980. Drought, Stress and the Origin of Adaptations. In: Adaptations of Plants to Water and High Temperature Stress (Eds: Turner, N.C., Kramer, P.J.). John-Wiley & Sons, New York. pp.7-20.

Kramer, P.J., Boyer, J. S., 1995. Water Relations of Plants and Soils. Academic Press, San Diego.

Larcher, W., 1995. Physiological plant ecology: Ecophysiology and stress physiology of functional groups into plant morphology, physiology and pathology. Ph.D., Dissertation, University of California, Riverside.

Lousada, Junior, J.E., Costa, J.M.C., Neiva, J.M.N., Rodriguez, N.M., 2006. Caracterização físico-química de subprodutos obtidos do processamento de frutas tropicais visando seu aproveitamento na alimentaçao animal. Revista Ciencia Agron. 37 (Abstract).

Mecca Climate and Weather Averages, Saudi Arabia. 2014. Weather 2 Travel. Retrieved 2014-10-14.

Mensah, J.K., Obadoni, B.O., Eruotor, P., Onome-Trieguna, F., 2006. Simulated flooding and drought effects on germination, growth and yield parameters of sesame. Afr. J. Biotechnol. 13, 1249-1253.

Navary, I.F., Ricci, F.,Vazzana, C., Quartacci, M.F., 1995. Unusual composition of thylakoid membranes of the resurrection plant. Physiol. Plantar. 94, 135-142.

Nezami, A., Khazae, H. R., Boroumand, R., Hossein, A., 2008. Effect of drought stress and defilation on sunflower (*Helianthus annuus*) in controlled conditions. Desert. 12, 99-104.

Nishiyama, R., Watanabe, Y., Leyva-Gonzalez, M. A., Van Ha, C., Fujita, Y., Tanaka, M., 2013. *Arabidopsis* AHP2, AHP3, and AHP5 histidine phosphotransfer proteins function as redundant negative regulators of drought stress response. Proc. Natl. Acad. Sci. U.S.A. 110, 4840–4845.

Osakabe, K., Osakabe, Y., 2012. Plant Light Stress. In: Encyclopedia of Life Sciences (Ed.: Robinson, S.A.). Nature Publishing Group, London.

Osakabe, Y., Arinaga, N., Umezawa, T., Katsura, S., Nagamachi, K., Tanaka, H., 2013. Osmotic stress responses and plant growth controlled by potassium transporters in *Arabidopsis*. Plant Cell. 25, 609–624.

Osakabe, Y., Kajita, S., Osakabe, K., 2011. Genetic engineering of woody plants: current and future targets in a stressful environment. Physiol. Plant. 142, 105–117.

Pinto, G.A.S., Brito, E.S., Andrade, A.M.R., Fraga, S.L.P.,Teixeira, R.B., 2005. Fermentação em estado sólido: Uma alternativa para o aproveitamento e valorização de resíduos agroindustriais tropicais. Comunicado técnico 102(1), 1-5.

Reich, B.R., Schoettle, A.W., 1988. Role of phosphorus and nitrogen in photosynthetic and whole plant carbon gain and nutrient use efficiency in eastern white pine. Oecol. 77(1), 25-33.

Seki, M., Umezawa, T., Urano, K., Shinozaki, K., 2007. Regulatory metabolic networks in drought stress responses. Plant Biol. 10, 296-302.

Shahniyar, M.B., Hesen, G.B., Minakhanim, K.N., Christine, A.R., 2010. Effect of water stress on protein content of some Calvin cycle enzymes on different wheat genotypes. Proc. Biol. Sci. 56(5), 106-111.

Shamim, A.R., Rashid, A., Muhammad, Y.A., Ashraf, M., Ejaz, A.W., 2009. Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. Pak. J. Bot. 41(2), 647-754.

Shashi, K., Godara, A.K., 2011. Effect of moisture stress on Leaf total protein, proline and free amino acids content in commercial cultivars of *Ziziphus mauritiana*. J. Scientific Res. 55, 65-69.

Stewart, E.A., 1983. Chemical Analysis of Ecological Materials. Black Well Scientific. London.

Tahar, B., Abdellah A., Abdulkhaliq A., Al-Shoaibi, A., Alhejeli, M., 2010. Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabia. J. Taibah University Sci. 3, 39-40.

Timpa, J.D., Burke, J.B., Queensberry, J.E., Wendt, C.W., 1986. Effects of water stress on the organic acid and carbohydrate compositions of cotton plants. Plant Physiol. 82, 724-728.

Yardanov, I., Velikova, V., Tsonev, T., 2003. Plants responses to drought and stress tolerance. Bulg. J. Plant Physiol. (Special Issue), 187–206.

Zhang, J., Maeda, M., Kotobuki, N., Hirose M., Ohgushi,H., Jiang, D., Iwasa, M., 2006. Aqueous processing of hydroxyapatite. Materials Chem. Phy. 99, 398–404.