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Effect of Water Stress on Yield and Bioactive Chemical Constituents of *Tribulus* Species

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Abstract: *Tribulus terrestris* L. and *Tribulus pentandrus* Forssk syn= *T. longipetalus* (is rare species) were brought into cultivation system, to determine the water regime conditions for growth, biomass production and chemical constituents (steroidal saponins), in addition to the biochemical responses of water stress for two growing seasons 2005 and 2006. *T. terrestris* plants exposed to different water stress regimes in open field experiment, plants irrigated every 7days (control plants), 15 days (Mod.WS) and 21 days (VSWS). While, *T. terrestris* L. and *T. pentandrus* plants were subjected to five water stress regimes of field capacity, control (100% FC), Mil.WS (80% FC), Mod.WS (60% FC), SWS (40% FC) and VSWS (20% FC) in pot experiment. In both two experiments either in open field or in pots, the growth and biomass production measurements i.e (plant height, aerial parts of fresh and dry weight /plant, leaves, stems, fruits dry weight and relative growth rates) were highly significantly decreased under different water stress in comparison with the control plants. In contrast, the active constituents (total carbohydrates, soluble sugars, free proline and total saponins) were highly significantly increased under water stress conditions (moderate water stress) comparing with control plants. Leaves subjected to water stress produced the highest concentration of saponins, an average of 25 to 37% greater than fruits, stems and roots. Generally, water stressed plants produced from 25 to 42 % of different chemical constituents yield greater than the control plants. On the other hand, the active constituents of the plants harvested at end the growing season raised up to 70 % greater than the first date of harvest (45 days from planting) under all water treatments in field experiment. Generally, it could be concluded that production of Tribulus plants under water stress conditions is a good techniqe for increasing levels of steroidal sapogenins or saponins through manipulation of water stress. For this need, from all obtained results, it could be recommended to optimise steroidal saponins of *Tribulus terrestris* L. and *Tribulus pentandrus* Forssk. by using moderate water stress condition.

Key words: *Tribulus tersstris* L., *Tribulus pentandrus* Forssk, Water stress, total steroidal saponins, growth, biomass production, carbohydrate, soluble sugars, proline.

INTRODUCTION

Tribulus L. (Zygophyllaceae) comprises about 25 species. *Tribulus terrestris* L. and *Tribulus pentandrus* Forssk syn= *T. longipetalus* (is rare plant) growing in Egyptian Dessert.

Tribulus terrestris L. is a famous pharmaceutical herb in therapeutically uses that has been a 5,000 year-old history, especially in India and China and the herb traditionally used by different culture for different purposes, as an aphrodisiac and as a treatment sexual dysfunction and this plant said to possess various pharmacological activates[16] and clinical trials, such as anticancer[37], anticholinergic[6], antifilarial[9], anti-malarial[24], CNS depressant and stimulant[38], hypoglycemic effect[20], immunologic effect[35], smooth muscle relaxant and stimulant activity[2] and other important activates[31].

Tribulus plants is extremely rich in substances having potential biological significance, including steroidal saponins[13], flavonoids[32], alkaloids[40], amides and lignanamids[40], cinammic acid amids[29], nitrate[8] and phytosterol[10] that have been isolated.

Cultivation of medicinal plants under water stress conditions is an important factor for controlling levels of phytochemicals[44]. Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rates and crop yields. However, some studies on the use of water stress to enhance or increased the production of useful natural products in medicinal plants, including essential oil in Satureja hortensis L.[3], antioxidants in St Jonn's wort Hypericum performatum[45], phenolic and flavonoids compounds in Hypericum brasiliense and harwathorn Crataegus spp.[1] and artemisinin in Artemisia[7].

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The objective of this study was to increase and determine the major phytoproducts concentrations in *Tribulus* plants and corelated its alteration with the changes in physiological and biochemical processes of plants under water stress conditions.

MATERIALS AND METHODS

Location and Duration: This work was carried out during two successive seasons (2005-2006) at the Experimental Farm of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza and pot experiment was conducted at National Research Centre, Cairo, Egypt.

Plant Material and Growth Conditions: Seeds of *Tribulus terrestris* L. and *Tribulus pentandrus* Frossk (syn= Tribulus longipetalus) have been kindly provided from the Department of Botany, Faculty of Science, South Valley University, Aswan, Egypt.

Tribulus cultivated by seeds and these is considerable dormancy therefore, seeds were soacked in running tap water for 8 hours then the seeds were sown in black polyethylene bags (10 cm in diameter). The experiment was performed in the first of June 2005 and 2006. Seeds were germinated after 10 days. The seedlings (about 5.0 cm high) were transplanted in open field (clay soil) on rows 100 cm apart and 50 cm between plants, also the rows were covered with mulching. The area of every main plot was $54m^2$ while it was 6 m^2 for every sub-plot which contain two rows, each row was 3 meters long and one meter wide, each sub-plot contain 12 plants on pot exp. The seedlings were transplanting in pots (30 cm in diameter, one plant per pot) containing a mixture of sand and loamy soil (2:1), seedlings of similar initial size were assigned to each treatment. The plants were equally well watered for one month prior to exposure to water stress treatment, on the second week of July, the plants assigned to water stress under the similar environmental conditions. *Tribulus pentandrus* Forssk and *T. terrestris* plants were grown in pot experiment and *T. terrestris* cultivated in open field experiment.

Water Stress Treatments and Experimental Design

Field Experiment: The statistical design was a complete randomized block design. This experiment included three treatments with three replicates for *T. terrestris*:

- Control (irrigated every 7 days).
- Moderate water stress"Mod.WS" (irrigated every 15 days).
- Very severe water stress "VSWS" (irrigated every 21 days).

Pot Experiment: The design was completely randomize design; this experiment included five treatments of water regimes at field capacity (FC) with ten replicates (ten plants) for *T. pentandrus* and *T. terrestris*:

- Control (100% FC).
- Mild Water Stress "Mil.WS" (80% FC).
- Moderate Water Stress"Mod.WS"(60% FC).
- Severe Water Stress "SWS" (40% FC).
- Very Severe Water Stress "VSWS" (20% FC).

The soil water was kept at the predetermined levels by weighting the individual pots in each treatment; the plants were irrigated to field capacity every 2 days. The amount of water lost through evapotranspiration was calculated and add in order to maintain the correct moisture level by hand-irrigation on soil surface, all additions of water were recorded for all treatments. The plants in all water stress conditions (open field and pot experiment) were fertilized once a month with fertilizers containing NPK about five grams for every plant throughout the experiment. Two programs including harvesting date, for open field experiment, the plants from each treatment were harvested at 45 days from applying water treatments, while all plants in pot experiment was harvested at the end of experiment. The water stress treatments starting after one month from transplanting.

Growth Measurements: The following data were recorded in all experiments

Growth Parameters: Plant height (cm), number of main shoots/plant, aerial parts fresh weight (APFW; g/plant), aerial parts dry weight (APDW; g/plant), leaves dry weight (LDW; g/plant), stems dry weight (SDW; g/plant), fruits dry weight (FDW; g/plant), aerial parts fresh weight per Fed. (APFW; ton/Fed.), aerial parts dry weight per Fed. (APDWF; ton/Fed.), leaves dry weight per Fed. (LDW; kg/ Fed.), stems dry weight per Fed. (SDW; kg/ Fed.) and fruits dry weight per Fed. (FDW; kg/ Fed.).

Dry Weight and Relative Growth Rate (RGR) (g per day/ plant): Dry weight (DW) of aerial leaves, stems and fruits were measured by drying plant material in a forced hot-air oven at 60 C until a constant weight was obtained. For the determination of relative growth rate, three plants from

each treatment were harvested; measurements started 30 days after transplanting. The RGR was derived by the following equation[12]. RGR = {in (final weight)-in (initial weight)}/ date of harvested.

Biochemical Determination:

- Determination of total carbohydrates and total soluble sugars were determined according to[23].
- Determination of free proline content (mg/100 mg

plant) was measured according to[30].

- Determination of total steroidal saponins content (mg/100mg plant) as method described by[29] with some modifications.

Statistical Analysis: Data were analyzed with the Analysis of Variance (ANOVA) according to (Snedecor and Cochran, 1980) using Mstatc program. When significant differences ($P<0.05$) were detected, the least significant difference (LSD) test was used to separate the mean values according to[36].

RESULTS AND DISCUSSION

Increased tolerance to abiotic stress in crop plants is necessary in order to increase productivity under cropping conditions with limited water supplies, high salinity and low temperature. Tolerant plants respond to abiotic stress with complex changes in their physiological and molecular status.

Vegetative Growth: Water stress lead to high significant effects on growth attributes as plant height, fresh and dry of aerial parts, dry weight of leaves, stems and fruits and relative growth rates, except number of main shoots for two seasons 2005 and 2006 in field and pot experiments.

Field Experiment: Data in Table (1) showed that water stress application had insignificant effect on plant height, but only it had significant differences in the 1st harvest in the second season. In general, in the second harvest it was found to be the best as it produced the highest mean values reached 181 cm/plant in the 1st season and 176 cm in the 2nd season. Results recorded on main shoots of plant revealed that, water regimes insignificantly affected the number of main shoots/plant in both seasons.

Table 1: Growth measurments and biomass production of T terrestris L plants under water stress conditions in field during two seasons (2005 and 2006).

	Growth measurements and biomass production												RGR
Treatments	No. Pof shoots	p.hight cm	APFW/ plan	APDW/ plant	LDW/ plant	SDW/ plant	FDW/ plant	APFW ton/plant	APDW ton/plant	LDW kg/plant	SDW kg/plant	FDW kg/plant	RGR (g/ plant/day)
1st season (2005)													
1st harvest													
Control	7.66a	166.66a	567.3a	144.3a	35.34a	64.18a	43.94a	4.53a	1.15a	283.5c	513.5a	351.5a	11.93a
Mod.WS	7.00a	173.33a	515.4b	144.6a	37.37a	63.99a	43.77a	4.12b	1.15a	511.9a	511.9a	350.1a	10.78b
VSWS	7.33a	164.66a	433.6c	127.0b	32.87b	50.43b	40.54b	3.46c	1.01b	403.5b	403.5b	324.3b	8.96c
2nd harvest													
Control	6.66a	185.33a	774.0a	207.8a	60.87a	90.34a	73.15a	6.18a	1.63a	486.9c	722.7a	585.1a	9.91a
Mod.WS	7.00a	178.33a	755.6b	196.5b	57.87b	86.16b	67.61b	6.01b	1.53b	689.3a	689.3b	540.8b	9.67b
VSWS	7.50a	179.33a	610.6c	173.4c	45.19c	64.00c	53.80c	4.88c	1.32c	512.0b	512.0c	430.4c	7.73c
1st harvest	7.33a	168.2b	505.4a	138.63b	35.22b	59.53b	42.75	4.04b	1.10b	396.0b	476.2b	341.9b	10.56a
2nd harvest	7.05a	181.0a	713.3a	192.38a	53.64a	80.16a	64.85	5.69a	1.53a	567.7a	661.3a	518.7a	9.01b
2nd season (2006)													
1st harvest													
Control	7.66a	166.3a	558.4a	141.9a	34.90a	62.77a	45.01a	4.46a	1.13a	279.0a	502.1a	360.1a	11.72a
Mod.WS	6.00a	162.3a	507.3b	143.2a	35.60a	62.63a	44.80a	4.05b	1.14a	284.8a	501.1a	358.4a	10.60b
VSWS	5.66a	123.4b	426.5c	123.5b	30.23b	48.70b	38.81b	3.40c	0.910b	241.9b	389.6b	310.5b	8.80c
2nd harvest													
Control	6.00a	176.40a	764.8a	204.9a	59.59a	85.18a	70.48a	6.11a	1.50a	476.7a	681.4a	563.7a	9.79a
Mod.WS	7.00a	174.16a	755.8a	192.9b	53.97b	82.43b	66.70b	6.04a	1.50a	431.7b	659.5b	533.6b	9.71a
VSWS	6.66a	177.66a	600.7b	166.0c	43.43c	64.81c	52.37c	4.80b	1.43b	347.4c	518.7c	418.9c	7.60b
1st harvest	6.44a	150.6b	487.4b	136.1b	32.5b	58.02b	42.87b	3.77b	1.04b	268.5b	464.2b	342.9b	10.37
2nd harvest	6.55a	176.0a	707.0a	187.9a	52.3a	77.48a	63.18a	5.65a	1.49a	418.6a	639.8a	505.4a	9.12b

Table 2: Growth measurements and biomass production of Tribulus terrestris Land Tribulus pentandrus Forssk plants under water stress conditions in field during two growing seasons (2005 and 2006).

	Growth measurements and biomass production							RGR
Treatments	No. of main shoots	Plant height (cm)	A P F W (g/plant)	APDW (g/plant)	LDW (g/plant)	SDW (g/plant)	FDW (g/plant)	RGR (g/plant per day)
1st Season (2005)								
Water stress								
Control	6.33a	61.6ab	143.6a	36.0a	8.35a	16.86a	12.82a	2.14a
Mil.WS	6.66a	64.17a	141.8a	35.38a	8.57a	16.41a	12.10b	2.11a
Mod.WS	6.83a	45.50b	127.2b	31.88b	6.13b	14.53b	9.90c	1.86b
SWS	4.66b	38.50c	71.33c	21.50c	4.55c	11.18c	6.73d	0.935c
VSWS	4.66b	32.67c	42.83d	16.38d	3.66d	8.61d	5.11e	0.461d
Species								
T. terrestris	6.26a	54.53a	131.8a	34.07a	7.79a	16.51a	10.80a	1.94a
T.pentandrus	5.40a	46.07b	78.87b	22.39b	4.91b	10.52b	7.86b	1.06b
Interactions								
Control X Tt	6.66a	67.66a	182.1a	43.66a	11.30a	21.13a	15.00a	2.78a
Control X Tp	6.00a	55.66a	105.0c	28.33c	6.40c	12.58d	10.63bc	1.49c
Mil.WS X Tt	7.00a	68.33a	179.7a	43.100a	10.57a	19.70b	14.40a	2.74a
Mil.WS X Tp	6.33a	60.00a	104.0c	27.66c	6.40c	13.13d	9.80c	1.48c
Mod.WS XTt	7.00a	61.66a	164.0b	39.26b	7.49b	18.12c	10.97b	2.48b
Mod.WS XTp	6.66a	47.33a	90.33d	24.50d	4.76e	10.93e	8.83d	1.25d
SWS X Tt	5.66a	41.33a	87.00d	26.66c	5.58d	13.85d	8.00d	1.19d
SWS X Tp	3.66a	35.66a	55.67e	16.33ef	3.53f	8.51fg	5.46ef	0.673e
VSWS X Tt	5.00a	33.66a	46.33f	17.66e	3.86f	9.76ef	5.63e	0.516f
VSWS X Tp	4.33a	31.66a	39.33f	15.100f	3.46f	7.46g	4.60f	0.406f
2nd Season (2006)								
Water stress								
Control	7.50a	72.50a	146.7a	36.63a	9.55a	17.32a	13.86a	2.19a
Mil.WS	6.33ab	68.17a	142.4a	35.58a	8.84b	16.26b	12.80b	2.12a
Mod.WS	7.00a	59.00b	125.8b	31.70b	7.63c	14.79c	10.53c	1.84b
SWS	5.00bc	45.83c	69.75c	21.06c	4.42d	11.22d	7.82d	0.917c
VSWS	4.00c	35.17d	45.33d	17.37d	3.83e	8.93e	6.47e	0.503d
Species								
T. terrestris	6.06a	58.80a	133.5a	35.06a	8.57a	16.94a	11.73a	1.97a
T.pentandrus	5.86a	53.47b	78.53b	21.88b	5.14b	10.47b	8.86b	1.05b
Interaction								
Control x Tt	7.33a	77.66a	188.0a	46.10a	11.97a	20.78a	15.90a	2.88a
Control x Tp	7.66a	67.33a	105.3c	27.17c	7.13c	13.85a	11.81b	1.50c
Mil.WS x Tt	6.33a	72.66a	180.3a	43.50a	10.54b	19.32a	15.64a	2.75a
Mil.WS x Tp	6.33a	63.66a	104.5c	27.67c	7.14c	13.19a	9.94c	1.48c

Table 2: Continued

Mod.WS xTt	7.00a	61.33a	160.7b	39.57b	10.12b	17.89a	12.37b	2.42b
Mod.WS xTp	7.00a	56.66a	91.00d	23.83d	5.15de	11.69a	8.69d	1.26d
SWS x Tt	5.33a	47.00a	85.67d	25.9cd	4.67e	14.79a	7.86de	1.17d
SWS x Tp	4.66a	44.66a	53.83e	16.17f	3.27f	7.64a	7.78e	0.650e
VSWS x Tt	4.33a	35.33a	52.67e	20.17e	4.67e	11.89a	6.87f	0.626e
VSWS xTp	3.66a	35.00a	38.00f	14.57f	3.00f	5.98a	6.08f	0.380f

Aerial parts (fresh weight g/plant & ton /fedd) increased considerably and gradually by increasing levels of water regimes. Plants irrigated every 7 days and 15 days had a high significant increase of aerial parts (fresh weight with mean values 657,3 & 515 g/plant and 4.53& 4.12 ton/fedd.as compared with plants which irrigated every 21 days produced which 433 g/plant and 3.46 ton/ fedd. in the first season. Similar trend was observed in the second one.

The aerial parts dry weight/ plant & ton /fed. increased considerably and gradually by increasing levels of water regimes. Plants irrigated every 7 days (control) and 15 days (moderate water stress), significant increasing aerial parts dry weight with mean values 144.3 and 144.6 g/plant and 1.15& 1.15 ton/ Fed. as compared with plants irrigated every 21 days that produced 127.0 g/plant and 1.01 ton/ fedd. in the first season. On the other hand, increasing irrigation level (control) significantly increased leaves, stems and fruits dry weight in both seasons compared with stressed plants and similar trend was observed with aerial parts dry weight.

Extend water stress significantly decreased of the relative growth rate (RGR), relative growth rate decreased from 11.93 g per day in control plants to 10.78, 8.96g per day in plants irrigated every 15 and 21 days in the first harvest and the same trend was observed in 2nd one, while RGR decreased from 9.91g per day in the control to 9.67 and 7.73 g per day in plants irrigated every 15 and 21 days in the second harvest in 2005 and the same trend shows that in plants were grown of season 2006.

Pot Experiment: Data reported in Table (2) showed that the effect of different water stress levels on different plant parameters of *T. terrestris* L. and *T. pentandrus* during the two successive seasons 2005 and 2006.

It is clear that water stress conditions had a high significant effect on the average of plant height of *T. terrestris* L. and *T. pentandrus* Forssk plants. Control and Mil.WS showed the highest mean values of plant height (61.67 and 64.17 cm in the first season and 72.50 and 68.17 cm during the second one. There was significant difference between *T. terrestris* L. and *T. pentandrus* with values 54.53 & 46.07 cm of *T. terrestris* L and *T. pentandrus* respectively in the 1st season and 58.80 & 53.47 cm of *T. terrestris* L. and *T. pentandrus* Forssk., respectively in the 2nd one. Results revealed that, water regimes insignificantly affect the number of main shoots/ plant in both seasons.

Aerial parts of fresh weight had significant response to water stress application, the maximum values were found to be in plants received 100, 80 and 60% of field capacity comparing with the plants growing at 40 and 20% of field capacity which gave the minimum values in both seasons and similar trend was observed in the second season.

There is no significant effect between control and Mild.WS on aerial parts dry weight /plant, while there was a significant effect between the control and Mod.WS. But there was significant effect between the control and VSWS. Water stress showed significant differences between aerial parts dry weight between *T. terrestris* and *T. pentandrus* in both seasons.

leaves, stems and fruits dry weight of plant have similar trend as with the data recorded on aerial parts dry weight in both Tribulus plants and both season. The stems dry weight was more than fruits and leaves dry weight of *Tribulus species* in both seasons.

Extend water stress decreased relative growth rate with a significant differences as compared with the control, relative growth rate decreased from 2.14 g/plant per day in control plants (100% FC) to 2.11, 1.86, 0.935 and 0.462 g/plant per day in plants under 80%, 60%, 40% and 20% of field capacity respectively in the first season, while RGR decreased from 1.94 g/plant per day in *T. terrestris* L. to 1.062 g/plant per day in *T. pentandrus* Forssk The interaction effect between water stress conditions and *Tribulus species* was significant in both seasons, except for the number of main shoots and plant height of the 1st and 2nd seasons.

Table 3: Biochemical profils of T. terrestris L plants under water stress conditions in field during 2005 season.

	Total carbohydrates% (mg/ 100mg DW)			Total soluble sugars% (mg/ 100mg DW)			Total free proline% (mg/ 100mg DW)		
Treatments	Leaves	Stems	Fruits	Leaves	Stems	Fruits	Leaves	Stems	Fruits
1st Harvest									
Control	14.15c	36.85b	27.88b	0.839b	0.672c	4.11a	1.12c	0.517b	0.73ab
Mod.WS	21.55b	42.98a	32.18b	0.985b	0.777b	4.08a	1.93b	1.17a	1.14a
VSWS	23.02a	40.84a	36.79a	1.087a	1.005a	4.66a	2.34a	1.19a	1.16a
2nd Harvest									
Control	28.50b	40.38c	32.92b	4.18b	8.63c	4.32b	1.39c	0.70b	1.33a
Mod.WS	34.78a	48.73a	40.48a	7.72a	9.90b	7.51a	2.37b	1.56a	1.31a
VSWS	33.05a	46.80b	42.18a	7.90a	11.84a	7.78a	2.60a	1.54a	1.41a
Harvest									
1st harvest	19.56a	40.22b	29.50b	5.61a	7.02b	4.28b	1.80b	0.963a	1.01b
2nd harvest	17.87a	45.30a	38.52a	6.62a	10.12a	6.54a	2.12a	1.27a	2.35a

In general, it could be noticed that the vegetative growth and biomass production measurements in field and pot experimental, shows that, the best treatment was irrigation every 15 days and 60% of field capacity.

In agreement with[42] on *Ocimum basilicum*, increases in plant height, number of branches per plant and herb yield papered to associated with the increasing levels of irrigation as compared to water stress conditions. In a previous study[15] on *Dracoceephalum moldavica* plants, used the water stress treatments at 100, 85, 70 and 55 % of field capacity. The results showed that the water stress had significant effects on growth, herb yield and essential oil yield. As the soil water content decreased, the growth parameters, fresh and dry herb and essential oil yield decreased.

A portion of the impact of water stress on reproductive growth is due to the inhibition of cell enlargement and cell expansion from drought-induce photosynthetic depletion, which can occur after only a few days of dehydration[18]. At the whole plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth associated with alteration in carbon and nitrogen metabolism[19].

Biochemical Indicators: Plants and other organisms cope with osmotic stress by synthesizing and accumulating some compatible solutes, which are termed as osmoprotectants or osmolytes. These compounds are small, electrically neutral molecules, which are non-toxic even at molar concentrations[28].

Carbohydrate Accumulation: Carbohydrates are a major category of compatible solutes, which accumulated during stress. Table (3) shows the effect of water stress at different levels (irrigation every 7, 15 and 21 days) on total carbohydrates of *T. terrestris* grown in field. From the obtained results it is clear that the total carbohydrate increased by the effect of water stress, for example at the first harvest, the total carbohydrates of leaves and fruits significantly increased by increasing the period of irrigation in comparison to the control plants.

On the other hand, the total carbohydrates of stems increased at the first level of water stress and begain to decrease by increasing the period of irrigation. In the second harvest, total carbohydrates of leaves and stems, significantly increased by the effect of water stress (irrigation every 15 days) and then declined at the second level of water stress.

The effect of water stress at the two harvest dates was clear on the total carbohydrates of the fruits, the total carbohydrates was significant increased by increasing the period of irrigation. Again, here it is noted that the total carbohydrates varies in the different parts of the plants in two both harvests either in the control or in stressed plants. In the first harvest, the stems contained high percentage of total carbohydrate more than leaves and then fruits contained the lower percentage. In contrast, in the second harvest stems contained the highest percentage of total carbohydrates, followed by fruits and leaves.

Referring to the effects of harvesting, it was observed that the stems and fruits contained high percentage of total carbohydrate in the second harvest more than the first harvest. Moreover, no dramatic change in the total carbohydrates of leaves in the two harvests. The results of total carbohydrates of different parts (leaves, stems and fruits) of *T.terrestris* and *T.pentandrus* grown under control, mild, moderate, severe and very severe water

Table 4: Biochemical profiles of Tribulus terrestris L and Tribulus pentandrus forssk plants under water stress conditions in pot during 2005 season.

	Total carbohydrates% (mg/ 100mg DW)			Total soluble sugars% (mg/ 100mg DW)			Total free proline% (mg/ 100mg DW)		
Treatments	Leaves	Stems	Fruits	Leaves	Stems	Fruits	Leaves	Stems	Fruits
Water stress									
Control	19.44c	23.49d	22.93b	3.51d	2.70d	2.44c	0.844d	0.489c	0.386d
Mil.WS	27.42b	32.47c	23.11b	4.59c	5.10b	4.21b	1.10c	0.583c	0.522c
Mod.WS	31.18a	58.96a	31.77a	7.30a	6.41a	5.28d	1.46b	0.875b	0.856b
SWS	25.76b	43.34b	31.80a	7.04ab	5.45b	5.54a	1.76a	1.02ab	1.15a
VSWS	20.26c	35.03c	23.39b	6.43b	4.44c	5.53a	1.67a	1.06a	1.22a
Species									
T. terrestris	25.75a	40.28a	25.86a	5.83a	4.46b	4.48a	1.37a	0.813a	0.873a
T.pentandrus	23.88b	37.03b	23.79b	5.71a	5.18a	4.65a	1.36a	0.800a	0.783b
Interaction									
Control xTt	15.66f	25.19a	28.2ab	3.08a	2.13e	2.61a	0.748a	0.496a	0.305e
Control x Tp	23.2df	21.77a	23.5de	3.95a	3.28d	2.28a	0.941a	0.483a	0.46de
Mil.WS x Tt	28.58b	33.40a	25.1cd	5.10a	5.41bc	3.93a	1.17a	0.670a	0.527d
Mil.WS x Tp	26.2bc	31.55a	25.3cd	4.08a	4.80c	4.49a	1.03a	0.497a	0.518d
Mod.WS xTt	33.87a	61.69a	24.30d	7.52a	6.21a	5.13a	1.45a	0.884a	0.797c
Mod.WS xTp	28.48b	56.22a	27.2bc	7.08a	6.61a	5.43a	1.48a	0.868a	0.91bc
SWS x Tt	28.79b	45.51a	29.95a	7.37a	4.99c	5.45a	1.83a	0.958a	1.29a
SWS x Tp	22.7cd	41.17a	23.6de	6.71a	5.91ab	5.62a	1.69a	1.08a	1.01b
VSWS x Tt	21.1de	35.62a	21.6ef	6.11a	3.58d	5.28a	1.66a	1.05a	1.44a
VSWS x Tp	18.7ef	34.44a	19.17f	6.76a	5.31bc	5.43a	1.67a	1.06a	0.999b

stress in pots experiment was illustrated in Table (4). The data showed that the percentage of total carbohydrates of different parts of the two plant species increased by the effect of Mod.WS then declined by increasing water stress. Generally, the results reported in this study showed that, carbohydrates content increased in response to water stress (moderate and severe) conditions in *Tribulus species*.

The results obtained here are in agreement with that obtained by Carvalho *et al.*,[4] who reported that the water stress led to an increase in sugar and total carbohydrates concentrations in seed dry weight as compared with non-stressed control plants of two *Lupinus species*. Also,[17] pointed that, total carbohydrate of *Ocimum spp*. increased under water stress.

Soluble Sugars Accumulation: Total soluble sugars were determined in different parts of *T. terrestris* L. either in the control or under different levels of water stress in field Table (3). From obtained data it was observed that there is no significant change in the amount of total soluble sugars in the three plant parts (leaves, stems and fruits) exposed to water stress in comparison to the control, especially in the first harvest, but these amounts were found to be significant increased by increasing the level of water stress in the second harvest. So the effect of water stress on the concentration of soluble sugars was clear in the second harvest than the first one. Again here, the effect of water stress in pot experiment on total soluble sugars content is shown in Table (4). The concentration of soluble sugars of different parts grown under different stress significantly increased in response to water stress treatments up to 60% of field capacity (moderate water stress), then the concentration began to decline by increasing water stress level.

Similar findings are observed with Li *et al.*[21] who mentioned that, under water stress conditions the soluble sugars content increased in *Pistacia chinesis*

Table 5: Total steroidal sapogenins and saponins content of T. terrestris L plants under water stress conditions in field during 2005 season.

Treatments	Total steroidal sapogenins % (mg/ 100mg DW)				Total steroidal saponins % (mg/ 100mg DW)			
	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots
1st Harvest								
Control	0.839b	0.672c	0.682a	1.12a	3.92b	3.93a	3.77a	4.37a
Mod.WS	0.985ab	0.777b	0.756a	1.23a	4.04b	3.92a	3.91a	4.40a
VSWS	1.08a	1.005a	0.712a	1.27a	4.35a	4.04a	3.80a	4.47a
2n Harvest								
Control	2.52b	2.15b	1.67b	1.58c	5.81b	4.79c	4.44b	4.80c
Mod.WS	4.52a	2.96a	2.38a	2.12b	7.62a	5.98b	5.51a	5.37b
VSWS	4.60a	2.86a	2.30a	2.38a	7.65a	6.36a	5.30a	5.65a
Harvet								
1st harvest	0.970b	0.818b	1.30b	1.21b	4.10b	3.97b	3.83b	4.32
2nd harvest	3.88a	2.66a	2.13a	2.02a	7.03a	5.71a	5.08a	5.27

Bung. However, Zhao *et al.*[43] demonstrated that a high soluble sugars concentration increased in seedling of *Nitraria sphaerocarpa* under water stress as compared with the control plants.

Free proline accumulation: Water stress induced the accumulation of free proline in plants may be part of a general adoption to water stress[14]. Free proline content was measured in *Tribulus terrestris* L. subjected to water stress and compared to the control plants as shown in Table (3). Free proline content of different parts of *T. terrestris* grown in field was significantly increased under water stresses in comparison to the control especially in the first harvest. Similar results were noticed at the concentration of proline of leaves and stems in the second harvest. On the other hand, the second harvest was characterized by high content of free proline than the first harvest.

The effect of water stress in pot experiment on total free proline of *T. terrestris* and *T. pentandrus* was recorded in Table (4). The concentration of free proline of different parts of *T. species* stressed plants was, significant increased in response to water stress compared to the control plants. Current study showed that the effect of water stress either in field or pots experiments lead to significant increase in the total free proline.

The obtained data were in agreement with[41] who stated that the content of free proline significantly increased under water stress of *Rosmarinus officinalis* L. As will as, *Tanacetum parthenium* maintained at 50% of field capacity was recorded higher content of free proline compared to plants grown at 90% of field capacity[5]. Also the data obtained are in agreement with many results of Misra *et al.*[25] came to the same conclusion on *Cymbopogon* spp. and Nik *et al.*,[27] on *Satureja hortensis*.

Amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses.

In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions. It may also function as a protein compatible hydrotrope[34].

Steroidal Saponins Content: Saponins are natural products (secondary metabolites) of plant cell, whose accumulation is usually stimulated in response to challenges by environmental stresses. Total saponins were hydrolyzed and determined as diosogenin then the results were corrected by multiplied in factor to obtain steroidal saponins content.

Current study showed that the effect of water stress on total sapogenins or saponins of different plant parts (leaves, stems, fruits and roots) of *T. terrestris* grown in field experiment was low in the first harvest but the effect of water stress was clear in the second harvest, the total sapogenins or saponins, significantly increased by increasing the period of irrigation Table (5).

Timing of harvest as opposed to water stress was the more important factor deterring the chemical concentrations, plants harvested at the end water stress period was in general significantly raised up to 70 % over the first date of harvest under all water treatments in field experiment. On the other hand, the leaves are characterized by high content of saponins in comparison to other parts. Similar effect of water stress was observed on the total sapogenins or saponins of different parts of *T.terrestris* and *T. pentandrus* Table (6). Increasing water stress up to severe water stress in comparison to the

Table 6: Total steroidal sapogenins and saponins content of T. terrestris L and T. pentandrus Forssk plants under water stress conditions in pot during 2005 season.

	Total steroidal sapogenins % (mg/ 100mg DW)				Total steroidal saponins % (mg/ 100mg DW)			
Treatments	Leaves	Stems	Fruits	Roots	Leaves	Stems	Fruits	Roots
Water stress								
Control	1.47d	0.981	0.808b	0.891c	4.54c	4.14d	3.96d	3.89d
Mil.WS	2.05c	1.57c	0.948b	1.01c	4.76c	4.71c	4.22c	4.38c
Mod.WS	4.64a	2.52b	1.98a	1.68b	7.81a	5.88b	4.95b	4.72b
SWS	4.55a	3.54a	2.16a	2.15a	8.14a	6.35a	5.30a	5.21a
VSWS	4.00b	2.73b	2.00a	2.11a	7.12b	5.74b	5.15a	5.11a
Species								
T. terrestris	3.39a	2.26a	1.59a	1.65a	6.60a	5.41a	4.65b	4.64a
T.pentandrus	3.29a	2.28a	1.56a	1.49b	6.35b	5.32a	4.78a	4.68a
Interaction								
Control xTt	1.6a	0.977a	0.683a	0.960ef	4.62a	4.15e	3.77f	3.91a
Control x Tp	1.3a	0.987a	0.933a	0.823f	4.46a	4.13e	4.15de	3.86a
Mil.WS x Tt	1.8a	1.53a	0.937a	1.04e	4.75a	4.65d	4.08e	4.22a
Mil.WS x Tp	2.2a	1.61a	0.960a	0.990ef	4.78a	4.77d	4.37d	4.53a
Mod.WS x Tt	4.7a	2.73a	1.94a	1.85c	7.84a	6.29ab	4.83c	4.67a
Mod.WS x Tp	4.5a	2.32a	2.02a	1.51d	7.78a	5.48c	5.07bc	4.76a
SWS x Tt	4.4a	3.39a	2.28a	2.32a	8.39a	6.48a	5.42a	5.23a
SWS x Tp	4.6a	3.70a	2.04a	1.98bc	7.88a	6.22ab	5.19ab	5.19a
VSWS x Tt	4.2a	2.69a	2.13a	2.07b	7.40a	5.45c	5.19ab	5.17a
VSWS x Tp	3.7a	2.78a	1.88a	2.15ab	6.83a	6.00b	5.11b	5.05a

control plants increased total sapogenins or saponins. present study also indicated that, the degree of total steroidal sapogenins or saponins accumulation correlated strongly with water stress conditions, the total steroidal sapogenins or saponins content of different parts of the plants significantly increased with increasing water deficient in moderate and very severe water stress conditions in comparison with the control treatment.

In general, observations in *Tribulus terrestris* L. and *Tribulus pentandrus* Forssk, water stressed plants produced from 25 to 42 % of the chemical constituents greater than the control plants in two experiments. As will as, leaves chemical concentrations were subjected to water stress produced the highest concentration of sapogenins or saponins, an average of 25 to 37 % greater than fruits, stems and roots.

The results of the present study are in agreement with[22] who reported that ginsenoides content of *Panax quinquefolium* increased by the effect of water stress. On the other hand, the content of the saponins of the berries of *Phytolacca dodecundra* was determined by HPLC and results showed that, the highest saponins content were of these harvested during the dry season and just before the onset of the rainy season,[26].

CONCLUSION

In conclusion, increasing levels of water stress progressively reduced vegetative growth and biomass production and increased carbohydrate, soluble sugars and free proline content. The chemical concentrations of steroidal saponins increased under water stress conditions. Researches attempting to enhance the biological active constituents concentrations of medicinal plants used in pharmaceutical preparations must take into consideration both parameters of chemical yield (chemical concentration and dry weight). For this need, from all obtained results, it could be recommended to optimize steroidal saponins yield of *Tribulus terrestris* L. and *Tribulus pentandrus* Forssk by use moderate water stress condition.

REFERENCES

1. Abreu, I.N. and P. Mazzafera, 2005. Effect of water and temperature stress on the content of active constituents of Hypericum brasiliense Choisy. Plant Physiology and Biochemistry, 43(3): 241-248.
2. Arcasoy, H.B., A. Erenmemisoglu, Y. Tekol, S. Kurucu and M. Kartal, 1998. Effect of *Tribulus terrestris* L. saponin mixture on smooth muscle preparations: a preliminary study. Boll. Chim. Farm., 137: 473-475.
3. Baher, Z.F., M. Mehdi, G. Mahlega and M.B. Rezaii, 2002. The influence of water stress on plant height, herbal and essential oil yield and composition in Satureja hortensis L. Flavour and Fragrance, 4: 275-277.
4. Carvalho, I.S., M. Chaves and C.P. Ricardo, 2005. Influence of Water Stress on the Chemical Composition of Seeds of Two Lupins *Lupinus albus* and *Lupinus mutabilis*. J. Agronomy & Crop Sci., 191: 95-98.
5. Carvalho, L.M.D., V.W.D. Casali, M.A.D. Souza and P.R. Cecon, 2003. Soil water availability and growth of feverfew. Horticultura-Brasileria, 21(4): 726-730.
6. Chae, B.Y., N.D. Hong; N.J. Kim and J.S. Kim, 1990. Studies on the efficacy of combined preparation of crude drug (XLI). Effects of tongkwan-san. Korean J Pharmacog, 21(2): 163-172.
7. Charles, D.J., J.E. Simon, C.C. Shock, E.B.G. Feibert and R.M. Smith, 1993. *Effect of water stress and post-harvest handling on artemisinin content in the leaves of Artemisia annua L*. In: Janick, J., Simon, J.E. (Eds.), New Crops. Wiley, New York, pp: 628-631.
8. Chemxcil, 1992. *Tribulus terrestris* Linn. (N.O.-Zygophyllaceae). In *Selected Medicinal Plants of India (A Monograph of Identity, Safety and Clinical Usage)*. Compiled by Bharatiya Vidya Bhavan's Swamy Prakashananda Ayurveda Research Centre for CHEMEXCIL. Bombay, India: Tata Press, pp: 323-326.
9. Comley, J.C.W., V.P.K. Titanji, J.F. Ayafor and V.K. Singh, 1990. In vitro antifilarial activity of some medicinal plants. Acta Leide-nsia, 59(1/2): 361-363.
10. Deepak, M., G. Dipankar, D. Prashanth, M.K. Asha, A. Amit and B.V. Venkataraman, 2002. Tribulosin and b-sitosterol-Dglucoside, the anthelmintic principles of *Tribulus terrestris*. Phytomedicine, 9: 753-756.
11. El-Hadidi, M.N. and A. Fayed, 1994. Materials for excursion flora of Egypt. Tackholmia 15, 118. (c.f Hamed *et al*., 2004).
12. Gardner, F.P., R.B. Pearce and R. Mitchell, 1985. Growth and development. In: *physiology of crop plants*. Iowa State University Press, Ames, pp: 187-208.
13. Hamed, A.I., A. Oleszek, A. Stochmal, C. Pizza and A. Piacente, 2004. Steroidal saponins from the aerial parts of *Tribulus pentandrus* Forssk. Phytochem., 65: 2935-2945.
14. Hare, P.D., W.A. Cress and J.V. Staden, 1998. Dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ, 21: 535-553.
15. Hassani, A., 2006. Effect of water stress on growth, yield and essentisl oil content of *Dracocephalum moldavica*. Iranian J. Med. and Aroma. Plants, 22(3): 256-261.
16. Kery, B., 2004. Tribulus for sexual dysfunction in men and women. Townsend Letter for Doctors & Patients, pp: 49-52.
17. Khalid, A.K., 2006. Influnce of water stress on growth, essential oil and chemical compostion of herbs *Ocimum spp*. Int. Agrophysics, 20: 289-296.
18. Kramer, P. and J. Boyer, 1995. Water relations of plants and soils. Academic Press, San Diego, CA., USA. pp: 495.
19. Law, R.D. and S.J. Crafts-Brandner, 2001. High temperature stress increases the expression of wheat leaf ribulose-1,5- bisphosphate carboxylase/ oxygenase activase protein. Arch Biochem Biophys, 386: 261-267.
20. Li, M., W. Qu, Y. Wang, H. Wan and C. Tian, 2002. Hypoglycemic e.ect of saponin from Tribulus terrestris. Zhong Yao Cai, 25: 420–422.(c.f. Ross 1996).
21. Li, Z., P.S. Leng and Z.F. Cong, 2006. The physiological response of *Pistacia chinensis* Bunge to drought stress. J. Plant Resources and Environ., 15(3): 47-50.
22. Lim, W.S., K.W. Mudge and J.W. Lee, 2006. Effect of water stress on ginsenoside production and growth of American ginseng. Hort Technology, 16(3): 517-522.
23. Mecozzi, M., 2005. Estimation of total carbohydrate amount in enviromental samples by the phenol-sulphuric acid method assisted by multivarite calibration. Chemometrics and Intelligent Laboratory Systems, 79: 84-90.
24. Misra, P., N.L. Pal, P.Y. Guru, J.C. Katiyar and J.S. Tandon, 1991. Antimalarial activity of traditional plants against erythrocytic stages of Plasmodium berghei. Int. J. Pharmacog, 29(1): 19-23.
25. Misra, H.O., J.R. Sharma, R.K. Lal and A.A. Naqvi, 1999. Potential of genotyp for tolerance to water stress in aromatic grasses cymbopogon spp. J. Medicinal and Aromatic plant Sci., 21(4): 950-958.

26. Ndamba, J., E. Lemmich and P. Molgaarad, 1994. Investigation of the durnal, ontogenetic and seasonal variation in the mollusicidal saponin content of *Phytolacca dodecandra* aqueous berry extracts. Phytochem., 35(1): 95-99.
27. Nik, Z.B., M.B. Rezaee, M. Ghorbanli, F. Asgari and M.K. Araghi, 2004. Research on the changes of metabolism in response to water stress in *Satureja hortensis* l. Iranian J. Med. and Aroma. plants Research, 20(3): 263-275.
28. Reddy, A.R. and K.V. Chaitanya, 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol., 161: 1189-1202.
29. Ren, Y.J., H.S. Chen, G.J. Yang and H. Zhu, 1994. Isolation and identification of a new derivative of cinnamic amide from Tribulus terrestris. Acta Pharm. Sin., 29: 204-206.
30. Ringel, C., S. Siebert and O. Wienhaus, 2003. Plotometric determination of proline in quartz microplates: remarks on specificity. Anal. Biochem., 313: 167-169.
31. Ross, I.A., 1996. Medicinal plants of the world: chemical constituents, traditional and modern medicinal uses.Humana press Inc., Totowa, 2: 411-427.
32. Saleh, N.A.M., A.A. Ahmed and M.F. Abdalla, 1982. Flavonoid glycosides of *Tribulus pentandrus* and *Tribulus terrestris*. Phytochem., 21: 1995-2000.
33. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods 6th Ed. The Iowa State Univ. press, Amer. Iowa, USA, pp: 593.
34. Srinivas, V. and D. Balasubramanian, 1995. Proline is a protein-compatible hydrotrope. Langmuir, 11: 2830-2833.
35. Stanislavov, R. and V. Nikolova, 1999. *Tribulus terrestris* and human male infertility: immunological aspects. Presented at the European Meeting of Immunology and Reproduction, Rome, Italy, 28-29.
36. Steel, R.G.D. and J.H. Torrie, 1981. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Ed. McGraw-Hill Book Co., New York, NY.USA.
37. Sun, B., W. Qu and Z. Bai, 2003. The inhibitory effect of saponins from *Tribulus terrestris* on Bcap-37 breast cancer line in vitro. Zhong Yao Cai, 26: 104-106.
38. Tariq, M., A. Al-Yahya, J.S. Moos, I.A. Al-Meshal and A.A. Al-Badr, 1985. Phytochemical, pharmaconustical and pharmacological studies on CNS depressant plants of Saudi Arabia. Abstract 45th International Congress of Pharmaceutical Sciences FIP85 Montreal Canda, Abrtr-125.(c.f. Ross 1996).
39. Uematsu, Y., K. Hirata, K. Saito and I. Kudo, 2000. Spectrophotometric determination of saponin in Yucca extract used as food additive. J AOAC Int., 83(6): 1451-1454.
40. Wu, T.S., L.S. Shi and S.C. Kuo, 1999. Alkaloids and other constituents from *Tribulus terrestris.* Phytochemistry, 50: 1411-1415.
41. Wu, X.H., Z.H. Tang and Y.G. Zu, 2006. Effect of water stress on free amino acid content in Rosmarinus officinalis L. J. Northeast Fores. Univ., 34(3): 57-58.
42. Yassen, M., P. Ram, A. Yadav and K. Singh, 2003. Response of Indian basil *Ocimum basilicum* to irrigation and nitrogen schedule in central Uttar Pradesh. Annals-of-plant- physiol., 17(2): 177-181.
43. Zhao, J., H. Fu and C. Wu, 2006. Effects of water stress on biomass and osmosis-regulating substance accumulations in Nitraria sphaerocarpa seedlings. Acta Botanica Boreali-Occidentalia Sinica, 26(9): 1788-1793.
44. Zheng, Y., M. Dixon and P.K. Saxena, 2007. Growing and nutrient availability affect the content of some Phenolic compounds in Echinacea purpurea and *Echinacea angustifolia*. Planta Medica, 10: 1055.
45. Zobayed, S.M.A., F. Afreen and T. Kozai, 2007. Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition. Environmental and Experimental Botany, 59(2): 109-116.