INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 1, p. 168-177, 2018

RESEARCH PAPER OPEN ACCESS

Interactive effect of drought and sea water treatments on metabolic profile of two different *Moringa* species

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Key words: Salinity, Drought, Metabolites, *Moringa*

http://dx.doi.org/10.12692/ijb/12.1.168-177 Article published on January 12, 2018

Abstract

Current study deals with the assessment of interactive effect of drought and sea water treatment on primary and secondary metabolic contents of *Moringa peregrina* and *M. oleifera*. Different intervals (2, 7 and 14days) of drought and alternating concentrations (0, 10, 25, 50, 75 and 100%) of sea water were interactively integrated with both species of *Moringa* with three factorial arrangements in RCBD design. By following the set protocols the primary (sucrose, glucose, fructose, mannitol, raffinose, starch and proline) and secondary (phenols, alkaloids, flavonoids and tannins) metabolic contents were extracted. It was observed that with increasing levels of both drought and salinity a dynamic decline was noticed both in the concentration of primary and secondary metabolites. However this decline was more dynamic for *M. oleifera* as compared to *M. peregrina*. Besides a contrary trend was noticed for proline whose concentration was dramatically increased with increasing levels f both drought and salinity stress. Present study authenticates that both stresses negatively affect the primary and secondary metabolic processes of plant. Therefore, metabolite profiling for any plant can be considered as an important tool to monitor the consequences of abiotic stresses at molecular level.

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Introduction

The genus *Moringa* originated from Indian subcontinent comprises fast-growing plants of thirteen different species (Lalas *et al.,* 2002; Gomaa and Pico 2011). Afterward it was distributed into various tropical and subtropical regions of the world, including Saudi Arabia (Alaklabi, 2015). *Moringa oleifera* and *M. peregrina* are local species of south Asia that were introduced in other parts of the world where they became naturalized owe to multiple use and medicinal values as well as environmental significance (El-Batran *et al.,* 2005; El-Alfy *et al.,* 2011). The tree is known as Miracle Tree or Tree of Life (Alaklabi, 2015). In Saudi Arabia, *M. peregrina* is mainly distributed in South and North Hijaz. *M. oleifera* is a small to medium sized tree native to the south central Asia from India to Nepal (Muluvi *et al.,* 1999; Hegazi, 2015). The species is widely cultivated in many parts of the world including Tanzania (Munns, 1999). Soil salinity is a main environmental constraint effecting crop productivity drastically. Every year more than million hectares of the fertile land are subjected to salinization (Munns, 1999). Soil salinity impacts the plant growth by various biochemical and physiological ways such as nutritional imbalance, ion toxicity, osmotic stress, photosynthesis and other metabolic activities (Jin *et al.,* 2016). Moreover abiotic stresses lead toward reduction in number of branches and leaves in addition to stunted shoot growth (Talebnejad *et al.,* 2016). Accumulation of Na^+ in leaf cells results in reduced photosynthesis as well as inhibited uptake of essential minerals like Mg, Ca and Zn (Al-Karaki, 2000). Tolerance to drought and saline stress in plants is a complicated phenomenon that renders developmental alterations in addition to biochemical and physiological mechanisms. (Frosi *et al.,* 2017). The yield and soil salinity estimation of Moringa can serve as a valuable tool to manage the soil salinity caused by shallow ground water (Nouman *et al.,* 2012). Moreover, this feature can also be used for better handling of salinity in irrigation water under varying ground water depths (Hegazi, 2015). Numerous studies has explicated that the lowest seawater ratios in *Moringa oleifera* irrigation water provided the best outcomes for germination%, growth parameter's and some chemical and mineral contents as total green color total carbohydrate. Apart from this, under water deficit and saline conditions woody plants face dynamic changes in their leaf metabolites, water potential, pigments and organic solute contents (Frosi *et al.,* 2017). The objective of current study is to interrogate the integrated effect of drought and salinity stresses on metabolite profiling of *Moringa* leaves.

Material and methods

Current study was conducted on two *Moringa* species *M. oliefira* and *M. peregrina* inside green house. One month old seedlings were evaluated against the combined effect of drought (2, 7 and 14 days) and salinity (sea water) treatments (0, 10, 25, 50, 75 and 100%). The pot experiment was conducted within green house in tri-replicate using 3 level factorial arrangement in RCBD design , with species as factor A , drought as factor B and salinity as factor C.

Assessment of primary metabolites

Extraction of carbohydrates was done by the protocol followed by Boussadia *et al.*, 2010. They were extracted using ethanol (80%) at 45°C, followed by centrifugation at 5000 x g for 10 minutes. Some contents such as sucrose, glucose, fructose, mannitol and raffinose were traced by high pH anion-exchange chromatography with pulsed-amperometric detection. To detect starch acid hydrolysis method was used accordingly the left over precipitate was washed two time using ethanol (80%) and the pellet was treated with 1 molar HCl for 2 hour at 95°C. Finally starch content was recorded using spectrophotometer at 340 nm via the enzyme mediated reduction of $NADP^+$ (UV-VIS). Leaf samples from 35 days old seedlings were subjected to ninhydrin to record proline using spectrophotometer (Bates *et al.,* 1973).

Determination of Secondary metabolites

Total phenolic compounds in leaf were determined in the ethanolic extract using Bray and Thorpe (1954) procedure while flavonoids in aqueous extract of olive leaf were calculated using the procedure of Jia *et al.*, 1999.

Correspondingly tannins contents were analyzed in aqueous extract by following the methodology proposed by Bray and Thorpe (1954) while Alkaloid contents were estimated using the method of Harborne (1973). The quantities of all secondary metabolites was calculated as mg (g DW)$^{-1}$.

Statistical analysis

The data obtained were analyzed using SAS and Statistix software and the means were compared using LSD ($P \leq 0.05$; Steel *et al.*, 1997).

Results

Effect on primary metabolites

Mannitol: It was observed that drought treatments made no significant (P≤ 0.05) effect on leaf mannitol contents, however significant (P≤ 0.01) decline was noticed for cultivar type and salinity (Table 1).

Table 1. Effect of drought, sea water concentrations and Moringa species on primary metabolites of Moringa leaves.

Treatments days	Mannitol mg g^{-1} DW	Starch mg g^{-1} DW	Glucose mg g^{-1} DW	Fructose mg g^{-1} DW	Sucrose mg g^{-1} DW	Raffinose mg g^{-1} DW	Proline µg g^{-1} FW
Drought Treatment							
D_1 (14)	ns	0.34±0.051^{b}	1.05±0.098^{c}	0.030± 0.0028^{c}	0.30±0.030^{c}	2.38±0.15^{c}	43.00±2.11^{a}
D_2 (7)		0.54±0.061^{ab}	1.20±0.11^{b}	0.033±0.0034^{b}	0.42±0.040^{b}	3.14±0.22^{b}	40.72±1.77^{b}
D_3 (2)		0.63±0.059^{a}	1.40±0.13^{a}	0.036±0.0037^{a}	0.52±0.051^{a}	3.56±0.26^{a}	39.88±1.77^{b}
LSD		0.22	0.077	0.0022	0.067	0.24	1.92
Variety (V)							
V_1(*M. oleifera*)	1.074±0.10^{bc}	0.48±0.070^{b}	1.43±0.12^{a}	0.029±0.0037^{b}	0.43±0.053^{b}	3.12±0.27^{b}	42.00±2.15^{a}
V_2(*M. peregrina*)	1.27±0.12^{a}	0.69±0.078^{a}	1.21±0.16^{b}	0.040±0.0047^{a}	0.49±0.060^{a}	3.28±0.29^{a}	39.71±2.09^{c}
LSD	0.106	0.12	0.076	0.080	0.032	0.081	0.60
Salinity (S)							
Salinity							
S_5 (100%)	0.26±0.013^{f}	0.10±0.011^{f}	0.22±0.01^{f}	0.008±0.0004^{f}	0.06±0.005^{f}	1.24±0.024^{f}	49.67±0.63^{a}
S_4 (75%)	1.22±0.055^{c}	0.51±0.050^{c}	1.36±0.005^{c}	0.024±0.0022^{e}	0.44±0.027^{c}	3.10±0.144^{d}	47.54±0.63^{b}
S_3 (50%)	0.75±0.029^{e}	0.33±0.016^{d}	0.68±0.01^{e}	0.032±0.0021^{d}	0.27±0.010^{e}	2.74±0.14^{e}	44.38±0.62^{c}
S_2 (25 %)	0.95±0.029^{d}	0.27±0.014^{e}	0.84±0.016^{d}	0.041±0.0025^{b}	0.32±0.011^{d}	3.34±0.17^{c}	44.42±0.21^{c}
S_1 (10 %)	1.38±0.047^{b}	0.60±0.055^{b}	1.55±0.060^{b}	0.039±0.0028^{c}	0.53±0.031^{b}	3.52±0.149^{b}	44.12±0.61^{c}
S_0 (0 %)	1.57±0.036^{a}	0.81±0.056^{a}	1.73±0.062^{a}	0.051±0.0027^{a}	0.61±0.032^{a}	4.24±0.160^{a}	23.46±0.24^{d}
LSD	0.053	0.077	0.054	0.0031	0.023	0.093	0.711
Significance							
D	ns	**	*	**	*	**	*
V	**	**	**	*	**	**	**
S	**	**	**	**	**	**	**
D x V	ns	ns	ns	ns	*	*	ns
D x S	**	**	**	**	**	**	**
V x S	ns	**	**	**	**	**	ns
D x V x S	ns	ns	ns	ns	ns	ns	ns

dry weight; FW, fresh weight

Means followed by the same letter (s) in each column and treatment showed no significant difference

*, ** indicate significant differences at 0.05, 0.01 probability levels respectively while 'ns' indicate non-significant difference.

Among cultivars, *M. peregrina* attained statistically significant higher value of 1.27 mg g^{-1} DW mannitol. A remarkable decline in mannitol was noticed for salinity level at 100 % as compared to other levels. Besides individual treatment, significant (P≤ 0.01) effect of interaction D x S was observed for mannitol, while no significant (P≤ 0.05) effect was observed for interactions D x V and V x S. Under the long drought treatment all salinity treatment considerably decreased the mannitol quantity (Table 3).

Starch: It was noticed that all treatments affected starch content significantly (P≤ 0.01) (Table 1). Mean comparison between different levels of drought showed that decrease in starch content by D_3 was

highest compared to D1. Among varieties, *M. oleifera* illustrated the lowest mean value for starch which was significantly different from the mean of *M. peregrina* . A dynamic reduction in starch was noticed for increasing levels of salinity; however 100 % salinity level revealed the lowest mean value as compared to control. Moreover, significant (P≤ 0.01) effect of interactions, D x S and V x S was observed on starch. The lowest values were noted at maximum concentration of salinity, in plants following drought treatments of 7 and 14 days respectively (Table 3). Similarly for V x S interaction minimum starch level was detected at 100% salinity concentration in cultivar *M. oleifera* (Table 4). Moreover no significant effect of interaction between drought and cultivar (D x V) was noticed on starch content. Likewise, no three way interaction was noticed among the treatments.

Table 2. Effect of drought, sea water concentrations and Moringa species on secondary metabolites of Moringa leaves.

Treatments days	Alkaloids mg g^{-1} DW	Flavonoids mg g^{-1} DW	Tannins mg g^{-1} DW	Phenols mg g^{-1} DW
Drought (D)				
D_1 (14)	1.53±0.075^{c}	1.36±0.068^{b}	ns	ns
D_2 (7)	1.76±0.078^{b}	1.45±0.078^{ab}		
D_3 (2)	2.01±0.10^{a}	1.61±0.083^{a}		
LSD	0.19	0.17		
Variety (V)				
V_1 (*M. oleifera*)	1.87±0.094^{a}	1.40±0.085^{b}	ns	1.14±0.050^{a}
V_2 (*M. peregrina*)	1.63±0.1^{b}	1.55±0.099^{a}		1.03±0.065^{b}
LSD	0.047	0.11		0.061
Salinity (S)				
S_0 (100 %)	1.03±0.32^{e}	0.85±0.034^{d}	0.69±0.015^{e}	0.74±0.030^{e}
S_1 (75 %)	1.81±0.051^{c}	1.47±0.030^{c}	0.85±0.025^{d}	1.10±0.042^{d}
S_2 (50 %)	1.49±0.055^{d}	1.41±0.055^{c}	1.00±0.033^{bc}	1.28±0.032^{a}
S_3 (25 %)	1.86±0.063^{c}	1.63±0.069^{b}	1.12±0.028^{a}	1.17±0.034^{bc}
S_4 (10 %)	2.01±0.058^{b}	1.64±0.036^{b}	0.97±0.036^{c}	1.15±0.043^{cd}
S_5 (0 %)	2.21±0.063^{a}	1.94±0.050^{a}	1.08±0.035^{ab}	1.31±0.041^{a}
LSD	0.054	0.079	0.073	0.056
Significance				
D	*	*	ns	ns
V	**	*	ns	**
S	**	**	**	**
D x V	ns	ns	ns	ns
D x S	**	ns	ns	ns
V x S	ns	ns	ns	ns
D x V x S	ns	ns	ns	ns

DW, dry weight; FW, fresh weight

Means followed by the same letter (s) in each column and treatment showed no significant difference

*, ** indicate significant differences at 0.05, 0.01 probability levels respectively while 'ns' indicate non-significant difference.

Glucose: All treatments significantly (P≤ 0.01) reduced the level of glucose in the leaves of *Moringa* (Table 1). Comparison between the means of different drought treatments disclosed that decrease in glucose by D3 was significantly dramatic than D1 . Among cultivars, *M. oleifera* showed the minimum mean content which was statistically different from the mean content of *M. peregrina*. A striking decrease in glucose was recorded for 100% salinity treatment, as compared to other levels. In addition, significant effect of interactions between D x S and D x V was noticed on glucose level. For interaction D x S, the lowest mean value for glucose content was noticed at 100% saline concentration for all drought treatments (Table 3). Additionally, both varieties depicted decrease in glucose with increased concentrations of

salinity as compared to control. The lowest glucose content, was isolated from the leaves of *M. peregrina* when its seedlings were exposed to salinity level of 100% (Table 4).

On the other hand no significant effect of interaction between drought and cultivar (D x V) was noticed on glucose. Moreover, no significant effect of three way interaction was observed.

Table 3. Effect of interaction between drought and salinity on primary and secondary metabolites of Moringa leaves.

		Primary Metabolites							Secondary Metabolites			
Drought (D) (days)	Salinity %	Mannitol mg g^{-1} DW	Starch mg g^{-1} DW	Glucose mg g^{-1} DW	Fructose mg g^{-1} DW	Sucrose mg g^{-1} DW	Raffinose mg g^{-1} DW	Proline µg g^{-1} FW	Alkaloids mg g^{-1} DW	Flavanoids mg g^{-1} DW	Tannins mg g^{-1} DW	Phenols mg g^{-1} DW
D1(14)	100	0.20±0.02	0.06±0.01	0.17±0.02	0.006±0.0008	0.03±0.002	1.19±0.04	47.8±0.4	0.86±0.04			
	75	1.02±0.12	0.24±0.064	1.16±0.07	0.017±0.003	0.31±0.022	2.26±0.08	45.6±0.27	1.59±0.04			
	50	0.60±0.03	0.34±0.064	0.59±0.011	0.021±0.003	0.22±0.004	1.96±0.1	43.1±0.91	1.43±0.06			
	25	0.83±0.021	0.31±0.041	0.75±0.012	0.029±0.003	0.26±0.005	2.44±0.13	23.9±0.38	1.59±0.07			
	10	1.22±0.09	0.46±0.094	1.35±0.08	0.028±0.005	0.39±0.022	2.67±0.06	43.0±0.53	1.74±0.58			
	0	1.48±0.07	0.60±0.092	1.51±0.06	0.039±0.0005	0.46±0.015	3.39±0.07	23.1±0.53	1.91±0.04			
D2(7)	100	0.28±0.01	0.11±0.01	0.23±0.006	0.009±0.0005	0.06±0.009	1.23±0.04	49.6±0.53	1.09±0.04			
	75	1.26±0.06	0.58±0.045	1.38±0.07	0.026±0.004	0.44±0.03	3.33±0.12	46.4±0.37	1.75±0.01			
	50	0.60±0.03	0.44±0.034	0.59±0.011	0.021±0.003	0.22±0.004	1.96±0.1	43.1±0.91	1.43±0.06	ns	ns	ns
	25	0.83±0.021	0.54±0.064	0.75±0.012	0.029±0.003	0.26±0.005	2.44±0.13	23.9±0.38	1.59±0.07			
	10	1.38±0.05	0.60±0.093	1.50±0.072	0.043±0.003	0.54±0.022	3.66±0.13	43.2±0.42	1.99±0.05			
	0	1.56±0.05	0.90±0.10	1.69±0.071	0.055±0.002	0.63±0.034	4.34±0.13	23.6±0.80	2.21±0.05			
D3(2)	100	0.29±0.02	0.14±0.02	0.24±0.009	0.008±0.0005	0.08±0.006	1.29±0.04	51.6±0.52	1.13±0.04			
	75	1.38±0.05	0.70±0.05	1.55±0.10	0.030±0.003	0.57±0.03	3.72±0.15	50.6±0.48	2.10±0.06			
	50	1.53±0.05	0.76±0.08	1.79±0.10	0.047±0.004	0.68±0.05	4.22±0.21	46.1±1.03	2.30±0.07			
	25	0.89±0.03	0.74±0.07	0.76±0.010	0.038±0.003	0.33±0.009	3.50±0.094	46.1±0.85	1.95±0.05			
	10	1.10±0.043	0.84±0.06	0.92±0.012	0.051±0.003	0.37±0.012	4.22±0.173	23.6±0.40	2.21±0.04			
	0	1.68±0.06	0.91±0.052	1.98±0.11	0.060±0.004	0.74±0.06	4.96±0.24	23.6±0.85	2.51±0.10			
	LSD	0.1329	0.1320	0.0930	0.0050	0.0404	0.1572	1.2147	0.0927			

DW, dry weight; FW, fresh weight

Means having difference greater than LSD are significant at $P \leq 0.05$.

Fructose: All treatments affected fructose synthesis significantly (P≤ 0.01, P≤ 0.05) in the leaves (Table 1). Means comparison of different levels of drought treatments revealed that decrease in fructose was significantly lower for D_3 as compared to D_1. Furthermore, among cultivars M. *peregrina* showed significantly (P≤ 0.05) higher fructose content compared to other cultivar. A remarkable decline in fructose was noticed for all salinity levels; however 100% salinity depicted the lowest mean content. The interactions (D x S and V x S) were significant (P≤ 0.05) while interaction of D x V was non-significant (P≤ 0.05) on fructose. The lowest mean value was recorded at 100% salinity concentration for interaction of D x H (Table 3). Also, for interaction (V x S), the lowest mean values were recorded at 100% concentration for cultivar *M. oleifera* (Table 4). Overall, salinity interaction with drought and variety showed relatively higher means of fructose. No effect of three way interaction was noticed on fructose content.

Sucrose: Synthesis of sucrose was affected significantly (P≤ 0.01) by all levels of treatments (Table 1). Mean comparison between different levels of drought depicted that decrease in sucrose content by D3 was statistically significant (P≤ 0.05) compared to D1. Among varieties, *M. peregrina* showed the highest sucrose content as compared to other. A decrease in sucrose was monitored for all levels of salinity; however 100% level has depicted the lowest mean content as compared to other levels.

Apart from individual treatment, significant effect of interactions between D x V and V x S were noticed on sucrose content. For interaction (D x S), the lowest mean values were found at 100% concentration as indicated in table 3. Moreover, for interaction (V x S), the lowest mean values were recorded at 100% salinity concentration for both cultivars (Table 4).

Table 4. Effect of interaction between varieties and salinity on primary and secondary metabolites of *Moringa* leaves.

		Primary Metabolites							Secondary Metabolites			
Varieties (V)	Salinity	Mannitol mg g^{-1} DW	Starch mg g^{-1} DW	Glucose mg g^{-1} DW	Fructose mg g^{-1} DW	Sucrose mg g^{-1} DW	Raffinose mg g^{-1} DW	Proline mgg^{-1} FW	Alkaloids mg g^{-1} DW	Flavanoids mg g^{-1} DW	Tannins mg g^{-1} DW	Phenols mg g^{-1} DW
V1 (*M. oleifera*)	100		0.06±0.01	0.20±0.02	0.007±0.0009	0.04±0.009	1.14±0.05		0.94±0.07	0.82±0.05		
	75		0.08±0.01	0.21±0.02	0.007±0.0008	0.06±0.01	1.24±0.01		0.98±0.06	0.95±0.06		
	50		0.38±0.09	1.18±0.08	0.018±0.003	0.36±0.04	2.75±0.25		1.30±0.11	1.07±0.08		
	25		0.44±0.07	1.28±0.10	0.020±0.003	0.42±0.05	3.05±0.31		1.44±0.09	1.18±0.07		
	10		0.58±0.09	1.38±0.11	0.033±0.005	0.43±0.03	3.17±0.20		1.49±0.11	1.25±0.08		
	0		0.70±0.06	1.55±0.07	0.042±0.006	0.49±0.03	3.83±0.29		1.66±0.14	1.42±0.08		
V2 (*M. peregrina*)	100		0.12±0.02	0.24±0.02	0.008±0.001	0.06±0.01	1.25±0.06		1.03±0.04	0.95±0.06		
	75	ns	0.50±0.12	1.60±0.08	0.024±0.004	0.44±0.06	3.18±0.28	ns	1.59±0.10	1.24±0.05	ns	ns
	50		0.48±0.10	1.45±0.08	0.035±0.004	0.49±0.06	3.48±0.34		1.55±0.10	1.35±0.06		
	25		0.52±0.15	1.83±0.09	0.037±0.006	0.57±0.06	3.67±0.35		1.74±0.08	1.48±0.03		
	10		0.69±0.11	1.62±0.09	0.049±0.004	0.59±0.05	4.13±0.26		1.80±0.14	1.54±0.06		
	0		0.77±0.10	2.03±0.09	0.048±0.005	0.65±0.06	4.39±0.28		1.93±010	1.72±0.04		
	LSD		0.1524	0.1063	0.0061	0.04638	0.1829		0.0492	0.0811		

DW, dry weight; FW, fresh weight

Means having difference greater than LSD values are significantly different at $P \leq 0.05$.

As a whole, salinity interaction with drought and variety showed comparatively lower means of sucrose. Furthermore, interaction between drought and cultivar (D x V) revealed statistically significant differences in leaf sucrose content. For all drought treatments, cultivar *M. oleifera* showed the minimum mean values of sucrose, however the lowest was reported at the duration of 14 days. No significant affect was noticed for three way interaction.

Raffinose: Significant (P≤ 0.01) effect of all treatments was observed on the raffinose level in *Moringa* leaves (Table 1). Mean comparison between different levels of drought depicted more decrease in raffinose content by D_3 compared to D1. Among cultivars, *M. oleifera* illustrated statistically lower sucrose content compared to other cultivar. A notable decrease in sucrose was determined for 100% (S5) salinity, as compared to other concentrations. Besides the individual treatments, significant effect of two way interactions between drought, varieties and salinity were noticed on raffinose content.

For interaction (D x S), the minimum mean values were detected at 100% concentration for all drought treatments (Table 3). Moreover for interaction (V x S), the minimum mean values were noticed at 100% level for cultivar *M. oleifera* (Table 4). The interaction between drought and cultivar (D x V), revealed statistically significant decrease in leaf raffinose content. For all drought treatments, cultivar *M. oleifera* depicted the lowest mean values; however the lowest mean was reported at the interval of 14 days. No significant affect was noticed for three way interaction.

Proline: It was observed that drought, varieties and salinity significantly (P≤ 0.01) affected proline content (Table 1). Mean comparison between different durations of drought showed that increase in proline content by D_3 was statistically significant compared to D_2 and D1 among cultivars *M. peregrina* revealed maximum increase in proline level compared to other cultivar. A considerable increase in proline was noticed for 100% salinity concentration,

as compared to other levels. Other than individual treatments, interactions (D x S) depicted significant, while interactions (D x V) and (V x S) showed no significant effect on proline content. For interaction (D x H), maximum mean values were detected at 100 % salinity concentration as indicted in Table 3. No significant affect was noticed for three way interaction.

Effect on secondary metabolites

Alkaloids: It was found that all treatments significantly (P≤ 0.01) affected alkaloid contents (Table 2). Mean comparison between different durations of drought illustrated that decrease in alkaloids by D3 was significantly higher than D1 as compared to other cultivars. A promising decline in alkaloids was noticed for all concentrations of salinity. Furthermore, significant effect of interactions between D x S and V x S was recorded on alkaloids. For interaction (D x S), the lowest mean values were reported at 100 % salinity concentration (Table 3). Correspondingly, interaction of V x S significantly (P≤ 0.01) affected alkaloids—where minimum values were noticed at 100% level concentration for both cultivars (Table 4). Besides, no significant effect of interaction between drought and cultivar (D x V) was noticed on alkaloids. Similarly, no significant affect was noticed for three way interaction.

Flavonoids: It was observed that drought, varieties and salinity significantly (P≤ 0.01) reduced the amount of flavonoids (Table 2). Mean comparison for all levels of drought treatments showed that flavonoids were significantly reduced by D_3 as compared to D1 (1.61 mg g^{-1} DW). Both cultivars depicted statistically significant decrease in flavonoids. A dramatic fall in flavonoids was observed at 100% salinity level, as compared to other levels. Besides individual treatments significant effect of two way interactions between drought, varieties and salinity was observed on flavonoids content. The mean values of D x S interaction (Table 3) indicated that the lowest values for flavonoids in case of drought treatments were at 100% salinity concentration. No effect of three way interaction was observed.

Tannins: It was found that drought treatment and cultivars did not significantly (P≤ 0.05) decreased tannins contents; however salinity affected significantly (P≤ 0.01) as indicated in Table 2. Comparisons of means revealed a statistically significant decrease in tannins for all salinity treatments; however 100% salinity demonstrated the highest mean value. Apart from individual treatment affect, no significant effect of interactions between drought, varieties and salinity was noticed on tannins contents.

Phenols: It was observed that both variety and salinity significantly (P≤ 0.01) altered the levels of phenols; however no significant (P≤ 0.05) alteration was observed for drought treatments (Table 2). Mean comparison for all cultivars revealed that phenolic contents were significantly higher in *M. oleifera* as compared to other cultivar. A statistically significant decrease in phenolic content was noticed for salinity treatments, with 100% being the most effective one. No two way significant interaction was noticed except D x S (Table 3) where 100% salinity concentration showed maximum value while the maximum values were observed for control. Moreover, no effect of three way interaction was observed.

Discussion

In present work, we found that the level of primary and secondary metabolites has increased in parallel fashion in both cultivars of *M. peregrina* and *M. Oleifera*. Therefore, primary growth mechanisms compete with secondary metabolism for common substrates like proteins and sugars. Ghasemzadeh *et al.* (2010), reported that high level of carbohydrate content might increases the synthesis of the substrates of shikimic acid pathway that ultimately stimulate the production of secondary metabolites. In fact the deposition of carbohydrates can be labeled as an indicator of secondary metabolite formation (Ghasemzadeh *et al.,* 2010; Wenzel *et al.,* 2014). Matyssek *et al.* (2005) reported that under favorable circumstances, primary metabolism commonly depicts precedence of resource utilization over secondary metabolism.

Perhaps, this was the reason the level of secondary metabolites under low salinity was high which necessitates the secondary metabolism as an accessory tool to balance the cellular deficit. These outcomes contradict with carbon nutrient theory, which states if plants have plenty of carbon and nutrients, optimal growth takes place that suppress secondary metabolism correspondingly (Matyssek *et al.* 2005; Guo *et al.,* 2011). Moreover, our results explicated that the content of both primary and secondary metabolites can be significantly altered by optimizing different concentrations of salinity and drought levels. On the other hand, contrary to all trends proline concentration decreased with increasing concentration of salinity; however this decrease was more dramatic for the lowest concentration of salinity. Proline not only shields the enzymes from ion inhibitory effects but also balances the subcellular machinery and facilitates the specific functioning of carbon and nitrogen (Ejaz *et al.,* 2012). Somehow catabolism of proline is associated with marked incline in the levels of flavonoids, polyphenols and primary metabolites as supported by previous researchers (Szabados and Savoure, 2010; Mahmoudi *et al.,* 2013; Aslam *et al.,* 2016). Stress like drought and salinity triggers various complicated reactions that are associated with enzymatic and metabolic changes (Selmar *et al.,* 2013). Hence, metabolic activities are guided to produce highly reduced secondary metabolites, such as, (poly) phenols or alkaloids. However contrary to their findings we observed a dramatic fall in the concentration of secondary metabolites when drought and salinity stressed applied together. On the other hand a correlation between salinity stress and increase in proline concentration can be supposed as stress marker (Goas *et al.,* 1982; Zhu, 2001; Diggelen *et al.,* 2006). Complementary results were found in current study, where dynamic increase in proline under high drought and salinity stress shield plants enzymatic machinery. According to Xu *et al.* (2011) high level of primary metabolites is also associated with catabolism of proline. Therefore in current study under low levels of drought and salinity stress high level of primary metabolites was noticed along with decline level of proline.

In current study variations in the quantities of both secondary and primary metabolites have been noticed in both cultivars of *Moringa* under different levels of drought and salinity, which authenticate that under stress directly affect both primary and secondary metabolic processes of plant negatively. Therefore, metabolite profiling for any plant can be considered as an important dynamic to monitor the consequences of abiotic stresses at molecular level.

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