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EFFECTS OF MAGNETIZED WATER ON PHENOLIC COMPOUNDS, LIPID PEROXIDATION AND ANTIOXIDANT ACTIVITY OF *MORINGA* SPECIES UNDER DROUGHT STRESS

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ABSTRACT

Drought stress is one of the major abiotic stresses and serious problem for global crop production. Magnetic water technology is supposed to be an environment-friendly tool used for alleviating abiotic stress in the plants. The current study investigates the application of magnetized or magnetic water (MW) on the two *Moringa* species (*Moringa oleifera* and *Moringa peregrina*) under drought conditions. Magnetic field was applied to magnetize the irrigation water. Both species were exposed to three irrigation regimes including, 100% Field capacity (FC, as Control), 50%Field capacity (FC, as Moderate drought stress) and 20% Field capacity (FC, as Severe drought stress). The pot experiment was conducted in November 2015 under randomized complete block design with three replications in the greenhouse of King Abdulaziz University, Jeddah, Saudi Arabia. The results indicated that the total phenolic compounds, flavonoids, Malondialdehyde (MDA), H_2O_2 and proline content increased during all the levels of drought stress. In addition, the total antioxidant capacity increased during the drought stress in both the species. However, seedlings with magnetic water treatment (MWT) decreased the phenolic and flavonoid compounds, MDA, H_2O_2 and proline content significantly. The *M. oleifera* and *M. peregrina* seedlings exposed to drought stress (MS, SS) exhibited 15%, 10% and 21%, 22% decrease in MDA content under MWT. In DPPH assay, the higher antioxidant activity was found in *Moringa peregrina* (63%) whereas; lower antioxidant activity was found in *Moringa oleifera* (40%) during drought stress. This study indicates that the MWT helps the plants to become more tolerant during the drought stress by improving their antioxidant capacity.

Keywords: Flavonoids; H_2O_2; MDA; Proline; Reactive oxygen species

Abbreviations: MW=Magnetic water, NW=Normal water, MWT=Magnetic water treatment, MDA=Malondialdehyde, ROS=Reactive oxygen species, OD=Optical density, FC=Field capacity, MS=Moderate drought stress, SS=Severe drought stress, TP=Total phenolic content, TF=Total flavonoid content, TBA=Thiobarbituric acid, H_2O_2=Hydrogen peroxide, DPPH =1-diphenyl-2-picrylhydrazyl.

INTRODUCTION

The evaluation of new antioxidant compounds from medicinal plants has been growing interest in recent times (Sökmen *et al*., 2004). The amount of secondary metabolites is affected by the biological, physiological, environmental and ecological factors (Ramakrishna and Ravishankar, 2011). One of the major abiotic stresses is the drought, which is affecting the plant growth and enhancing the concentration of their secondary metabolites (Selmar and Kleinwächter, 2013; Nahar *et al*., 2015; Tamburino *et al*., 2017). The phenolic and flavonoid compounds, synthesized from the shikimate-phenylpropanoid biosynthetic pathway, are generally regarded as the extensive groups of plant secondary metabolites (Torras-Claveria *et al*., 2012; Ma *et al*., 2014). In plants, phenolic compounds are designated as markers of biotic and abiotic stress tolerance (Balasundram *et al*., 2006; Lattanzio *et al*., 2006). Higher amounts of phenolic compounds were found under abiotic stress condition as compared to non-stress condition of the plants (Selmar, 2008). In tobacco, most of the phenolic compounds were identified in drought stress condition (Torras-Claveria *et al*., 2012).

The extent to which reactive oxygen species (ROS) cause damage to cells is correlated to a balance between ROS synthesis and scavenging system of antioxidants (Azooz *et al*., 2009; Nahar *et al.,*2015; Hasanuzzaman *et al.*, 2017). Drought stress supposedly affects MDA and H_2O_2 in some plants (Chandra *et al.,* 2012; Mirzaee *et al*., 2013).It is stated that the plant cell membrane is more prone to leakage under drought stress caused by increased ROS concentrations, which lead to lipid peroxidation (Azooz *et al*., 2009). Lipid peroxidation, protein oxidation and inhibition of enzyme activities and nucleic acid damages lead to cell death due to increase in ROS levels (Tamburino *et al*., 2017). Lipid peroxidation also causes degradation of fatty acids in the cell membrane leading to the formation of MDA. Since, MDA is considered as the final product of lipid peroxidation, it is also designated as a marker for cellular membrane damage under drought stress (Cunhua *et al*., 2010). Drought stress also affects the proline content in plants (Mirzaee *et al*., 2013). Proline acts as an osmoprotectant,

plays a vital role in maintaining osmoregulation and decreasing stress-induced cellular acidification, which can prevent water loss (Hasegawa *et al*., 2000; Ahmed *et al*.,2011). It is also helpful to recover stress condition of the plants and serves as a component of signaling molecule (Khedr *et al*.,2003; Ashraf and Foolad, 2007).

Magnetic water technology, as one of the new, eco-friendly and cost effective techniques is used for the agricultural as well as environmental management (Ali *et al*., 2014). Magnetic water (MW) is generated by treating the normal water with magnetic field, resulting the rearrangement of water structure into a new hexagonal form in various ways (Ali *et al*., 2014).The beneficial effects of MWT in agriculture have been reported by many workers (Aladjadjiyan, 2002; Maheshwari and Grewal, 2009; Grewal and Maheshwari, 2011).It is reported that static magnetic field (MF) can ameliorate the soil water stress in the *Zea mays* (Anand *et al*., 2012). Magnetic field has been reduced the adverse effects of pathogenic microbes (Galland and Pazu, 2005) and had a protective role against the salt stress (Radhakrishnan and Kumari, 2013).

Moringa oleifera and *Moringa peregrina* are well-known species of the family Moringaceae (Katayon *et al*., 2006). *Moringa oleifera* is a recognized medicinal plant in many developing countries (Katayon *et al.*, 2006). Similarly, *Moringa peregrina* is an economically important plant and a source of various minerals, proteins and essential amino acids (Osman and Abohassan, 2012).

Although various scientific studies have been carried out to understand the effects of exogenous application of chemical compounds, plant growth promoting bacteria and mycorrhiza to mitigate the impacts of drought stress on various plants. However, a little information is available about the application of MW to reduce the drought stress in plants. Consequently, to the best of our knowledge, studies were absent regarding the application of MW on the antioxidant activity and other physiological parameters in the both *Moringa* species under drought stress. Hence, the present study was carried out to assess the antioxidant activity, measures the major changes of phenolic and flavonoids, MDA, proline and H_2O_2 under Magnetic water treatment (MWT) during drought stress condition, and categorize the species based on drought tolerance features.

MATERIALS AND METHODS

Experimental materials and design: The greenhouse experiment was carried out in November 2015 at the King Abdulaziz University, Jeddah. The seeds of the two *Moringa* species were collected from Abha region, Saudi Arabia. The seeds were identified by the renowned taxonomist at King Abdulaziz University Herbarium (KAUH) and double-checked using the herbarium specimen at KAUH. The seeds of the both species were transferred to the green house and were sown in the pots under randomized completely block design with three replications. The pots were filled up with sandy loam mixed with compost and peat moss (1:1:1) and were kept under greenhouse controlled conditions light/dark regime about 12/12 h, at 25/15±3^{0}C and relative humidity (RH) 30-50%, respectively. Magnetic instrument was used (150d magnetic technologies L.L.C) with power 30mT for magnetizing the water by following the methods of Selim and El-Nady (2011). Half of the pots were irrigated with normal tap water and remaining pots were irrigated with the MW.

Drought stress treatments: Plants were allowed to grow for 60 days for the plant establishment. After the plant establishment, both species were subjected to drought treatments for 30 days including 100% FC (Control), 50% FC (Moderate stress, MS), and 20% FC (Severe Stress, SS). Field capacity were maintained by weighing the pot every day. The leaf samples were collected during harvest time at 90 days.

Methanol extract preparation: For the preparation of leaf extract, 2.0-gram leaves were shaken on the orbital shaker (150 rpm) along with 20 ml methanol (80%) for 12h and filtered by using filter paper.

Estimation of total phenolic content: Phenolic content was measured according to procedures mentioned by Velioglu *et al.,* (1998). The prepared methanol extract (50 µl) was mixed with Folin–Ciocalteu reagent (100 µl), methanol (850 µl). The mixture was allowed to stand for 5 min at ambient conditions. Then 500 µl of 20% sodium carbonate was added to the mixture, allowed the reaction to occur for another 30 min. Optical density (OD) was measured at 750 nm. Total phenolic concentration was quantified using a calibration curve that was made by measuring the absorbance of known concentrations of gallic acid. The results were expressed as mg of gallic acid equivalent/g tissues.

Estimation of total flavonoids concentration: The total flavonoids content was determined by using a modified colorimetric method as described previously by Zhishen *et al*., (1999). The methanolic extract, also called as standard solution (250 µl) was mixed with distilled water (1.25 ml) and 5 % Sodium Nitrite solution (75 µl) and allowed for 6 min reaction. 10% $AlCl_3$ solution (150 µl), 1 M NaOH (0.5 ml) and distilled water (275 µl) were added to the mixture after 5 min later. Absorbance was measured at 510 nm. The flavonoids were quantified using a calibration curve obtained by measuring the optical density (OD) of catechin of known concentrations. The results were expressed as mg of catechin equivalent/g tissues.

DPPH radical scavenging assay: By using 1-diphenyl-2-picrylhydrazyl (DPPH), free radical scavenging activity

of methanol extract was determined, which was described by Ao *et al.,* (2008). 0.1 ml methanolic extract was added to 0.9 ml freshly prepared DPPH methanol solution (0.1mM). An equivalent amount of methanol was used as control. The reacted mixture was incubated for half an hour in the dark at room temperature. The optical density (OD) was measured at 517nm and the radical scavenging activity (%) was calculated through the following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{(\text{OD Control} - \text{OD Sample})}{\text{OD Sample}} \text{X100}$$

Measurement of H_2O_2: Hydrogen peroxide (H_2O_2) was measured by following the procedures described by Yu *et al*., (2003). A homogenate was prepared from 0.5 g leaf sample and was added 3 mL of 50 mM potassium-phosphate buffer (pH 6.5) at 4 °C. At 11,500xg speed and 15 min. time, the homogenate was centrifuged, from which 3 ml supernatant was collected and reacted with 1 mL of 0.1 % titanium tetrachloride in 20% H_2SO_4. The mixture was centrifuged again at 11,500xg for 15 min, after allowing the mixture to stand in room temperature for 10 min. The absorbance was quantified at 410 nm to determine the concentration of H_2O_2 and expressed as nanomoles/gram fresh weight.

Measurement of Lipid Peroxidation: Malondialdehyde (MDA) concentrations act as an indicator for the level of lipid peroxidation, the methods described by Heath and Packer (1968). Thiobarbituric acid (TBA) was used as the reactive material for measuring the MDA. 0.5 g leaf samples were homogenized in 3 ml 5 % (w/v) trichloroacetic acid (TCA). The homogenate was allowed for centrifugation at 11,500×g for 10min. 1 ml supernatant was collected and mixed with 4 ml TBA reagent, which consisted of 0.5% TBA dissolved in 20% TCA. A hot water bath was used to heat the reacting mixture up to 95 °C for 30 min, followed by rapid cooling in an ice bath. The cool mixture was centrifuged at 11,500×g for 15 min, which produced coloured supernatant, whose optical density was measured at 532 nm. In addition, correction was done for non-specific absorbance at 600nm. Malondialdehyde (MDA) concentration was expressed as nanomoles /gram fresh weight, using extinction coefficient as $155 mM^{-1} cm^{-1}$.

Proline content: Free proline content was measured according the procedures described by Bates *et al*., (1973). Fresh leaf tissues (0.5 g) was taken and homogenized in 10 ml of 3% sulfosalicylic acid in ice. The homogenate was centrifuged at 11,500×g speed for 15 min. 2 ml of the filtrate was collected, which was allowed to react with 2 ml acid ninhydrin and 2ml glacial acetic acid. The mixture was incubated at 100°C for an hour. Once the mixture was cooled, 4 ml toluene was added. The absorbance was taken at 520nm. From the standard curve, the amount of proline was determined and expressed as µg/g FW.

Statistical analysis: Analysis of variance (ANOVA) and the mean differences of data were tested by Fisher's LSD test using Minitab (17) statistical software. The differences between the data at $P \leq 0.05$ were regarded as significant.

RESULTS

Total phenolic content (TP): In the present study, it was observed that magnetic water treatment (MWT) had a statistically significant effect on the TP in both species (*Moringa oleifera* and *Moringa peregrina*) (Table 1). *M. peregrina* exhibited the higher amount of TP in the drought level as compared to *M. oleifera*. The *M. oleifera* and *M. peregrina* seedlings exposed to MS, SS level exhibited 20%, 30% and 13%, 29% increased TP under normal irrigated water (Table 1). The MW application in *M. oleifera* and *M. peregrina* results decreased in TP by 11%, 15% and 16%, 21% under drought stress (MS, SS).

Total flavonoid content (TF): The total flavonoid contents (TF) were significantly increased under drought stress condition. Under normal water treatment, the *M. oleifera* and *M. peregrina* seedlings exposed to MS, SS level exhibited 16%, 24% and 9%, 23% increase in TF (Table 1). The total flavonoid content (TF) significantly decreased under MWT in both of the species.

DPPH Assay: DPPH was increased in *M. oleifera* and *M. peregrina* seedling by 14%, 27% and 8%, 15% exposed to MS, SS level under NW (Figure 1). The *M. oleifera* and *M. peregrina* seedlings exposed to drought stress (MS, SS) showed 10%, 10% and 7%,6% decline in DPPH under MWT.

Lipid peroxidation: The *M. oleifera* and *M. peregrina* seedlings exposed to drought stress (MS, SS) showed 27%, 48% and 6%, 27% increase in MDA content in the normal water irrigation. A significant decrease in MDA content was observed in the *Moringa* seedlings exposed to MW (Figure 2).

H_2O_2 Content: A significant increase in H_2O_2 content was observed under drought stress. The higher amount of H_2O_2 was found in the *Moringa oleifera* exposed to MS level. The *M. oleifera* and *M. peregrina* seedlings exposed to drought stress (MS, SS) showed 23%, 24% and 8%,21% decrease in H_2O_2 under MWT (Figure 3).

Proline content: The proline content was significantly increased in both of the species of *Moringa* under drought stress (Figure 4). The higher accumulation of proline content was observed in *M. peregrina* whereas, lower accumulation of proline content was found in *M. oleifera* at the all level of drought stress. Under drought

stress (MS, SS), MWT with *M. oleifera* and *M. peregrina* resulted in decreased proline by 8%, 7% and 21%,13% respectively.

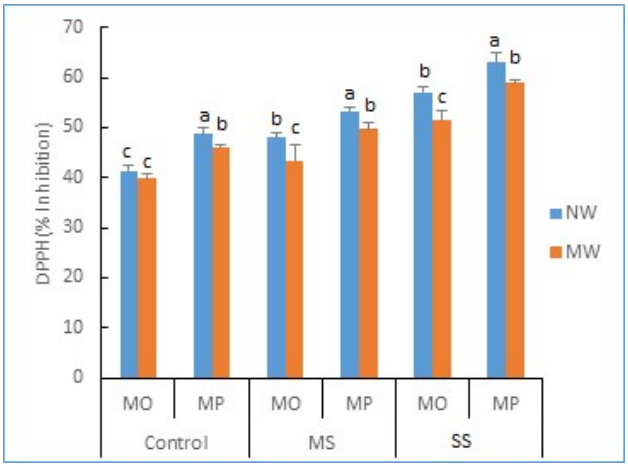

Figure 1. DPPH(% Inhibition) of the two *Moringa* species (*Moringa oleifera*, MO*;Moringa peregrina,* MP) with two treatment (Normal water, NW; Magnetic water, MW) in the different irrigation regimes(Control, MS, SS).Dissimilar letters with mean are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test.

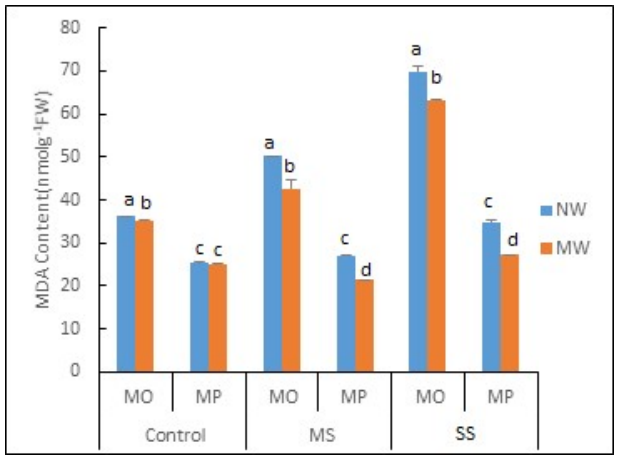

Figure 2. MDA content of thetwo *Moringa* species (*Moringa oleifera*, MO*; Moringa peregrina,* MP) with two treatment (Normal water, NW; Magnetic water, MW) in the different irrigation regimes (Control, MS, SS). Dissimilar letters with mean are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test.

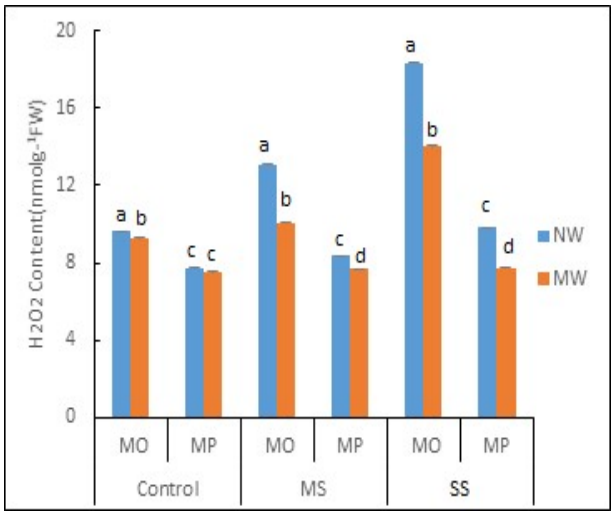

Figure 3.H_2O_2 content of thetwo *Moringa* species (*Moringa oleifera*, MO*; Moringa peregrina,* MP) with two treatments (Normal water, NW; Magnetic water, MW) in the different irrigation regimes (Control, MS, SS). Dissimilar letters with mean are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test.

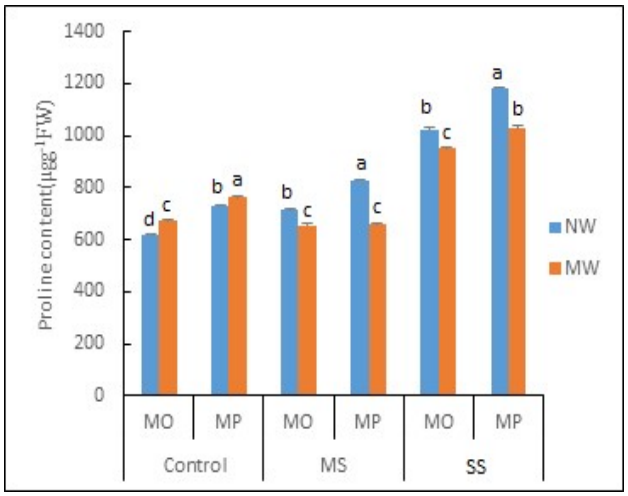

Figure 4. Changes of proline content of the two *Moringa* species (*Moringa oleifera*, MO*; Moringa peregrina,* MP) with two treatments (Normal water, NW; Magnetic water, MW) in the different irrigation regimes (Control, MS, SS). Dissimilar letters with mean are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test.

Table 1. Effects of drought stress on the total phenolic (TP) and flavonoid content (TF) under magnetic water treatment (MWT) in the different irrigation regimes: 100% FC (Control), 50% FC (MS), 20% (SS).

Species	Treatments	Total phenolic content (TP) (mg/gallic acid/gFW)	Total Flavonoid content (TF) (mg/ catechin /g FW)
Moringa oleifera	Control±NW	6.45±0.11^{d}	3.24±0.017^{e}
	Control±MW	6.7±0.15^{c}	3.33±0.009^{d}
	MS±NW	7.73±0.01^{b}	3.87±0.06^{b}
	MS±MW	6.86±0.04^{c}	3.16±0.1^{f}
	SS±NW	9.24±0.01^{a}	4.28±0.05^{a}
	SS±MW	7.88±0.09^{b}	3.69±0.02^{c}
Moringa peregrina	Control±NW	7.95±0.06^{d}	3.84±0.03^{e}
	Control ±MW	8.21±0.007^{cd}	3.97±0.04^{d}
	MS±NW	9.19±0.02^{b}	4.23±0.02^{c}
	MS±MW	7.68±1.12^{d}	3.45±0.03^{f}
	SS ±NW	11.28±0.05^{a}	5.02±0.06^{a}
	SS ±MW	8.88±0.1^{bc}	4.53±0.03^{b}

Means within each column followed by the same letter are not significantly different at level $P \leq 0.05$.

DISCUSSION

Drought stress affects the total phenolic compounds and flavonoids content (Quan *et al.*, 2016). In our study, phenolic and flavonoids content were increased significantly in both of the species under drought stress (Table 1). Similar studies were reported by some of the authors in many of the crops like *Hypericum brasiliense*, *Silybum marianum* and *Pisum sativum* (De Abreu and Mazzafera, 2005; Selmar and Kleinwächter, 2013).

Under drought stress condition, the accumulation of soluble carbohydrates in the plant cells were attributed by the phenolics and flavonoids due to the reduction of soluble sugar transportation (Ibrahim and Jaafar, 2011; Jaafar *et al*., 2012). Phenolic compounds were biosynthesized with shikimic and malonic acid pathways and responsible for the carbohydrates precursors into amino acid (Ghasemzadeh *et al*., 2010).

The rising of phenolic compounds are highly related to the between carbohydrate sources and sinks (Jaafar *et al*., 2012). MWT rearranges the water molecule, generally each made up of six ordered molecules. This tiny and hexagonal structure can easily move in passageways in plant cell membrane (Ali *et al*., 2014). MW particle might be helpful for transporting the soluble sugars resulting the decreases the accumulation of phenolic compounds under drought stress.

M. peregrina had higher radical scavenging capacity (higher antioxidant capacity) as compared to *M. oleifera* (Figure 1). Generally, Drought tolerant plants have higher antioxidant capacity than drought sensitive plants (Herbinger *et al*., 2002; Lin *et al*., 2006). Antioxidant improves the plant defense system by protecting the cells from free radicals (Espinozaa *et al*., 2013). Higher antioxidant capacity under drought stress in both of the species were minimized by the MWT (Figure 1). Cakmak *et al*. (2012) reported that the static magnetic field (MF) with low intensities had an effect on the antioxidant system in the plants leaves. The magnetic field (MF) is involved in antioxidant mediated reactions in the apoplast, which plays an important role in overcoming a redox imbalance.

MDA and H_2O_2 content significantly increased in the *M. oleifera* as well as *M. peregrina* under drought stress, possibly due to insufficient antioxidant defense system. The similar outcome was found in *Achillea* species (Gharibi *et al*., 2016). The free radicals were scavenged by the natural antioxidants and antioxidant enzymes (Jain *et al*., 2004). The MWT seedlings exhibited the lower MDA and H_2O_2 content as compared to NW treated seedlings under drought stress condition. Chen *et al*., (2011) reported that the MF helps to lowering the MDA and H_2O_2 content during cadmium stress condition that supported our outcome.

Proline content was increased with the rising up the drought level in both of the species (Figure 4).The accumulation of proline content in plants is the sign of stress initiation (Rampino *et al.*,2006). Application of MW in both of the species counteracted the adverse effects of drought stress, may be due to the decrease of osmotic stress. Higher amounts of proline under drought stress with (MWT) might also have a beneficial role in decreasing oxidative damage.

Conclusion: Magnetized water significantly assisted to alleviate the drought stress on phenol, flavonoid, proline

content and antioxidant activity of the *Moringa* species. It could be used to enhance the yield of *Moringa* production by mitigating the drought stress.

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