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# LEAF GAS EXCHANGE, Fv/Fm RATIO, ION CONTENT AND GROWTH CONDITIONS OF THE TWO *MORINGA* SPECIES UNDER MAGNETIC WATER TREATMENT

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**Abstract**

The current greenhouse experiment investigates the role of magnetic water on the two *Moringa* species (*Moringa oleifera* and *Moringa peregrina*). Both species were exposed to the magnetic field (30 mT). The magnetic water increased the plant height, leaf number, leaflet number, and internode distances in both the species, respectively. Relative water content (RWC) and leaf area in both the species showed changes under magnetic water treatment. The results showed in magnetic water treatment, the leaf gas exchange parameters such as assimilation (A), stomatal conductance (gs), transpiration rate (E), and vapor pressure deficit (VPD) were increased. Similarly, Photosynthetic pigments (Chl a, Chl b, Chl (a+b), Carotenoids), photosynthetic water use efficiency (WUE) were also increased significantly. Magnetized water had also significant effects on the maximal efficiency of PSII photochemistry (Fv/Fm). Our study suggested that magnetic water treatment could be used as an environment-friendly technology for improving the growth and physiology of *Moringa* species. In addition, this technology could be further incorporated into the traditional methods of agriculture for the improvement of crop plants, particularly in the arid and sub-arid areas of the world.

**Key words:** Relative water content, Leaf area, Water use efficiency, Photosynthetic pigments, Magnetic water treatment (MWT).

## Introduction

Currently, farms and other agricultural techniques are far sophisticated than what was a few decades ago, surely due to the improvements in technology e.g., the use of sensors, different types of machines and better use of information. Such great improvements have allowed better precision in measurements as well as businesses to be more successful in terms of profit, efficiency, safety and eco-friendly manners. Magnetic water technology (MWT), though not so common, is one of the successful environment-friendly advanced application for the improvement of agricultural yields (Ali *et al*., 2014). MWT methods have displayed potential use over the years in many disciplines, in-particular agriculture, which has several advantages over traditional water treatment methods. For example, it is safer and more compatible with simple design. Using MWT methods in agriculture would mean improved irrigation, increased crop production and quality, enriched soil and water saving (Ali *et al*., 2014).

Generally, Magnetic water is generated by treating water with different types of magnetic fields (mT).Water passes through the specialized type of machine and it creates a special form of water (Kronenberg, 1993). Many reports have indicated that MWT affects the molecular and other physicochemical properties of water (Cai *et al*., 2009). This would be due to alteration of water nucleus under magnetic treatment (Gehr, 1995; Coey *et al*., 2000; Cai *et al*., 2009). In agriculture, magnetic treatment on irrigation water increases the number of crystallization centers and affects the free gas content, which is known to improve water quality (Bogatin,1999). Many studies have shown that magnetic field has significant effects on the germination of seeds, plant growth, and yield (Martínez *et al*., 2000; Carbonell *et al*.,2000; Souza *et al*., 2005). In radish seedlings, MWT raises the plant metabolism in provisions of water uptake and photosynthesis (Yano *et al*., 2004), it enhances nutrient movement in soil, and uptake of some ion content (P, Fe, and N) by plants (Ali *et al*., 2014). Many studies have revealed that it has a helpful outcome on the activity of the enzyme, photochemical and respiration ratio (Phirke *et al*., 1996; Martinez *et al*.,2000; Carbonell *et al*., 2002). Magnetic water treatment (MWT) increases transpiration, stomatal conductance, respiration during different growth stages of plants. In corn plants, photosynthesis rates have improved under MWT compared to normally irrigated water (Anand *et al*.,2012). Permanent magnetic field induces substantial changes in the fluorescence spectra and leaf temperature (Jovanic & Sarvan, 2004).The fresh weight (FW) of plants increases with the substantial increase of the magnetic field. In corn plants, the highest fresh weight has been obtained by increasing magnetic field to 125 or 250mT (Florez *et al*., 2007). Magnetic water helps in increasing the chlorophyll content substantially (Namba *et al*.,1995; Atak *et al*., 2003; Qados & Hozayn, 2010; Hozayn *et al*., 2014).

*Moringa* species are commonly used as a source of food and medicine (Olson, 2002).The leaves of *Moringa* have antibiotic and antithelmintic activities. These are used as detoxifier and for water purification in some countries (Thilza *et al*., 2010). About thirteen (13) species of *Moringa*, belonging to the family Moringaceae, have been documented so far. *Moringa oleifera* and *Moringa peregrina* are the two most commonly used species. This research study was carried out to assess the impact of MWT on the growth and physiology of these two *Moringa* species and to develop the cost effective and eco-friendly tactics for the crop improvement.

## Materials and Methods

**Experimental material and design:** The research was carried out at 9 m long and 4m wide greenhouse of King Abdul-Aziz University, Jeddah, Saudi Arabia. *Moringa* *oleifera* Lam. and *Moringa peregrine* Forssk. ex Fiori. seeds were collected from the Abha region, Saudi Arabia and their authenticity was confirmed by taxonomists at King Abdulaziz University Herbarium (KAUH), Jeddah. A special magnetic instrument (model A150d magnetic technologies L.L.C) was used with power 30mT for magnetizing the water. The seeds of the both species were transferred to the greenhouse and sown in pots filled with sandy loam, mixed with peat moss and compost (1:1:1). The experiments were set up under randomized completely block design with 7 replications. All seeds were sown on the same day. The emergence of seeds was considered when the exposing radical reached about 2mm.The duration of the experiment was for 70 days for an analysis of the growth parameters and physiology.

**Growth parameters:** Growth parameters were précised by the analysis of plant height, leaf number, leaflet number, internode distances, fresh weight and dry weight**.** All these were measured every week until harvest time, from the surface to the top of the plant. At the harvest day, plants were cut from the soil surface, stem and leaves were separated and fresh weight was determined. Two leaves were taken from each plant, put into sealed vials, frozen in liquid $N_2$ and then stored in a freezer preset at -80ºC. Roots were collected carefully by removing the soil from the pot and washing with tap water, these were dried with a thick tissue, weighed and collected in bag paper. For dry weight measurements all plant samples were oven dried at 65ºC for 72h.

**Leaf area and relative water content:** The leaf area of the two species was measured with the help of Leaf Area Meter (LICOR-3000A, USA). For measuring the relative water content, leaf discs were taken and fresh weight (FW) weight recorded. The discs were floated on the deionized water in a petri dish for 8 h in dark. After drying excess surface water with paper towels, turgid weights (TW) were attained. The samples were finally dried at 80°C for 48 h to record the dry weight.

Relative Water Content calculation was done using the following formula:

$$\text{Relative water content, RWC (\%)} = \frac{FW - DW}{TW - DW} \times 100$$

**Leaf gas exchange:** Leaf gas exchange was measured by CIRAS III photosynthesis system (model 2012 inc.USA).The leaf gas exchange parameters included assimilation rate ($A, CO_2 m^{-2} s^{-1}$), stomatal conductance (gs, mol $CO_2$ m $^{-1}$ $s^{-1}$), transpiration rate (E, mmol $CO2m^{-2}s^{-1}$),vapor pressure deficit (VPD, kPa) and water use efficiency (WUE, $\mu mol CO_2 mmol^{-1} H_2O$). The young, fully developed and healthy leaves were selected for the measurement. The leaf was measured between 12:00 and 14:00h (when solar radiation is at maximum intensity) at ambient condition. Three readings from each treatment were taken carefully using randomly chosen leaves.

**Fv/Fm:** The maximum quantum yield of PSII photochemistry (Fv/Fm) was measured during the experiment at altered period and at the end of relief on the same leaves used for gas exchange measurements. The leaves of both the species under study were measured after dark adaption of leaf for 30 min with leaf cuvette. CIRAS III photosynthesis system (model 2012 inc.USA) machine was used to generate the data.

**Photosynthetic pigments:** Homogenized leaf samples (0.5 g) were taken for measuring the photosynthetic pigments. The samples were crushed carefully using mortar and pestle. 10 milliliters (ml) of acetone (80% v/v) were added to the sample, which was followed by centrifuging at 5,000×g for 10 min. The absorbances were measured with a UV-visible spectrophotometer (UV-1900) at 663, 645 and 470 nm respectively, according to Lichtenthaler & Wellburn (1983).

**Determination of ion content:** The plant samples (root, shoot and leaves) were prepared using Humpherics (1956) method. The dried samples under study were crushed into very fine powder by a grinder, put in the digestion tubes and 1 ml Sulphuric acid ($H_2S0_4$) was added to each tube before transferring them to the sand heater. The digestion process was completed within 15-20 min until the dark colour occurs. These samples were cooled and one ml mixture of Sulphuric acid ($H_2SO_4$) and Percloric acid ($HCLO_4$) (1:1) was added and again heated for 30-40 min. When the transparent colour appeared, the distilled water was added up to 100 ml into the each sample containing tubes. The total ion contents (sodium, potassium, magnesium, zinc, nickel, phosphorus, copper, manganese) were determined using Optima ICP-OECS machine (PerkinElmer Inc.,UK).

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

## Results

**Growth conditions:** Magnetic water treatment (MWT) had a statistically significant impact on the plant height of both species. (Fig. 1A). MWT with *M. oleifera* and *M. peregrina* resulted in an increased leaflet number, leaf number, and internode distances from 1$^{st}$ week to 10$^{th}$ week (Fig. 1B, C, D).The results clearly exhibited that MWT had a significant effect on the leaf area of *M. oleifera* (Fig. 2A). MWT resulted in an increased leaf area of *M. oleifera* and *M. peregrina* seedlings by 4.4 percent and 10.66 percent respectively. (Fig. 2A) shows that the larger leaf area ($cm^2$) is found in *M. oleifera* than *M. peregrina* .The fresh weight of leaf, shoot and root of *M. oleifera* and *M. peregrina* was significantly increased under MWT. Magnetic water treatment (MWT) in *M. oleifera* species increased fresh weight of the root, shoot and leaf by 14.1, 22.66 and 6.4 percent respectively. Similarly, MWT caused in increased fresh weight of root, shoot, and leaf in *M. peregrina* by 22.66, 17.5, and 27.21 percent (Table 1). The lateral root number increased in *M. oleifera* and *M. peregrina* by 17.2 and 6.2 percent under MWT (Table 1).The root texture of *M. peregrina* was slightly smoother than *M. oleifera*. *M. peregrina* had the lower lateral root number as compared to *M. oleifera* (Table 1).

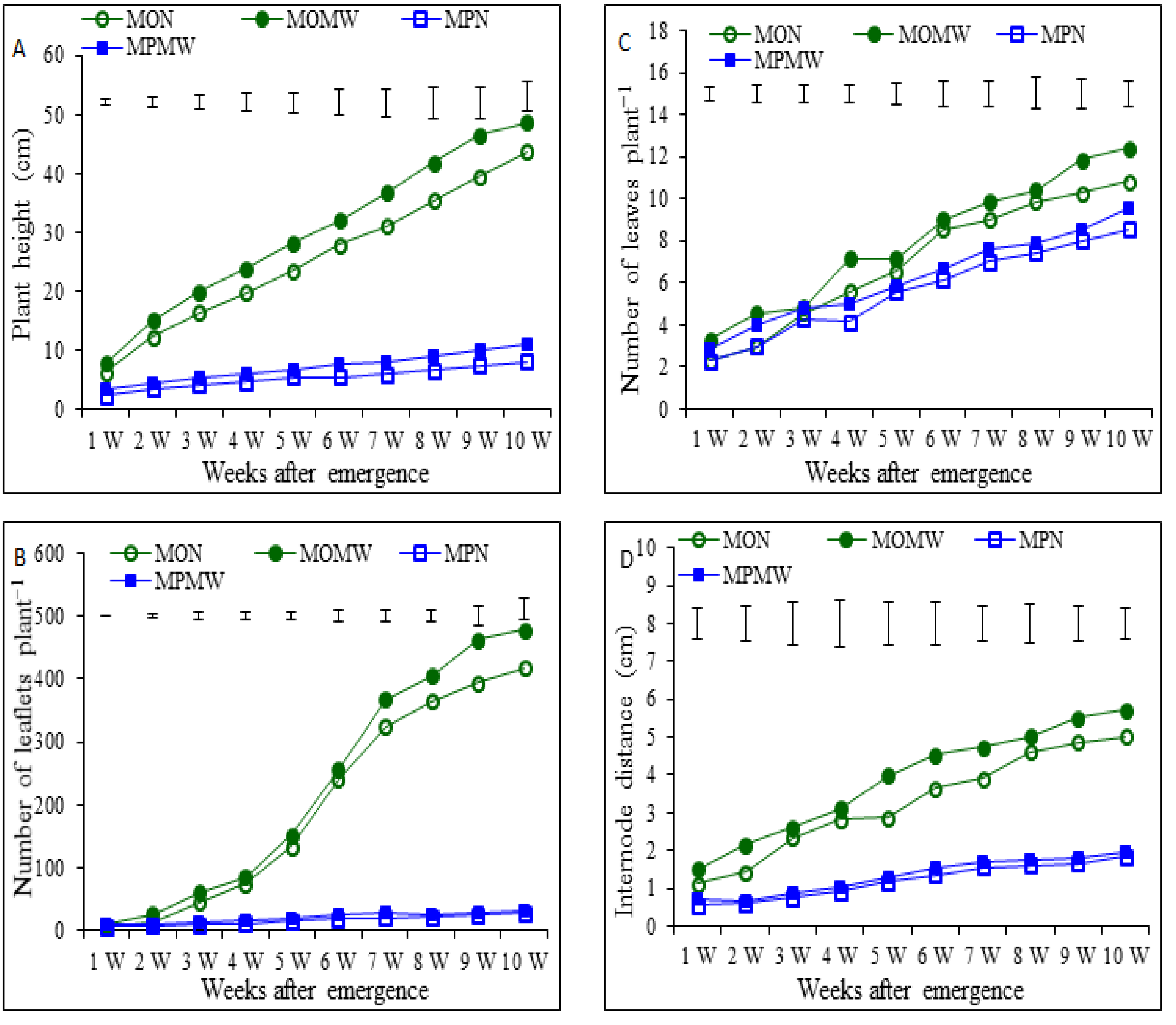


Fig. 1. Interaction effects between species vs. water (Normal water, N; Magnetic water, M) on the Plant height (A), Number of leaflets (B), Number of leaves(C) and internode distances (D) of *Moringa oleifera* (MO) and *Moringa peregrina* (MP) from emergence to harvest time;1 W(week)-10 W(week).Vertical bars at top figure represent LSD value at the p≤0.05 level.

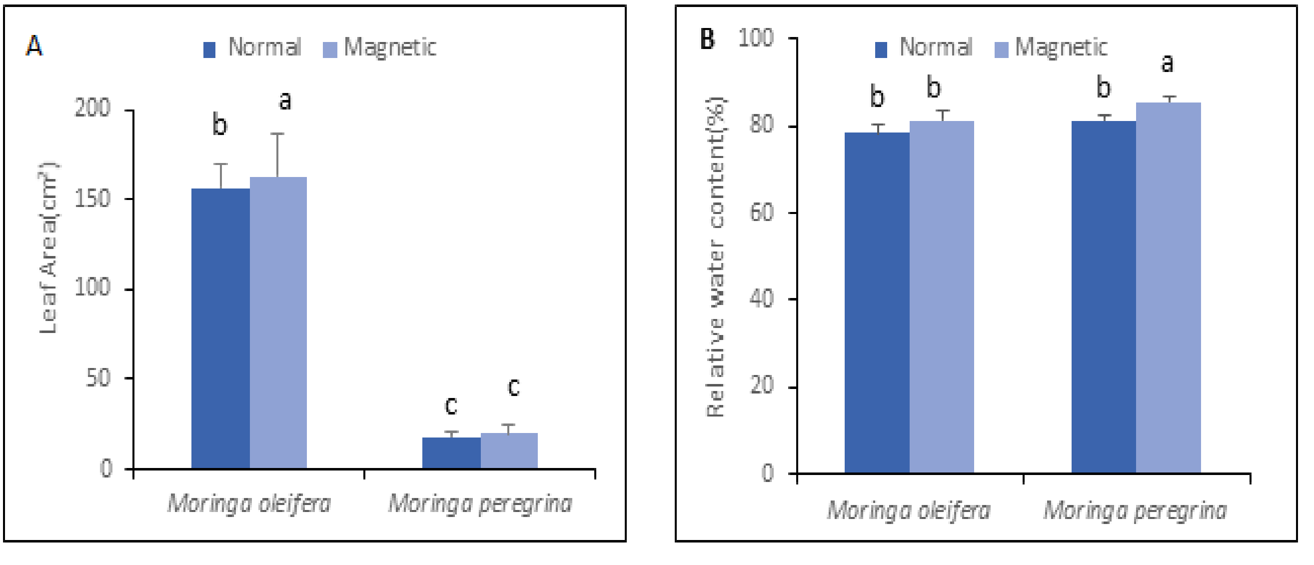


Fig. 2. Leaf area (A) and Relative water content (B) of the two *Moringa* species at harvest time. Dissimilar letters with mean are significantly different at p≤0.05 level of significance by applying Fisher's LSD Test.

**Table 1. The interaction effect between two *Moringa* species vs. water on fresh weight and dry weight of root, stem, leaf, lateral root number.**

| Treatment combinations | Root | | Shoot | | Leaf | | Lateral root number |
|---|---|---|---|---|---|---|---|
| | FW | DW | FW | DW | FW | DW | |
| MON | 11.12 ± 7.87a | 2.6 ± 1.8ab | 11.8 ± 5.2a | 2.7 ± 1.3a | 22.94 ± 2.49b | 3.84 ± 0.4a | 29.35 ± 4.45b |
| MOMW | 12.96 ± 4.7a | 3.02 ± 1.08ab | 13.8 ± 5.4a | 3.2 ± 1.2a | 25.31 ± 1.97a | 4.17 ± 0.7a | 35.45 ± 5.1a |
| MPN | 11.27 ± 6.3a | 2.02 ± 1.07b | 0.8 ± 0.3b | 0.1 ± 0.01b | 4.38 ± 0.6c | 0.78 ± 0.1b | 6.66 ± 0.89c |
| MPMW | 14.5 ± 4a | 3.49 ± 0.97a | 1.1 ± 0.4b | 0.2 ± 0.12b | 5.3 ± 1.4c | 1.02 ± 0.2b | 7.12 ± 1.8c |

Dissimilar letters within mean and between columns are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test

**Table 2. The interaction effect between two *Moringa* species vs. water on the ion content.**

| Treatments combinations | Root (ion content, mg/L) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ca | Mg | Ni | P | Zn | Mn | Cu |
| MON | 14.56±0.01d | 8.4±0.001d | 0.15±0.002c | 14.2±0.005d | 0.89±0.0005c | 0.09±0.000d | 0.05±.005c |
| MOMW | 20.54±0.01b | 17.56±0.02a | 0.18±0.0005a | 15.73±0.2c | 1.09±0.005a | 0.14±0.002b | 0.2±0.004a |
| MPN | 20.38±0.01c | 9.81±0.001c | 0.13±0.0005d | 15.86±0.5b | 0.69±0.001d | 0.13±0.001c | 0.03±0.0005d |
| MPMW | 23.21±0.001a | 14.07±0.001b | 0.17±0.0005b | 17.86±0.5a | 0.9±0.001b | 0.18±0.001a | 0.07±0.004b |
| **Treatments combinations** | **Shoot (ion content ,mg/L)** | | | | | | |
| | **Ca** | **Mg** | **Ni** | **P** | **Zn** | **Mn** | **Cu** |
| MON | 36.82±0.02d | 14.12±0.005c | 0.09±0.001d | 19.45±0.01d | 0.85±0.0005b | 0.16±0.001c | 0.02±0.001d |
| MOMW | 37.74±0.02c | 13.82±0.02d | 0.1±0.001c | 19.7±0.005c | 0.8±0.001c | 0.1±0.001d | 0.06±0.001a |
| MPN | 55.84±0.005b | 19.2±0.02b | 0.33±0.001a | 23.8±0.01a | 1.44±0.0.01a | 0.24±0.001b | 0.046±0.001b |
| MPMW | 73.24±0.001a | 25.63±0.03a | 0.24±0.001b | 22.2±0.01b | 0.76±.01d | 0.4±0.001a | 0.043±0.001c |
| **Treatments combinations** | **Leaf (Ion content, mg/L)** | | | | | | |
| | **Ca** | **Mg** | **Ni** | **P** | **Zn** | **Mn** | **Cu** |
| MON | 60.6±0.04d | 17.32±0.01d | 0.24±0.001c | 28.2± 0.01b | 0.93±0.001b | 0.74±0.003b | 0.03±0.001b |
| MOMW | 66.2±0.02c | 20.94±0.1c | 0.08±0.001d | 36.24±0.03a | 1.4±0.03a | 0.4±0.002d | 0.01±0.005c |
| MPN | 67.19±0.01b | 23.07±0.01b | 0.25±0.05b | 23.88±0.01c | 0.53±0.001c | 0.5±0.0005c | 0.01±0.005c |
| MPMW | 103.4±0.4a | 32.02±0.01a | 0.41±0.001a | 14.22±0.1d | 0.93±0.001b | 0.9±0.006a | 0.05±0.001a |

Dissimilar letters within mean and between columns are significantly different at p≤0.05 level of significance by applying Fisher's LSD Test

**Relative water content (RWC):** Relative water content (RWC) increased in *M. oleifera* and *M. peregrina* seedling by 3.61 percent and 5.03 percent under MWT (Fig. 2B).

**Leaf gas exchange:** The assimilation (A), stomatal conductance (gs), transpiration rate (E) and vapor pressure deficit (VPD) increased significantly at 40(DAS) under MWT in *M. oleifera* and *M. peregrina* (Fig. 3). However, at 55 DAS, assimilation rate (A) significantly increased in *M. oleifera* whereas, remaining parameters did not alter significantly in both the species. At 70 DAS, only the stomatal conductance increased significantly in *M. peregrina* under MWT.

**Photosynthetic pigments:** *M. oleifera* seedlings with MWT resulted in increased chl a, chl b, chl (a+b) and carotenoid content by 43, 52, 45 and 41 percent respectively as compared to normal water treated plants. In *M. peregrina*, the chl a, chl b, chl (a+b) and carotenoid contents increased by 17, 1.5, 11 and 13 percent individually (Fig. 5).The higher amount of chlorophyll pigments were observed in the *M. peregrina* as compared to *M. oleifera*.

**Water use efficiency and Fv/Fm ratio:** Photosynthetic or intrinsic water use efficiency showed a significant increase by MWT as compared to normal water treatment. *M. oleifera* had the higher water use efficiency as compared to *M. peregrina.* MWT increased the water use efficiency in *M. oleifera* by 22.1, 2, and 41.2 percent at 40 DAS, 55 DAS and 70 DAS respectively (Fig. 4A). Results in Fig. 4B show that MWT has a significant impact on the Fv/Fm ratio. MWT increased Fv/Fm ratio in *M. oleifera* and *M. peregrina* by 2.73 and 5.06 percent respectively.

**Ion content**: A marked increase in the K+ content was observed in the case of MWT as compared to normal water treatment in the leaf, stem, and root of both the species (Fig. 6). MWT decreased the Na+ uptake in the root, shoot and leaf of the two species. MWT resulted in the treatment decreased Na+/K+ ratio by 56.20, 33, 55.2 percent in the leaf, shoot and root of *M. oleifera*. It lead to a decrease in the Na+ /K+ ratio by 4.7,44,and 50 percent in the leaf , stem and root of *M. peregrine* respectively. It was found that application of magnetic water significantly increases the ion content and the high nutrients amounts of calcium, copper, magnesium, nickel, phosphorus, zinc and manganese as well (Table 2). The two species possess the large amount of calcium, magnesium and phosphorus contents in the root, shoot and leaf as compared to nickel, zinc and copper. Magnetic water treatment with *M. oleifera* and *M. peregrina* resulted in an increase in the calcium ion in root, shoot and leaf by 29.1, 2.43, 8.4 percent and 12.21, 23.75 and 35.1 percent respectively (Table 2).

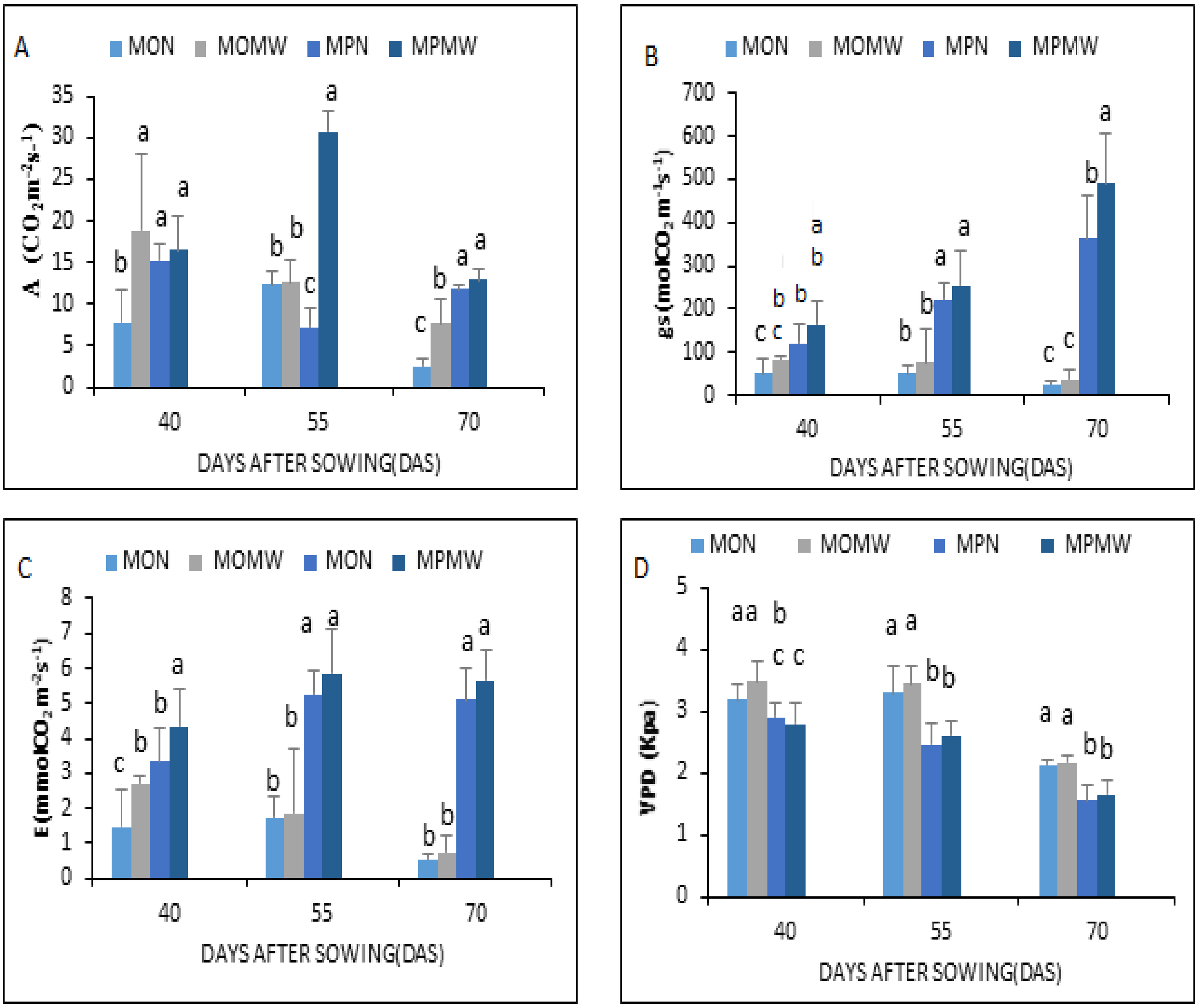


Fig. 3. Interaction effects between species vs. water on the leaf gas exchange of the two *Moringa* species (*Moringa oleifera*, MO; *Moringa peregrina*, MP) with two treatment (Normal water, N; Magnetic water, MW) in different time at 40DAS, 55DAS, and 70DAS. Days after sowing, DAS. (A)Assimilation, A ; (B) Stomatal conductance, gs ;(C)Transpiration E;(D) Vapour Pressure deficit, VPD. Dissimilar letters with mean are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test.

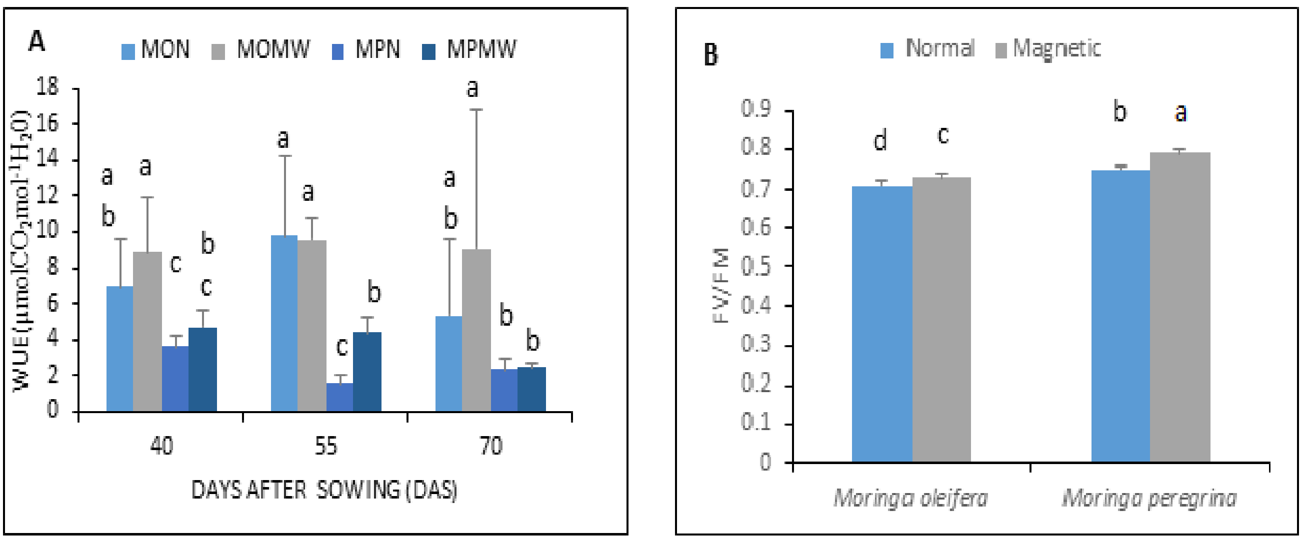


Fig. 4. Water use efficiency (WUE) (A) and Fv/Fm (B) of the two *Moringa* species (*Moringa oleifera*, MO*; Moringa peregrina*, MP) with two treatment (Normal water, N; Magnetic water, MW).Dissimilar letters with mean are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test.

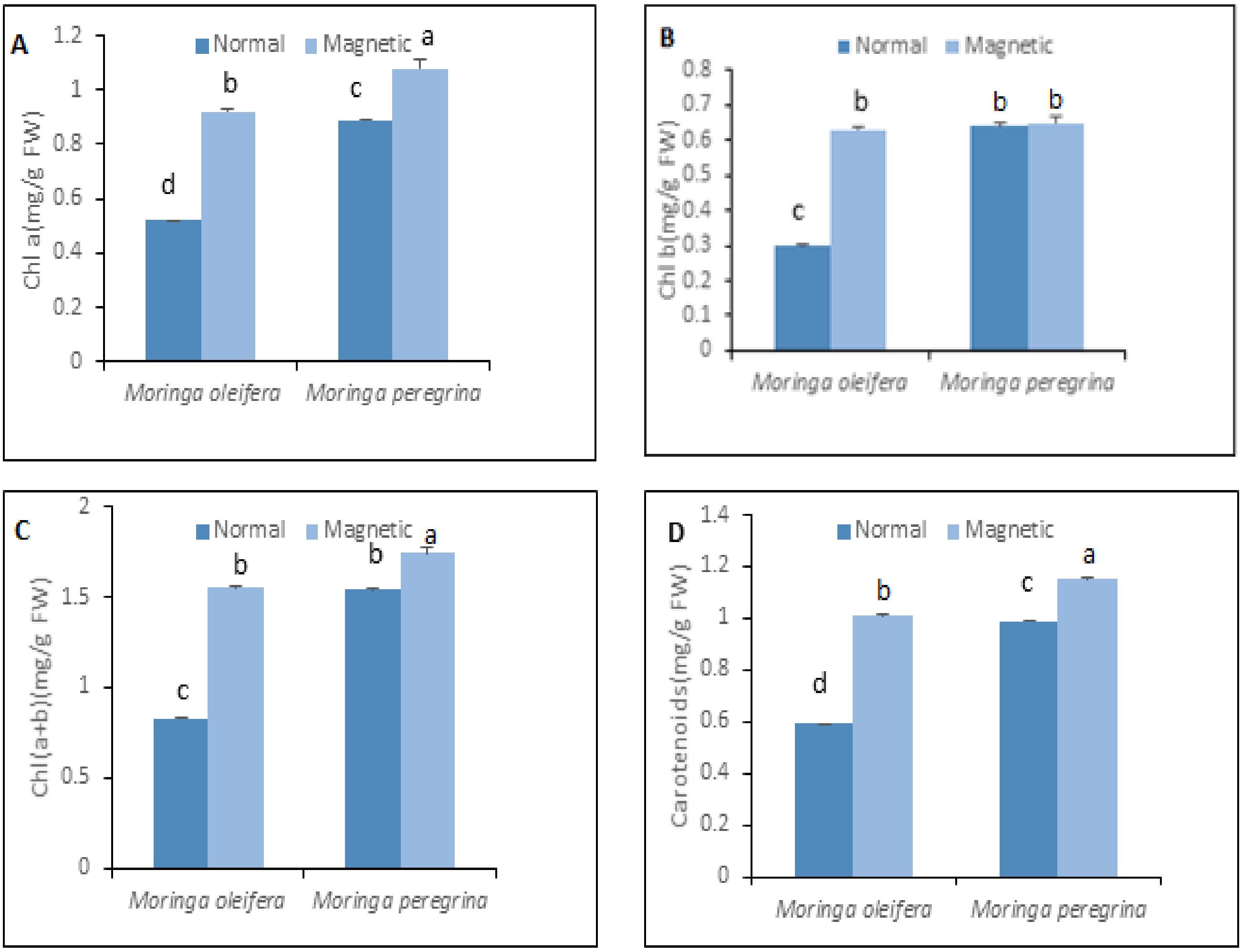


Fig. 5. Photosynthetic pigments of the two *Moringa* species under normal and magnetic water treatment. (A) Chl a, (B) Chl b, (C) Chl (a+b), (D) Carotenoids. Dissimilar letters with mean are significantly different at p ≤0.05 level of significance by applying Fisher's LSD Test.

**Discussion**

MWT as an alternative cost effective crop improvement technique is not so common in practice (Ali *et al*., 2014). However, this technique has shown some positive results in plant growth and development (Maffei, 2014). Our results revealed that plant height, leaf number, leaflet number and internode distances increased significantly under MWT, possibly by improving cell division and cell expansion. Some substantial work in *Beta vulgaris* has been reported that root and leaf yield increase under MWT, which evidently supports our findings (Rochalska, 2005 & 2008). There was an increase in the fresh weight of *Moringa* seedlings, similar result have been reported for *Helianthus annuus* under MWT (Fischer *et al*., 2004).The lateral root number of *M. oleifera* increased more as compared to *M. peregrina*. Similar outcome has been recorded in *Dioscorea opposite* under MWT (Li, 2000). Leaf area of *M. oleifera* is significantly influenced by the MWT, probably due to the larger and increased number of cells supplied by leaf meristems. El-Yazied *et al*. (2011) has reported that MWT increases the leaf area in the tomato which also supports our results.

Relative water content (RWC) increased significantly in *M. peregrina* under MWT. Leaf relative water content was higher under MWT as compared to normal water treatment; perhaps it accelerates the swelling pressure in plant cells, which leads to increase in the plant growth. The increase or decrease of RWC is probably related to the assimilation, intercellular $CO_2$ concentration, stomatal conductance and transpiration. Similar results have been reported in the *Simmondsia chinensis* by Al-Khazan *et al*. (2011).

Assimilation (A), stomatal conductance (gs), transpiration rate (E) and vapour pressure deficit (VPD) increased simultaneously due to MWT in both the species. In corn plants, Anand *et al*. (2012) reported that photosynthesis and stomatal conductance increased at 200 mT. Perhaps, the chlorophyll pigments increased in all species and are responsible for improving the photosynthesis rate under MWT. The higher photosynthetic water use efficiency was found under MWT in both the species, which possibly helps to increase the water use efficiency by improving the opening of stomata.

The improvement of chlorophyll fluorescence in *M. oleifera* and *M. peregrina* may be due to the high-efficiency use of radiation in the photosynthesis and because no damage occurs in the Photosystem II under MWT. Magnetic water probably helps to alter the photochemical efficiency of photosystem II in the two *Moringa* species. . The Fv/Fm value was modified using radiations by carbon assimilation reactions.

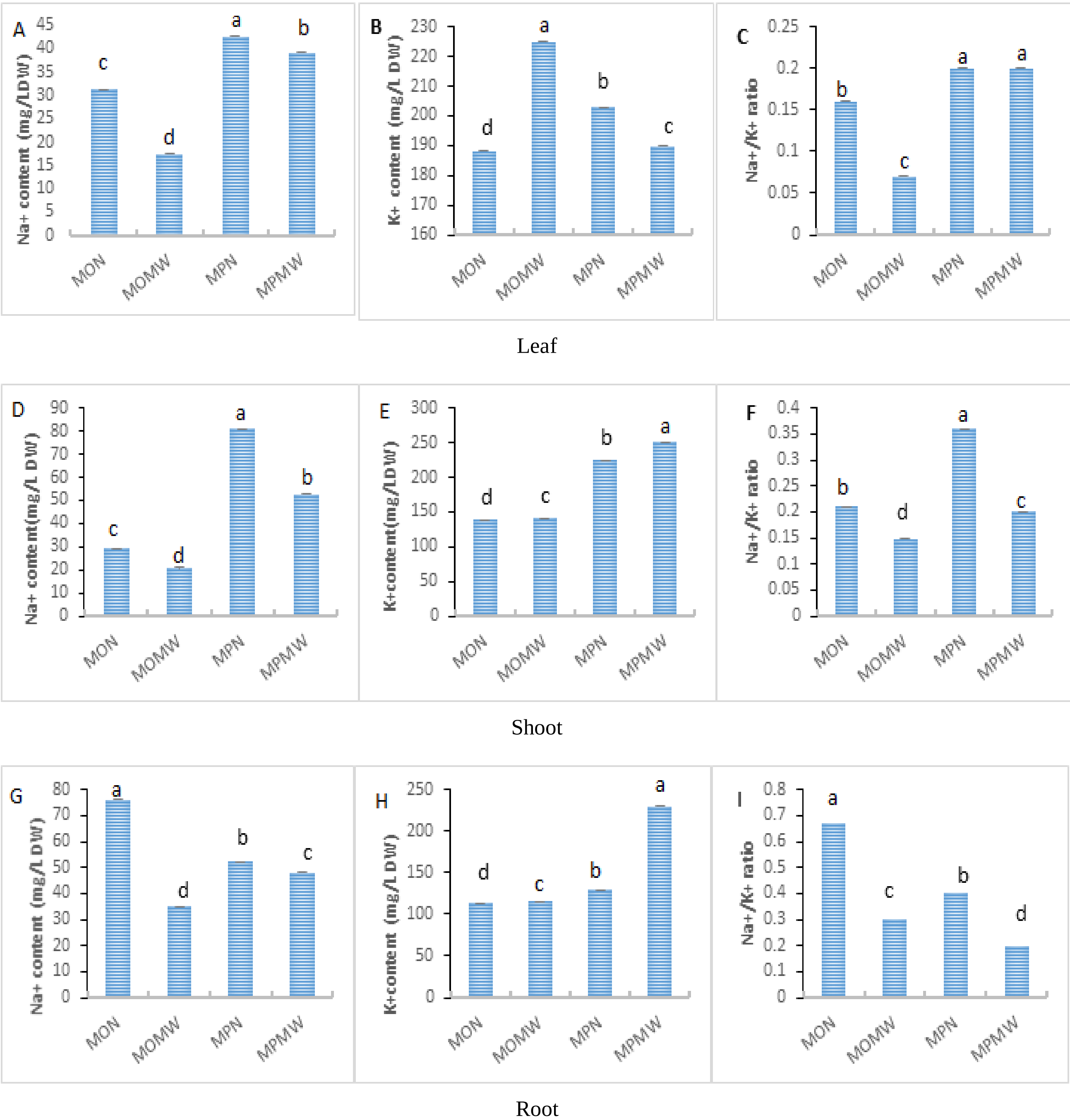


Fig. 6. Interaction effects between species vs. water to the $Na^+$ and $K^+$ content and their ratio in leaf, shoot, and the root. Dissimilar letters with mean are significantly different at p≤0.05 level of significance by applying Fisher's LSD Test.

The chlorophyll content increased significantly due to MWT in both the species, as reported for some other plant species as well (Rochalska, 2005; Turker *et al*., 2007; Radhakrishnan & Kumari, 2013).The amount of essential elements required for photosynthesis reaction is high and perhaps, the magnetic water helps to increase a number of essential elements which help to generate more chlorophyll content. Magnesium is significant essential element found in the chlorophyll molecule center, it leads to generate more plant growth (Bohn *et al*., 2004).

We found that MWT helps in lowering the accumulation of $Na^+$ ion and Na+/K+ ratio in both *M. oleifera* as well as *M. peregrina*. A higher accumulation of $Na^+$ in the roots, shoot and leaf results in a disruption in the ion homeostasis. Possibly, MWT helps to open stomata while sunlight, $CO_2$ enter to the cell and $O_2$ is released during the photosynthesis process and $Na^+$ and $K^+$ homeostasis help to stabilize and regulate the internal systems of the *Moringa* species and alter their internal systems adaptation to environmental situations.

In our study, a higher amount of the ions was found with MWT except sodium. Perhaps, magnetic flux helps to speed up cells to intake ion content. Here, some elements were diamagnetic which is prevented by the magnetic field. It has been regarded that the Sodium is paramagnetic element and small and positive susceptibility to magnetic field has been described by Nave (2008).

## Conclusion

Our findings have shown that an irrigation with magnetic field plays significant role in *Moringa* species in the production. It may help to improve the production of other crops as well. Yet, more studies are needed to ensure the crop production using magnetic water treatment under field conditions under different levels of magnetic field.

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