

Morphological and Physiological Responses of *Cucumis sativus* L. to Water with Micro-Nanobubbles

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ABSTRACT

The scarcity of water, along with the concern of safe production of food, emphasizes the need for new agricultural techniques. Increasing dissolved oxygen concentration in water promotes the growth of plants in many ways. The aim of the present research was to investigate how cucumbers (*Cucumis sativus* L.) morphologically and physiologically respond to water enriched with air Micro-NanoBubbles (MNBs) as an oxygen saturating measure. The plants from early stage of seed planting (two groups, 32 plants in each) were cultured either with air-nanobubbles water or with tap water for 12 weeks, and the steric stability of MNBs in water was confirmed through zeta potential measurements (-20.47 mV). The number of blossoms in the plants irrigated by air MNBs water was almost 3.8 times more than the number of blossoms in those that were irrigated by tap water. MNBs water increased leaf area up to an average of 77%. Physiological indices such as chlorophylls a, b, and carotenoids were, respectively, 1.34, 1.44, and 1.35 times greater in the plants watered with MNBs than those with tap water. Overall, this study demonstrated that water with air micro-nanobubble had a positive effect on cucumber plants and is potentially an effective tool for the environmental friendly, economical, and profitable production of the plant.

Keywords: Air micro-nanobubble, Carotenoid, Chlorophyll, Cucumber, Plant growth.

INTRODUCTION

The current water crisis encourages researchers to find safe, achievable, and economical methods for agricultural production; nevertheless, using chemicals to enhance the agricultural production has received a negative feedback due to the adverse effects of those chemicals on the environment and human health. Finding a way to enhance the morphological and physiological properties of plants by lowering or limiting the use of chemicals with the same amount of water or even less, could be a way to face the water crisis around the world. Enrichment of water with oxygen may prove to be a key solution to the process of growing more agricultural

product with the same amount of water (Yoshida *et al.*, 1996; Park and Kurata, 2009).

Micro-NanoBubbles (MNBs) are micro-nano sized fluids that form in a liquid media, the various aspects of these bubbles have been studied and given much importance during recent years. MicroBubbles (MBs) and NanoBubbles (NBs) are characterized as having diameters of less than 50 micrometers and 200 nanometers, respectively (Agarwal *et al.*, 2011; Li and Tsuge, 2006). Small bubbles of this size show special properties such as large specific surface area and higher inside pressure that results in large gas dissolution (Bredwell and Worden, 1998). Air MNBs are able to saturate water and keep it

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saturated for a long period of time. The other significant property of micro-nanobubbles is the negative charge on their surface (Mozaffari, 2013); due to these charges, there is no possibility of the coalescence of bubbles so they remain in solution for a longer period, while macrobubbles with diameter larger than 0.1 mm, grow larger and move upwards faster, then burst on the surface (Takahashi, 2015). In other words, MNBs increase gas solubility in aquatic environments by increasing the driving force of dissolution. It was also proved that the explosion of MNBs resulted in free radical production (Ebina *et al.*, 2013).

MNB technology in industries including food processing, purification of polluted water, and oyster farming has attracted much attention (Takahashi, 2005, as cited in Park, 2009). Successful application of MNBs in biological processes helped to improve aeration processes in irrigation systems for agricultural purposes and accelerated physiological activities in living organisms (Endo *et al.*, 2008). Also, a better quality in size and taste of farming oysters was achieved using air MBs (Onari, 2001). Using MBs in hydroponic solutions for growing lettuce could increase the size of the plant by 2.1 times (Park and Kurata, 2009). Ebina *et al.* (2013) studied the effects of air MNBs on the growth of plants, fish, and mice. In their research, *Brassica campestris* was hydroponically cultured for 4 weeks, in both air-nanobubble water and tap water, sweetfish for 3 weeks, and rainbow trout for 6 weeks. Also, 5 weeks old male mice were bred with either oxygen-nanobubble water or tap water for 12 weeks. Results showed that the use of air-nanobubble water significantly promoted the height (19.1 vs. 16.7 cm) and length (24.4 vs. 22.4 cm) of the leaves, and also increased the aerial fresh weight (27.3 vs. 20.3 g) of *Brassica campestris* compared to when watered with tap water. Total weight of sweetfish increased from 3.0 to 6.4 kg in tap water, whereas it increased from 3.0 to 10.2 kg in air-nanobubble water. In addition, total weight of rainbow trout increased from

50.0 to 129.5 kg in tap water, whereas it increased from 50.0 to 148.0 kg in air-nanobubble water. Using oxygen-nanobubble water significantly promoted the weight (23.5 vs. 21.8 g) and the length (17.0 vs. 16.1 cm) of mice compared to that of tap water.

Since cucumber is a major part of foods and salads throughout the world, researches have been conducted on many aspect of cucumber production, for instance, in the effect of growing medium (Samadi, 2011), the diseases and cures (Hassanzadeh *et al.*, 2012; Anand *et al.*, 2010), and in the application of modern technologies (Pahlavan *et al.*, 2012). This research was performed to investigate the effect of Air Micro-NanoBubbles (AMNBs) on the growth of cucumber (*Cucumis sativus L.*) in greenhouse conditions. In order to distinguish the effect of AMNB water on the plants, the growth of two groups of cucumbers that were irrigated by water enriched with AMNBs or tap water were compared with each other.

MATERIALS AND METHODS

The water used to conduct this study was taken from water supply system of the City of Shahrood, Iran, for both tap water and the MNB watering. Physicochemical characteristics of tap water are shown in Table 1. The air MNBs were produced hydro-dynamically by using a micro-nanobubbles generator (Minab Toos T1A-375, Iran). The generator works based on hydrodynamic cavitation that occurs in a Venturi tube which makes the gas-liquid mixture possible. This device has three main parts: gas inlet, pressure enforcer on the liquid, and pressure drop zone. The gas inlet regulates the type and the flow rate of the gas, the pressure enforcer increases the pressure to facilitate the fast saturation of liquid by the injected gas, and the pressure drop zone forms the bubbles. As dissolved gas reaches the saturation, the additional gas attaches to the existing bubbles. In this

study, air was used as the injected gas since it is freely available, so the results of the experiment could be directly used for all kinds of users around the world. In this apparatus, fluid was introduced to the system with a turbine pump. Air valves were used to control the amount of intake air in order to supply the sufficient volume of air for the formation of bubbles while inhibiting the formation of microbubbles. Figure 1 shows a schematic of the air micro-nanobubbles generator.

Properties of MNBs, which have particle size distribution, zeta potential, mobility, and dissolved oxygen concentration in water, were measured. Particle size distribution and the number of MNBs in water were measured one day after production by Particle Size Analyzer (VASCO3, CORDOUAN, France) which is designed based on the Mai theory. This instrument works with a Dynamic Light Scattering (DLS) method and is able to measure particles in the size range of 1 nm to 6 microns. Zeta potential and mobility of air MNBs in water were measured with Zeta Potential meter (CAD Instruments) at the condition of 8.36 V/cm electric field. Also, the Dissolved Oxygen concentration (DO) of water enriched with air MNBs was measured by a DO meter (TPS smartCHEM-Lab ion meter) periodically up to 50 hours after production.

The seeds of the cucumbers (*Cucumis Sativus L.*) were obtained from Hakim Agricultural Co., Iran. They were planted in two groups, 32 plants in each group, for 12 weeks. They were planted under greenhouse conditions, i.e. daytime temperature of 25°C and nighttime temperature of 21°C, and the humidity ranged from 75 to 85%. Each of

Table 1. Physicochemical characteristics of tap water.

Parameter	Values
pH	8.15
Temperature (°C)	22-23
EC ($\mu\text{S cm}^{-1}$)	581
TDS (mg L^{-1})	274
Turbidity (NTU)	0.41
Dissolved Oxygen (mg L^{-1})	3

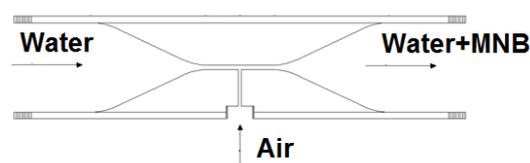


Figure 1. Schematic of micro-nanobubbles generator.

the samples was watered 48 hours to reach the saturation in soil. Both groups were watered at the same time bi-daily, either with air MNBs water or with tap water. The characteristics of the soil are presented in Table 2. The total amount of water used for irrigation was 124 liters for each group during the course of the study.

In order to assess the effects of air MNBs on plant growth, morphological parameters, namely, length of the stem, number of leaves and each leaf's width, length, number of blossoms, and the root length were measured on a weekly basis. The physiological parameters including amounts of chlorophyll a and b, and carotenoids of leaves were measured towards the end of the experiment. Physiological parameters were measured using the Arnon method with UV-VIS Spectrophotometer (JENWAY, UK). According to this method, 300 mg of the leaves were grinded with acetone of 80%

Table 2. Characteristics of the soil.

Parameter	Values	Measurement method
pH	8.73	EPA SW-846 METOD 9045
EC ($\mu\text{S cm}^{-1}$)	277	1:5 Soil to Water, EPA 2006
Organic matter (%)	2.41	Standard method 209f, (APHA, 1995)
Calcium carbonate (%)	9.03	Carver, 1971
Sand (%)	85.90	ASTM, D422
Silt and clay (%)	7.84	ASTM, D422-63



purity. This mixture was filtered with Whatman paper and the remainder of it on the filter paper was washed with the same kind of acetone. After re-filtering, its volume was increased to 15 mL. Then, chlorophyll concentration was measured with spectrophotometer at 663 and 645 nm wavelength (Shams *et al.*, 2011).

RESULTS

Characteristics of Air MNBs in Water

The concentration and stability of dissolved oxygen in air MNBs water was measured. Earlier researches on the features of MNB water reported that the amount of DO in air MNB water is more than in tap water (Ebina *et al.*, 2013; Matsuki *et al.*, 2014; Park and Kurata, 2009). The present data shows that DO concentration declined from 9 to 7 mg L⁻¹ within 10 hours of generation and remained at this level for at least 50 hours (Figure 2).

Since nanobubble stability is an important parameter for practical purposes, changes in the size distribution of bubbles was determined through time. In the DLS measurement techniques used in this research, the intensity of the scattered light from the media was detected and based on Mai theory, the intensity of each frequency translated to a specific bubble diameter. The size distribution and the volumes are secondary outcomes from the intensity distribution, thus, the ordinate of both graphs are of arbitrary unit (Mozaffari, 2013).

To guarantee the stability of air, the water sample was kept under lab conditions in an open lid container. Size distribution of air MNBs by number, volume, and intensity are shown in Figure 3. Bubble size ranged from approximately 80 to more than 1,000 nm. Average diameter of MNBs in water was 286.36 nm, while the average volume and intensity was 998.74 and 782.64, respectively. The results are similar to those reported by Takahashi (2015). Bubbles with the size of 400-700 nm had the highest

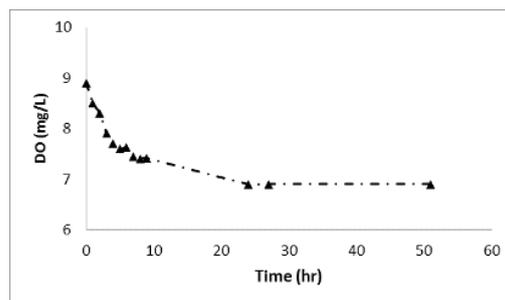


Figure 2. The solubility and stability of dissolved oxygen in air MNBs water in 50 hours.

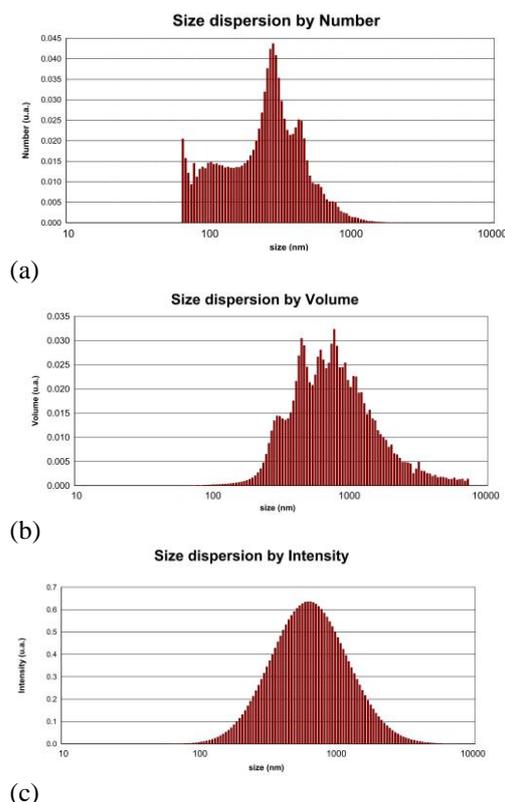


Figure 3. Size distribution of air MNBs in water 1 day after production, by number (a), volume (b), and intensity (c).

frequency, and there were also a number of bubbles smaller than 200 nm that confirmed the existence of nanobubbles. Figure 4 shows the change in distribution according to the number and the diameter of the air MNBs in water in a chronological manner. The results indicate that the average size of air MNBs were 364.24, 405.18, 441.37, and 1347.25 nm after 1, 3, 6, and 30 days, respectively.

Zeta potential and mobility of air micro-nanobubbles were measured in order to determine the stability of the MNBs in water. The zeta potential and mobility represent the stability of colloids, in this case micro-nanobubbles, in water. The mean of zeta potential was -20.47 mV (Figure 5). This value shows a steric stability for micro-nanobubbles in water. As seen in Figure 6, the mean for air MNBs mobility was $-1.54 \mu\text{m s}^{-1} \text{V}^{-1} \text{cm}^{-1}$.

Effects of Air MNBs on Morphological Parameters of Cucumber

The effects of irrigating the plant with either air MNBs water or tap water was studied. According to the average values of leaf area, number of blossoms, length of stem, and root length, a significant increase occurred in the growth of plants irrigated by air MNBs water (Figure 7).

Stem length, leaf width, and leaf length in the plants irrigated with air MNBs water were, respectively, 1.38, 1.1, and 1.05 times more than that of the plants irrigated by tap water 4 weeks after planting the seeds.

The Analysis Of Variance (ANOVA) showed that the indicators of growth for plants irrigated with MNB water significantly increased over time as presented in Figure 8, Tables 3, and 4. The leaf area was calculated by product of width and length.

In the twelfth week, the number of leaves and blossoms in the plants irrigated with air MNBs water was increased by approximately twice compared to those by tap water. In order to have a clear understanding of the results, the minima, averages and maxima for Leaf Length (LL), Leaves Width (LW), leaf numbers, and stem length are presented in Table 4. The data indicates that there were significant differences between the two types of watering as presented in Table 4.

Watering plants with air MNBs water always led to better functionality. Differences in leaf number in the groups at

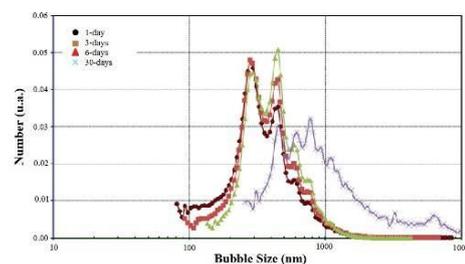


Figure 4. Sequential changes of number and diameter of generated air MNBs in water.

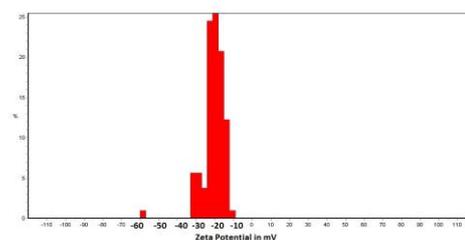


Figure 5. Zeta potential of air micro-nanobubbles water 1 day after production.

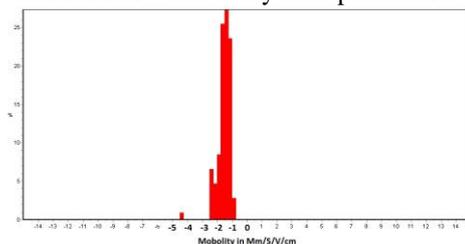


Figure 6. Mobility of air micro-nanobubbles in water, 1 day after production.

the beginning of growth cycle (0-4 weeks) were inconsiderable, while it did grow during the next 9 weeks and the effects were seen by the end of week 12, whereas those that were irrigated by tap water lost some of their leaves. The number of leaves on the plants irrigated by air MNBs water remained stable or even showed a slight increase (Figure 8).

It was clear that the number of blossoms in the plants irrigated by air MNBs water were significantly greater than those irrigated by tap water (Figure 9). The number of blossoms in each plant irrigated by air MNBs water was almost 3.8 times greater than the number of blossoms in those

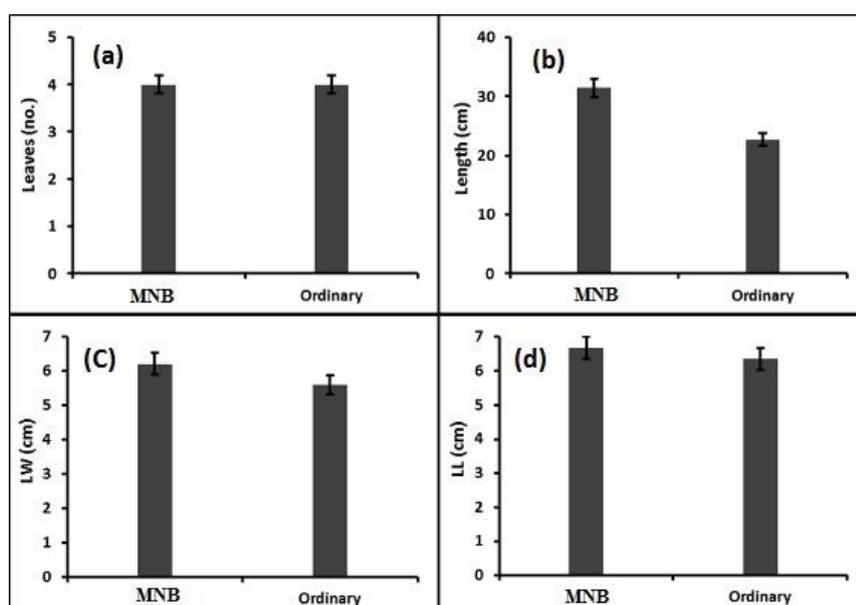


Figure 7. Leaf number (a), stem length (b), Leaf Width (LW) (c), and Leaf Length (LL) (d) of cucumber cultured for 4 weeks in greenhouse conditions with micro-nanobubbles water or with tap water.

Table 3. Analysis Of Variance (ANOVA) results on the morphological parameters of cucumber irrigated with air MNBs water or with Tap Water (TW) for a weeks. Twelve plants and 48 leaves from each group were randomly examined.

Indicator	No of samples ^a		Average		SD		Significance			
	MNB*	TW*	MNB	TW	MNB	TW	F	P	SS	MS
Stem length	12	12	31.42	22.67	5.71	4.30	17.98	0.00036	1021.46	459.37
Leaf width	48	48	6.22	5.61	1.42	1.70	3.61	0.060	240.45	8.88
Leaf length	48	48	6.67	6.36	1.83	1.52	0.83	0.365	267.73	2.3437
Leaf area	48	48	44.31	37.46	21.84	16.28	3.04	0.087	35996.2	1125.94

^a MNB= Watered by MNB water, TW= Watered by Tap Water.

Table 4. Morphological parameters of cucumber irrigated with air MNBs water or with tap water for 12 weeks.

		Leaf width (cm)	Leaf length (cm)	Leaf (no)	Stem length (cm)
MNABs Water	Min	2.6	3	12	125
	Ave	6.2	6.7	16.6	199.86
	Max	9.5	11	23	300
	SD	1.68	2.01	3.58	46.97
Tap Water	Min	2.5	3.5	5	120
	Ave	5.6	6.3	5.83	166.16
	Max	8.5	9.5	6.5	200
	SD	1.41	1.75	0.55	29.15
<i>F</i>		0.977	98.188	133.127	4.372
<i>P</i>		0.332	0.000	0.000	0.047

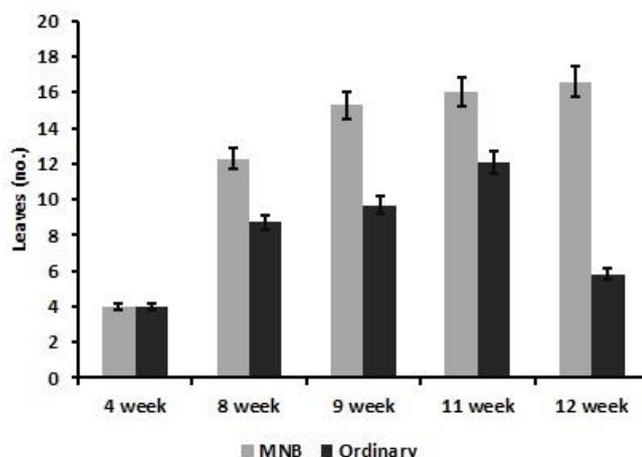


Figure 8. Sequential changes in average number of cucumber leaf irrigated with MNBs water or with tap water during growth period.

irrigated with tap water; thus, it could be economical and profitable for fruit production. As seen in Figure 9, after the eleventh week, the number of blooms in plants that were irrigated by tap water significantly declined; while those plants irrigated by air MNBs water continued to produce leaves. According to plant root measurements at the end of cultivation period (after 12 week), plants irrigated with air MNBs water had more split roots and their root hairs were longer (Figure 10).

High levels of productivity and operation can be reached when using air MNBs.

Effects of Air MNBs on Physiological Parameters of Cucumber

In addition to evaluation of plant morphological properties, some physiological features including chlorophyll a and chlorophyll b content, and the amount of carotenoids in leaves, were evaluated and

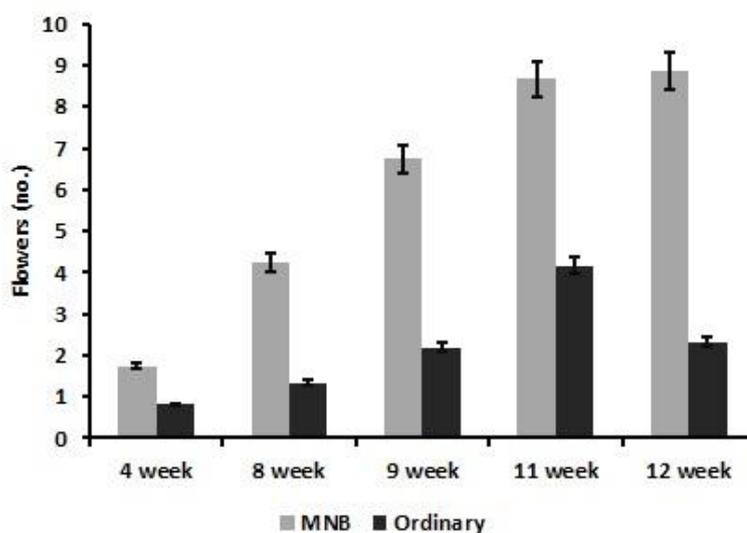


Figure 9. Sequential changes in the number of blossom on cucumber irrigated with MNBs water or with tap water during growth period.

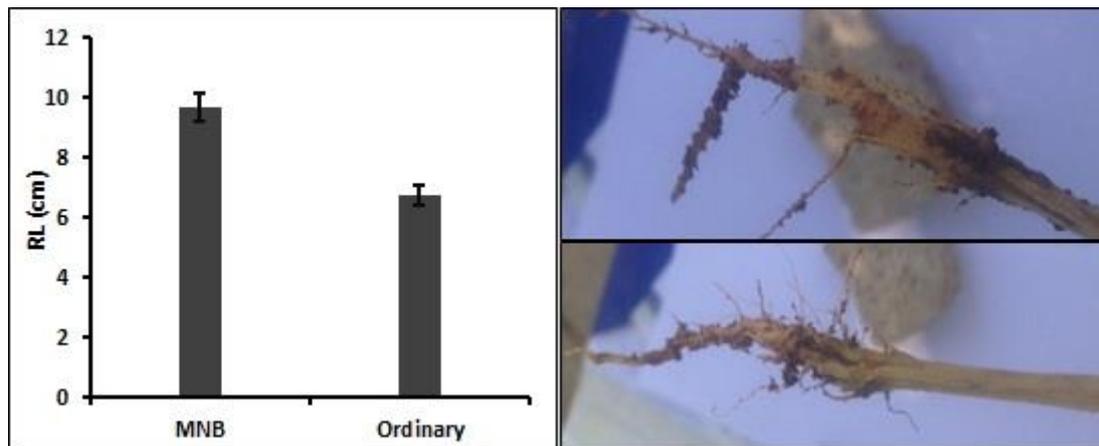


Figure 10. The growth of root of cucumber (RL: Root Length) irrigated with MNBs water (above) or with tap water (bottom) for 12 weeks.

the results are presented in Table 5.

Air MNBs water caused 1.34 and 1.44 times increase in, respectively, chlorophyll a and b in the leaf of the plant. The analysis of variance indicates a significant increase in all three photosynthesis indicators.

DISCUSSION

This is the first study performed on the effects of watering cucumber plants with water enriched with air MNBs. Previously, the effects of the concentration of dissolved oxygen in the water on cucumber were thoroughly investigated (Ehret *et al.*, 2010; Yoshida *et al.*, 1996; Asao *et al.*, 1999; Rong and Tachibana, 1997). Improvements in plant growth and physiological

characteristics after application of MNBs to water has been studied for other plant species (Ebina *et al.*, 2013; Park and Kurata, 2009; Lee *et al.*, 2014; Yoshida *et al.*, 1997; Bonachela *et al.*, 2010). The results of this study confirm the available data and provide new insights to the phenomenon. Recently, Jiang *et al.* (2016) reported that MNBs of oxygen in water could improve lettuce growth and quality, which is in agreement with the findings of the present research.

Chlorophyll is the main factor in adsorbing light energy (Lichtenthaler, 1988). Carotenoids are the pigments responsible for the color of plants and also play an effective role in photosynthesis (Young and Britton, 1993). Chlorophyll a is the main pigment in photosynthesis and chlorophyll b, which can be found in some plants, especially the green

Table 5. The concentration of photosynthesis pigments in cucumber leaves.

		Chlorophyll a	Chlorophyll b	Carotenoids
MNABs water	Min	0.7676	0.261	0.5021
	Max	0.8617	0.3393	0.5498
	Ave	0.8084	0.3044	0.5297
	SD	0.0394	0.0325	0.0201
Tap water	Min	0.482	0.1824	0.3189
	Max	0.6689	0.2353	0.4292
	Ave	0.6048	0.2117	0.3922
	SD	0.0868	0.0219	0.0518
f	56.373	71.385	89.590	
p	0.000	0.000	0.000	

ones, is another factor which helps chlorophyll a in photosynthesis. The increase of 1.34 and 1.44 fold in chlorophyll a and b, respectively, shows that using air MNBs water could be an effective factor in improving plants photosynthetic function and vegetative and reproductive growth and production. Carotenoids do not have fluorescence property outside the living material while they show such behavior in chlorophyll a in a way they adsorb the light wavelength that chlorophyll a cannot adsorb. Some researchers believe that carotenoids adsorb the light wavelength which causes photo oxidation of chlorophylls to protect them (Demming-Adams, 1990). Increasing 1.35 fold of carotenoids in irrigated plants with air MNBs water in comparison with the plants irrigated with tap water could have an effective role in increasing photosynthesis and chlorophylls protection.

There is no clear knowledge about the mechanism of improvement of cucumber growth by air MNBs water. The large surface area of MNBs in comparison to macrobubbles and the considerable increase in the capacity of air holding in water even after irrigation could be the reason for these improvements. In the presence of air MNBs, a direct supply of oxygen through a large number of small air bubbles sticking to the roots helps the plants to grow more efficiently. In addition, negative electrical surface charges of MNBs can increase the cation exchange capacity (CEC) of the plants roots and help them to adsorb more nutrients. This means that the elevated negative charged root can absorb the main growth factors with positive electrical charge nutrients, such as calcium, magnesium, and potassium, more easily from the soil (Park and Kurata, 2009; Ebina *et al.*, 2013).

In terms of the number of leaves in the first 4 weeks, the results are in agreement with Yoshida *et al.* (1996) who did not record any significant differences in the number of leaves of cucumber by change in oxygen level in water after 10 days. However, after week 4 through the rest of

the experiment in the present research, watering with MNBs promoted leaf number and area which is in agreement with Lee *et al.* (2014) who used root zone aeration (0.5 L min⁻¹ per plant) for cucumber plants. They reported that the total area of leaves, i.e. summation of the area of individual leaves, increased by applying 0.5 L min⁻¹ oxygen to the plant, while the results were inverted by addition of the oxygen to 1, 1.5, and 2 L min⁻¹ of oxygen per plant. Yoshida *et al.* (1996) reported 75% expansion of leaf area of cucumber after 10 days of watering with solution of 0.2 mM oxygen. The same trend was reported by Rong and Tachibana (1997) for both cucumber and tomato. The results of an experiment on cucumber by Holtman *et al.* (2005) showed 73% increase in leaf area after using oxygen saturated water. Lee *et al.* (2014) reported an almost identical change in cucumber growth when water was aerated at 0.5 L min⁻¹ for 65 days. In our research, by watering cucumber with MNBs, a 66% increase in leaf number and 17% expansion in individual leaves (total of 77%) was achieved. Since MNBs communicate with the ambient water through the water-bubble interface, it keeps oxygen at the ambient saturation level. This could prevent the undesirable outcome of over-aeration over the long term as Lee *et al.* (2014) reported this could result in decrease of the number and area of leaves.

In this research, stem length increased when MNBs were applied to water. Yoshida *et al.* (1996) reported that the length of stem of cucumber plants was not affected by level of DO in water. In fact, over-aeration of water could negatively affect the length of stem in cucumber (Lee *et al.*, 2014). In contrast, the current study showed that MNBs in water helped cucumber plant gain 50% more in stem height. It should be mentioned that Ebina *et al.* (2013) observed a 20 times increase in the length of stem of *Brassica compestris* when water was enriched with MNBs. The early conclusion could be that MNBs are responsible for the elongation of the stem. The matter should be investigated more fully for confirmation.



Asao *et al.* (1999) reported that the number of cucumber flowers slightly increased if water was continuously aerated instead of 15 min h^{-1} or 6 h d^{-1} , although they did not provide data for plants in non-aerated water. A similar observation was reported by Ehret *et al.* (2010) while comparing the effects of oxygen enriched water with ambient oxygen. However, Lee *et al.* (2014) reported a maximum 27% increase in the number of fruits per plant by aerating the root zone. The present research showed that MNBs are capable of enhancing the productivity of cucumber by drastically increasing the number of flowers. The number of flowers constantly increased throughout the experiment (12 weeks). It is worth noticing that this finding was not limited to the use of MNBs to water cucumber. Park and Kurata (2009) added MicroBubbles (MBs) as the oxygen source to water and used it to water *Lactuca sativa* which led to the promotion of plant growth. Oshita and Liu (2013) reported a higher germination of barley seeds if MNBs were supplied.

These findings confirm the positive effect of existence of high concentration or saturation dissolved oxygen in water for cucumber growth. It also shows that air MNBs could successfully replace ordinary aeration technologies. While oxygen, not the whole air, is needed by plant root, MNBs are more economical since they are more stable and water would not be oversaturated, which could be harmful to the plant.

CONCLUSIONS

In this research, the feasibility of promoting cucumber (*Cucumis sativus L.*) growth by using air micro-nanobubble water instead of tap water for irrigation was examined. Evaluating the morphological and physiological parameters of the plant supplied with air micro-nanobubbles in water, it was concluded that, generally, the stem length, leaf number, blossom number, chlorophyll a, chlorophyll b, and carotenoids

were increased 1.14, 2.86, 3.80, 1.34, 1.44 and 1.35 times, respectively, in the plant after 12 weeks. Since air MNBs water increased the amount of chlorophyll a, chlorophyll b and carotenoids in plant leaves, it could be concluded that the improvement in plant growth is a result of increase in photosynthesis phenomena in the plant. Generally, results showed a significant improvement of the plant growth using air micro-nanobubbles in cucumber. In order to determine the mechanism of action of micro-nanobubbles on the plants more fully, further research is needed.

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اثر آب دارای میکرو-نانو حباب هوا بر خواص مورفولوژیکی و فیزیولوژیکی خیار

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چکیده

کمبود آب و همچنین دغدغه تولید غذا، بر نیاز به استفاده از تکنولوژی‌های جدید کشاورزی تاکید میکند. افزایش غلظت اکسیژن محلول در آب، رشد گیاهان را از طرق مختلف ارتقا می‌دهد. هدف تحقیق حاضر بررسی اثر آب غنی شده با میکرو-نانو حباب هوا (MNBS) بر خواص مورفولوژیکی و فیزیولوژیکی خیار (*Cucumis sativus L.*) است. گیاهان از مرحله اولیه کاشت بذر (دو گروه، ۳۲ گیاه در هر گروه) به مدت ۱۲ هفته در خاک و با آب دارای میکرو-نانو حباب و آب معمولی پرورش داده شدند. پایداری میکرو-نانو حباب‌های هوا در آب با استفاده از اندازه‌گیری‌های زتاپتانسیل (-) (20.47 mV) مورد تایید قرار گرفت. افزایش تعداد شکوفه‌ها در گیاهان آبیاری شده با آب دارای MNBS تقریباً ۳۸ برابر تعداد شکوفه‌ها در گیاهان آبیاری شده با آب معمولی بوده است. آب دارای MNBS مساحت برگ گیاه را بطور میانگین بیش از ۷۷٪ افزایش داد. شاخص‌های فیزیولوژیکی از قبیل کلروفیل a، b، و کاروتنوئیدها در گیاهان آبیاری شده با آب دارای MNBS به ترتیب ۱.۳۴، ۱.۴۴، و ۱.۳۵ برابر بیشتر از گیاهان آبیاری شده با آب معمولی بوده است. به طور کلی، تحقیق حاضر نشان داد که آب دارای میکرو-نانو حباب هوا تاثیر مثبت برای گیاه خیار داشته و از جنبه‌های محیط زیستی و اقتصادی یک ابزار موثر و دارای پتانسیل مناسب برای تولید گیاه است.