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Diatomite Improves Productivity and Quality of *Moringa oleifera* Grown in Greenhouse

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Research Article

Abstract

Moringa oleifera Lam. is a tropical perennial, soft-wooded tree. Different parts of the tree have been used as traditional medicine, food, plant growth enhancer, animal feed, water coagulant, cosmetic ingredient, and biodiesel raw materials. In this study, we have investigated the effects of diatomite, a rich source of silicate on the growth and active compounds content in *Moringa oleifera*. We have irrigated our plants with 3 different concentrations (2.5, 5.0 and 10.0 g/l), of diatomite solution. Our results indicated that plants irrigated with 2.5 g/l of diatomite shown highest rate of improvement in growth parameters such as plant height, number of branch, leaves, and roots, as well as fresh and dried weight of stem, leaves, and roots as compared to the control (irrigated with water only) and plants irrigated with 5 or 10 g/l diatomite. The enhancement in growth parameters is supported by the increased in photosynthetic pigments. The amount of total phenolic and flavonoid as well as tocopherol and apigenin in the leaves and roots samples shown slight increment in all the diatomite treatment groups as compared to the control.

Keywords: Growth and yield; Plant pigments; Total phenolics; α-tocopherol; Total flavonoids; Apigenin.

1. Introduction

Moringa oleifera Lam. commonly known as the drumstick or ben oil tree is a widely cultivated species of the monogeneric Moringa genus from the Moringaceae family [1]. It is a fast-growing, soft wood, perennial tree, native to the Himalayan region. Currently, it is widely cultivated in the tropical and sub-tropical regions [2]. Beside its medicinal and culinary usage [3-5], Moringa is also used as plant growth enhancer and animal feed in agricultural, as well as water coagulant, cosmetic ingredient and biodiesel source in industrial sectors [6,7]. Moringa leaves, flowers and immature seed pods are good source of protein. In addition, its leaves also contain high level of minerals (iron, calcium, potassium, zinc, magnesium, and copper) and vitamins such as vitamin A (beta carotene), B (folic acid, pyridoxine, nicotinic acid), C (ascorbic acid), D and E (tocopherol). Essential fatty acids such as palmitic, linolenic, linoleic and oleic acids are found in the immature pods and flowers. Various phenolic acids and flavonoid compounds were also found in Moringa. All these valuable compounds have contributed to the various bioactivities such as anti-oxidants, anti-inflammatory, antipyretic; anti-epilepticanti-tumor, anti-ulcer, antispasmodic, circulatory stimulants, diuretic, anti-hypertensive, cholesterol-lowering, anti-diabetic and hepato-protective properties [2-5,7].

Diatoms are the most diverse group of phytoplankton, ranging in size from a few micrometers to a few millimeters and exist either as single cells or chains of cells [8]. All diatoms have siliceous cell walls (frustules) made of polymerized silicic acid (biogenic silica, bSi), but there is considerable variation in the Si content among different species [9]. Diatomite, also known as diatomaceous earth, is the fossil remains of diatoms. It contains 80-90% of highly soluble silica (SiO_2) readily available to plants. Sayyari-Zahan et al. [10] have proposed that diatomic can be a good fertilizer as it can increase the electric conductivity, and contents of potassium and phosphorus of the soil. Diatomite application has been shown to enhance the growth of several plant species such as *Vicia faba* [11], strawberry [12], wheat [13], rice [14] and *Antirrhinum majus* [10-16]. Angin et al. [12] proposed that diatomite enhanced the growth of strawberry by improving the hydro-physical properties of soil through increasing the macro, meso, micro and ultra-pores, which are important for plant growth and roots distribution as well as water supplementation to the plant. Nevertheless, the high concentration of SiO_2 in diatomite might be one of the factors that contributed to the growth enhancement effects in many of the studied plant species. Silicon, even though is not listed as an essential element for plant growth, evidences from studies on various plant species have indicated that it alleviates the negative effects on plant growth under different biotic and abiotic stress conditions. These studies have suggested that insoluble silicon could strengthen the physical barrier of plant cell structure, while soluble silicon regulates the network of signal pathways, activates the defense-related and antioxidant enzymes activities and genes expression, stimulates antimicrobial compounds production, modifies gas exchanges attributes and maintains nutrient and osmotic homeostasis under various stress conditions [17-22]. Indeed, Abdalla [11] and Ahmed et al. [23] have shown that diatomite improved the growth and mitigate the negative effects of salinity stress on *Trifolium alexandrinum* and *Stevia rebaudiana*, respectively. In addition, Abdalla [15] also shown that diatomite also mitigated the negative effects of water stress on *Lupinus albus* grown under drought stress. The main objectives of this study were to examine the roles of diatomite as silicon source on the vegetative growth and phytochemicals compounds in *Moringa oleifera* Lam. tree grown on sandy soil.

2. Materials and Methods

2.1 Plant material and diatomic treatments

Seeds of *Moringa oleifera* Lam. were obtained from the Medicinal and Aromatic Plants Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The experiment was conducted under greenhouse conditions during the two successive seasons in 2015 and 2016 at AL Sahab farm, Al-Hassa, Saudi Arabia. During the experiment, temperature between 32-36ºC and relative humidity of 47-56%, with 14 h average photoperiod was recorded. Seeds were sown on March 1st in both 2015 and 2016. The germination trial was carried out by sowing seeds in germination trays (depth of 1.0-2.0 cm) filled with a moist mixture of (1:1 v:v) sand and peat moss. After one month, the seedlings were transplanted into plastic pots of 20 cm in diameter and 15 cm in depth, containing 4.5 kg of moist mixture of (1:1 v:v) sand and peat moss per pot. The experiments contained four treatment groups, namely the control (tap water) (Table 1) and diatomite solutions at 2.5, 5 and 10 g/l. Each treatment consisted of 10 replicates (pots). The pots were arranged in complete randomized block design. Every two months, the plants were irrigated with one of the above-mentioned treatment solutions to raise the soil water holding capacity. Other agricultural practices such as weeding and fertilization were carried out as recommended. Diatomite is characterized by 89.00% silicon (SiO_2), 0.20% magnesium (MgO), 0.32% sodium (Na_2O), 0.88% iron (Fe_2O_3), 0.63% potassium (K_2O), 5.95% aluminum (Al_2O_3) and other trace minerals such as 0.29% titanium (TiO_2) and 0.10% carbonate (CaO) (Sekem company, Cairo, Egypt).

2.2 Vegetative growth

In both seasons, after eight months from the planting date, the plants were harvested. The tree growth parameters, such as plant height (cm), number of branches/plant (n), leaves number/plant (n), roots number/plant (n), longest root length/plant (cm) and leaves, roots and stem fresh and dry weight (g) were recorded from 8 random plants selected from each of the treatment group.

2.3 Plant pigments determination

The plant pigments [chlorophyll a (Chl-a), chlorophyll b (Chl-b) and carotenoid] were extracted from the leaves of the 8 months old Moringa trees by 80% acetone. These pigments were determined spectrophotometrically according to A.O.A.C (1984) and then calculated as mg/100 g on a fresh weight basis.

2.4 Quantitative determination of total phenolic, α-tocopherol, total flavonoids and epigenins

Total phenolics, α-tocopherol, total flavonoids and epigenin were extracted from 1 g of the homogenized air-dried leaves and roots of Moringa plants per each treatment group (control and 2.5, 5.0 and 10.0 g/L diatomite) and determined by using HPLC (Shimadzu, CTO-10ASvp, Japan) according to the methods described by Öztürk et al. [24], Carpenter [25], Rajanandh and Kavitha [26] and Leone et al. [27], respectively.

2.5 Statistical analysis

Experiments were set up in complete randomized design. Data were statistically analyzed using ANOVA/MANOVA of Statistica 6 software (Statsoft, 2001). The significance of differences among means was carried out using the Least Significant Test (LSD) at $p=0.05$.

3. Results and Discussion

3.1 Vegetative growth and yield

The effects of diatomite concentrations on the plant height, the number of branches, leaves and roots, the longest root length, and the fresh and dried weight of stem, leaves and roots of *Moringa oleifera* Lam. tree are presented in Tables 2-4. Among all the experimental groups, highest measurements in all the above mentioned parameters were observed in plants irrigated with 2.5 g/l of diatomite. As compared to the control groups, a two season's average of 5, 51, 28, 26 and 38 percent increase were observed for the plant height, the number of branches, leaves and roots and the longest root length respectively. Stem, leaves and roots showed similar percentage increase in dried weight, with an average of 43, 53 and 40 percent respective increment as compared to control group. Further increase in diatomite concentration to 5 g/l indeed reduced

Table 1. Some chemical composition of the experimental water.

Salinity Level (mS cm^{-1})	Cations (meq L^{-1})				Anions (meq L^{-1})				SAR
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	SO_4^{2-}	Cl^{-}	
1.35	5.72	2.02	7.27	0.38	0.28	2.68	4.03	8.4	3.43

Table 2. Effect of diatomite concentration (g/L) on plant height, branch number, leaves and roots number and longest root length of *Moringa oleifera* Lam.

Diatomite concentration (g/L)	Plant height (cm)		Branch number (n)		Leaves number (n)		Roots number (n)		Longest root length (cm)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Control	1.63a*	1.55ab	9.4a	10b	52.33a	55.3ab	23.5a	23bc	21a	28a
2.5	1.703a	1.65a	13.33a	16a	70.5a	67.5a	24a	34.67a	34.33a	31.67a
5	1.64a	1.57a	9.5a	10.67b	69a	60ab	24.33a	25.5b	35.67a	25a
10	1.52a	1.35b	6.66b	6.33b	55.5a	46b	23.67a	17c	25a	23a

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to LSD test

Table 3. Effect of diatomite concentrations (g/L) on fresh weight of stem, leaves and roots of *Moringa oleifera* Lam.

Diatomite concentrations (g/L)	Fresh weight of Stem (g)		Fresh weight of leaves (g)		Fresh weight of roots (g)	
	2015	2016	2015	2016	2015	2016
Control	250ab*	245b	75ab	60a	105a	85ab
2.5	322a	320a	106.67a	75a	100a	120a
5	235b	266.67 b	65ab	66.67a	53.33b	76.67ab
10	180b	186.67 c	60b	50a	55b	60b

*Mean followed by the same letter within a column are not significantly different at 0.05 level of probability according to LSD test

Table 4. Effect of diatomite concentrations (g/L) on dry weight of stem, leaves and root of *Moringa oleifera* Lam.

Diatomite concentrations (g/L)	Dry weight of stem (g)		Dry weight of leaves (g)		Dry Weight of Roots (g)	
	2015	2016	2015	2016	2015	2016
Control	95ab*	120a	15a	10.3b	35a	20b
2.5	153.33a	150a	16.67a	20a	36.67a	35a
5	105ab	120a	15.3a	10.b	10.5b	20b
10	53.33b	63.33b	6.66a	3.33b	10.2b	10c

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to LSD test

Table 5. Effect of diatomite concentrations (g/L) on the contents of chlorophyll a (mg/100 g F.W.), chlorophyll b (mg/100 g F.W.) and carotenoids (mg/100 g F.W.) in the leaves of *Moringa oleifera* Lam.

Diatomite concentrations (g/L)	Chl a (mg/100 g F.W.)		Chl b (mg/100 g F.W.)		Carotenoids (mg/100 g F.W.)	
	2015	2016	2015	2016	2015	2016
Control	39.71b	20.42b	15.16b	8.9a	79.82b	54.43b
2.5	45.21ab	22.76b	18.07ab	21.41a	109.40ab	69.27ab
5	45.62ab	43.70a	18.13ab	16.87a	104.78ab	92.72a
10	53.38a	43.33a	21.41a	10.21a	125.71a	57.82ab

*Mean followed by the same letter within a column are not significantly different at 0.05 level of probability according to LSD test

the diatomite growth enhancement effects. Lower percentage increase as compared to control in all the vegetative growth parameters and some of the yield measurements were observed in plants irrigated with 5 g/l diatomite. In addition, negative effects were observed in the dried weight of leaves and roots, in which a two season's average of 1 and 35 percent respective reduction were observed in these plants as compared to the control group. Plants irrigated with 10 g/l diatomite showed decrease in all growth and yield parameters as compared to control plants.

Vladimir et al. [28] have reported that silicate from pro-silica slag significantly increased the height and length of tree branches of Valencia orange trees. The vegetative growth and yield enhancement effects observed in the Moringa is consistent with the reports in other plant species treated with diatomite, such as *Vicia faba* [11], strawberry [12], wheat [13], rice [14], and *Antirrhinum majus* [16]. In our study, we have shown that 2.5 g/l of diatomite is the optimal concentration, which give the highest vegetative growth and yield enhancement in Moringa. On the other hand, irrigation with 10 g/L diatomite was found to reduce the vegetative growth and yield of Moringa.

3.2 Plant pigments analysis

As indicated in Table 5, the application of diatomite at all concentrations (2.5, 5.0 and 10.0 g/l) increased the chlorophyll a and b and carotenoids contents of the *Moringa oleifera* Lam. leaves as compared to the control treatment. Similar to the effects on the vegetative growth parameters and yields, increase in the concentration of diatomite reduced the percentage increase as compared to the control group for both chlorophyll a and b contents. However, unlike the vegetative growth and yield parameters, plants irrigated with high concentration of diatomite (10 g/l) still contained higher chlorophyll a and b contents as compared to control plants. Taken together, our results have suggested that the increased in photosynthetic pigments (chlorophyll a and b) is one of the factors that contributed to the growth and yield enhancement effects for plants irrigated with diatomite.

Increased of photosynthesis rate or photosynthetic pigment resulted from the application of diatomite were also described in *Lupinus albus* [11] and wheat [13]. In addition, silicate application also increased the photosynthetic rate or pigment in maize [29,30], cucumber [31,32], rice [33,34], cotton [35], *Lonicera japonica* [36] and *Zygophyllum xanthoxylum* [37] grown in ordinary or various stress conditions.

3.3 Phytochemical analyses

The total amount of phenolic (mg/g), flavonoid (mg/g), tocopherol (μg/g) and apigenin (μmole/g) in the leaf and root samples were presented in Figures 1-4, respectively. Our results indicated that both leaf and root samples accumulated similar amount of phenolic compounds and tocopherol (Figures 1 and 3), while the amount of flavonoid and epigenin were respectively 80 (Table 2) and 4-6 times (Table 4) higher in the leaf samples as compared to the root samples. The amount of total phenolic, flavonoid, tocopherol and epigenin increased in a diatomite dose dependent manner. In contrast to the growth and yield parameters as well as the chlorophyll a and b contents, the highest percentage increase for all the above phytochemicals were recorded in the plants irrigated with 10 g/l diatomite instead of those irrigated with 2.5 g/l diatomite. Higher percentage increase was shown in the root samples as compared to the leaf samples for the total phenolic, tocopherol and flavonoid contents. However, for epigenin, the leaves samples showed much higher percentage increase as compared to the root samples. The percentage increase for total phenolic, tocopherol, and flavonoid were 5, 9 and 5 percent, respectively in the leaf samples as compared to the corresponding values of 6, 22 and 13 percent in the root samples. Interestingly, we have found that the application of diatomite at 2.5 g/l increase the epigenin content up to 45% as compared to the control. Further increase in the diatomite concentration to 5 and 10 g/l only showed a slightly higher percentage increase than that observed in the 2.5 g/l treatment group.

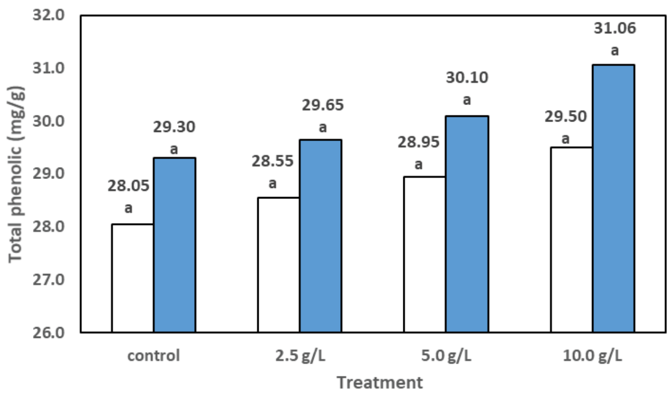

Figure 1. The average phenolic compound (mg/g) in the leaves (clear rectangle) and roots (dark rectangle) of plant samples from the control, 2.5, 5.0 and 10.0 g/L diatomite treatment groups. The mean values with the same letter are not statistically different are not significantly different at 0.05 level of probability according to the LSD test.

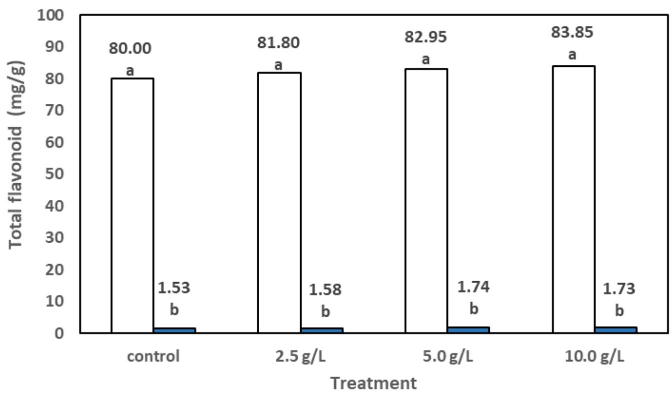

Figure 2. The average flavonoid compound (mg/g) in the leaves (clear rectangle) and roots (dark rectangle) of plant samples from the control, 2.5, 5.0 and 10.0 g/L diatomite treatment groups. The mean values with the same letter are not statistically different are not significantly different at 0.05 level of probability according to the LSD test.

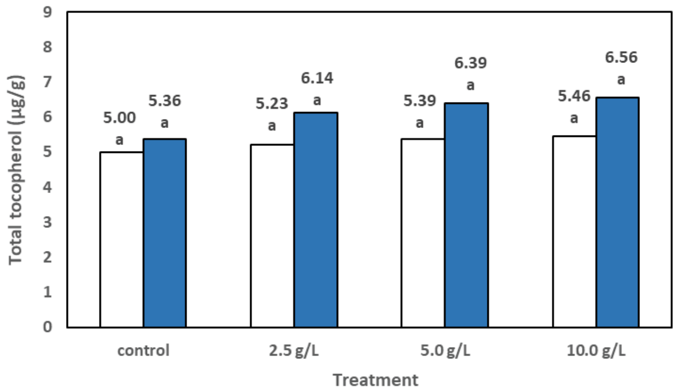

Figure 3. The average tocopherol (µg/g) in the leaves (clear rectangle) and roots (dark rectangle) of plant samples from the control, 2.5, 5.0 and 10.0 g/L diatomite treatment groups. The mean values with the same letter are not statistically different are not significantly different at 0.05 level of probability according to the LSD test.

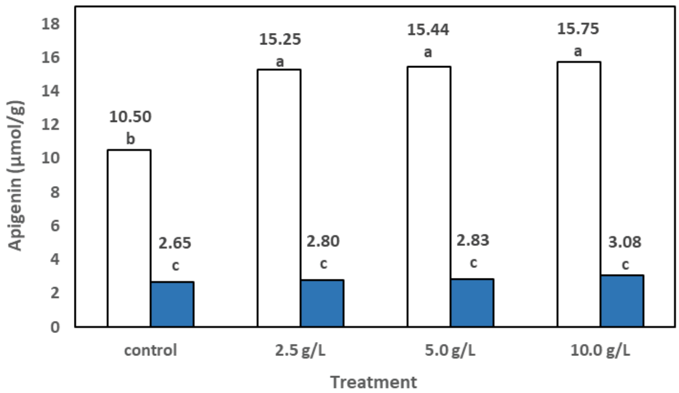

Figure 4. The apigenin compound (µmol/g) in the leaves (clear rectangle) and roots (dark rectangle) of plant samples from the control, 2.5, 5.0 and 10.0 g/L diatomite treatment groups. The mean values with the same letter are not statistically different are not significantly different at 0.05 level of probability according to the LSD test.

Epigenin is a flavonoid, and recently it has gained high attention due to its potential neuro-protective [38] and anti-cancer [39] effects in *in vivo* and *in vitro* studies. The presence of tocopherol and various phenolic and flavonoid compounds in Moringa have been associated with its various biological activities; therefore, our results suggested that the increase of these compounds resulted from the application of diatomite might contribute to the higher medical and nutritional potential of this plant.

4. Conclusion

Morphological changes were observed in the 8 months old *Moringa oleifera* Lam. after irrigated with different concentrations (2.5, 5 and 10 g/l) of diatomite solutions at a 2 months interval. Diatomite application increased the vegetative growth and yield, as well as the contents of chlorophyll a and b and carotenoids, total phenolic, flavonoid, tocopherol and apigenin in *Moringa oleifera*.

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