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Effect of Moringa Leaf Extract on the Performance of Lettuce Cultivars

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Abstract

Lettuce is an important crop that is grown commercially for salad purposes. To increase production of lettuce, synthetic fertilizers are applied. However, an excess of synthetic fertilizers is hazardous for the human body and also affects soil and environmental conditions. Alternative methods are available to enhance the production of lettuce, e.g., application of moringa leaf extract (MLE), which is also environmentally friendly. As MLE is a plant-based organic product, there are no side effects. The research was conducted at the Ornamental Nursery, Department of Horticulture, University of Agriculture Peshawar, during the 2020–21 season. The study was laid out in two-factor randomized complete block design in a split-plot arrangement with three replications. The two factors were MLE concentration (0, 2, 4, 6, and 8% v/v) and five lettuce cultivars (Red Laurel, Red Oakleaf, Milky White, Romaine, and Large Speed). Lettuce cultivars were sprayed with the required MLE concentrations at 2, 4, and 6 weeks after transplantation, while an extra application of MLE was given before bolting to the plants left for seed production. It was observed that lettuce cv. Red Laurel produced maximum fresh and dry head weight. Maximum plant height was recorded for cv. Red Oakleaf. Maximum leaves, head diameter, head height, root length, seed yield, and leaf area were noted in cv. Milky White. Cultivar Romaine took the maximum number of days to flowering and seed production. It also provided maximum chlorophyll content and the best taste. Moreover, MLE also had substantial effects on the growth and yield of lettuce cultivars. Here, maximum plant height, leaf area, number of leaves, head diameter, days to flowering, root length, head height, seed yield, days to seed production, chlorophyll content, and fresh and dry head weights were produced by plants sprayed with 8% (v/v) MLE. It is concluded that MLE, a useful growth promoter, has a considerable effect on the growth and development of lettuce cultivars and is thus recommended for organic production of the crop.

Keywords Organic farming · Biostimulants · Cytokinin · *Lactuca sativa* · *Moringa oleifere*

Lettuce (*Lactuca sativa* L.) is an important herbaceous leafy vegetable belonging to the Compositae family (Karam et al. 2002). It is one of the most important salad vegetables, being a healthy food consumed in large amounts (Dupont et al. 2000). Lettuce is an economically important salad vegetable with the highest consumption rate worldwide (Coelho et al. 2005). It is a vital crop commonly grown as a greenhouse vegetable, with high production among vegetables grown throughout the world (Křístková et al. 2008). Consumption of lettuce can provide proteins, fatty acids, carbohydrates, and dietary fiber to the human body (Hooper and Cassidy 2006). It has many essential components and vitamins for human health such as calcium, iron, and vitamin A. Lettuce is low in calories, so it is suitable for reducing human body weight (Maboke and Du-Plooy 2009), and 94% moisture, 1.3 g protein, 0.3 g lipid, 3.5 g carbohydrate, 68 mg calcium, 25 mg P, 1.4 mg Fe, 264 mg K, and 18 mg ascorbic acid are contained in 100 g of the edible portion (Hooper and Cassidy 2006). A vegetable-rich diet decreases the risk of heart disease and cancer. These valuable effects are because of ascorbic acid, vitamins, carotenoids, phytochemicals, fiber, and polyphenols that protect important biological constituents such as membranes, DNA, and lipoproteins (Szeto et al. 2004). One of the important health benefits of lettuce is that it prevents heart disease in humans (Nicolle et al. 2004; Serafini et al. 2002). Lettuce is healthy because it is packed with large amounts of antioxidants, vitamin C,

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polyphenols, and fiber. Polyphenols have been shown to have higher levels of antioxidant activity than vitamins E and C (Nicolle et al. 2004; Serafini et al. 2002). Nutritionally, lettuce is placed 26th out of 39 vegetables and fruits, and ranks fourth for salad consumption (Karam et al. 2002).

Moringa oleifera and belongs to the Moringaceae family. Its common name is drumstick or horseradish tree. Moringa tree can be grown successfully in subtropical, semiarid, and tropical areas. Moringa grows well in dry sandy soil, along the coast, and in poor soil; it is a fast-growing, drought-resistant tree. This magic tree is native to Pakistan, Bangladesh, Afghanistan, and India (Fahey 2005). Currently, it is grown extensively in America, Africa, Mexico, Malaysia, India, Sri Lanka, the Philippines, and Indonesia. Moringa is also used in emerging countries as a source of micronutrients, a natural insecticide, and as a metabolic conditioner to prevent endemic disorders (Foidle et al. 2001). It is a most nutritious plant, providing antioxidants and amino acids with antiaging and anti-inflammatory properties. This miracle tree is a rich source of calcium, iron, vitamin C, and highly digestible proteins (Fahey, 2005). Moringa leaf extract (MLE) is a powerful natural growth stimulant because it contains zeatin, a natural derivative of cytokine. MLE is also a source of minerals, vitamins, amino acids, protein, and phenolics, which makes it a potential plant growth enhancer (Emonger 2012; Rady et al. 2013; Howladar 2014). MLE contains zeatin—a plant hormone cytokine that plays an important role in stress resistance, plant growth, and development—in abundance. MLE contains endogenous cytokines such as zeatin, isopentyladenine, and dihydrozeatin, which promote growth (Andrews and Zija international 2006; Price 2007). MLE functions as a plant hormone that supports seed germination, proliferation, and increases plant yield. Foliar application of MLE helps to improve the overall performance of crops. It also has a vital role in maintaining the optimal tissue water content, enhancing the stability of membranes, and maximizing antioxidants, thus ultimately ensuring plant vigor (Yasmeen et al. 2012; Rehman et al. 2014). Research keeps in view the importance of lettuce for salad purposes and the problem of increasing production via use of synthetic fertilizers, i.e., urea, DAP, etc., which are hazardous for humans, soil, and the environment. A possible solution would be use of organic fertilizer to increase lettuce production. The present study was conducted to find out the effect of MLE on the production of lettuce cultivars.

Materials and Methods

Experimental Design and Plant Material

The experiment was conducted during the 2020–21 season. The research was performed at the Ornamental Horticultural Nursery, Department of Horticulture, University of Agriculture Peshawar Pakistan, in randomized complete block design with two factors, a split-plot arrangement, and three replications. The MLE (0, 2, 4, 6, and 8% v/v) was assigned to main plots and the lettuce cultivars (Red Laurel, Red Oakleaf, Milky White, Romaine, and Large Speed) were assigned to subplots.

Plant Culture and Transplantation

Seeds were sown in pots with media for nursery raising. The pots were immediately sprinkled with water after sowing the seeds. Pots were kept in a plastic tunnel to protect them from harsh environmental conditions. When the seeds had emerged and reached the three to four leaf stage, they were transplanted to the field. Plants were irrigated immediately after transplantation. Two weeks after transplantation, plants were sprayed with MLE concentrations of 2%, 4%, 6%, 8% (v/v). Later, the second and third MLE treatments were applied at 4 and 6 weeks after transplantation. To the plants that were left for seed production, an extra MLE treatment was applied 1 week before the bolting stage.

Moringa Leaf Collection and Preparation of MLE

Fully grown, young moringa leaves (less than 30 days old) were obtained from a mature moringa tree grown at Malakandher Farm, University of Agriculture Peshawar. MLE was made by blending (10000 rpm) 100 g of fresh leaves with 10 mL of water (Yasmeen et al. 2014). A cheesecloth was used to separate the solids from the juice. After collecting the pure juice in a separate glass container, four MLE dilutions were made.

Characteristics of MLE

Moringa leaf extract (MLE) is a powerful natural growth stimulant because it contains zeatin, a natural derivative of cytokine. MLE is also a source of minerals, vitamins, amino acids, protein, and phenolics, which makes it a potential plant growth enhancer (Emonger 2012; Rady et al. 2013; Howladar 2014). MLE also contains endogenous cytokines such as zeatin, isopentyladenine, and dihydrozeatin, which promote growth (Andrews 2006; Price 2007). Zeatin, a plant cytokine that plays important role in stress resistance, plant growth, and development, is present in MLE in abundance. MLE thus functions as a plant hormone source that supports seed germination, proliferation, and an increase plant yield.

Studied Parameters

Data were recorded on head diameter, head height, number of leaves plant^{-1}, leaf area (with a CI-201 leaf area meter; CID Bio-Science, Camas, WA, USA), chlorophyll content (chlorophyll content was measured by SPAD meter; Konica Minolta SPAD-502 plus, Tokyo, Japan), taste, head fresh weight, head dry weight (fresh and dry head weight was measured with a digital balance, Hytek Sf-400C), days to flowering, days to seed production, plant height, root length, and seed yield.

Statistical Analysis

The collected data were analyzed with the help of STATISTIX 8.1 statistical software (Analytical Software, Tallahassee, FL, USA) using the analysis of variance (ANOVA) technique (Steel and Torrei 1980). In case of significant differences, the means were separated further using the least significant difference (LSD) test (Williams and Abdi 2010).

Results and Discussion

The experiment was performed to assess the effect of MLE concentrations on the performance of lettuce cultivars. The data of all experimental parameters were recorded. The results are discussed, with possible clarifications, in the following sections.

Head Diameter

The data relating to head diameter (centimeters) are presented in Table 1. Statistical analysis shows that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of head diameter. The interaction between MLE concentration and lettuce cultivar was nonsignificant. Among the lettuce cultivars, maximum head diameter (31.10 cm) was recorded for cv. Milky White, followed by cv. Red Laurel, cv. Large Speed, and cv. Red Oakleaf, with 26.88, 23.86, and 21.14 cm head diameter, respectively. Minimum head diameter was produced by cv. Romaine (19.32 cm). Regarding MLE concentrations, maximum head diameter (26.14 cm) was recorded in plants sprayed with 8% MLE, closely followed by those sprayed with 6% MLE (25.55 cm). The rest of the treatments behaved similarly, with minimum head diameter (22.76 cm) observed in plants sprayed with distilled water (control). Compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce head diameter by 1.7%, 8.8%, 12.3%, and 14.9%, respectively.

Lettuce cultivars are grouped into crisphead, butterhead, romaine, and loose-leaf based on their shape. These differences in the head diameter of lettuce cultivars might be due to the genetic variation among them. Crisphead lettuce has compact, round heads, forming crispy, curved, and overlap-

Table 1 Head diameter, head height, no. of leaves plant^{-1}, leaf area, chlorophyll content, and taste of lettuce cultivars as affected by moringa leaf extract concentrations

Treatment	Attribute					
MLE concentration	Head diameter (cm)	Head height (cm)	No. leaves plant^{-1}	Leaf area (cm^2)	Chlorophyll content	Taste
0	22.7b	32.2c	28.0b	1339.7c	32.5c	5.4
2	23.1b	33.2bc	28.1b	1347.7bc	34.1bc	5.5
4	24.7ab	34.6ab	28.3b	1354.5bc	35.1abc	5.5
6	25.5a	34.9ab	29.2a	1367.8ab	36.3ab	5.6
8	26.1a	35.2a	29.3a	1380.4a	36.8a	5.8
LSD ($P \leq 5\%$)	2.00	1.79	0.83	20.89	1.12	NS
Lettuce cultivar						
Red Laurel	26.8B	33.8C	27.2C	1356.6C	26.9E	5.4C
Red Oakleaf	21.1D	30.2D	23.4E	1297.2D	32.6D	3.7D
Milky White	31.1A	41.6A	34.6A	1417.7A	38.2B	3.6D
Romaine	19.3E	37.1B	32.5B	1381.8B	41.3A	7.8A
Large Speed	23.8C	27.6E	25.3D	1336.8C	35.8C	7.1B
LSD ($P \leq 1\%$)	1.02	1.10	1.16	24.01	2.64	0.28
Interactions MLE × cultivar	NS	NS	NS	NS	NS	NS

Mean value followed by different letters are significantly different at $P \leq 0.05$ (lower case) and $P \leq 0.01$ (upper case)
MLE moringa leaf extract, *LSD* least significant difference, *NS* nonsignificant

ping leaves. Butterhead also produces small heads of loose leaves. The lettuces of romaine cultivars produce a long, loaf-shaped head, with elongated dark green leaves. The cultivars of loose-leaf lettuces do not form heads and produce large and loosely packed leaves. Oakleaf lettuce has a loose leaf arrangement, and produces leaves at its base (Gosh 2019).

Head diameter significantly increased with an increase in MLE concentration. This increase in head diameter might be due to the presence of macro- and micronutrients and cytokine in moringa leaves. MLE contains cytokine (zeatin) as well as other growth-promoting chemicals such as phenols and ascorbic acid (Foidle et al. 2001; Fuglie 2000). Moreover, MLE contains key plant pigments such as carotenoids and chlorophyll, which play an important role in improving photosynthesis and hence increasing plant growth (Iqbal 2014; Owusa 2006). Abd-Elkafie et al. (2016) observed maximum vegetative growth of coriander in plants sprayed with MLE.

Head Height

Data regarding the head height (centimeters) of lettuce are presented in Table 1. The ANOVA showed that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of head height. The interaction between lettuce cultivar and MLE concentration was nonsignificant. The mean values of head height show that cv. Milky White attained maximum head height (41.6 cm), followed by cv. Romaine (37.1 cm). Minimum head height (27.6 cm) was recorded for cv. Large Speed. Mean head height values reveal that maximum head height (35.2 cm) was recorded in plants treated with 8% MLE, closely followed by those treated with 6% MLE concentrations (34.9 cm). Minimum head height (32.2 cm) was seen in plants sprayed with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce head height by 0.3%, 7.4%, 8.3%, and 9.3%, respectively.

Lettuce cultivars are categorized into different groups. Each of these cultivars possesses unique characteristics of growth and adaptability to the environment (Grant 2018). Romaine varieties grow upright, with tightly folded leaves up to 20–25 cm. The current results showed maximum head height in cv. Milky White, which could be due to the cultivars' genetic potential. Our result is in agreement with Afton (2008), who reported that romaine lettuce resulted in taller heads, while crisphead lettuce produced smaller heads.

MLE is a most valuable plant hormone source because it contains sufficient gibberellic acid and cytokine. In addition, MLE contains a number of additional micronutrients that enhance plant growth by functioning in several physiological processes (Makkar et al. 2007). The presence of cytokine and gibberellin in MLE could increase the gain in head height. Matthew (2016) found that foliar application of MLE increased pepper (*Capsicum annuum* L) vegetative growth.

Number of Leaves per Plant

Data regarding the number of leaves plant^{-1} are given in Table 1. The statistical analysis shows that there were significant differences among lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of leaf number plant^{-1}. The interaction between lettuce cultivar and MLE concentration was nonsignificant. Regarding cultivars, the mean data show that cv. Milky White resulted in the maximum number of leaves (34.6), followed by cv. Romaine (32.5). The minimum number of leaves (23.4) was observed in cv. Red Oakleaf. Regarding MLE concentration, the maximum number of leaves (29.3) was recorded in plants treated with 8% MLE, which was statistically similar to the number of leaves plant^{-1} observed in plants treated with 6% MLE concentration of 29.2. Control, 2%, and 4% MLE concentrations gave similar results, with the control providing the minimum number of leaves (28.0). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased the no. of leaves in lettuce by 0.3%, 1.0%, 4.2%, and 4.6%, respectively.

Lettuce cultivars have different responses regarding growth and yield parameters. The increase in leaves is related to cultivar and also contributes to leaf fresh and dry mass. Genetic variation might be the reason for the difference in number of leaves plant^{-1} of lettuce cultivars. Reshma et al. (2007) reported that cultivars' genetic makeup causes plants to absorb nutrients more efficiently and makes cultivars more adaptable to changing environmental conditions.

An increase in MLE concentration increased the number of leaves in various lettuce cultivars. The presence of nutrients and cytokine in MLE may be responsible for the increase in leaf number. Moringa leaf is rich in cytokine and micro- and macronutrients (Taiz and Zeiger 2010). MLE is a plant growth promoter that contains a many essential nutrients, phenols, ascorbic acid, and antioxidants that are important for plant development and growth (Yasmeen et al. 2013). Alkuwayti et al. (2020) found that when basil plants were sprayed with MLE, they produced the maximum numbers of leaves as compared to control plants.

Leaf Area

The data relating to leaf area (square centimeters) of lettuce cultivars are presented in Table 1. The ANOVA showed that regarding leaf area, there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$), whereas the interaction between MLE concentration and lettuce cultivar was nonsignificant. Maximum leaf area was exhibited by cv. Milky White (1417.7 cm^2) followed by cv. Romaine (1381.8 cm^2), while minimum leaf area (1297.2 cm^2) was recorded in cv. Red Oakleaf. The means table of leaf area shows that maximum leaf area (1380.4 cm^2) was recorded in plants treated with 8% MLE followed by those treated with 6% MLE (1367.8 cm^2). Plants sprayed with distilled water (control) had the minimum leaf area (1339.7 cm^2). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce leaf area by 0.5%, 1.1%, 2.0%, and 3.0%, respectively.

In the comparison of various lettuce cultivars regarding leaf area, cv. Milky White showed the maximum leaf area as compared to the rest of the cultivars. This variability in leaf area could be due to the cultivars' hereditary and genetic makeup. According to Hay and Porter (2006), leaf area is also a trait that is determined during plant growth, involving the expression of genes via metabolic pathways.

MLE foliar application increased leaf area, which could be influenced by the presence of endogenous cytokines such as zeatin, dihydrozeatin, and isopentyladenine in MLE (Andrews 2006). Tissue growth, cell elongation and division, the development of new shoots, and chlorophyll synthesis are all regulated by cytokine. It also enhances the uptake of nutrients in plants and delays senescence (Taiz and Zeiger 2006, Anwar et al. 2007). The increased leaf area of lettuce plants could be due to the presence of plant growth stimulants in MLE. Abdel-Rahman and Abdel-Kader (2020) showed that foliar application of MLE increased the growth of fennel (*Foeniculum vulgure*).

Chlorophyll Content

The data pertaining to chlorophyll content (SPAD) of lettuce are presented in Table 1. The analyzed data reveal that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of chlorophyll content. The interaction between MLE concentration and lettuce cultivar was nonsignificant. The mean values of the chlorophyll content show that cv. Romaine had maximum chlorophyll content (41.3 SPAD), followed by cv. Milky White (38.2 SPAD). Minimum chlorophyll content (26.9 SPAD) was recorded in cv. Red Laurel. Maximum chlorophyll content (36.8 SPAD) was recorded in plants treated with 8% MLE, closely followed by those treated with 6% MLE (36.3 SPAD). Chlorophyll content was minimum (32.5 SPAD) in plants sprayed with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce chlorophyll content by 4.9%, 8%, 11.6%, and 13.2%, respectively.

In the comparison of various lettuce cultivars regarding chlorophyll content, cv. Romaine showed maximum chlorophyll content as compared to the rest of the cultivars. This variation in chlorophyll content could be due to genetic differences among cultivars. Cassetari et al. (2015) reported that chlorophyll content varies between different lettuce cultivars. Similar results were also reported by Caldwell and Britz (2006), who found differences in the chlorophyll content of lettuce cultivars.

According to the current findings, the chlorophyll content of lettuce cultivars was likely enhanced by MLE. The presence of zeatin in MLE, which stops green leaves from ageing prematurely and hence increases plant photosynthesis, is likely to be the cause of this increase in leaf chlorophyll concentration. Exogenous cytokine is produced by foliar application of MLE, which encourages the production of cytokine-dependent enzymes like isopentenyl transferase, which increases chlorophyll content in leaf and prevents chlorophyll breakdown (Tatley and Thimam 1974; Emonger 2002; Fletcher et al. 1982). The result of Yasmeen et al. (2014) and Waqas et al. (2017) showed that foliar application of MLE increased chlorophyll content significantly in tomato and maize.

Taste

The data pertaining to taste of lettuce are presented in Table 1. Statistical analysis indicates that there were significant differences among the lettuce cultivars ($P \leq 0.01$). The MLE concentrations were nonsignificant in terms of taste, and the interaction between MLE concentration and lettuce cultivar was also nonsignificant. The means table showed that cv. Romaine had the best taste, with a score of 7.8 out of 10, followed by cv. Large Speed with a taste score of 7.1 out of 10. Cultivar Red Laurel had a 5.4 out of 10 taste score, while cultivars Red Oakleaf and Milky White behaved similarly, resulting in the minimum taste scores of 3.7 and 3.6 out of 10, respectively. Moreover, cv. Milky White had a bitter taste.

Lettuce cultivars are categorized into five different groups. Each of these cultivars possesses unique characteristics of flavor, texture, and adaptability to the environment (Grant 2018). Crisphead lettuce has a crunchy texture and mild flavor that lends itself to salads and sandwiches. Romaine lettuce has a slightly sweeter, bolder flavor. Red Oakleaf lettuce has a crisp, mild flavor (Kim et al. 2016). In our study, the taste of Romaine and Large Speed cultivars was sweet and desirable, and that of cv. Milky White was bitter. The differences between lettuce cultivars' taste might be due to genetic variation.

Table 2 Head fresh weight, head dry weight, days to flowering, days to seed production, plant height, root length, and seed yield (kg ha^{-1}) of lettuce cultivars as affected by moringa leaf extract concentration

Treatment	Attribute						
MLE concentration	Head fresh weight (g)	Head dry weight (g)	Days to flowering	Days to seed production	Plant height (cm)	Root length (cm)	Seed yield (kg ha^{-1})
0	359.9c	24.5b	108.7b	130.6c	131.4c	39.8b	1275.7b
2	375.7bc	24.7b	111.4ab	134.3bc	132.4bc	41.3a	1321.1ab
4	379.3bc	25.7ab	113.8a	136.9abc	133.5bc	41.4a	1339.1a
6	385.5ab	26.0ab	114.9a	137.4ab	135.1ab	42.2a	1368.0a
8	403.7a	27.0a	116.2a	144.8a	136.7a	42.3a	1373.5a
LSD ($P \leq 5\%$)	21.09	1.66	4.68	8.86	2.98	1.42	59.28
Lettuce cultivar							
Red Laurel	462.8A	29.3A	111.2C	137.0C	133.6C	43.0B	1621.4B
Red Oakleaf	256.7D	20.3D	108.7C	133.5D	147.8A	41.4C	1148.7D
Milky White	385.6C	23.8C	101.4D	127.0E	138.5B	48.5A	1657.0
Romaine	388.2BC	25.9B	126.0A	147.8A	131.6D	34.2E	718.1E
Large Speed	410.7B	28.6A	117.6B	141.6B	118.2E	39.9D	1532.2C
LSD ($P \leq 1\%$)	23.80	1.32	3.97	2.74	1.69	1.09	32.65
Interaction MLE × cultivar	NS	NS	NS	NS	NS	NS	NS

Mean value followed by different letters are significantly different at $P \leq 0.05$ (lower case) and $P \leq 0.01$ (upper case)
MLE moringa leaf extract, *LSD* least significant difference, *NS* nonsignificant

Fresh Head Weight

The data pertaining to fresh head weight (grams) of lettuce are presented in Table 2. The ANOVA reveals that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of fresh head weight, whereas the interaction between lettuce cultivar and MLE concentration was nonsignificant. The mean values regarding fresh head weight show that cv. Red Laurel produced heads with maximum fresh weight (462.8 g), followed by cv. Large Speed (410.7 g). Minimum fresh head weight (256.7 g) was recorded for cv. Red Oakleaf. Among the MLE concentrations, maximum fresh head weight (403.7 g) was recorded in plants treated with 8% MLE, followed by those treated with 6% MLE (385.5 g). Minimum fresh head weight (359.7 g) was recorded in plants treated with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce fresh head weight by 4%, 5.3%, 7.1%, and 12.1%, respectively.

The response of different lettuce cultivars differs regarding the growth and yield parameters. Some lettuce cultivars have more leaves, which ultimately increases leaf fresh and dry mass. As mentioned above, the possible reason for these variations is their genetic characteristics. Reshma et al. (2007) reported that different lettuce cultivars had different genetic makeups, due to which their nutrient uptake and adaptation to the environment was different.

The results showed that increasing MLE concentrations significantly improved fresh head weight. This is most likely due to the presence of ascorbates, protein, zeatin, phenols, vitamins, and several other necessary macro- and micronutrients in MLE, which make it a powerful biostimulant (Emonger 2012; Rady et al. 2013). Furthermore, MLE is important for regulating tissue water status, stabilizing membranes, and enhancing secondary metabolism in plants, all of which promote vigorous plant growth (Yasmeen et al. 2012, 2013). Mathew (2016) studied exogenous MLE application, which resulted in maximum growth in pepper (*Capsicum annuum* L), while Culver et al. (2012) showed similar results in tomato plants.

Dry Head Weight

The data prevailing to head dry weight (grams) of lettuce are presented in Table 2. The ANOVA indicates that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of dry head weight. The interaction between MLE concentration and lettuce cultivar was nonsignificant. The means regarding head dry weight indicate that cv. Red Laurel exhibited maximum dry head weight (29.3 g), closely followed by cv. Large Speed (28.6 g). Minimum dry weight (20.3 g) was recorded for cv. Red Oakleaf. Regarding MLE concentration, maximum dry head weight (27.0 g) was observed in plants treated with 8% MLE, closely followed by those treated with 6% (26.0 g) and 4% (25.7 g) MLE. Minimum dry head weight (24.5 g) was produced in plants treated with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce dry head weight by 0.8%, 4.8%, 6.1%, and 10.2%, respectively.

The increase in dry weight of lettuce cultivars was influenced by an increase in the number of leaves and head fresh weight. The dry weight of the plant is the result of photosynthetic accumulation in the form of plant biomass and moisture content in leaves (Atikah and Widyawati 2019). Cultivar Red Laurel had maximum head fresh weight. Therefore, head dry weight was also higher in this cultivar. The difference in head dry weights of the cultivars might be due to their hereditary makeup.

Among the MLE concentrations, higher concentrations of MLE resulted in plants with higher head dry weights. The increase in head dry weight may be because of the presence of cytokine, macro- and micronutrients, and ascorbic acid in MLE. The presence of macro- and micronutrients in MLE increased vegetative growth, resulting in an increased head dry weight (Simirnoff 1996). MLE is also a powerful biostimulant because it includes enough zeatin, protein, vitamins, phenols, ascorbates, and other important micro- and macronutrients (Emonger 2012; Rady et al. 2013). Zeatin in MLE plays a role in plant nutrition by enhancing nutrient absorption and delaying premature senescence (Taiz and Zeiger 2006). Abd-Elkafie et al. (2016) found that coriander vegetative growth was enhanced by exogenous application of MLE.

Days to Flowering

Data for days to flowering are given in Table 2. The ANOVA reveals that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of days to flowering, whereas the interaction between lettuce cultivar and MLE concentration was nonsignificant. Mean data regarding various cultivars showed that cv. Romaine took the maximum number of days (126.0) to flowering, followed by cv. Large Speed (117.6 days). Cultivars Red Laurel and Red Oakleaf behaved similarly, taking 111.2 and 108.7 days to flowering, respectively, whereas cv. Milky White took the minimum number of days (101.4) to flowering. Among the MLE concentrations, maximum (116.0) days to flowering was recorded for plants sprayed with 8% MLE, although this was on par with 6%, 4%, and 2%, with means of 114.9, 113.8, and 111.4 days to flowering, respectively. Minimum (108.7) days to flowering were taken by plants sprayed with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased days to flowering in lettuce by 2.4%, 4.6%, 5.7%, and 6.8%, respectively.

The results showed that lettuce cultivars took different days to flowering. Cultivars Milky White and Red Oakleaf took the minimum number of days to flowering as compared to other cultivars. The selection of cultivar is of great importance, as it determines successful cropping (Olasantan 2007). These differences could be due to genetic variation between cultivars. The current results are similar to the findings of Al-Harbi (2001), who reported variation in flowering for various lettuce cultivars.

Delayed flowering was observed in plants with an increase in MLE concentration. The presence of a high level of zeatin, vitamins, and several other growth-promoting components such as Ca, Fe, and K in MLE could be the cause of this flowering delay (Basra et al. 2011), which promoted lettuce cultivars' vegetative growth. The primary function of zeatin in plants is to promote cell division and nutrient absorption (Emonger 2015; Taiz and Zeiger 2006). MLE contained protein and growth-promoting hormones, which stimulated cell elongation and division, resulting in greater plant growth (Makkar and Becker 1996). Moyo et al. (2011) reported that plants sprayed with moringa showed the maximum number of days to flowering. Mathur (2006) and Fuglie (2000) both found that increasing MLE concentrations increased growth.

Days to Seed Production

Data regarding days to seed production of lettuce are presented in Table 2. The analyzed data show that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of days to seed production. The interaction between MLE concentrations and lettuce cultivars was nonsignificant. The means comparison regarding the cultivars shows that cv. Romaine took the maximum number of days to seed production (147.8) followed by cv. Large Speed (141.6 days), while cv. Milky White took the minimum number of days to seed production (127.0). Among different concentrations of MLE, the maximum number of days to seed production (144.8) was recorded for plants sprayed with 8% MLE, though this was on par with 6%, 4%, and 2% concentrations that took 136.9, 137.4, and 134.3 days to seed production, respectively. The minimum number of days to seed production (130.6) was taken by plants sprayed with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased days to seed production in lettuce by 2.8%, 4.8%, 5.2%, and 10.8%, respectively.

According to the findings of this study, significant differences were observed among lettuce cultivars regarding days to seed production. Days taken to flowering by lettuce cultivars were different, and as per Welbaum (2010), seed formation occurs between 12 and 21 after anthesis. The cultivars Milky White and Large Speed took the minimum number of days to flowering, which also reduced the time taken to seed production in these cultivars. On the other hand, a delay in the flowering of the other cultivars also delayed their seed production. The differences in the number of days it takes to produce seeds could be due to genetic makeup and environmental conditions. Zhao and Carey (2009) reported on different lettuce varieties taking different days to seed production.

According to the results, an increase in MLE significantly increased the number of days to lettuce seed production. The presence of zeatin, protein, acrobats, polyphenols, vitamins, and several other necessary macro- and micronutrients in moringa may increase the number days to seed production. MLE increased vegetative growth and postponed flowering, resulting in delayed seed production (Emonger 2012; Rady et al. 2013). The results of Mathew (2016) showed that foliar application of MLE increased pepper (*capsicum annuum* L) growth and yield. Culver et al. (2012) found the same thing in tomato plants.

Plant Height

The data pertaining to plant height (centimeters) of lettuce are presented in Table 2. The ANOVA revealed that there were significant differences between lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of plant height, whereas the interaction between MLE concentration and lettuce cultivar was nonsignificant. The mean values for plant height of cultivars show that cv. Red Oakleaf attained maximum plant height (147.8 cm) followed by cv. Milky White (138.5 cm). Minimum plant height (118.2 cm) was recorded for cv. Large Speed. Mean values of MLE concentrations regarding plant height reveal that maximum plant height (136.7 cm) was observed in plants treated with 8% MLE, closely followed by those treated with 6% MLE (135.1 cm). Minimum height (131.4 cm) was observed in plants treated with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased plant height in lettuce by 0.7%, 1.5%, 2.8%, and 4.0%, respectively.

Culitivar Red Oakleaf resulted in maximum plant height as compared to other cultivars. Lettuce cultivars are categorized into different groups, which possess unique characteristics of flavor, texture, color, growth, and adaptability to the environment (Grant 2018). Our results are supported by those of Baloch et al. (1991) and Rostamforoudi et al. (1999), who reported variations in plant height for the studied lettuce cultivars.

Among the various MLE concentrations, 8% MLE resulted in maximum plant height. This increase in height might be due to the availability of numerous beneficial elements such as Ca, K, Mn, P, Zn, Mg, and Fe, which helped to improve development and growth and resulted in good production (Aslam et al. 2005). Furthermore, the presence of a biostimulant (zeatin) in MLE may be responsible for enhancing other physiological processes and increasing plant height by stimulating cell division (Price 2007). Aslam et al. (2005) achieved maximum height in mint (*Menthe arvensis L*) with the use of MLE. Fuglie (2000) also reported similar results, where foliar application of MLE increased growth of young plants.

Root Length

The data pertaining to root length (centimeters) of lettuce cultivars are presented in Table 2. The ANOVA showed that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of root length, whereas the interaction between MLE concentration and lettuce cultivar was nonsignificant. The mean values of root length reveal that cv. Milky White attained maximum root length (48.5 cm), followed by cv. Red Laurel producing 43.0 cm long roots. Minimum root length (34.2 cm) was recorded for cv. Romaine. Mean values reveal that maximum root length (42.3 cm) was recorded in plants sprayed with 8% MLE. However, this was on par with the other MLE concentrations, i.e., 2%, 4%, and 6% MLE, which developed 41.3, 41.4, and 42.2 cm long roots, respectively. Root length was minimum (39.8 cm) in plants sprayed with distilled water (control). An overall trend was seen in which root length increased with increased MLE concentration. As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce root length by 3.7%, 4.0%, 6.0%, and 6.2%, respectively.

The maximum root length was recorded in cv. Milky White and Red Laurel, which could be due to the genetic makeup of the cultivars. The root of lettuce plants consists of a main taproot and smaller subsidiary roots. These taproots of lettuce are long and slender with a series of secondary roots. Selective breeding has resulted in various modifications in lettuce over the centuries, delayed bolting, larger seeds, larger leaves and heads, superior taste and texture, lengthy roots, and a variety of leaf shapes and colors (Davey et al. 2007).

The results show that MLE application significantly enhanced root length. The availability of phosphorus, calcium, vitamins, and various other essential micro- and macroelements in MLE could be the cause of the increase in root length. Phosphorus, calcium, vitamins, ascorbic acid, and phenols are abundant in moringa leaves (Emonger 2012; Rady et al. 2013). Culver et al. (2012) found that foliar spraying of MLE resulted in maximum tomato growth and yield. When the pepper plant (*Capsicum annuum* L) was sprayed with MLE, Mathew (2016) observed maximum growth.

Seed Yield

The data pertaining to lettuce seed yield per hectare (kg ha^{-1}) are presented in Table 2. The data analysis revealed that there were significant differences among the lettuce cultivars ($P \leq 0.01$) and MLE concentrations ($P \leq 0.05$) in terms of seed yield. The interaction between MLE concentration and lettuce cultivars was nonsignificant. The means data regarding the cultivars show that cv. Milky White resulted in the maximum seed yield (1657.0 kg ha^{-1}) followed by cv. Red Laurel (1621.4 kg ha^{-1}), while the minimum seed yield (718.1 kg ha^{-1}) was observed in cv. Romaine. In terms of MLE concentration, the means reveal that maximum seed yield (1373.5 kg ha^{-1}) was recorded for plants treated with 8% MLE. However, this was also on par with 6%, 4%, and 2% MLE concentrations, which provided 1368.0 kg ha^{-1}, 1339.1 kg ha^{-1}, and 1321.1 kg ha^{-1} seed yield, respectively. Minimum (1275.7 kg ha^{-1}) seed yield was recorded for plants sprayed with distilled water (control). As compared to control, MLE concentrations of 2%, 4%, 6%, and 8% increased lettuce seed yield by 3.5%, 4.9%, 7.2%, and 7.6%, respectively.

In the comparison of various lettuce cultivars regarding seed yield per hectare, cv. Milky White showed the maximum seed yield. Genetic variability might be a possible reason for the variation in seed yield per hectare of the cultivars. Seed yield depends on the cultivar, as some cultivars have greater capability for producing higher yield than others (Zhao and Carey 2009). Another reason for the different seed yields is the growing period: lettuce cultivars vary in terms of growing time, which influences their seed yield (Zhao and Carey 2009). The present results corroborate those of Welbaum (2010), who reported that the seed yield of lettuce cultivars varied from 0.5 to 2 ton ha^{-1}.

According to the results of this study, an increase in the seed yield per hectare occurred with increased MLE concentrations. The presence of various essential macro- and micronutrients in MLE may be the reason for the increased seed yield. MLE also has a high concentration of antioxidants, ascorbic acid, phenolics, and amino acids. Plant hormones such as gibberellic acid, indole acetic acid, and zeatin are present in MLE (Muthalagu et al. 2018). MLE has an important role in increasing plant growth and development due to these essential nutrients, and, as a result, more seeds are produced (Yasmeen et al. 2013). Nouman et al. (2012) and Yasmeen et al. (2014) found that MLE treatment enhanced tomato productivity as compared to control.

Conclusion

This experiment confirmed that plants sprayed with 8% MLE showed better results for all the studied attributes as compared to controls. Maximum fresh head weight was produced by cv. Red Laurel. Maximum number of leaves and maximum seed yield were produced by cv. Milky White. However, Milky White was not liked due to its shape and bitter taste. Cultivars Romaine and Large Speed had the best taste. Moreover, cultivar Red Oakleaf was low in head weight and seed yield, but had attractive red leaves due to high anthocyanin content. The present research will benefit leafy vegetable (lettuce) growers of Pakistan as well as around the world. This organic fertilizer not only enhances the production of leafy vegetable (lettuce) but is also friendly to the environment and humans. Future research should be conducted to study the effect of higher concentrations of moringa leaf extract on lettuce cultivars.

Conflict of interest A. Farooq, A. M. Khattak, G. Gul, W. Habib, S. Ahmad, M. Asghar, and T. Rashid declare that they have no competing interests.

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