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EFFICACY OF MORINGA LEAF EXTRACT HORMESIS AS NATURAL BIOSTIMULANT ON YIELD AND BIOACTIVE COMPOUNDS OF LETTUCE

S.K. Pramanik[1]*, D. Saha[1], S. Mou[1], S. Ahammed[1], T. Siddiquea[1], B.S. Bandhan[2], L. Roy[1] and R. Hasan[1]

[1]Department of Crop Physiology and Ecology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh
[2]Department of Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh
*Corresponding Author, Email: pramaniksk100@gmail.com

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Abstract

This study was conducted to assess the effectiveness of moringa (*Moringa oleifera* Lam.) leaf extract (MLE) as a natural biostimulant on yield and bioactive compounds of lettuce. The trial was conducted during November 2023 to February 2024 with lettuce var. BARI lettuce-1. Twenty plastic pots following complete randomized design with four replications was followed. Four concentrations (3%, 4%, 5% and 6%) of MLE was foliar sprayed into lettuce where a control treatment (tap water) was also considered. Leaf number, leaf length, fresh and dry weight of leaves, SPAD value, total chlorophyll, total phenol, flavonoid and vitamin C were assessed. Correlation and regression analysis among the traits were also carried out. The results show that there was significant improvement in yield traits and bioactive compounds of lettuce except vitamin C due to foliar spray of MLE, where 6% MLE was found to maximize the value of the all studied traits of lettuce. Plants treated with 6% MLE provided maximum improvement by 25.01% in leaf number, 14.11% in leaf length, 20.61% in leaf fresh weight, 36.10% in leaf dry weight, 13.13% in SPAD value, 53.72% in total chlorophyll, 34.17% in total phenol and 19.29% in flavonoid compared to that of control. Correlation analysis among leaf traits and bioactive compounds showed positive connection with each other. Similarly, regression analysis among bioactive compounds exhibited positive correlation regression among the traits. Therefore, MLE could play a significant role as safe and natural biostimulant in improving yield and quality of lettuce through improving the vegetative and bioactive traits of lettuce leaf.

Introduction

Lettuce (*Lactuca sativa* L.), a member of the Asteraceae family, is cultivated worldwide and one of the most consumed rosette leafy vegetables in the raw form for its taste and high nutritive value. It is considered as one of the most important salad vegetables and an excellent source of vitamins, iron, folate, caffeic acid, carotenoids, and other antioxidants (Sonmez *et al.,* 2017; Malejane *et al.,* 2018).

Lettuce is a soil nutrient-depleted vegetable which produces a very shallow root system and requires adequate levels of fertilizer during its growing season (Thorup-Kristensen, 2001). Excessive fertilization of the lettuce field may cause many issues to the environment as water quality throughout leaching and runoff, eutrophication, greenhouse effect and acid rain (Heckman, 2007; Wang *et al.,* 2013) and harmful effects on human health (Ikemoto *et al*., 2002; Liu *et al*., 2014). The rising awareness of consumers on the importance of consuming sustainable, safe, and healthy foods could be met by improving the quality of leafy vegetables with the use of natural biostimulants (Corbo *et al.,* 2015). Safe and environmentally friendly natural products, such as natural biostimulants have essential role in sustainable agriculture through improving plant productivity and food quality while reducing environmental pollution (Shalaby, 2024).

Biostimulants are the products derived from biological materials that improve plant productivity including yield, quality and production efficiency due to the presence of plant growth regulators, essential nutrients and plant protective compounds (Yakhin *et al.,* 2017, Ho). Among natural biostimulants, moringa (*Moringa oleifera* Lam.) leaf extract (MLE) has attained massive attention due to its notable effect on plant productivity (Merwad, 2018; Zulfiqar *et al.,* 2020). Moringa (*Moringa oleifera* Lam.) leaf extract is a recently concerned plant extract that is applied to the plants as an environmentally friendly and safe biostimulant, since it contains many beneficial bioactive compounds, macro and micronutrients, minerals, plant hormones, vital amino acids and vitamins (Sohaimy *et al.,* 2015; Yaseen and Hájos, 2021). Moringa (*Moringa oleifera* Lam.) leaf extract is also considered as an alternative to inorganic fertilizer (Saini *et al.,* 2016). The benefits of MLE as a growth enhancer in improving yield and quality has been found in lettuce (Yaseen and Hájos, 2021) and many other crops.

No research information is available on the influence of MLE in improving the vegetative yield and quality of Bangladeshi lettuce cultivars. Therefore, the study was aimed to assess the feasibility of applying MLE to improve leaf yield and bioactive compounds of Bangladeshi lettuce variety, BARI lettuce-1.

Materials and Methods

Location, duration and growing conditions

A pot experiment was conducted at Department of Crop Physiology and Ecology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during November 15, 2023 to February 15, 2024. The experimental area is located under the Agro-ecological zone-1 of Old Himalayan Piedmont Plain with an elevation of 37.58 m above the sea level. The experimental area belongs to the subtropical climate characterized by rainfall during the month of last April to October and scanty rainfall in the rest of the year. The average temperature and humidity during the lettuce growing season is presented in Fig. 1. Twenty plastic pots (20 cm diameter and 25 cm height) were filled with equal amount (10 kg pot^{-1}) of a combined medium of soil and compost (weight ratio = 3:1). The soil was characterized by sandy loam texture with pH 5.50, organic carbon 0.24%, organic matter 0.413%, salinity 0.37 dS m^{-1}, total N 0.021%, available P 29.22 µg g^{-1} and exchangeable K 0.117 meq 100 g^{-1}. Inorganic fertilizers were applied on the basis of mass of soil per hectare area @ 200 kg Urea, 75 kg TSP and 75 kg MOP as per recommendation of Bangladesh Agricultural Research Institute (Azad *et al.,* 2019).

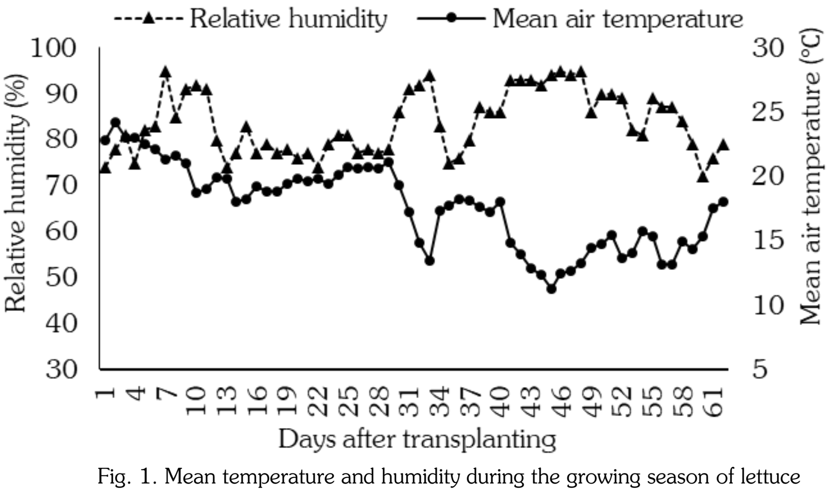

Fig. 1. Mean temperature and humidity during the growing season of lettuce

Raising of plant materials and transplanting in the experimental pots

Seeds of BARI Lettuce-1 were sown in seedbed on November 15, 2023. After 25 days of the seed sowing, 3 seedlings were transplanted to each experimental pot and light irrigation was given immediately after transplanting and continued up to the seedling's establishment and development in the pots. When the seedlings attained good development and hard enough then one healthy seedling was selected to remain in each pot and others were thinned out. Necessary measures were taken to protect the crop from various insect-pest and diseases during the growing period of the crop when and as necessary. For better growth and development of the plants, irrigation was provided when and as required.

Experimental design and treatments

The single factor experiment was performed using completely randomized design and replicated quarce. Four concentrations of MLE (3, 4, 5 and 6%) were sprayed on lettuce plants as foliar treatment along with a control treatment (normal tap water).

Preparation and application of moringa (*Moringa oleifera* Lam.) leaf extract

Moringa (*Moringa oleifera* Lam.) leaf extract was prepared by extraction of fresh young leaves obtained from mature moringa trees. First, the leaves were collected then washed and placed in a refrigerator at 4°C for 24 h as described by Yaseen and Takacs-Hajos (2022). The stored leaves were grinded with the amount of 100 ml water kg^{-1} fresh material using a kitchen blender, thereafter the mixture was squeezed and passed through a locally fabricated extraction machine and filtered twice using Whatman No. 1 filter paper based on the method by Foidl *et al.* (2001). The filtrate was placed in 8000 × g centrifuge for 15 min and supernatant was collected. The collected supernatant was considered as 100% MLE and saved as a stock extract for preparing desirable concentration of MLE (Yasmeen *et al.,* 2013). The extract was subject to keep in the refrigerator at 4℃ till the plants ready to be foliar sprayed. The required concentration of 3, 4, 5 and 6% MLE were prepared for the foliar spray from stock extract and mixed with 0.1% (v/v) surfactant (Tween 20) for optimal penetration. Two weeks after transplanting, the plants were foliar sprayed with respective concentrations of MLE by a hand sprayer at every 10 days intervals where the control plants were sprayed with water only.

Harvesting and data collection

All lettuce plants were harvested at 50 days after transplanting and data were collected on foliage yield (leaf number $plant^{-1}$, leaf length, fresh weight and dry weight of leaves $plant^{-1}$) and bioactive compounds (SPAD value, total chlorophyll, total phenol, flavonoid and vitamin C content of leaf). SPAD value of leaf was recorded just before harvesting the plants with a hand held SPAD meter (Model: SPAD-502 Plus, Konica Minolta, Inc.).

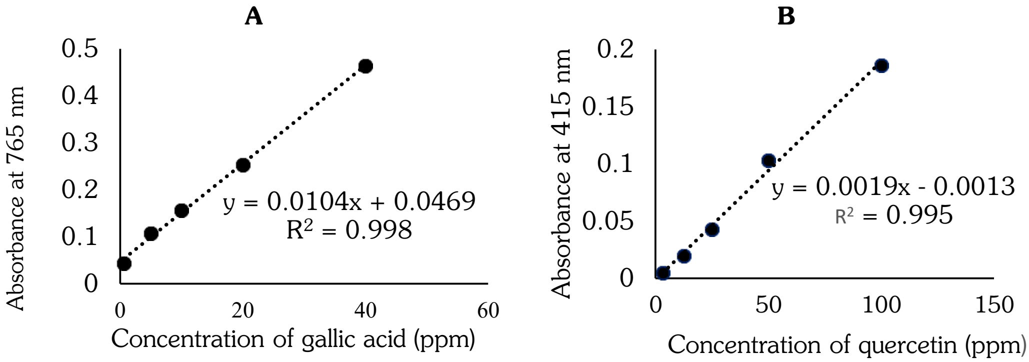

Fig. 2. Standard calibration curve of gallic acid (A) and quercetin (B) for determination of total phenol and flavonoid, respectively.

Total chlorophyll and vitamin C were measured on fresh weight basis but total phenol and flavonoid were determined on dry weight basis after harvesting the leaves. Total chlorophyll was estimated according to Witham *et al.* (1986) using the formula: total chlorophyll (mg g^{-1} FW) = [20.2 (D_{645}) + 8.02 (D_{663})] × [V/ (1000 × W)], Where, V = Volume of 80% aqueous acetone (ml), W = Weight of fresh leaf (g), D_{645} = Absorbance at 645 nm wavelength and D_{663} = Absorbance at 663 nm wavelength. Phenolic content of the dry extract of leaf was measured by the modified Folin-Ciocalteu's method as described by Zilani *et al.* (2016). Flavonoid content of dry extract of leaf was estimated using aluminium chloride colorimetric assay as described by Mahmud *et al.* (2017). Vitamin C content was determined by redox titration using iodine solution following the method described by Ciancaglini *et al.* (2001).

Statistical analyses

The recorded data were analyzed by partitioning the total variance with the help of software package "Statistix 10" program and the treatment means were compared by using Tukey's test at 5% level of probability.

Results and Discussion

Leaf traits of lettuce

The result showed that leaf traits of lettuce plant (leaf number $plant^{-1}$, leaf length, fresh weight and dry weight of leaves $plant^{-1}$) were significantly affected due to foliar application of different concentrations of MLE (Table 1).

Table 1. Effect of foliar application of MLE on leaf traits of lettuce

Treatments	Leaf number $plant^{-1}$	Leaf length (cm)	Fresh weight of leaves $plant^{-1}$ (g)	Dry weight of leaves $plant^{-1}$ (g)
Control	16.95 c	24.38 b	207.03 c	15.54 b
3% MLE	18.96 bc (+11.86)	24.45 a (+0.29)	215.49 bc (+4.09)	15.64 b (+0.64)
4% MLE	20.85 ab (+23.01)	25.14 a (+3.12)	236.20 ab (+14.09)	16.34 b (+5.15)
5% MLE	20.96 ab (+23.66)	26.10 a (+7.05)	239.30 ab (+15.59)	20.24 a (+30.24)
6% MLE	21.19 a (+25.01)	27.82 a (+14.11)	249.71 a (+20.61)	21.15 a (+36.10)
$LSD_{(0.05)}$	**	*	**	**
CV (%)	4.02	5.01	4.01	5.05
CD	2.1382	3.3958	24.734	2.4135

In a column, means having similar letter(s) did not differ significantly at $p \leq 5\%$ level by Tukey. ** and * indicate significant at 1% and 5% level of probability, respectively. Values in parenthesis indicate % improvement over control in respective treatment.

The minimum values of the leaf traits of lettuce were recorded under control condition, whereas the maximum values of the traits were measured under 6% MLE treated condition. Results showed that exogenously applied MLE meaningfully improved all the leaf traits studied as compared to control (normal water) at different extends. The degrees of improvement in leaf number $plant^{-1}$ of lettuce were 11.8, 23.01, 23.66 and 25.01% for 3, 4, 5 and 6% MLE treatments, respectively. An increase of 0.29, 3.12, 7.05, and 14.11% were observed in leaf length of lettuce due to application of 3, 4, 5 and 6% MLE, correspondingly when compared to control. In terms of fresh mass of leaves $plant^{-1}$, statistically similar enhancement (14.09, 15.59 and 20.61%) was observed in 4, 5 and 6% MLE treated lettuce, respectively, whereas 4.09% increment under 3% MLE as compared to that of control. The dry weight of leaves $plant^{-1}$ of lettuce also showed substantial progression under MLE application with the increment of 0.64, 5.15, 30.24 and 36.10% for respective MLE concentrations (3, 4, 5 and 6% MLE) than that of non-treated control condition. Overall results clarified that the highest magnitude of enhancement across all the leaf traits was observed under 6% MLE treatment, indicating that this concentration provided the most significant improvement regarding leaf traits compared to the other concentrations of MLE applied.

It is noticeable that foliar application of MLE had significant positive effect on forecited vegetative growth characters of lettuce plant. Foliar application of MLE significantly increased the leaf number and leaf length of each leaf of lettuce which consequently resulted in increased fresh and dry mass plant^{-1} as compared to control (normal tap water). The enhancement of lettuce leaf traits using MLE may be due to as moringa leaf is a rich source of amino acids, potassium, calcium, iron, vitamin E, ascorbates, phenolic compounds and growth regulating hormones like zeatin (Jiang and Asami, 2018) that reflected on an increase in growth characters of plants. The presence of phytohormones (auxin, GA_3 and especially cytokinin) (Latif and Mohamed, 2016) in biostimulant like MLE influence the physiological processes in plants (Rodrigues *et al.,* 2020) where cytokinin plays a vital role in increasing sink capacity (Zwack and Rashotte, 2013). This findings are in a line with the findings of Chanthanousone *et al.* (2022) who had been reported that the supplementation of MLE @ 200 mg L^{-1} enhanced the fresh mass and quality of lettuce leaves. Similar responses of lettuce plant to MLE were also observed by Admane *et al.* (2023) on hydroponic lettuce and Elbagory (2018) on head lettuce.

Bioactive compounds of lettuce leaf

The analysis of variance of collected data on bioactive compounds of lettuce leaf indicates that, foliar application of MLE significantly influenced the SPAD value ($p \leq 0.05$), total chlorophyll ($p \leq 0.01$), total phenol ($p \leq 0.01$) and flavonoid content ($p \leq 0.05$) of lettuce leaf except vitamin-C content of leaf (Table 2).

Table 2. Effect of foliar application of MLE on SPAD value and bioactive compounds of lettuce leaf

Treatments	SPAD value	Total chlorophyll (mg g^{-1} FW)	Total phenol (mg GAE g^{-1} DW)	Flavonoid (mg QE g^{-1} DW)	Vitamin C (mg g^{-1} FW)
Control	42.12 b	2.55 c	5.18 d	6.48 b	0.0347
3% MLE	43.59 ab (+3.49)	2.81 bc (+10.20)	5.52 cd (+6.56)	7.10 ab (+9.57)	0.0349 (+0.58)
4% MLE	43.76 ab (+3.89)	3.12 b (+22.35)	6.12 bc (+18.15)	7.35 a (+13.43)	0.0357 (+2.88)
5% MLE	45.43 ab (+7.86)	3.78 a (+48.26)	6.53 ab (+26.06)	7.49 a (+15.59)	0.0351 (+1.15)
6% MLE	47.65 a (+13.13)	3.92 a (+53.72)	6.95 a (+34.17)	7.73 a (+19.29)	0.0363 (+4.61)
LSD $_{(0.05)}$	*	**	**	*	NS
CV (%)	4.01	5.14	5.08	3.99	4.98
CD	4.7908	0.4468	0.8270	0.7753	0.4731

In a column, means having similar letter(s) did not differ significantly at p ≤5% level by Tukey. ** and * indicate significant at 1% and 5% level of probability, respectively. NS indicates not significant at 5% level of probability. Values in parenthesis indicate % improvement over control in respective treatment.

The findings revealed that, foliar application of MLE notably increased the bioactive compounds of lettuce leaf as compared to control condition but the degrees of increment were different for different concentrations of MLE. Though, there was no significant variation among the vitamin C content of lettuce leaf under different treatments. The degrees of augmentations in these bioactive traits were maximum with the 6% MLE application, followed by the 5, 4, and 3% MLE treatments, respectively. Among the treatments, the 3% MLE treatment improved the bioactive compounds of lettuce leaf by 3.49% in SPAD value, 10.20% in total chlorophyll content, 6.56% in total phenol content, 9.57% in flavonoid content and 0.58% in vitamin C content. An increasing level of 3.89% in SPAD value, 22.35% in total chlorophyll content, 18.15% in total phenol content, 13.43% in flavonoid content, and 2.88% in vitamin C content of lettuce leaf was observed when the lettuce plant was treated with 4% MLE. Under the treatment of 5% MLE, 7.86, 48.26, 26.06, 15.59 and 1.15% improvement were recorded in SPAD value, total chlorophyll content, total phenol content, flavonoid content and vitamin C content of lettuce leaf, respectively. The 6% MLE caused the uppermost augmentations with the increasing level of 13.13% in SPAD value, 53.72% in total chlorophyll content, 34.17% in total phenol content, 19.29% in flavonoid content and 4.61% in vitamin C content, making it the most effective treatment for enhancing these attributes as compared to other treatments.

As expected, the MLE application provides higher photosynthetic pigments that contribute to the enhancement of greenness (SPAD index) and chlorophyll content of leaf (Khan *et al.* 2022). This ability to increase pigment content is in line with other reports on plant-based biostimulants, whose application may lead to the up-regulation of nitrogen and carbon metabolism by enhancing N uptake efficiency and limiting chlorophyll degradation as well as leaf senescence (Colla *et al.,* 2017). Elzaawely *et al.* (2017) further mentions that the quality enhancement in plants treated with MLE is related to the hormone concentration in moringa leaves, particularly gibberellins (GA7). Under a glasshouse experiment, increments in polyphenols of different lettuce cultivars were found in plants treated with 6% MLE (Yaseen and Takacs-Hajos, 2022). Moringa (*Moringa oleifera* Lam.) leaf extract application at a concentration of 20% displayed the maximum phenolic and flavonoid content in the stevia leaf (Sardar *et al.,* 2021) that indicates the positive responses of MLE as the present study. The increment in vitamin C of lettuce leaf due to 6% foliar MLE was also reported by Yaseen and Takacs-Hajos (2022).

Correlation analysis of the leaf traits and bioactive compounds of lettuce

Correlation analysis among different leaf traits and bioactive compounds of lettuce presented in Table 3 reveals that all the traits showed significant positive correlation with each other except the relation of vitamin C with leaf dry weight plant^{-1} and total chlorophyll content.

Table 3. Correlations (Pearson) among leaf traits and bioactive compounds of lettuce

	LNP	LL	FWLP	DWLP	SPAD	TCCL	TPCL	FCL	Vit C
LNP	1.0000***								
LL	0.7640***	1.0000***							
FWLP	0.9574***	0.8417***	1.0000***						
DWLP	0.7512**	0.7789***	0.8625***	1.0000***					
SPAD	0.8123***	0.9078***	0.9042***	0.8839***	1.0000***				
TCCL	0.8722***	0.7633***	0.9284***	0.9717***	0.8741***	1.0000***			
TPCL	0.9275***	0.8219***	0.9857***	0.9298***	0.9215***	0.9745***	1.0000***		
FCL	0.9687***	0.8349***	0.9600***	0.8088***	0.9197***	0.8910***	0.9477***	1.0000***	
Vit C	0.6081*	0.8919***	0.6911**	0.4959 NS	0.8044***	0.4829NS	0.6249*	0.7082**	1.0000***

LNP = Leaf number plant^{-1}, FWLP = Fresh weight of leaves plant^{-1}, DWLP = Dry weight of leaves plant^{-1}, SPAD = SPAD value of leaf, TCCL = Total chlorophyll content of leaf, TPCL = Total phenol content of leaf, FCL = Flavonoid content of leaf, Vit C= Vitamin C content of leaf. *, ** and *** indicate correlation is significant at the 0.05, 0.01 and 0.001 level of probability (1-tailed), respectively. NS indicates correlation is not significant at the 0.05 level of probability.

The strongest significant positive correlation was observed in FWLP-TPCL ($r = 0.9857^{***}$) followed by TCCL-TPCL ($r = 0.9745^{***}$), DWLP-TCCL ($r = 0.9717^{***}$), LNP-FCL ($r = 0.9687^{***}$), FWLP-FCL ($r = 0.9600^{***}$), LNP-FWLP ($r = 0.9574^{***}$), TPCL-FCL ($r = 0.9477^{***}$), DWLP-TPCL ($r = 0.9298^{***}$), FWLP-TCCL ($r = 0.9284^{***}$), LNP-TPCL ($r = 0.9275^{***}$), SPAD-TPCL ($r = 0.9215^{***}$), SPAD-FCL ($r = 0.9197^{***}$), LL-SPAD ($r = 0.9078^{***}$) and FWLP-SPAD ($r = 0.9042^{***}$). Moderate significant and positive connection was discovered in LL-Vit C ($r = 0.8919^{***}$), TCCL-FCL ($r = 0.8910^{***}$), DWLP-SPAD ($r = 0.8839^{***}$), SPAD-TCCL ($r = 0.8741^{***}$), LNP-TCCL ($r = 0.8722^{***}$), FWLP-DWLP ($r = 0.8625^{***}$), LL-FWLP ($r = 0.8417^{***}$), LL-FCL ($r = 0.8349^{***}$), LL-TPCL ($r = 0.8219^{***}$), LNP-SPAD ($r = 0.8123^{***}$), DWLP-FCL ($r = 0.8088^{***}$) and SPAD-Vit C ($r = 0.8044^{***}$). Comparatively weak but significant positive association was found in LL-DWLP ($r = 0.7789^{***}$), LNP-LL ($r = 0.7640^{***}$), LL-TCCL ($r = 0.7633^{***}$) and LNP-DWLP ($r = 0.7512^{**}$). Among the correlations, comparatively weaker but positively significant connection was detected in FCL-Vit C ($r = 0.7082^{**}$) and FWLP-Vit C ($r = 0.6911^{**}$) and the weakest but significant positive correlation was found in LNP-Vit C ($r = 0.6081^{*}$) closely followed by TPCL-Vit C ($r = 0.6249^{*}$), whereas the non-significant but positive correlation was observed in DWLP-Vit C ($r = 0.4959^{NS}$) and TCCL-Vit C ($r = 0.4829^{NS}$).

Regression analysis of the bioactive compounds and SPAD value of lettuce

Regression analysis results of the bioactive compounds of lettuce in Fig. 3 reveals that, vitamin C had positive correlation regression equation with phenol content ($R^2 = 0.6861$) and flavonoid content ($R^2 = 0.6383$) of lettuce leaf. Even so, positive correlation regression equation was found between total chlorophyll content and SPAD value as well as between phenol content and flavonoid content of lettuce leaf at $R^2 = 0.8917$ and $R^2 = 0.8967$, respectively.

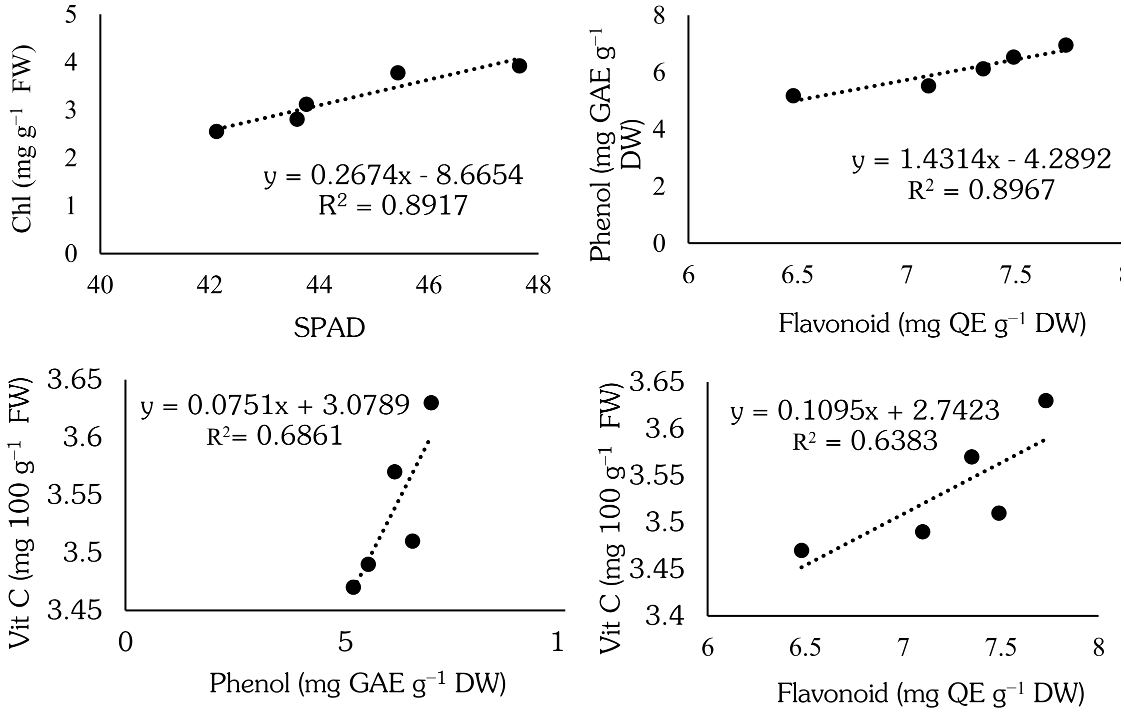

Fig. 3. Regression equation results of the studied bioactive compounds and SPAD value of lettuce

Conclusion

Moringa (*Moringa oleifera* Lam.) leaf extract as natural biostimulant improved yield traits and certain bioactive components of lettuce leaf. Therefore, MLE can be the reliable source of safe and natural biostimulant for stimulating yield and quality of lettuce. However, further studies will be effective to reach a conclusion about the efficacy as well as optimum concentration of MLE for improving productivity and quality of lettuce, ensuring its practical application in sustainable crop management.

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