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Exogenous application of bio-stimulants and growth retardants improve nutrient absorption and fiber quality in upland cotton

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Abstract

Background Natural and synthetic plant growth regulators are essential for plant health, likewise these regulators also play a role in increasing organic production productivity and improving quality and yield stability. In the present study, we have evaluated the effects of foliar applied plant growth regulators, i.e., moringa leaf extract (MLE) and mepiquat chloride (MC) alone and in combination MC and MLE on the conventional cotton cultivar (CIM 573) and transgenic one (CIM 598). The growth regulators were applied at the start of bloom, 45 and 90 days after blooming.

Results The application of MC and MLE at 90 days after blooming significantly improved the relative growth rate, net assimilation rate, the number of bolls per plant, and seed cotton yield. Likewise, the combined application of MLE and MC at 90 days after blooming significantly boosted the nitrogen uptake in locules, as well as the phosphorus and potassium uptake in the leaves of both cotton cultivars. The application of MLE alone has considerably improved the nitrogen uptake in leaves, and phosphorus and potassium contents in locules of Bt and conventional cotton cultivars. Similarly, Bt cotton treated with MLE at 90 days after blooming produced significantly higher ginning out turn and oil contents. Treatment in combination (MLE + MC) at 90 days after blooming produced considerably higher micronaire value, fiber strength, and staple length in conventional cultivar.

Conclusion The natural growth enhancer, MLE is a rich source of minerals and zeatin, improving the nutrient absorption and quality of cotton fiber in both conventional and Bt cotton cultivars.

Keywords Bio-stimulant, Cotton, Fiber quality, Mepiquat chloride, Moringa leaf extract, Potassium accumulation, Oil contents

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Introduction

Gossypium hirsutum L. is a cash crop cultivated for fiber and oil seed in many countries/territories, playing a vital role in the socio-economic activities. The textile industry relies on cotton plants to yield high-quality fiber (Dehghanisanij et al., 2022). Fiber quality is a range of assessable fiber properties that enhance the spinning performance during textile processing. Among these properties, fiber strength is the key fiber quality index and is measured as one of the imperative features defining yarn quality, particularly yarn strength (Sief et al., 2021). Fiber strength is primarily constituted during the thickening of the secondary fiber wall and is associated with the cellulose deposition (Mehran et al., 2023). The physiological effects of growth stimulants are diverse on various crops. They can directly affect the physiology and metabolism of the plant (Mosa et al., 2023) as well as growth and development from seed germination to maturity in various ways (Gupta et al., 2023).

Application of growth regulators enhances the plant metabolism efficiency, resulting in improved yield and product quality. These regulators also enhance the resistance to biotic and abiotic stresses, facilitate the absorption, transfer, and use of nutrients, improve the water consumption efficiency, and refine the physio-chemical properties of soil while promoting the growth of soil microorganisms (Irani et al., 2021). An exogenous application of different growth regulators boosted the phenolic, sugar, and total protein contents as well as chlorophyll biosynthesis (Arif et al., 2022a; Mashamaite et al., 2022). Furthermore, the foliar spray of various organic osmolytes improves the source-sink relationship which results in higher lint yield and oil seeds. Amongst various organic growth enhancers, leaves of moringa are rich sources of plant growth promoters (ascorbates and zeatin), minerals (K^+ and Ca^{2+}), and pigments (phenols and carotenoid), proved an ideal growth promoter (Shafiq et al., 2021). Foliar spray of moringa leaf extract (MLE) significantly increased the chemical composition, enzyme activities, growth, productivity, and fiber quality attributes (Ibrahim et al., 2021).

The growth pattern of the cotton crop is unique because it is a perennial with an indeterminate growth nature (Murtza et al., 2022). Excessive vegetative growth, poor bud development, shedding of squares and flowers, and imbalance between source and sink are reasons for the indeterminate growth behavior of cotton (Hussain et al., 2021). Numerous tactics have been tried to break the yield plateau, among which the use of plant growth retardants, especially mepiquat chloride (MC) has received greater attention among cotton researchers. Mepiquat chloride prevents cell expansion but not cell division. Its application channels the carbohydrates into reproductive organs and therefore diminishs vegetative growth (Abbas et al., 2022). This allows the plants to transfer the desired amount of photo-synthetase from the vegetative organs to the reproductive ones, enhancing the cotton yield and quality parameters (Hussain et al., 2021).

Considering the role and importance of plant growth stimulants in plant nutrition and health, especially in the production of healthy and organic products, an understanding of the characteristics and mechanisms of their effect on the plant, as well as the challenges to their application in field conditions, is necessary to optimize their use for cotton production. The worldwide use of growth-promoting substances has increased significantly in recent years, but there is still not enough documented information about these compounds, especially on the influence of moringa leaf extract and mepiquat chloride on cotton fiber quality parameters. This study was conducted to evaluate the role of MLE as a natural growth promoter and MC as a vegetative growth retardant for improving the seed cotton yield, fiber quality characteristics, and oil contents of both Bt and non-Bt cotton cultivars. The research was based on the hypothesis that combined application of MLE and MC might be more effective in obtaining optimum seed cotton yield and fiber quality attributes.

Results

Data regarding the relative growth rate was recorded at various stages of both cotton cultivars. Foliar spray of MLE and MC significantly improved the relative growth rate during 101–130 and 131–160 days after sowing (Fig. 1). A foliar spray of only MLE and combined application of MLE + MC on CIM 598 at 45 days after blooming produced a significantly higher relative growth rate than the control treatment with distilled water during 101–130 and 131–160 days after sowing.

The net assimilation rate followed an increasing trend in the primary stages of cotton growth and then reduced subsequently (Fig. 2). Data regarding the net assimilation rate was recorded at various stages of both cotton cultivars. Foliar spray of MLE and MC significantly improved the net assimilation rate during 101–130 and 131–160 days after sowing. A foliar spray of MLE on CIM 598 at 45 days after blooming produced a significantly higher net assimilation rate than the control treatment with distilled water during 101–130 and 131–160 days after sowing.

The number of bolls per plant was recorded at different stages, and foliar spray with the growth regulators after blooming significantly affected the number of bolls per plant and ultimately the final seed cotton yield during both years (Table 1). The combined application of MLE and MC at 90 days after blooming produced a significantly greater number of bolls per plant which ultimately produced the highest seed cotton yield per hectare.

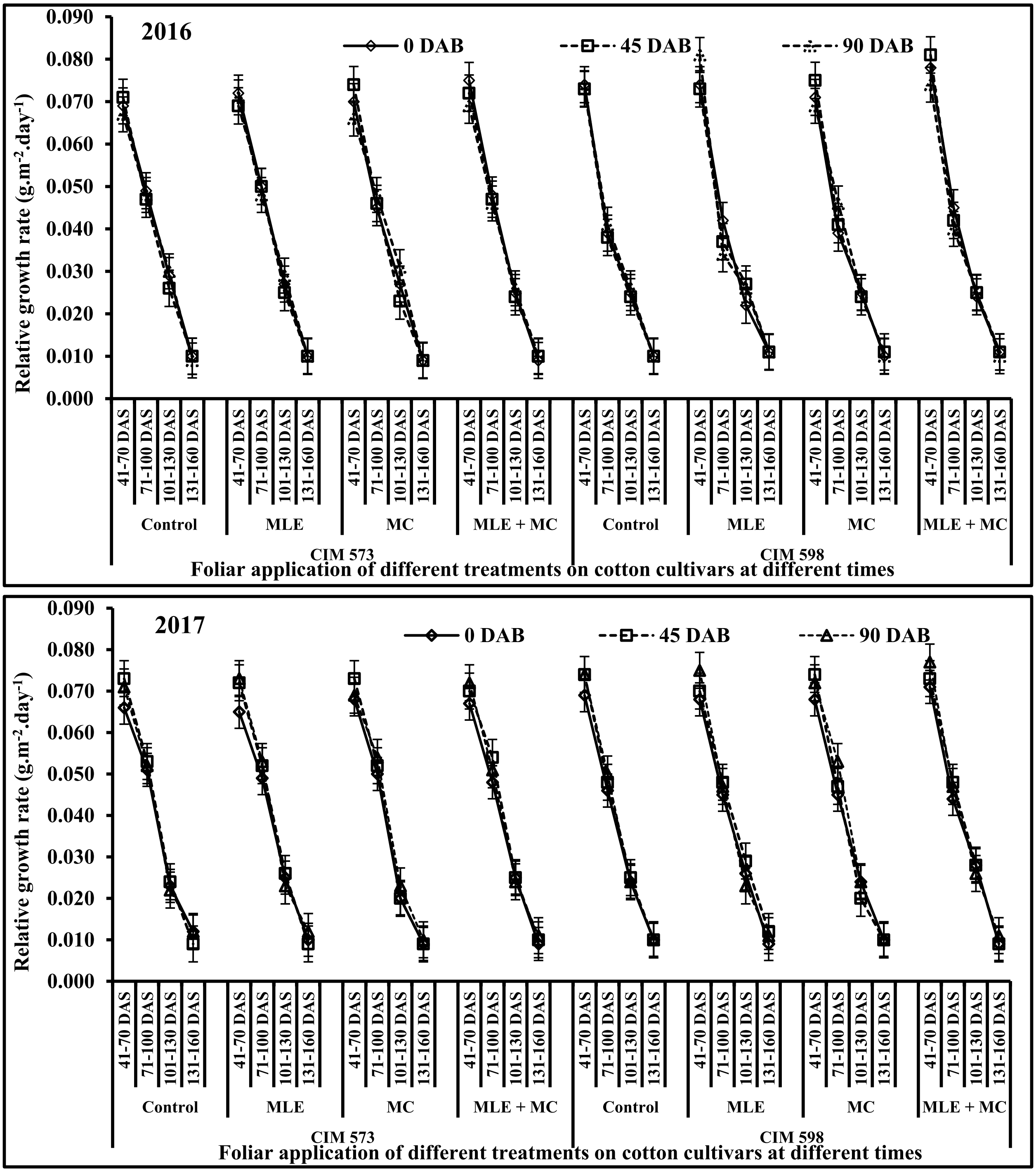

Fig. 1 Effect of foliar spraying with MLE (moringa leaf extract) and MC (mepiquat chloride) on the relative growth rate of two cotton cultivars during 2016 and 2017. DAB: days after blooming; DAS: days after sowing

The growth regulators significantly affected the nutrient accumulation and fiber quality characteristics of the varieties during both growing seasons. Spraying on CIM 598 with MLE at 90 days after blooming significantly increased the nitrogen percentage in leaves in both growing seasons (Table 2). The combined spray of MLE and MC at 90 days after blooming increased the uptake of phosphorus and potassium

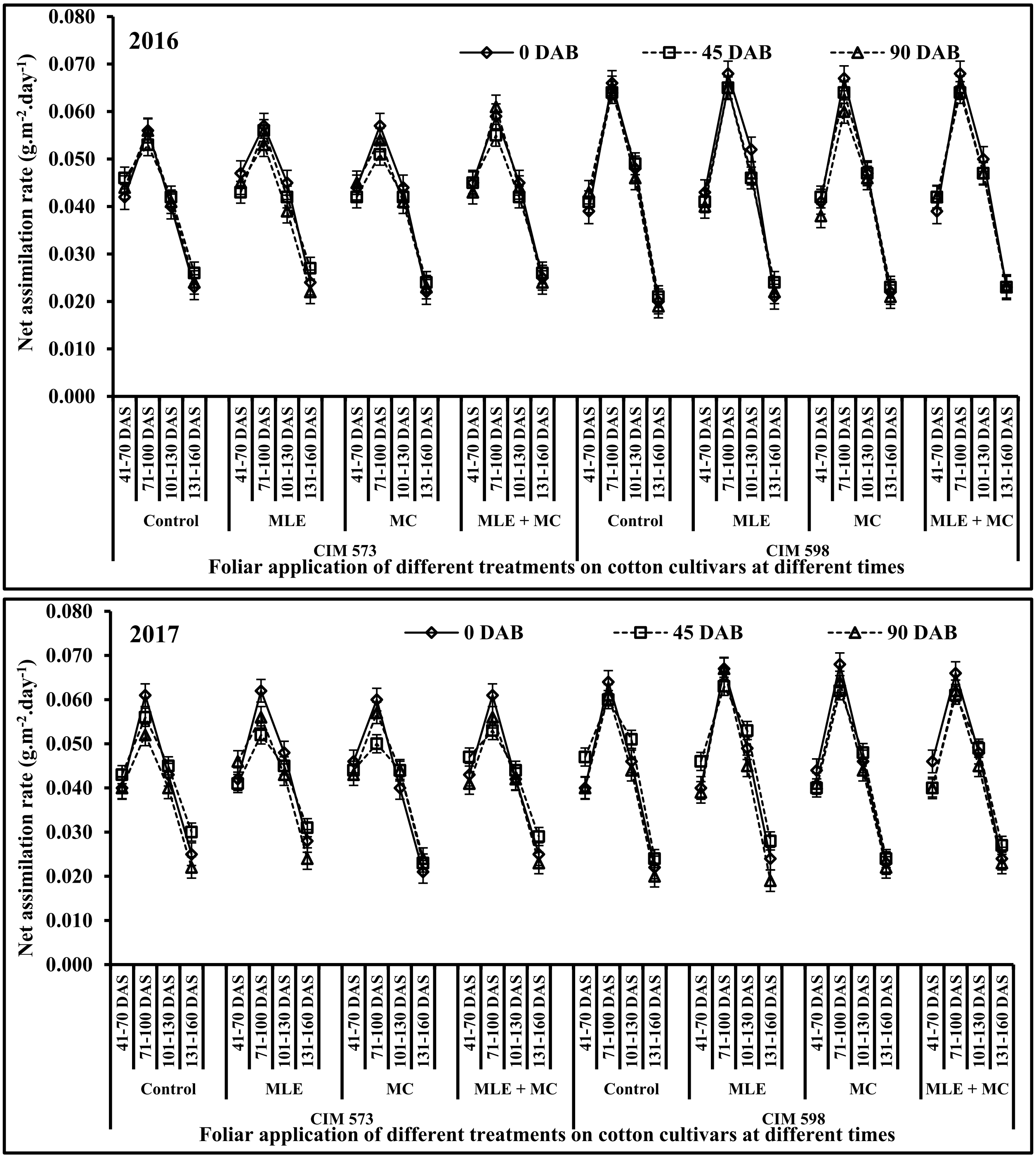

Fig. 2 Effect of foliar spraying with MLE (moringa leaf extract) and MC (mepiquat chloride) on net assimilation rate of two cotton cultivars during 2016 and 2017. DAB: days after blooming; DAS: days after sowing

in leaves of CIM 598 during both growing seasons (Table 2).

The MLE + MC treatment at 90 days after blooming significantly increased the locule nitrogen percentage in the Bt cotton during both growing seasons (Table 3), while the conventional cotton (CIM 573) sprayed with distilled water showed the lowest locule N, P, and K uptake. Foliar spray with MLE at 90 days after blooming showed a significantly higher percentage of phosphorus in the locule of CIM 598 (Table 3). Treatment of CIM 573

Table 1 Effect of foliar spray with MLE and MC on number of bolls per plant and seed cotton yield of two cotton cultivars

Treatments			No. of bolls per plant		Seed cotton yield/ (kg·ha^{-1})	
Cultivar	Foliar spray	Application time (DAB)	2016	2017	2016	2017
CIM 573	Distilled water	0	20.47m	22.37g	2 707.2k	2 676.9k
		45	21.27lm	22.94fg	2 919.7jk	2 707.7jk
		90	22.48jm	23.91cg	3 007.5ik	2 838.8ik
	MLE	0	24.69fi	24.38ag	3 103.0gk	2 961.9hk
		45	25.83dg	24.53ag	3 337.5ej	3 153.9ci
		90	26.46cf	25.73ac	3 395.7ei	3 350.9ae
	MC	0	22.09km	23.43cg	2 779.6k	2 990.9gk
		45	24.67fi	24.35ag	2 915.7jk	3 158.9ci
		90	24.85ei	24.35ag	3 162.4fk	3 071.8di
	MLE + MC	0	24.13gj	24.42ag	3 068.0hk	3 249.9ah
		45	26.11cg	24.91ag	3 344.9ej	3 309.4ag
		90	26.81be	25.71ad	3 367.3ej	3 397.6ac
CIM 598	Distilled water	0	22.51jl	23.98cg	3 519.8dh	2 995.5gk
		45	23.75hk	23.19dg	3 550.6dg	3 042.6ei
		90	23.26il	24.01cg	3 580.9cf	3 175.7bh
	MLE	0	25.94dg	25.23af	3 626.5ce	3 291.3ag
		45	28.04ac	25.04af	3 920.4ad	3 375.4ad
		90	28.03ac	25.82ac	4 022.5ac	3 489.0ab
	MC	0	22.07km	23.11eg	3 577.8cf	3 010.4fj
		45	24.14gj	24.20bg	3 734.6be	3 192.7bh
		90	25.69eh	25.10af	3 660.4ce	3 238.2bh
	MLE + MC	0	27.76ad	25.49ae	3 974.2ad	3 326.6af
		45	28.83ab	26.72ab	4 181.0ab	3 435.8ac
		90	29.06a	26.76a	4 279.4a	3 563.8a

Values are means of three replications with ten plants per replication. Values with the same letter in a column do not differ significantly at $P \leq 0.05$. *MLE* Moringa leaf extract, *MC* Mepiquat chloride, *DAB* days after blooming

with MLE + MC at 90 days after blooming significantly increased the potassium percentage in the locule.

Foliar spray on CIM 598 with MLE at 90 days after blooming produced the highest ginning percentage. The lowest ginning percentage was observed in CIM 573 control plots in 2016 (Table 4). However, the treatment with MLE + MC showed a non-significant impact on the ginning percentage in 2017.

Foliar spray on CIM 573 with MLE + MC at 90 and 45 days after blooming produced significantly higher micronaire values than other treatments (Table 4), while spraying on CIM 598 with distilled water and MC at the start of bloom produced the lowest micronaire values. Fiber strength is a key parameter that determines the yarn's spinnability. The combined application of MLE + MC and MLE on CIM 573 at 90 and 45 days after blooming produced significantly higher fiber strength while distilled water produced the lowest fiber strength in CIM 598 (Table 4). Foliar spray on CIM 573 with MLE at 45 and 90 days after blooming produced the highest fiber uniformity ratio in 2016 and 2017, respectively, whereas the lowest ratios were observed with MC and distilled water at 45 and 90 days after blooming in CIM 598 in 2016 and 2017, respectively (Table 4). Spraying on CIM 573 with MLE + MC at 90 days after blooming produced the longest fiber while the shortest was obtained from CIM 598 treated with distilled water at 0 days after blooming (Table 4). The MLE applied at 90 and 45 days after blooming produced the highest seed oil content for CIM 598 while distilled water applied to the conventional cultivar, CIM 573 at the beginning and 90 days after blooming produced the lowest seed oil content (Table 4).

Discussion

The use of bio-stimulants in agriculture altered the constituents of crop plants and enhanced their growth, yield, and quality parameters (Hu et al., 2023). In the current study, foliar application of MLE in combination with MC improved the relative growth rate and net assimilation rate and increased the number of bolls per plant which finally improved the seed cotton yield. This effect may be attributed to the foliar spray of MLE alters the endogenous cytokinin levels; the enhanced contents stimulate cell division

Table 2 Effect of foliar spray with MLE and MC on nutrient uptake in leaves of two cotton cultivars

Treatments			Nitrogen /%		Phosphorus /%		Potassium /%	
Cultivar	Foliar spray	Application time (DAB)	2016	2017	2016	2017	2016	2017
CIM 573	Distilled water	0	0.143gh	0.147c	0.213c	0.223c	1.753f	1.790g
		45	0.153fh	0.147c	0.230ac	0.243ac	1.853ef	1.837fg
		90	0.133h	0.157bc	0.220bc	0.243ac	1.927de	1.880bg
	MLE	0	0.177ch	0.180ac	0.233ac	0.277ac	2.057ad	1.987bg
		45	0.193ah	0.197ac	0.247ac	0.293ab	2.073ad	2.087ab
		90	0.210af	0.207ac	0.267ac	0.290ab	2.120a	2.027af
	MC	0	0.170eh	0.187ac	0.240ac	0.257ac	1.940be	1.857dg
		45	0.183bh	0.190ac	0.243ac	0.267ac	1.933ce	1.843eg
		90	0.180bh	0.183ac	0.237ac	0.267ac	1.937be	1.917bg
	MLE + MC	0	0.183bh	0.193ac	0.247ac	0.283ac	2.087ad	2.077ac
		45	0.193ah	0.197ac	0.253ac	0.283ac	2.090ad	2.093ab
		90	0.203ag	0.207ac	0.253ac	0.300a	2.107ac	2.233a
CIM 598	Distilled water	0	0.197ag	0.180ac	0.240ac	0.230bc	2.030ad	1.790g
		45	0.173dh	0.193ac	0.253ac	0.220c	2.057ad	1.863cg
		90	0.183bh	0.190ac	0.250ac	0.233bc	2.070ad	1.857dg
	MLE	0	0.230ae	0.220a	0.270ac	0.267ac	2.113ab	2.057e
		45	0.233ad	0.213ab	0.270ac	0.273ac	2.083ad	2.070ad
		90	0.247a	0.237a	0.280ab	0.283ac	2.147a	2.093ab
	MC	0	0.223ae	0.197ac	0.270ac	0.243ac	2.037ad	1.937bg
		45	0.220ae	0.193ac	0.263ac	0.233bc	2.060ad	1.973bg
		90	0.230ae	0.203ac	0.277ab	0.243ac	2.070ad	1.953bg
	MLE + MC	0	0.237ac	0.223a	0.283ab	0.270ac	2.123a	2.053ae
		45	0.237ac	0.227a	0.280ab	0.277ac	2.120a	2.083ab
		90	0.240ab	0.233a	0.293a	0.283ac	2.150a	2.093ab

Values are means of three replications with ten plants per replication. Values with the same letter in a column do not differ significantly at $P \le 0.05$. *MLE* Moringa leaf extract, *MC* Mepiquat chloride, *DAB* days after blooming

resulting in significantly higher growth and yield (Khan et al., 2021; Shafiq et al., 2021). Moreover, the foliar spray of MLE prevents the abscission of squares and bolls and stimulates the mobilization and accumulation of photosynthates in newly formed bolls (Arif et al., 2019). Foliar spray with MC, being anti-gibberellic acid when absorbed by the plants, leads to a decrease in cell elongation, thereby inhibiting vegetative growth but improving the retention of early buds and bolls with higher productivity, particularly during the first pick period. This may be associated with higher availability of plant nutrients to reproductive parts through favorable photo-assimilate partitioning from the vegetative to the reproductive organs (Arif et al., 2022b). Moreover, foliar application of MC in combination with MLE maintains the balance between the vegetative organs and the reproductive ones and hence the overall productivity. They also play an important role in maintaining internal hormonal balance and effective source-sink relationship, which improves yield contributing parameters depending on the enhanced photosynthetic activity (Hussain et al., 2021).

Exogenously applied MLE significantly improved the nutrient uptake in cotton leaves and locules. Similarly, Yuniati et al. (2022) observed higher uptake and accumulation of various nutrient elements such as nitrogen, phosphorus, potassium, calcium, iron, and magnesium in different parts of numerous crop plants with the application of MLE, which was considered to enhance the absorption of mineral nutrients and translocation throughout the plant by improving the membrane permeability of roots for electrolytes, averting nutrient fixation, and enhancing their mobility in soil. Foliar spray of MLE remarkably improved the N, P, and K contents in a snap bean pod (Elzaawely et al., 2016) and brinjal fruit (Hoque et al., 2020).

The variation in fiber quality attributes between the two cotton cultivars is attributed to the use of different types of genetic material and efficient utilization of inputs and natural resources (Arif et al., 2019). Previous studies indicated similar reports and they concluded that conventional cultivars have produced better fiber quality attributes than Bt cultivars, possibly due to the

Table 3 Effect of foliar spray with MLE and MC on nutrient uptake in locules of two cotton cultivars

Treatments			Nitrogen uptake in locule /%		Phosphorus uptake in locule /%		Potassium uptake in locule /%	
Cultivar	Foliar spray	Application time (DAB)	2016	2017	2016	2017	2016	2017
CIM 573	Distilled water	0	0.313ij	0.353f	0.313bc	0.323fg	0.723g	0.770be
		45	0.303j	0.373ef	0.310c	0.340eg	0.757af	0.760ce
		90	0.323hj	0.380df	0.330ac	0.310g	0.750cg	0.787ae
	MLE	0	0.390dg	0.380df	0.340ac	0.360dg	0.763ae	0.790ae
		45	0.407bg	0.410bf	0.360ac	0.377bf	0.773ac	0.803ad
		90	0.427ad	0.417be	0.370ac	0.390ae	0.787a	0.803ad
	MC	0	0.353gj	0.367ef	0.340ac	0.333eg	0.747cg	0.767be
		45	0.363ei	0.380df	0.350ac	0.360dg	0.763ae	0.783ae
		90	0.357fj	0.370ef	0.340ac	0.387ae	0.777ac	0.800ae
	MLE + MC	0	0.393dg	0.397cf	0.347ac	0.387ae	0.767ad	0.810ac
		45	0.430ad	0.400bf	0.350ac	0.423ac	0.777ac	0.827a
		90	0.413ag	0.437ad	0.373ac	0.420ad	0.783ab	0.830a
CIM 598	Distilled water	0	0.387dg	0.410bf	0.357ac	0.367cg	0.737dg	0.747e
		45	0.400cg	0.410bf	0.360ac	0.390ae	0.727fg	0.750de
		90	0.380dh	0.423ae	0.377ab	0.387ae	0.733eg	0.783ae
	MLE	0	0.420ae	0.440ad	0.363ac	0.403ad	0.753bg	0.787ae
		45	0.437ad	0.440ad	0.377ab	0.427ac	0.767ad	0.800ae
		90	0.467ab	0.460ab	0.380a	0.443a	0.773ac	0.813ac
	MC	0	0.417af	0.420be	0.347ac	0.410ad	0.740dg	0.760ce
		45	0.403cg	0.427ae	0.360ac	0.410ad	0.747cg	0.777ae
		90	0.413ag	0.443ac	0.353ac	0.413ad	0.763ae	0.770be
	MLE + MC	0	0.440ad	0.443ac	0.357ac	0.427ac	0.773ac	0.790ae
		45	0.457ac	0.450ac	0.367ac	0.413ad	0.777ac	0.803ad
		90	0.473a	0.483a	0.367ac	0.430ab	0.783ab	0.820ab

Values are means of three replications with ten plants per replication. Values with the same letter in a column do not differ significantly at $P \leq 0.05$. *MLE* Moringa leaf extract, *MC* Mepiquat chloride, *DAB* days after blooming

superior genetic potential and optimum agroecological aspects (Yasmeen et al., 2018). Foliar spray of MLE and MC had a realized role in fiber development and hence the foliar spray of this combination improved the fiber properties of cotton and produced longer cellulose molecules and better cross-linkages between the fibers that enhance their quality traits (Hussain et al., 2021). It might be due to the fact that the combined application of MLE + MC plays an important role in enzymatic activities, cell division, hormonal balance, photosynthesis, and translocation of photosynthates from leaves to bolls thus improving the fiber quality attributes (Arif et al., 2022b).

Conclusions

It is concluded from this study that the foliar application of MLE alone, and in combination with MC at 90 days after blooming can improve nutrient absorption and thereby improve plant growth, yield, and quality attributes of upland cotton.

Materials and methods

Experimental site and plot management

Two field experiments were carried out at the Agricultural Research Farm of Bahauddin Zakariya University Multan, Pakistan. Each experiment was triplicated by following a randomized complete block design with a factorial arrangement. Two genotypes, namely CIM 598 (Bt cotton) and CIM 573 (non-Bt), were selected based on different genetic characteristics. CIM 598 is a Bt cultivar having high yield potential, early maturity, heat tolerance, good boll opening, and tolerance to jassid incidence. It was developed by Central Cotton Research Institute, Multan, Pakistan in 2012. CIM 573 is a non-Bt high-yielding cultivar having excellent fiber characteristics. It was also developed by Central Cotton Research Institute, Multan, Pakistan in 2012. The major difference among these cultivars lies in the presence of the *Bacillus thuringiensis* gene. These cultivars had semi erect growth habits and are adopted in agro-ecological conditions of the Multan region of Pakistan. Foliar spray with two growth

Table 4 Effect of foliar spray with MLE and MC on the ginning percentage, fiber quality parameters and oil contents of two cotton cultivars

Treatments			Ginning outturn/ %		Micronaire value		Fiber strength/ (cN·tex^{-1})		Fiber uniformity ratio		Staple length / mm		Oil contents /%	
Cultivar	Foliar spray	Application time (DAB)	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
CIM 573	Distilled water	0	39.72ac	41.54a	3.63bf	3.73a	35.32b	35.20a	84.97ad	85.33ac	29.84ef	29.64b	17.63i	17.54ik
		45	39.07bc	38.98a	3.60bg	3.65ac	35.63b	35.71a	85.27ac	84.89ad	29.78f	29.44b	17.80hi	17.20jk
		90	38.83c	41.27a	3.69ad	3.68a	35.71b	34.93a	85.33ab	84.74be	29.94ef	29.50b	17.95hi	17.11k
	MLE	0	39.91ac	41.65a	3.68ad	3.68a	35.79b	35.49a	85.82a	86.12ab	30.23ce	29.90ab	18.46fg	18.10ej
		45	39.83ac	41.05a	3.74bc	3.66ab	36.28ab	35.56a	86.51a	86.21a	30.38ac	30.04ab	18.62ef	18.73bh
		90	40.57ac	41.85a	3.79ab	3.70a	36.20ab	35.42a	85.96a	85.62ab	30.64ab	30.11ab	18.78ef	18.05fk
	MC	0	41.20ac	40.77a	3.62bf	3.67ab	35.62b	34.78a	85.72a	85.57ab	29.89ef	29.72b	17.94hi	17.77hk
		45	40.90ac	40.22a	3.65be	3.72a	36.29ab	35.39a	85.75a	84.75be	29.97df	29.89ab	18.06gi	18.19ej
		90	40.24ac	42.16a	3.59bg	3.69a	36.26ab	35.01a	85.27ac	85.22ac	29.94df	29.61b	18.15gh	17.81gk
	MLE + MC	0	40.34ac	40.65a	3.74ac	3.72a	36.30ab	35.22a	84.94ad	84.76be	30.15cf	29.81ab	18.47fg	18.14ej
		45	39.96ac	41.26a	3.78ab	3.73a	36.87a	34.75a	86.01a	86.04ab	30.34bd	30.01ab	18.76ef	18.10ek
		90	40.41ac	41.63a	3.85a	3.63ad	36.96a	34.91a	85.82a	86.20a	30.76a	30.42a	18.86ef	17.96gk
CIM 598	Distilled water	0	40.99ac	41.21a	3.37h	3.55de	29.86cd	28.87b	82.64e	83.21fh	27.97k	27.67e	18.80ef	19.03af
		45	40.82ac	39.37a	3.43fh	3.57be	29.45d	29.10b	82.70e	82.55gh	27.98jk	28.31ce	18.91ef	18.61ch
		90	41.16ac	41.23a	3.42gh	3.52e	30.04cd	28.73b	82.55e	82.30h	28.20hk	28.43cd	19.02de	18.35di
	MLE	0	41.58ac	41.32a	3.44fh	3.52e	30.08cd	29.15b	82.69e	82.69gh	28.38gj	28.05ce	19.44cd	19.11ae
		45	41.14ac	40.55a	3.49dh	3.54de	30.22cd	29.00 b	83.77ce	83.10fh	28.51gh	28.33ce	19.88ac	19.88a
		90	41.87a	41.48a	3.50dh	3.57be	30.37cd	28.79b	82.69e	82.69gh	28.53gh	28.53cd	20.04a	19.38ac
	MC	0	41.68ab	40.90a	3.41gh	3.50e	29.92cd	28.90b	83.25e	83.85dg	28.10ik	28.43cd	18.69ef	18.43ci
		45	41.38ac	40.76a	3.44eh	3.51e	30.35cd	29.04b	82.44e	83.24fh	27.92k	28.25ce	18.74ef	18.40ci
		90	40.65ac	40.02a	3.47eh	3.55ce	30.29cd	28.95b	82.88e	83.21fh	28.14hk	28.47cd	18.91ef	18.37ci
	MLE + MC	0	41.26ac	38.09a	3.53dh	3.53de	30.35cd	28.99b	83.18e	83.51eh	28.21hk	27.87de	19.46bd	18.79bg
		45	40.98ac	40.18a	3.53ch	3.57be	30.81c	28.83b	83.91be	84.11cf	28.47gi	28.66c	19.68ac	19.68ab
		90	41.57ac	41.40a	3.59bg	3.52e	30.86c	28.94b	83.50de	82.94fh	28.64g	28.68c	19.91ab	19.34ad

Values are means of three replications with ten plants per replication. Values with the same letter in a column do not differ significantly at $P \leq 0.05$. *MLE* Moringa leaf extract, *MC* Mepiquat chloride, *DAB* days after blooming

Table 5 Physi-chemical properties of the experimental soils

Year	pH_{water}	EC_{water}/ ($dS\cdot m^{-1}$)	BD/ ($g\cdot cm^{-3}$)	PWP/ ($cm^3\cdot cm^{-3}$)	FC/ ($cm^3\cdot cm^{-3}$)	OC/ %	pH_{soil}	Texture	EC_{soil}/ ($dS\cdot m^{-1}$)	Silt/%	Sand/%	Clay/%
2016	7.3	0.89	1.34	0.11	0.28	0.34	9.3	Silty loam	0.99	67	20	13
2017	7.6	0.77	1.38	0.11	0.28	0.64	7.9	Silty loam	1.11	72	17	11

pH Potential of hydrogen, *EC* Electrical conductivity, *BD* Bulk density, *PWP* Permanent wilting point, *FC* Field capacity, *OC* Organic carbon regulators, i.e. moringa leaf extract (MLE, 30 times diluted) and Mepiquat hloride (MC, 42 g·ha^{-1} in active ingredient), either alone or in combinations and distilled water was taken as control. The foliar spray was applied at three stages, i.e. the start of bloom, 45 and 90 days after blooming. Pre-sowing physicochemical characteristics of soil and water analyzed are presented in Table 5.

Crop husbandry

The seedbed was prepared with a tractor-mounted ridge, and 6 m×4.5 m beds were properly shaped. Seeds of CIM 573 and CIM 598 were dibbled manually, keeping a plant-to-plant distance of 30 cm and a row-to-row of 75 cm, resulting in an approximate plant population of 43 000 per hectare. The furrows were irrigated followed by dibbling to achieve the highest germination percentage. Following irrigations were applied with 6–10 days intervals depending on crop requirement, until the first week of September.

Nitrogen fertilizer at 140 and 115 kg·ha^{-1} for CIM 598 and CIM 573 was applied in three identical splits at the time of seedbed preparation, the start of flowering, and the peak flowering phase. Phosphorus and potassium fertilizers were broadcast at 55 and 60 kg·ha^{-1} during seedbed preparation.

Data collection

Ten plants were randomly selected from each experimental unit and labeled to record the number of bolls per plant in each experimental plot. Manual harvesting of seed cotton was performed twice in the middle two rows. Cotton picking was first carried out at 60% boll opening, while the last picking was performed on 24th and 27th November of 2016 and 2017, respectively.

Relative growth rate (RGR) and net assimilation rate were computed using the standard procedures of Radford (1967). Leaf samples (the 6th and 7th from the top) were collected from the central part of ten randomly selected plants at 210 days after sowing. Locule samples (the 4th and 5th from the top) were collected from ten randomly selected plants at 225 days after sowing. Both the leaf and locule samples were washed with distilled water, air-dried, and then oven-dried at 72 °C till constant weight in the oven (SLN 32, POLEKO-APARATUR A). Dry ashing was used to determine the nitrogen contents in cotton leaves and locules by the micro Kjeldahl method and phosphorus content by the Vanadomolybdophosphoric yellow colour method (Shimadzu UV–Vis spectrophotometer, model: UV-1280, wavelength range: 190 to 1 100 nm, Japan) on spectrophotometer after digestion in triacid (Jackson, 1967). Leaves and locules tissue were digested in a diacid mixture (HNO_3 and $HClO_4$ in 3:1, v/v), and the potassium content of aqueous extracts was determined by a flame photometer (FP910-4) (Miller, 1997). Ginning out turn was calculated using the following equation.

$$\text{Ginning out turn} = (\text{Weight of lint}) / (\text{Total weight of seed cotton}) \times 100\%$$

Seed cotton acquired from the net plot area of each treatment was uniformly mixed and a sample of 300 g was separated from each treatment, and then ginned to determine the fiber quality parameters. Lint samples were used to evaluate the fiber quality parameters using HVI-900, a computerized High-Volume Instrument that provides a comprehensive profile of raw fiber according to the International Trading Standards (Sundaram et al., 2002).

Oil extraction was carried out using n-hexane (1 000 mL) according to the method proposed by Atolani et al. (2016). A sample of cottonseed of 200 g was used for oil extraction using Soxhlet extractor at 55 °C for 7 h. The oil was obtained using a rotary evaporator at 40 °C, according to standard methods described by Zubair et al. (2018).

Statistical analysis

The data of each variable were subjected to analysis of variance (ANOVA) using the statistical package of MSTAT-C (Fareed et al., 1991). Treatment mean differences of each parameter were conducted using the least significant difference (LSD) (Steel et al., 1997).

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Authors' contributions

Al-Khayri JM, Arif M, and Kareem SHS conceptualized, designed the experiments, wrote the first draft of the manuscript. Arif M, Anwar A, and Aftab K performed the experiments. Yasmeen A supervised the experiments. Dehghanisanij H, Emami S reviewed and edited the manuscript. Arif M, Kareem SHS, and Anwar A analyzed the data. Arif M and Yasmeen A contributed the reagents and materials. Negm M, Arif M, and Kareem SHS reviewed the final manuscript for improvements. All authors read and approved the final manuscript.

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Availability of data and materials

All the data related to the present study are included in the article. Any further details related to the experiments conducted can be made available by requesting the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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