Mepiquat chloride-mediated modulation of agronomic, physiological and quality traits of cotton (*Gossypium hirsutum* L.) sown under varying plant spacings

Running Title: Foliar spray of mepiquat chloride in cotton under different plant spacings

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Abstract

Different crop management practices significantly alter agronomic and physiological traits of cotton. Excessive vegetative growth results in low seed cotton yield as reproductive growth is suppressed. Mepiquat chloride (MC) is a plant growth regulator capable of suppressing vegetative growth and enhancing reproductive growth of cotton. Optimum plant spacing improves planting density and physiological attributes of crop plants. The current study was laid out according to split-plot arrangement with three replications at research area of Department of Agronomy, University of Agriculture Faisalabad. Treatments comprised of two factors, i.e., five levels of MC (0, 30, 60, 90, 120 g ha^{-1}) and two plant spacings (23 cm and 30 cm). Effect of plant spacing was non-significant for most of the studied agronomic, physiological and quality characters of cotton. Likewise, its interaction with MC was also non-significant except for seed cotton yield and ginning out turn. Application of MC helped the cotton plants to achieve optimal plant height which was reduced by 3 to 13%. Application of 90 and 120 g ha^{-1} MC resulted in statistically similar plant height. Increments of 35 and 46% in sympodial branches were recorded in response to 90 and 120 g ha^{-1} MC, respectively. Upper limit of seed cotton yield was noticed under wider plant spacing of 30 cm and application of 120 g ha^{-1} MC. Among physiological characters' nitrogen contents, chlorophyll *a* and *b* contents and relative water contents were increased by increasing MC levels regardless of plant spacing. Fiber quality traits also responded positively to MC application. The application of 120 g ha^{-1} proved optimum level of MC for better growth and yield of cotton plants.

Keywords: *Crop Stature, Canopy Architecture, Gossypium hirsutum; Mepiquat chloride; Plant spacing; Fiber Quality*

Introduction

Cotton (*Gossypium hirsutum* L.) is an important fiber crop that provides raw material for textile industry. However, infestation of insects and pests, low water availability and excessive vegetative growth have significantly decreased its production in the last decade. Indeterminate growth habit and perennial nature of cotton plant pose serious challenge while managing it as an annual crop in a rotation. Increased application of inputs such as irrigation and fertilizers, cultivation of tall growing varieties, increased planting densities to compensate for unforeseen biotic and abiotic stresses, and excessive rains during Moonsoon may cause excessive vegetative growth of cotton plants [1,2]. In Pakistan, cotton is mostly grown on sandy loam soils and thus requires frequent irrigations during hot months of June and July, which triggers vegetative growth. More vegetative growth can lead to shedding of squares, flowers and bolls, production of fewer nodes and reproductive branches, and shortened internodes, thereby reducing lint yield and delaying maturity. Loss of reproductive sinks can alter dynamics of source-sink relationship and more photo-assimilates move towards vegetative plant parts thereby stimulating main stem growth. There is a trade-off between excessive vegetative growth and reproductive growth in cotton [3]. A dense crop canopy provides congenial microclimate for boll rot fungi and might interfere with other crop husbandry practices and harvesting operations [4].

Cotton growers depend on plant regulators for example mepiquat-chloride to control extensive growth of vegetative parts. Different practices are being adapted by the farmers' community to enhance the productivity of agronomic and horticultural crops [5-8] including the application mineral elements, synthetic compounds, plants extracts and biostimulants [9-12] via soil, seed priming agents and foliar spray [13-16]. Mepiquat-chloride (1, 1-dimethylpiperidinium chloride; MC) is a plant growth regulator an inhibitor of gibberellin biosynthesis that suppresses vegetative growth and favors the transport of assimilates towards reproductive parts [17]. The MC modifies plant stature by decreasing leaf area, length of internodes, increases light penetration and use efficiency and promotes setting of bolls at sympodial branches which collectively results in increased yield [18]. The effects of MC on seed cotton yield remain inconsistent [19] and studies report contrasting findings such as increment [20], decrease [21] and no effect as well [19,22]. The influence of MC on modulation of cotton crop growth and cotton seed yield is more pronounced under conditions (such as high temperature) which promote excessive vegetative growth [23].

Optimum plant population of cotton crop is vital to get higher yield [24]. The planting density significantly alters growth and development of plant, fiber quality and seed cotton yield [25]. Planting technique is key factor affecting crop development and growth and yield of crop plants. Optimum plant density depends on cultivar type, planting time and the growth environment [26], whereas fiber quality characters are also affected by plant population and spacing [25]. Fiber quality and seed cotton yield can be increased through increased plant density; however, better nutrient management is needed [20]. The early maturity of crops can be achieved by various management factors including, nitrogen application, cultivar, date of sowing, plant density, irrigation, growth regulators and insect control [27]. Much focused work has been undertaken to optimize dose and application timing of MC for cotton crop; while, its interaction with other crop management practices needs further research. Limited studies have been conducted on the interactive effect of MC and plant spacing on morpho-physiological attributes and seed cotton yield. Previous work reported significant interaction of planting density with MC application for plant height and seed cotton yield [18,28], while other studies reported a non-significant interaction [29,30]. This study assessed the interactive effect of MC and plant spacing on morpho-physiological attributes and seed cotton yield. It was hypothesized that growth and yield response of cotton to MC application will vary with plant spacing. Control of vegetative growth of cotton (grown at variable plant spacing) through the application of MC was the major objective of the current study. The results will be helpful to improve cotton productivity in the areas having excessive vegetative and low reproductive growth of cotton crop.

Materials and Methods

The field study to assess the interactive effect of plant spacing and MC on morpho physiological attributes and seed cotton yield of cotton crop was conducted at Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan during summer 2019. Average values of maximum and minimum temperatures (℃), rainfall (mm) and relative humidity (R.H) are given in Figure 1. The data relating to meteorological attributes were recorded during cotton growing season, i.e., May to November, 2019. The experiment was laid out according to randomized complete block design with split-plot arrangements. The experiment consisted of two factors. The main plot factor was plant spacing (PS), whereas, the MC application rate was the sub-plot factor. The net plot size was 15 m^2 and row-to-row spacing was 75 cm. Two plant spacings (23 and 30 cm) and five levels of MC application (0, 30, 60, 90, 120 g ha^{-1}) were used in the study. Each treatment had three replications. Seeds of variety 'FH-326' were sown as per treatment during May 2019. Recommended production technology [31] was followed throughout the growing period. The recommended amounts of nitrogen 198 kg ha^{-1}, phosphorus 87 kg ha^{-1} and potash 45 kg ha^{-1} were applied in the form of Urea (46% N), DAP (46% P_2O_5 and 18% N) and MOP (60% K_2O). Full dose of phosphorus and potash along with half of the nitrogen were applied as basal dose; whereas, the remaining nitrogen was applied 30 days after sowing. The MC was foliar applied three times at flowering, peak bloom and boll setting [32].

Data relating to plant height, number of bolls per plant, boll weight, seeds cotton yield per plant, seed index, lint index, ginning out turn, total nitrogen contents (%), chlorophyll *a* and chlorophyll *b* were collected from 10 randomly selected plants from each experimental unit. The first harvest of cotton was done at 60% bolls' opening, and final harvest was done once the 90% bolls were opened. Ginning out turn was recorded after ginning 10 g samples of seed cotton from each treatment and ginning out turn was calculated by using the formula of Singh [33].

$$\text{GOT} = \frac{\text{Weight of lint in sample}}{\text{Seed cotton weight of the sample}} \times 100$$

Chlorophyll *a* and *b* (mg g^{-1} FW) were measured according to the procedure of Zhao and Oosterhuis [34]. Regarding relative water contents, fresh leaf samples were collected from ten randomly selected plants at physiological cutout stage and their fresh weight was recorded on digital balance. The leaves were dipped in distilled water for 15-18 hours and then their turgid weights were recorded. The leaf samples were placed in a drying oven for 24 hours at 72℃ and their dry weight was recorded. Finally, relative water contents were measured using the formula of Barrs and Weatherly [35].

$$\text{Relative water content (\%)} = \frac{Wf - Wd}{Ws - Wd} \times 100$$

Total nitrogen contents (%) of leaf with petiole were determined following the procedure of Bremner [36].

After ginning 10 g lint sample was kept for conditioning for 6 hr with 65 to 68% relative humidity at 20℃ in the laboratory of Fiber Technology Department, University of Agriculture Faisalabad. Quality parameters, i.e., fiber length (mm), strength (g/tex), fineness (µg $inch^{-1}$), elongation (%) and uniformity (%) were observed on high volume instrument (HVI). Module-920 of HVI-900A provided the data to the CPU (Central Processing Unit of PC computer) for interpretation.

All the collected data were subjected to analysis of variance (ANOVA) (Statistics 8.1) and means were compared by Tukey's HSD at 0.05 probability only where ANOVA indicated significant differences [37].

Results

Plant height was significantly reduced due to MC application, while its interaction with cotton plant spacing was non-significant (Table 1). Application of MC reduced cotton plant height by 3 to 13%. Application of 90 and 120 g ha^{-1} MC resulted in statistically similar plant height, whereas no MC application resulted in the tallest plants. Plant spacing had a non-significant effect on plant height of cotton (Table 1). Sympodial branches were more numerous compared to the control where MC was applied in the range of 60-120 g ha^{-1}. Application of MC at 30 g ha^{-1} resulted in statistically similar number of sympodial branches as that of control treatment. The highest (23.50) and the lowest (16.17) number of sympodial branches were recorded for 120 and 0 g ha^{-1} MC application, respectively. Increments of 35 and 46% in sympodial branches were recorded in response to 90 and 120 g ha^{-1} MC, respectively. Sympodial branches remained unaffected by plant spacing as well its interaction with MC application rates. Boll weight was increased with an increase in plant spacing (Table 1). Positive influence of MC application on boll weight was statistically significant only when MC was applied above 30 g ha^{-1}. Boll weight remained statistically similar for pants treated with 90 and 120 g ha^{-1} MC. The interaction of MC application rates with plant spacing was significant for ginning out turn as well seed cotton yield per plant (Table 1). Upper limit of ginning out turn was realized where MC was applied at 120 g ha^{-1} under both plant spacings, and this corresponded to an increase of 47-53% compared with no MC application. The highest seed cotton yield (50 g per plant) was recorded for the plants sown at a spacing of 30 cm and receiving 120 g ha^{-1} MC, this treatment combination was superior over all treatment combinations. Same application rate under 23 cm plant spacing resulted in 14% less yield than 30 cm (Table 1).

Chlorophyll *a* and *b* contents were increased over control at MC concentrations of 60 and 90 g ha^{-1}, respectively; however, plant spacing had non-significant effect in this regard. The highest chlorophyll *a* contents were recorded for the plants treated with 120 g ha^{-1} MC (Table 2). This treatment stood at par with MC application rates of 60 and 90 g ha^{-1}. Chlorophyll b contents also remained statistically similar in response to 90 and 120 g ha^{-1} MC, however, these were significantly higher than control and other MC application rates. Total nitrogen contents were significantly affected by MC application; however, plant spacing and interaction of plant spacing by MC application remained non-significant in this regard. The highest (4.38%) nitrogen contents were recorded for the plants treated with 120 g ha^{-1} MC and no application of MC resulted in the lowest (2.03%) nitrogen content (Table 2). Relative water contents manifested an increase in response to MC application as well plant spacing. Nevertheless, the interaction between these two factors was non-significant. All application rates of MC improved leaf water content over control with the exception of 30 g ha^{-1} (Table 2). The highest (84.90%) relative water content was observed for the plants treated with 120 g ha $^{-1}$ MC. Wider plant spacing (30 cm) resulted in higher (10%) relative water content compared to narrow spacing (23cm; Table 2).

Fiber length responded positively to MC application and an increase of 5-10% was observed in response to varying application rates of MC. The longest fiber length (28.90 mm) was recorded for 120 g ha^{-1} MC application (Table 3). Although higher than control, fiber length remained similar for MC application rates of 60-120 g ha^{-1}. Plant spacing and interactive effect of plant spacing with MC application remained non-significant for fiber strength (Table 3). Fiber fineness was significantly affected by plant spacing and MC application, while their interaction was non-significant. The highest (4.82) micronaire value was observed for lint of plants treated with 120 g ha^{-1} MC. Wider plant spacing of 30 cm plant was more effective in improving this trait than 23 cm spacing. Fiber elongation was increased by 11-47% when MC concentration was increased from 0 to 120 g ha^{-1} (Table 3). Application of MC at 30-90 g ha^{-s} resulted in statistically similar values of fiber elongation. The results revealed that the fiber uniformity remained unaffected by individual and interactive effects of plant spacing and MC application (Table 3).

Discussion

The current study revealed that maximum reduction in plant height was recorded for most compact plants in response to MC application. This reduction was due to reduced height to node ratio. About 14-17% shorter plants were observed with the highest concentration of MC compared to the plants receiving no MC. A 11% reduction in plant height has been observed by Cook and Kennedy [38], 9% by Pettigrew and Johnson [39] and 10.0-14.6% by Siebert and Stewart [20]. The MC inhibits endogenous gibberellic acid biosynthesis that results in inhibition of cell elongation through reduction in cell wall plasticity [40]. Water status is very impotent for the stability of biosynthetic processes in plants. Mathur *et al*. [41] reported similar results for plant growth inhibitor on Japanese mint. Increased water contents might be due the increased water retention at tissue level (due to thicker leaves and small leaf area), and better uptake of water owing to enhanced lateral root initiation, elongation and proliferation in cotton due to MC application [42]. Application of plant extracts and organic fertilizers also influenced the growth and biochemical composition of crop plants and weeds [43-45].

The increase in the concentration of MC application and plant spacing increased boll weight [46]. The rise in average boll weight with increasing MC concentration has been attributed to increase in reproductive growth [34] and difference in plant density due to plant spacing [47]. A sympodial branch is fruit-bearing branch. It is generally thin in diameter and shorter as compared to monopodial branches, present at the top of main stem. Higher number of sympodial branches results in more seed cotton yield [48]. Reduced vegetative growth in cotton results in higher number of sympodial branches [17]. Seed cotton yield per plant is an important agronomic character used in the economic analysis. It is also replotted that exogenous application of mineral elements and farm yard manures significantly influenced the growth and productivity of field crops (49,50). Nuti *et al*. [51] reported that high seed cotton yield with higher concentration of MC is the result of better partitioning of photo-assimilates from vegetative to reproductive structures. Moreover, such an increase in seed cotton yield could be attributed to the increase in number of bolls per plant and higher boll weight owing to optimization of canopy structure, control of excessive vegetative growth and greater boll setting percentage [52].

The MC reduces the abscisic acid and ethylene which increases boll retention; thus, opened bolls are heavy. The previous work of Karthikeyan and Jayakumar [53] and Ahmed et al. [54] depicted similar findings for seed cotton yield. It has been suggested that trade-off between reproductive and vegetative growth might be a reason that contributed towards higher yield. Application of MC as a growth retardant can divert energy from leaf and stem growth to boll development and retention. Furthermore, change in maturity and fruit bearing pattern due to MC application could also be the reason for increased yield [55]. Similar results have been reported by Tung et al. [32] and Zhao and Oosteruis [34] for chlorophyll contents. The MC had a significant effect on total nitrogen contents of leaf with petiole; however, plant spacing and interactive effect of plant spacing, and MC application was non-significant for nitrogen contents. Similar results have been reported elsewhere [56,57] for MC. Mahdi [57] observed significant effect of wide plant spacing on nitrogen and chlorophyll contents. Zhao and Oosterhuis [34] suggested that increase in nitrogen and chlorophyll contents could be the result of specific leaf weight since MC application upregulates chlorophyll contents. Ginning out turn is very important character since it determines lint percentage in cotton seed that is important for textile industry. The results of the current study are contradictory to the finding of Ali *et al*. [24] who reported non-significant effect of plant spacing on GOT of cotton. However, Wilson *et al*. [58] reported increased GOT with increasing levels of MC and wider plant spacing.

Fiber quality parameters are important for the textile industry. The results of the current study revealed that MC application significantly altered fiber length, while plant spacing and its interaction with MC application were non-significant. The results of the current study for fiber length corroborate the findings of Hussain *et al*. [59]. Fiber strength reveals the force in grams, needed to halt a bundle of fibers by one text unit in size, fastened in 2 sets of jaws (1/8 inch apart). Strength percentages are remunerated for readings above 29.4, whereas discounts are incurred for readings below 25.5. Sturdier threads provide stronger tales which increase productivity by increasing processing speed with less end breakages. The results of the current study are contrary to Clawson *et al*. [60] who found inconsistent effect of MC application and plant spacing on fiber strength. Fiber fineness is the indirect measure of fiber maturity and fineness, and the results are in line with Sawan [52] for MC-treated plant. For fiber elongation, the obtained results are similar to that of Sawan *et al*. [61]. However, increase in fiber elongation is quite unpredictable under different levels of MC because it showed inconsistent results [62]. Fiber uniformity is the percentage (ratio) between the 50% and 2.5% span length of fiber length and was unaffected by any of the factor as reported by Wilson et al. [58] (2007). Khan *et al*. [63] also investigated that plant spacing and intercropping had positive impact on productivity of cotton crop.

Conclusion

Reproductive growth, yield and fiber quality traits of cotton were improved in response to MC application. Foliar applied MC helped in ameliorating the negative impacts of excessive vegetative growth and application of MC at 120 g ha^{-1} proved optimum in this regard.

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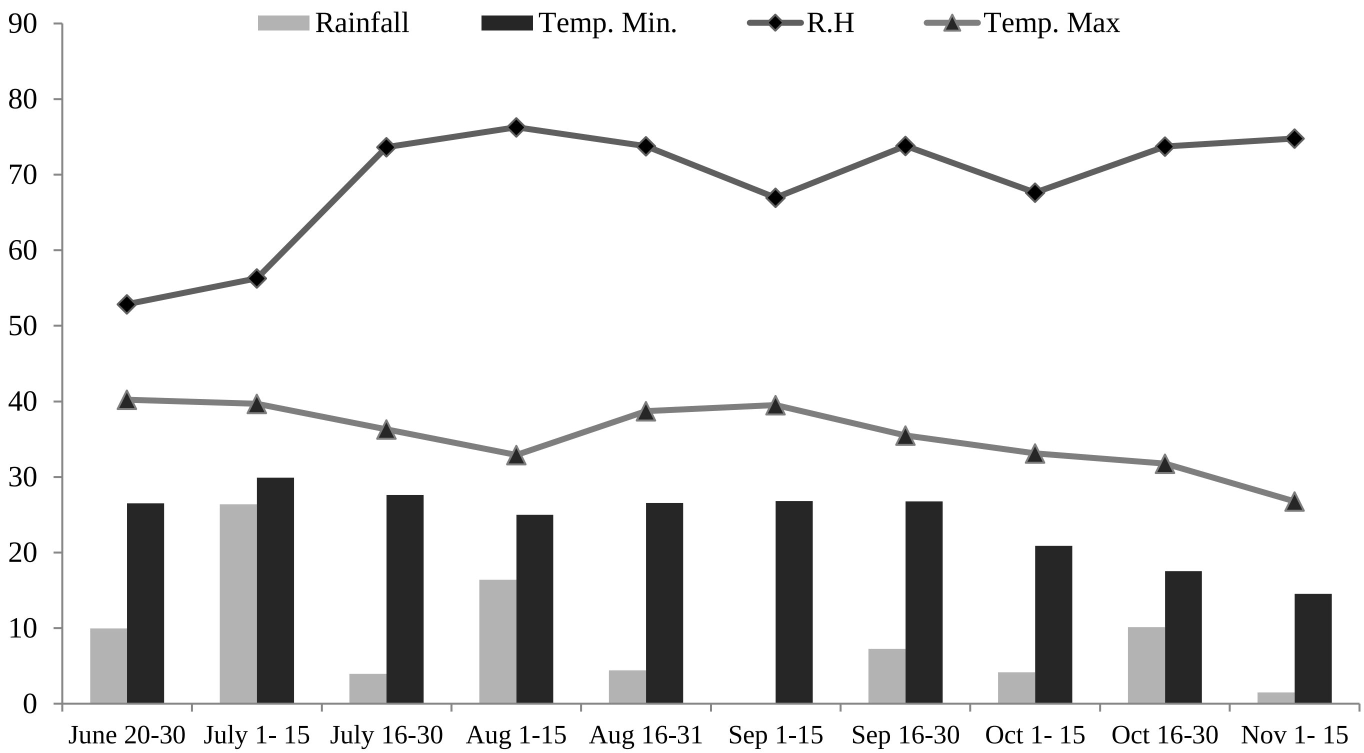

Figure 1: Weather data of the experimental site during the study period

Table 1 Impact of foliar applied mepiquat chloride and plant spacing on yield and quality attributes of cotton

Treatments	Plant height (cm)			Sympodial branches			Average boll weight (g)			Ginning out turn (%)			Seed cotton yield per plant (g)		
	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)
MC at 0 g ha^{-1}	123.7	118.7	121. 2 E	16.3	16.0	16.1 D	2.61	2.67	2.64 C	30.0 f	33.0 ef	31.5	20.00 h	24.0 g	22.00
MC at 30 g ha^{-1}	120.2	115.5	117.8 CD	18.7	17.3	18.0 CD	2.67	2.73	2.70 C	34.0 e	36.0 de	35.0	27.0 fg	30.0 ef	28.50
MC at 60 g ha^{-1}	118.0	113. 2	115.5 C	20.0	19.3	19.6 BC	2.92	2.98	2.95 B	38.0 cd	40.0 bc	39.0	32.0 de	35.0 cd	38.00
MC at 90 g ha^{-1}	109.3	108. 2	108.7 AB	22.6	21.0	21.8 AB	3.12	3.21	3.16 A	40.0 bc	42.5 b	41.0	36.0 c	40.0 b	38.00
MC at 120 g ha^{-1}	105.3	105.0	105. 1 A	23.0	24.0	23.5 A	3.20	3.28	3.24 A	46.0 a	48.5 a	47.5	43.0 b	50.0 a	46.50
Mean (PS)	115.4	112. 1		20.133	19.533		2.90 B	2.97 A		37.6 B	40.0 A		31.60 B	35.80 A	
HSD (0.05)	PS =ns, MC =1.73**, PS×MC=ns			PS =ns, MC =1.31**, PS×MC =ns			PS = 0.013*, MC =0.06**, PS×MC = ns			PS =1.29**, MC = 0.86**, PS×MC = 1.80**			PS =1.26**, MC = 1.31**, PS×MC = 1.98*		

Means sharing the same letter did not differ significantly at P = 0.05

PS = Plant spacing, **MC** = Mepiquat chloride, **PS × MC** = Interaction, **ns** = Non-significant, * = significant, ** = highly significant

Table 2 Impact of foliar applied Mepiquat chloride and plant spacing on biochemical attributes of cotton

Treatments	Chlorophyll a (mg g^{-1})			Chlorophyll b (mg g^{-1})			Total nitrogen content of leaf with petiole (%)			Relative water content (%)		
	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)
MC at 0 g ha^{-1}	0.79	0.90	0.85 C	1.38	0.92	1.15 B	1.9	2.2	2.03 B	61.7	67.9	64.8 C
MC at 30 g ha^{-1}	0.87	1.25	1.07 BC	1.62	1.43	1.53 B	2.4	2.7	2.5 AB	68.5	73.9	71.2 BC
MC at 60 g ha^{-1}	1.30	1.29	1.30 AB	1.74	1.49	1.62 B	3.2	2.8	3.0 AB	70.6	79.1	74.9 B
MC at 90 g ha^{-1}	1.42	1.75	1.48 AB	2.55	1.98	2.24 A	3.5	4.4	2.5 AB	72.2	84.1	77.8 AB
MC at 120 g ha^{-1}	1.76	1.87	1.73 A	2.75	2.35	2.41 A	3.9	4.9	2.0 B	80.5	88.8	84.9 A
Mean (PS)	1.23	1.34		1.57	2.01		2.9	3.3		70.7 B	78.7 A	
HSD (0.05)	PS = ns, MC =0.72**, PS×MC=ns			PS =ns, MC =0.90**, PS×MC = ns			PS = ns, MC = 0.91 **, PS×MC = ns			PS =6.28*, MC =5.55**, PS×MC =ns		

Means sharing the same letter did not differ significantly at P = 0.05

PS = Plant spacing, **MC** = Mepiquat chloride, **PS × MC** = Interaction, **ns** = Non-significant, ***** = significant, ****** = highly significant

Table 3 Impact of foliar applied Mepiquat chloride and plant spacing on quality attributes of cotton

Treatments	Fiber Length (mm)			Fiber Strength (g/tex)			Fiber Fineness (micronaire)			Fiber elongation (%)			Fiber uniformity (%)		
	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)	PS at 23 cm	PS at 30 cm	Mean (MC)
MC at 0 g ha^{-1}	26.4	26.6	26.4 C	18.8	18.9	18.9	3.30	3.60	3.45 E	7.96	8.33	8.15 C	81.70	85.36	83.95
MC at 30 g ha^{-1}	27.4	27.8	27.6 BC	20.8	20.9	20.8	3.50	3.86	3.68 D	9.06	9.00	9.03BC	83.70	83.73	83.85
MC at 60 g ha^{-1}	27.8	28.0	27.9 AB	22.0	22.0	22.0	3.80	4.03	3.91 C	9.50	9.70	9.60 B	84.53	83.16	83.80
MC at 90 g ha^{-1}	28.3	28.0	28.4 AB	22.1	27.6	22.5	4.26	4.35	4.28 B	10.10	10.85	10.40B	84.56	83.55	83.71
MC at 120 g ha^{-1}	28.8	28.7	28.9 A	22.9	24.9	22.9	4.56	5.35	4.81 A	11.53	13.45	12.00A	84.50	84.50	83.53
Mean (PS)	27.7	27.9		21.3	21.6		3.88 B	4.17 A		9.63	10.04		83.80	83.74	
HSD (0.05)	PS =ns, MC =2.30**, PS×MC=ns			PS =ns, MC = 4.97 ns, PS×MC =ns			PS = 0.23**, MC = 0.12**, PS×MC = ns			PS = 0.94 ns, MC = 0.83**, PS×MC =ns			PS =ns, MC = ns, PS×MC = ns		

Means sharing the same letter did not differ significantly at P = 0.05

PS = Plant spacing, **MC** = Mepiquat chloride, **PS × MC** = Interaction, **ns** = Non-significant, ***** = significant, ****** = highly significant