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Rajendra R Lipane
 Research Scholar, Department
 of Agricultural Botany,
 MPKV, Rahuri, Ahmednagar,
 Maharashtra, India

Dr. RS Wagh
 Ex-Associate Director of
 Research, MPKV, Rahuri,
 Ahmednagar, Maharashtra,
 India

Dr. VR Awari
 Assistant Professor,
 Department of Agricultural
 Botany, MPKV, Rahuri,
 Ahmednagar, Maharashtra,
 India

Dr. AR Aher
 Assistant Professor (SLBTC),
 State Level Biotechnology
 Centre, MPKV, Rahuri,
 Ahmednagar, Maharashtra,
 India

Dr. BM Bhalerao
 Assistant Professor,
 Department of Biochemistry,
 PGI, MPKV, Rahuri,
 Ahmednagar, Maharashtra,
 India

Dr. DV Deshmukh
 Assistant Breeder (JAF), All
 India Network project on Jute
 and Allied Fibers, MPKV,
 Rahuri, Ahmednagar,
 Maharashtra, India

Dr. VS Wani
 Head & Professor, Department
 of Statistics, PGI, MPKV,
 Rahuri, Ahmednagar,
 Maharashtra, India

Corresponding Author:
Rajendra R Lipane
 Research Scholar, Department
 of Agricultural Botany,
 MPKV, Rahuri, Ahmednagar,
 Maharashtra, India

Impact of canopy management practices on physiological and yield parameters of Bt cotton (*Gossypium hirsutum* L) under High-Density Planting Systems (HDPS)

Rajendra R Lipane, RS Wagh, VR Awari, AR Aher, BM Bhalerao, DV Deshmukh and VS Wani

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Abstract

At the All India Coordinated Cotton Improvement Project, MPKV, Rahuri, Dist. Ahmednagar, Maharashtra, India, a field experiment study on the “Impact of canopy management practices on physiological and yield parameters of Bt Cotton (*Gossypium hirsutum* L.) under high-density planting systems (HDPS)” was carried out during the *Kharif* season of 2022. Two factors, namely spacing *viz.*, S1 (60x30 cm), S2 (90x30 cm), S3 (120x30 cm) and S4 (120x60 cm) and canopy management practices *viz.*, T₁ (Pruning at 40 DAS and topping at 70 DAS), T₂ (Pruning at 50 DAS and topping at 80 DAS), T₃ (Mepiquat chloride @ 50 ppm at 55 and 75 DAS) and T₄ (No pruning and no topping - control), were used to set up the experiment, which was replicated three times.

The experiment was conducted using the RCH-659 Bt cotton hybrid seed. With the exception of 30 DAS, where only spacing had a significant effect, the results showed that the spacing and treatment (canopy management practices) at 60 and 90 DAS had a significant impact on physiological parameters, including the chlorophyll index (SPAD), rate of photosynthesis, leaf area index, and specific leaf weight. With treatment T₁ (pruning at 40 DAS and topping at 70 DAS) in 60 and 90 DAS, the spacing S4 (120x60 cm) had the significantly greatest chlorophyll index (SPAD), rate of photosynthesis, and specific leaf weight.

The maximum leaf area index was recorded by treatment T₁ (pruning at 40 DAS and topping at 70 DAS) with spacing S1 (60x30 cm), whereas the lowest leaf area index was obtained at both 60 and 90 DAS by treatment T₁ (pruning at 40 DAS and topping at 70 DAS) with spacing S4 (120x60cm). When treated with T₁ (pruning at 40 DAS and topping at 70 DAS) under spacing S4 (120 x 60 cm), the yield parameters, including seed cotton yield per ha, demonstrated better results. Treatment T₁ (pruning at 40 DAS and topping at 70 DAS) with spacing S2 (90 x 30 cm) had the highest seed cotton yield ha⁻¹.

Keywords: HDPS, Bt cotton, RCH-659, spacing, canopy management practices, physiological and yield parameters

Introduction

Cotton is known as white gold and queen of fibres is a vital cash crop in India, making a substantial contribution to economic activity and jobs in both rural and urban areas. About 90% of the world's cotton production comes from *Gossypium hirsutum* L. the most prevalent of the four types grown in the nation. India produced over 343.47 lakh bales (5.84 million metric tons) during the 2022–2023 cotton season, ranking second in the world and accounting for 23.83% of global production. However, with a yield of 447 kg/ha, India's productivity comes in at number 39. According to the COCPC meeting on June 1, 2023, Maharashtra, a major cotton-producing state, produced 84.09 lakh bales on 42.29 lakh hectares at that time, yielding 338 kg/ha. (Annexures IV and VII). Cotton farming in India faces many problems that make it less productive than in other countries. Some of the main issues are poor soil health, small farm size, limited access to technology, dependence on rainfall, low-quality seeds, climate change, not enough plants, fertilizer issues, and more pests and diseases. To tackle these problems, high-density planting systems have been suggested as a new way to grow cotton. This method could help increase productivity and profits by using resources more efficiently, lowering costs, and reducing risks.

But it necessitates prompt action and thorough planning. Given that research indicates that a greater number of plants can result in a larger output of cotton, this technique could greatly benefit farmers who grow cotton without irrigation, particularly those with less fertile soil. Plant population and seed cotton yield are positively correlated in cotton (Chavan et al., 2019) [6].

Cotton plants (*Gossypium hirsutum* L.) grow in a way that makes the top part of the plant dominate, which affects how well they produce. Farmers are currently dealing with problems from extra vegetative growth with bollworm and sucking pest infestations in Bt cotton, particularly due to the growth of the unproductive branches and upper part of the plant. This leads to lower yields and poorer quality of cotton. To help improve yields and profits, farmers are exploring High-Density Planting Systems (HDPS). Canopy management practices to prevent plants from falling over when they reach 5-6 feet, is manual and chemical pruning and topping, which uses growth regulators or manual method to manage plant canopy growth. This result in enhance or control physiological traits in response with yield.

Farmers in Maharashtra cultivate cotton hybrids with narrow spacing and without proper canopy management to control excessive vegetative growth, which is an important low-cost strategy to enhance cotton yields. To assess the optimal planting spacing combined with canopy management practices, an attempt has been made to study the impact of these practices on the physiological and yield parameters of Bt cotton (*Gossypium hirsutum* L.) under high-density planting systems (HDPS).

Materials and Methods

The experiment on “Impact of canopy management practices on physiological and yield parameters of Bt cotton (*Gossypium hirsutum* L.) under high-density planting systems (HDPS)” was carried out during *Kharif*, 2022 season to find out the influence of plant spacing and canopy management practices on physiological parameters of Bt cotton hybrid RCH 659 at All India Coordinated Cotton Improvement Project, MPKV, Rahuri, Ahmednagar (Dist.), Maharashtra, India. The geographical situation is on 19.38° N latitude and 74.65° E longitude, with an elevation of 511 metres above mean sea level. The soil is deep black with slightly alkaline in reaction. The average weekly maximum temperature during crop growing period was 38.14°C and minimum temperature was 17.34°C. Seed required for the experiment was collected from AICIP, Cotton Breeder, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra. The sowing was done by hand dibbling by adopting spacing as per treatment by placing 2-3 seeds per hill on 22nd June 2022 (*Kharif*- 2022).

The statistical design adopted for the experimentation was Split Plot design, with three replications and sixteen treatment combinations. The main plots were four spacing viz., S1 (60x30 cm), S2 (90x30 cm), S3 (120x30 cm) and S4 (120x60 cm). Each of these main plots were divided into four sub-plots. The sub plots consisted of four treatments (canopy management practices) viz., T₁ (Pruning at 40 DAS and topping at 70 DAS), T₂ (Pruning at 50 DAS and topping at 80 DAS), T₃ (Mepiquat chloride @ 50 ppm at 55 and 75 DAS) and T₄ (No pruning and no topping - control). Plot size of individual treatment was gross 6.30 x 6.00 m² and the net sizes were 5.70 x 4.80 m² for S1 (60 x 30 cm), 5.70 x

4.60 m² for S2 (90 x 30 cm), 5.70 x 3.60 m² for S3 (120 x 30 cm), and 5.40 x 3.60 m² for S4 (120 x 30 cm) spacing.

Results and Discussion

1. Physiological parameters

1.1 Chlorophyll index (SPAD)

The chlorophyll index (SPAD) determines the photosynthetic capacity. SPAD readings (chlorophyll content in leaves) increased from 30 DAS to 90 DAS. Data on chlorophyll Index (SPAD) as influenced by different spacing and treatment (Canopy management practices) are presented in Table 1. A perusal of the data reveals that, chlorophyll Index (SPAD) was significantly affected by the spacing and treatment in the experiment at 60 DAS and 90 DAS except at 30 DAS (where only spacing had a significant effect) and interaction, during the year 2022.

1.1.1 Effect of spacing

The data in Table 1. reveals that at 30, 60 and 90 DAS, significantly highest chlorophyll index (SPAD) recorded in spacing S4 (120x60 cm) as 35.9, 43.26 and 52.89, respectively during the year 2022, which was at par with S3 (120x30 cm) as 34.99, 41.20 and 51.57, respectively. While the lowest chlorophyll index (SPAD) was found in S1 (60x30 cm) as 31.42, 39.28 and 48.48, at 30, 60 and 90 DAS respectively, during the year 2022 irrespective of treatments. With respect to plant spacing, wide spacing provided higher chlorophyll content values this is due to the fact that, wide spacing provided with all the natural resources required for their growth and attributed in better performance of individual plant. Similar findings were also reported by Venkatkiran Reddy *et al.* (2023) [28].

1.1.2 Effect of treatment

Chlorophyll index (SPAD) in respect to treatment, were observed to be significant at 60 and 90 DAS, except 30 DAS during the year 2022 (Table 1).

At 30 DAS, numerically highest chlorophyll index (SPAD) was recorded in T₄ (No pruning and no topping- control) were (34.51), while the lowest chlorophyll index (SPAD) was recorded in T₁ (Pruning at 40 DAS and topping at 70 DAS) were (32.86) during the year 2022, irrespective of spacing.

At 60 and 90 DAS the highest chlorophyll index (SPAD) was recorded in T₁ (Pruning at 40 DAS and topping at 70 DAS) were (43.42 and 52.68) respectively, which was at par with T₂ (Pruning at 50 DAS and topping at 80 DAS) were (42.36 and 51.64) and T₃ (Mepiquat chloride @ 50 ppm at 55 and 75 DAS) were (40.94 and 50.98) during the year 2022, While the lowest chlorophyll index (SPAD) was recorded in T₄ (No pruning and no topping - control) were (37.67 and 47.51) respectively, during the year 2022, irrespective of spacing.

The treatment T₁ (Pruning at 40 DAS and topping at 70 DAS) shows highest chlorophyll index (SPAD) than other treatment might be due to early pruning or topping enhances light penetration to the lower parts of a plant. By redirecting nutrients and water from the upper leaves to the lower ones, these practices promote growth and increase chlorophyll production. Additionally, pruning encourages new shoots and expands the overall leaf area, allowing for greater light absorption. With the lower leaves receiving more light and nutrients, growth potential is maximized, thereby improving photosynthesis and chlorophyll synthesis. The results of the

present study support the previous findings in *Acer pseudoplatanus* L. Trees by Fini et al. (2015)^[9].

Interaction effect

The interaction effect on the chlorophyll index (SPAD) of Bt cotton was found not significant at 30, 60, and 90 DAS during the year 2022, but numerically highest chlorophyll index (SPAD) (37.11) was found in S4T₂ (120x60 cm with pruning at 50 DAS and topping at 80 DAS) at 30 DAS, while S1T₁ (60x30 cm with Pruning at 40 DAS and topping at 70 DAS) recorded the lowest chlorophyll index (SPAD) (29.85).

At 60 and 90 DAS, the interaction S4T₁ (120x60 cm with pruning at 40 DAS and topping at 70 DAS) recorded the highest chlorophyll index (SPAD) of 44.98 and 55.11, respectively during the year 2022. While S1T₄ (60x30 cm with no pruning and no topping -control)) recorded the lowest chlorophyll index of 35.87 and 45.68 at 60 and 90 DAS respectively, during the year 2022.

Similar reports of non-significant influence of spacing and treatment (canopy management practices) on chlorophyll index (SPAD) was reported by Venkatkiran Reddy *et. al* (2023)^[28].

Rate of photosynthesis (umol m⁻² sec⁻¹)

A perusal of the data reveals that, rate of photosynthesis was significantly affected by the spacing and treatment in the experiment at 60 DAS and 90 DAS except at 30 DAS (where only spacing had a significant effect) and interaction during 2022.

Effect of spacing

At 30 DAS, the data was significantly influenced by spacing but treatment and interaction were found to be not significant. However, significantly maximum rate of photosynthesis recorded in spacing S4 (120x60 cm) were (27.36 umol m⁻² sec⁻¹), which was followed by S3 (120x30 cm) were (25.01 umol m⁻² sec⁻¹) during the year 2022, while minimum rate of photosynthesis was found in S1 (60x30 cm) were (22.08 umol m⁻² sec⁻¹) during the year 2022, irrespective of treatments.

The data in Table 2, reveals that rate of photosynthesis at 60 and 90 DAS was recorded significantly maximum in spacing S4 (120x60 cm) were (31.81 and 32.58 umol m⁻² sec⁻¹) respectively, which was at par with S3 (90x30 cm) were (30.96 and 31.91 umol m⁻² sec⁻¹) and S2 (120x30 cm) were (29.66 and 30.80 umol m⁻² sec⁻¹) respectively, during the year 2022. While minimum rate of photosynthesis was found in S4 (120x60 cm) were (27.48, and 29.89 umol m⁻² sec⁻¹) respectively, during the year 2022, irrespective of treatment.

The investigation revealed that wide spacing in Bt cotton can lead to increased rate of photosynthesis primarily due to enhanced light availability, reduced competition for resources, better air circulation, and optimized growth conditions. Similar findings were reported by Ishrat et al. (2021)^[12].

Effect of treatment

Rate of photosynthesis in respect to treatment, were observed to be significant at 60 and 90 DAS, except 30 DAS during the year 2022.

At 30 DAS, effect of treatment shows not significant result on rate of photosynthesis but numerically highest rate of

photosynthesis was recorded in T₃ (Mepiquat chloride @ 50 ppm at 55 and 75 DAS) were (26.39 umol m⁻² sec⁻¹), while the lowest rate of photosynthesis was recorded in T₁ (Pruning at 40 DAS and topping at 70 DAS) were (22.93 umol m⁻² sec⁻¹) during the year 2022, respectively, irrespective of spacing.

At 60 DAS and 90 DAS the maximum rate of photosynthesis was recorded in T₁ (Pruning at 40 DAS and topping at 70 DAS) were (32.04 and 33.56 umol m⁻² sec⁻¹) respectively. which was at par with T₂ (Pruning at 50 DAS and topping at 80 DAS) were (30.40 and 31.62 umol m⁻² sec⁻¹) respectively, during the year 2022. While the minimum rate of photosynthesis was recorded in T₄ (No pruning and no topping - control) were (27.82 and 28.98 umol m⁻² sec⁻¹) respectively, during the year 2022, irrespective of spacing.

The highest result of rate of photosynthesis in T₁ (Pruning at 40 DAS and topping at 70 DAS) might be due to early pruning and topping allows a greater allocation of carbohydrates and resources directed towards the remaining leaves, potentially enhancing their photosynthetic rate and overall growth. The results of the present study support the previous findings of Junjun *et al.* (2021)^[13] that the removal of vegetative branches and plant topping enhance the canopy carbon assimilation rate in cotton, leading to an increase in the photosynthesis rate.

Interaction effect

The interaction effect on rate of photosynthesis of Bt cotton was found not significant at 30, 60, and 90 DAS during the year 2022, but numerically highest rate of photosynthesis (27.91 umol m⁻² sec⁻¹) was found in S4T₁ (120x60 cm with pruning at 40 DAS and topping at 70 DAS) at 30 DAS, while S1T₁ (60x30 cm with Pruning at 40 DAS and topping at 70 DAS) recorded the lowest rate of photosynthesis (18.45 umol m⁻² sec⁻¹) at 30 DAS.

At 60 and 90 DAS, the interaction S4T₁ (120x60 cm with pruning at 40 DAS and topping at 70 DAS) recorded the highest rate of photosynthesis of 33.67 and 34.51 umol m⁻² sec⁻¹, respectively during the year 2022. While S1T₄ (60x30 cm with no pruning and no topping -control)) found the lowest rate of photosynthesis (25.13 and 27.08 umol m⁻² sec⁻¹), at 60 and 90 DAS respectively, during the year 2022.

Leaf area index (mm)

Data on Leaf Area Index (LAI) as influenced by different spacing and treatment (Canopy management practices) are presented in Table 1. A perusal of the data reveals that, LAI was significantly affected by the spacing and treatment in the experiment at 60 DAS and 90 DAS except at 30 DAS (where only spacing had a significant effect) and interaction during the year 2022.

Effect of spacing

The data in Table 3, reveals that leaf area index at 30, 60 and 90 DAS was recorded significantly highest in spacing S4 (120x60 cm) as 0.247, 0.997 and 1.563, which was at par with S2 (90x30 cm) as 0.237, 0.954 and 1.519 respectively, during the year 2022. While minimum leaf area index was found in S4 (120x60 cm) as 0.146, 0.893 and 1.457, respectively at 30, 60 and 90 DAS, during the year 2022, irrespective of treatment.

The study found that wider plant spacing resulted in a lower leaf area index (LAI), while narrower spacing led to a

higher LAI. This might be due to increased plant density in narrower spacing allows for more efficient use of natural resources such as sunlight, moisture, and nutrients. This is in conformity with the earlier results that were obtained by Yadav *et al.* (2021) [30], Sisodia and hamparia (2007) [24], Shukla *et al.* (2013) [23], Venkatkiran reddy *et al.* (2023) [28] and Chapepa *et al.* (2020) [5] also confirms the current finding.

Effect of treatment

Leaf area index in respect to treatment (Canopy management practices) were observed to be significant at 60 DAS and 90 DAS except at 30 DAS during the year 2022 (Table 1).

At 30 DAS, the LAI was observed to be non-significant indicating that there is no effect of spacing on LAI. Numerically, the highest LAI recorded as 0.210, which was observed with treatment T₁ (Pruning at 40 DAS and topping at 70 DAS), While, lowest LAI was recorded with T₄ (No pruning and no topping - control) were (0.197) during the year 2022.

Data on LAI revealed that at 60 and 90 DAS, LAI was significantly influenced by treatment during the year 2022. Highest LAI of 0.971 (At 60 DAS); 1.535 (At 90 DAS), were recorded with treatment T₁ (Pruning at 40 DAS and topping at 70 DAS), which was at par with T₂ (Pruning at 50 DAS and topping at 80 DAS) as 0.947 (At 60 DAS); 1.513 (At 90 DAS), While, least LAI was found in T₄ (No pruning and no topping - control) as 0.916 (At 60 DAS); 1.482 (At 90 DAS) during the year 2022, irrespective of spacing. The highest Leaf Area Index (LAI) was recorded in T₁ (pruning at 40 DAS and topping at 70 DAS) compared to the other treatments. This may be due to the fact that this practice often temporarily reduces the leaf area. This reduction is caused by the immediate loss of biomass from the top of the plant and the vegetative branches. However, subsequent growth may result in the development of new leaves and branches, potentially increasing the LAI over time. Additionally, it leads to a more uniform distribution of leaves, enhancing the light capture efficiency of the plant. The results are in agreement with the results of Junjun *et al.* (2021) [13].

Interaction effect of spacing and treatment

The interaction effect of spacing and canopy management practices on the leaf area index of Bt cotton was not significant at 30, 60 and 90 DAS. At 30 DAS, the interaction of S4T₁ (120x60 cm with pruning at 40 DAS and topping at 70 DAS) had the lowest leaf area index (0.141 mm), while S1T₁ (60x30 cm with pruning at 40 DAS and topping at 70 DAS) exhibited the highest (0.258 mm).

At 60 and 90 DAS, the interaction S4T₄ (120x60 cm with no pruning and no topping - control) recorded the lowest leaf area index of 0.875 mm and 1.441 mm, respectively, while S1T₁ achieved the highest leaf area index (1.037 and 1.603), respectively, during the year 2022.

Specific leaf weight (g/dm²)

Data pertaining to specific leaf weight (g/dm²) as influenced by different spacing and canopy management practices are tabulated in Table 1, shows that specific leaf weight was significantly affected by the different spacing and canopy management practices at 60 and 90 DAS, except at 30 DAS and interaction, during the year 2022.

Effect of spacing

The data in Table 4, reveals that specific leaf weight at 30 DAS was not-significantly influenced by spacing. However, numerically the highest specific leaf weight of 0.641 g/dm² during the year 2022, was observed with S4 (120x60 cm) and the lower specific leaf weight of 0.526 g/dm² during the year 2022 was noticed with the spacing S1 (60x30 cm).

The data in Table 1, reveals that specific leaf weight at 60 and 90 DAS was recorded significantly highest in spacing S4 (120x60 cm) were (1.162 and 1.251 g/dm²) respectively, which was at par with S3 (90x30 cm) were (1.067 g/dm²) at 60 DAS and S3 (120x30 cm) were (1.221 g/dm²) and S2 (90x30 cm) were (1.189 g/dm²), at 90 DAS, during the year 2022. While minimum specific leaf weight was found in S1 (60x30 cm) were (0.933 and 1.105 g/dm²) at 60 and 90 DAS respectively, during the year 2022, irrespective of treatment. The highest specific leaf weight in narrow spacing may be due to plants competing for resources like light, water, and nutrients, leading to thicker leaves with higher specific leaf weight to maximize resource utilization. This adaptation helps plants efficiently capture light, reduce water loss, and optimize nutrient uptake in dense planting conditions. These results are consistent with the findings of Sultana *et al.* (2023) [26] and Venkatkiran Reddy *et al.* (2023) [28] reported that sufficient availability of nutrients, space, sunlight, and soil moisture can lead to thicker leaves resulting in higher leaf specific weight in lower plants densities. Higher plant densities were found to have less specific leaf weight due to lower production of photosynthates, as competition exists for nutrients, light, and moisture.

Effect of treatment

Specific leaf weight in respect to treatments, were observed to be significant at 60 and 90 DAS except at 30 DAS and interaction during the year 2022.

The data in Table 4, reveals that specific leaf weight at 30 DAS was not-significantly influenced by treatment. However, numerically the highest specific leaf weight of 0.588 g/dm², during the year 2022, was observed with T₄ (No pruning and no topping - control) and the lower specific leaf weight of 0.547 g/dm², during the year 2022 was noticed with the treatment T₁ (Pruning at 40 DAS and topping at 70 DAS). At 60 DAS and 90 DAS the maximum specific leaf weight was recorded in T₁ (Pruning at 40 DAS and topping at 70 DAS) were (1.067 and 1.226 g/dm²) respectively. which was at par with T₂ (Pruning at 50 DAS and topping at 80 DAS) were (1.047 and 1.205 g/dm²) and T₃ (Mepiquat chloride @ 50 ppm at 55 and 75 DAS) were (1.017 and 1.180 g/dm²) respectively at 60 and 90 DAS, While the minimum specific leaf weight was recorded in T₄ (No pruning and no topping - control) were (0.991 and 1.155 g/dm²) respectively at 60 and 90 DAS, during the year 2022, irrespective of spacing. This highest specific leaf weight at T₁ (Pruning at 40 DAS and topping at 70 DAS), might be due to photosynthate is diverted towards boll development and treatment differences were found significant. This shift in partitioning increased the ability of the plant to allocate more photosynthate towards reproductive structures. The results are in accordance with the findings of Kumari and George (2012) [14].

Interaction effect

The interaction effect of spacing and treatment on specific leaf weight at 30, 60 and 90 DAS of Bt cotton was non-

significant during the year 2022, but numerically highest specific leaf weight (0.669 g/dm^2) was found in S4T₂ (120x60 cm with pruning at 50 DAS and topping at 80 DAS) at 30 DAS, while S1T₄ (60x30 cm with no pruning and no topping -control) recorded the lowest specific leaf weight (0.510 g/dm^2) at 30 DAS.

At 60 and 90 DAS, the interaction S4T₁ (120x60 cm with pruning at 40 DAS and topping at 70 DAS) recorded the highest specific leaf weight of 1.214 and 1.286 g/dm^2 , respectively during the year 2022. While S1T₄ (60x30 cm with no pruning and no topping -control) found the lowest specific leaf weight (0.914 and 1.055 g/dm^2), at 60 and 90 DAS respectively, during the year 2022.

Yield parameters

Seed cotton yield plant⁻¹

The data regarding seed cotton yield plant⁻¹ at 140 and 165 DAS as influenced by species, treatments and their interaction are presented in Table 5.

Seed cotton yield plant⁻¹ at 140 and 165 DAS.

Effect of spacing

The results at 140 and 165 DAS, revealed that there was a significant difference among the spacing during the year 2022. The spacing, S4 (120x60 cm) was recorded highest seed cotton yield plant⁻¹ at 140 and 165 DAS were (116.97 and 118.10 (g)) respectively, which was followed by S3 (120x30 cm) were (69.90 and 70.61 g) respectively in the year 2022, while the lowest seed cotton yield plant⁻¹ at 140 DAS and 165 DAS was found in S1 (60x30 cm) were (36.27 and 37.31 g) respectively, in the year 2022.

The S4 spacing (120 cm × 60 cm) significantly increased boll production plant⁻¹ compared to other spacings. This improvement is attributed to reduced competition for resources, which enhances access to sunlight, water, and nutrients, leading to larger growth and more branches and flowering sites. Similar results were also observed by Suma (2019) [27], Pradeep Kumar *et al.* (2017) [19], Paslawar *et al.* (2017) [18], Hargriess and Saini (2018) [10], and Nehra and Chandra (2001) [15], who reported better development of individual plants in wider spacing, suggesting that widely spaced plants received a favourable microclimate.

Effect of treatment

Treatments showed significant differences for seed cotton yield plant⁻¹ at 140 and 165 DAS, in the year 2022. From the data, it was found that the T₁ (Pruning at 40 DAS and topping at 70 DAS) was recorded highest seed cotton yield plant⁻¹ at 140 and 165 DAS as 78.61 and 80.09 (g), which was at par with T₂ (Pruning at 50 DAS and topping at 80 DAS) as 76.13 and 76.46 (g) respectively. while the lowest seed cotton yield plant⁻¹ at 140 and 165 DAS which was found in T₄ (No pruning and no topping -Control) as 56.38 and 57.12 (g) respectively, in the year 2022.

Therefore, the treatment T₁ (pruning at 40 DAS and topping at 70 DAS) recorded the highest seed cotton yield plant⁻¹. This is mainly due to early pruning and topping practices, which decreased morphological and physiological characteristics such as plant height, internodal distance, stem girth, and leaf area index while increasing chlorophyll index (SPAD), rate of photosynthesis, and specific leaf weight. Additionally, these canopy management practice also increased the value of yield components, while reducing the boll shedding percentage plant⁻¹. The higher

values of yield components lead to an increase in seed cotton yield per plant. These results are consistent with the previous results of El-Sayed *et al.* (2023) [8].

Interaction effect

From the data, it was resulted that the interaction effects of species and treatments on seed cotton yield plant⁻¹ (g) of Bt cotton, were significant in the year 2022.

Among the interaction (S x T), the interaction S4T₁ (120x60 cm with pruning at 40 DAS and topping at 70 DAS) recorded highest Seed cotton yield plant⁻¹ at 140 and 165 DAS were (127.84 and 129.09 (g)), which was at par with S4T₂ (120x60 cm with pruning at 50 DAS and topping at 80 DAS) were (124.92 and 125.18 (g)) during the year 2022, whereas, the lowest seed cotton yield plant⁻¹ at 140 and 165 DAS was found in S1T₄ (60x30 cm with no pruning and no topping - control) were (25.87 and 26.17 g) respectively, in the year 2022.

Seed cotton yield ha⁻¹

Seed cotton yield ha⁻¹ at 140 and 165 DAS

The data on seed cotton yield ha⁻¹ at 140 DAS, as influenced by spacing, treatments and their interaction are presented in Table 6.

Effect of spacing

According to the data (Table 6) the seed cotton yield ha⁻¹ revealed substantial differences related to the different spacing in the year 2022. Among the spacing, S2 (90x30 cm) was found significantly highest seed cotton yield ha⁻¹ at 140 and 165 DAS were (2047.73 and 2071.95 kg), which was at par with S1 (60x30 cm) were (1955.27 and 2011.97 kg) and S3 (120x30 cm) were (1934.78 and 1954.14 kg) respectively in the year 2022, while the lowest seed cotton yield ha⁻¹ at 140 and 165 DAS was found in S4 (120x60 cm) were (1620.48 and 1636.17 kg) respectively during the year 2022 with independent of treatments.

Higher seed cotton yield per hectare was recorded under closer spacing S2 (90 x 30 cm) and S1 (60x30 cm) as compared to wider spacing. This might be due to it maintain higher values of yield attributes like number of bolls sympodia⁻¹, total number of boll plant⁻¹, number of bolls per square meter etc. and it promotes healthy growth with more plant population and effective farming practices, leading to increased seed cotton yield ha⁻¹. These findings corroborate the results of Solanki (2020) [25], Sarkar and Malik (2004) [21], Nehra *et al.* (2004) [16], Buttar and Singh (2006) [4].

Effect of treatments

Among the treatment, significantly highest seed cotton yield ha⁻¹ at 140 and 165 DAS, recorded in T₁ (Pruning at 40 DAS and topping at 70 DAS) were (2148.22 and 2193.52 kg), which was at par with T₂ (Pruning at 50 DAS and topping at 80 DAS) were (2068.05 and 2087.00 kg) respectively in the year 2022, while the lowest seed cotton yield ha⁻¹ at 140 and 165 DAS, recorded in T₄ (No pruning and no topping - Control) as (1443.13 and 1455.34 kg) respectively, during the year 2022 with independent of spacing.

Pruning and topping practices increased yield ha⁻¹ compared to the control, likely due to improved plant architecture, which allows better sunlight penetration into the canopy due to reduced foliage and lodging. This results in higher photosynthetic activity, as well as a more spread-out plant structure that captures more sunlight and enhances

photosynthesis. The result also confirms the findings of Virdia (2011) [29], Selvaraj *et al.* (1977) [22], Ahmed *et al.* (1992) [1], Rahman *et al.* (1992) [20] and Aleeva *et al.* (1992) [2].

Interaction effect

The interaction effects revealed that, significantly highest seed cotton yield ha⁻¹ at 140 and 165 DAS was observed in interaction S2T₁ (90x30 cm with pruning at 40 DAS and topping at 70 DAS) were (2340.59 and 2402.21 kg) respectively, during the year 2022, which was at par with S1T₁ (60x30 cm with pruning at 40 DAS and topping at 70 DAS) were (2312.39 and 2357.09 kg), S1T₂ (60x30 cm with pruning at 50 DAS and topping at 80 DAS) were (2183.22 and 2258.46 kg), S2T₂ (90x30 cm with pruning at 50 DAS and topping at 80 DAS) were (2252.99 and 2318.03 kg), S2T₃ (90x30 cm with mepiquat chloride @ 50 ppm at 55 and 75 DAS) were (2142.95 and 2162.37 kg), S3T₁ (120x30 cm with pruning at 40 DAS and topping at 70 DAS) were (2163.68 and 2221.87 kg) respectively, in the year 2022. The lowest seed cotton yield ha⁻¹ at 140 and 165 DAS was found in interaction S1T₄ (60x30 cm with no pruning and no topping - control) were (1376.15 and 1395.67 kg) respectively, during the year 2022.

Total seed cotton yield ha⁻¹

The data on total seed cotton yield ha⁻¹, as influenced by spacing, treatments and their interaction are presented in Table 6.

Effect of spacing

According to the data (Table 6) the seed cotton yield ha⁻¹ revealed substantial differences related to the different spacing in 2022. Among the spacing, S2 (90x30 cm) was found significantly highest total seed cotton yield ha⁻¹ were (4119.45 kg), which was at par with S1 (60x30 cm) were (3966.96 kg) and S3 (120x30 cm) were (3888.86 kg), during the year 2022, while the lowest total seed cotton yield ha⁻¹ was found in S4 (120x60 cm) were (3256.33 kg) during the year 2022 with independent of treatments.

Higher seed cotton yield per hectare was recorded under spacing S2 (90 x 30 cm) as compared to other spacing. This might be due to it maintain higher values of yield attributes like number of bolls sympodia⁻¹, total number of boll plant⁻¹, number of bolls per square meter etc. and it promotes healthy growth with more plant population and effective farming practices, leading to increased seed cotton yield ha⁻¹. These findings corroborate the results of Solanki (2020) [25], Sarkar and Malik (2004) [21], Nehra *et al.* (2004) [16], Buttar and Singh (2006) [4].

Effect of treatments

Among the treatments, significantly highest total seed cotton yield ha⁻¹ recorded in T₁ (Pruning at 40 DAS and topping at

70 DAS) were (4341.08 kg) which was at par with T₂ (Pruning at 50 DAS and topping at 80 DAS) were (4155.20 kg), during the year 2022, while the lowest total seed cotton yield ha⁻¹, recorded in T₄ (No pruning and no topping - control) were (2898.00 kg), during the year 2022, with independent of spacing.

In this investigation highest total seed cotton yield ha⁻¹ recorded in T₁ (Pruning at 40 DAS and topping at 70 DAS) this might be due to pruning and topping practices have a synergistic effect that maximizes seed cotton yield by reducing excess vegetative growth, which allows for more resources to be allocated to reproductive growth; this not only increases the number of bolls per plant but also enhances the flow of assimilates towards the fruit, resulting in heavier bolls and ultimately higher seed cotton yield ha⁻¹. Such findings were obtained by Obasi and Msaakpa (2005) [17], revealed that pruning, either alone or combined with topping, may redirect the excess flow of assimilates towards the fruit, allowing for more and heavier bolls. This practice also minimizes boll infestation, maximizes boll set, and enhances higher seed cotton yield.

Interaction effect

The interaction effects revealed that, significantly highest total seed cotton yield ha⁻¹ was recorded in interaction S2T₁ (90x30 cm with pruning at 40 DAS and topping at 70 DAS) were (4742.87 kg), in the year 2022, which was at par with S1T₁ (60x30 cm with pruning at 40 DAS and topping at 70 DAS) were (4669.06 kg), S1T₂ (60x30 cm with pruning at 50 DAS and topping at 80 DAS) were (4441.61 kg), S2T₂ (90x30 cm with pruning at 50 DAS and topping at 80 DAS) were (4571.02 kg), S2T₃ (90x30 cm with mepiquat chloride @ 50 ppm at 55 and 75 DAS) as 4304.90 kg, S3T₁ (120x30 cm with pruning at 40 DAS and topping at 70 DAS) were (4384.73 kg) in the year 2022. The lowest seed cotton yield ha⁻¹ was recorded in interaction S1T₄ (60x30 cm with no pruning and no topping - control) were (2770.67 kg) during the year 2022.

In the present investigation, the result might be due to a higher plant population with suitable spacing and early topping and pruning practice allows for better plant growth, enabling the plant to spread out its branches and grow vigorously. This results in improved accumulation and partitioning of photosynthates in the reproductive parts of the plant, contributing to higher seed cotton yield. The relatively higher biomass and enhanced partitioning of photosynthates towards reproductive structures are likely factors leading to increased seed cotton yield. This positive effect is reflected in the physiological parameters and seed cotton yield plant⁻¹. Therefore, seed cotton yield depends on the, higher values of physiological and yield components. This is in confirmation with the findings of Donald and Edgar. (2003) [17], El-Sayed (2023) [8] and Hussain *et al.* (2002) [11].

Table 1: Chlorophyll Index (SPAD) at 30, 60 and 90 DAS of HDPS Bt cotton influenced by different spacing and treatment

Spacing	2022														
	30 DAS					60 DAS					90 DAS				
Treatment	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T ₁	29.85	31.98	33.68	35.95	32.86	41.87	43.05	43.79	44.98	43.42	51.01	51.66	52.93	55.11	52.68
T ₂	32.12	33.22	35.54	37.11	34.50	41.01	42.35	41.85	44.24	42.36	49.34	50.96	52.91	53.35	51.64
T ₃	31.25	32.29	36.11	33.78	33.36	38.38	40.44	41.13	43.79	40.94	47.89	49.97	52.25	53.81	50.98
T ₄	32.46	34.15	34.66	36.78	34.51	35.87	36.77	38.04	40.01	37.67	45.68	46.88	48.18	49.29	47.51
Mean	31.42	32.91	34.99	35.91	33.81	39.28	40.65	41.20	43.26	41.10	48.48	49.87	51.57	52.89	50.70
	Spacing	Treatment	Interaction			Spacing	Treatment	Interaction			Spacing	Treatment	Interaction		
SEM ±	0.618	0.89	SEM ±1	1.78		0.198	0.92	SEM ±1	1.84		0.884	0.84	SEM ±1	1.69	
CD at 5%	2.138	NS	CD 1 at 5%	NS		0.684	2.69	CD 1 at 5%	NS		3.058	2.46	CD 1 at 5%	NS	
			SEM ±2	1.66				SEM ±2	1.61				SEM ±2	1.71	
			CD 2 at 5%	NS				CD 2 at 5%	NS				CD 2 at 5%	NS	

CD1 means between two subplots means at same level of main plot mean. CD2 Means between two main plots means at same level of subplot mean, Main factor: Spacing: - S1- 60x30 cm, S2- 90x30 cm, S3- 1200x30 cm and S4- 120x60 cm. Sub factor: Treatments (Crop management practices): - T₁- Pruning at 40 DAS and topping at 70 DAS, T₂- Pruning at 50 DAS and topping at 80 DAS, T₃- Mepiquat chloride @ 50 ppm at 55 and 75 DAS, T₄- No pruning and no topping (Control)

Table 2: Rate of photosynthesis (umol m⁻² sec⁻¹) (SPAD) at 30, 60 and 90 DAS of HDPS Bt cotton influenced by different spacing and treatment

Spacing	2022														
	30 DAS					60 DAS					90 DAS				
Treatment	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T ₁	18.45	22.11	23.24	27.91	22.93	29.75	31.72	33.02	33.67	32.04	32.79	32.80	34.15	34.51	33.56
T ₂	23.78	21.45	26.88	27.27	24.85	28.13	29.94	31.49	32.06	30.40	30.22	30.93	32.41	32.91	31.62
T ₃	24.22	26.20	27.01	28.12	26.39	26.92	29.18	30.88	31.65	29.66	29.47	30.72	31.42	32.49	31.03
T ₄	21.88	25.33	22.89	26.15	24.06	25.13	27.81	28.46	29.86	27.82	27.08	28.76	29.68	30.42	28.98
Mean	22.08	23.77	25.01	27.36	24.56	27.48	29.66	30.96	31.81	29.98	29.89	30.80	31.91	32.58	31.30
	Spacing	Treatment	Interaction			Spacing	Treatment	Interaction			Spacing	Treatment	Interaction		
SEM ±	0.628	1.02	SEM ±1	2.04		0.780	0.62	SEM ±1	1.23		0.527	0.55	SEM ±1	1.10	
CD at 5%	2.174	NS	CD 1 at 5%	NS		2.701	1.80	CD 1 at 5%	NS		1.824	1.60	CD 1 at 5%	NS	
			SEM ±2	1.88				SEM ±2	1.32				SEM ±2	1.09	
			CD 2 at 5%	NS				CD 2 at 5%	NS				CD 2 at 5%	NS	

CD1 means between two subplots means at same level of main plot mean. CD2 Means between two main plots means at same level of subplot mean, Main factor: Spacing: - S1- 60x30 cm, S2- 90x30 cm, S3- 1200x30 cm and S4- 120x60 cm. Sub factor: Treatments (Crop management practices): - T₁- Pruning at 40 DAS and topping at 70 DAS, T₂- Pruning at 50 DAS and topping at 80 DAS, T₃- Mepiquat chloride @ 50 ppm at 55 and 75 DAS, T₄- No pruning and no topping (Control)

Table 3: Leaf Area Index at 30, 60 and 90 DAS of HDPS Bt cotton influenced by different spacing and treatment (Canopy management practices)

Spacing	2022														
	30 DAS					60 DAS					90 DAS				
Treatment	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean
T ₁	0.258	0.243	0.196	0.141	0.210	1.037	0.983	0.951	0.912	0.971	1.603	1.549	1.517	1.471	1.535
T ₂	0.249	0.235	0.164	0.149	0.199	1.003	0.962	0.924	0.899	0.947	1.569	1.528	1.489	1.465	1.513
T ₃	0.228	0.244	0.188	0.143	0.201	0.982	0.943	0.907	0.884	0.929	1.548	1.509	1.473	1.450	1.495
T ₄	0.252	0.226	0.160	0.153	0.197	0.964	0.927	0.900	0.875	0.916	1.530	1.491	1.466	1.441	1.482
Mean	0.247	0.237	0.177	0.146	0.202	0.997	0.954	0.921	0.893	0.941	1.563	1.519	1.486	1.457	1.506
	Spacing	Treatment	Interaction			Spacing	Treatment	Interaction			Spacing	Treatment	Interaction		
SEM ±	0.010	0.010	SEM ±1	0.020		0.013	0.014	SEM ±1	0.027		0.019	0.013	SEM ±1	0.026	
CD at 5%	0.034	NS	CD 1 at 5%	NS		0.044	0.040	CD 1 at 5%	NS		0.064	0.039	CD 1 at 5%	NS	
			SEM ±2	0.020				SEM ±2	0.027				SEM ±2	0.029	
			CD 2 at 5%	NS				CD 2 at 5%	NS				CD 2 at 5%	NS	

CD1 means between two subplots means at same level of main plot mean. CD2 Means between two main plots means at same level of subplot mean, Main factor: Spacing: - S1- 60x30 cm, S2- 90x30 cm, S3- 1200x30 cm and S4- 120x60 cm. Sub factor: Treatments (Crop management practices): - T₁- Pruning at 40 DAS and topping at 70 DAS, T₂- Pruning at 50 DAS and topping at 80 DAS, T₃- Mepiquat chloride @ 50 ppm at 55 and 75 DAS, T₄- No pruning and no topping (Control)

Table 4: Specific Leaf Weight (g/dm²) at 30, 60 and 90 DAS of HDPS Bt cotton influenced by different spacing and treatment

Spacing	2022															
	30 DAS					60 DAS					90 DAS					
Treatment	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	
T ₁	0.512	0.525	0.529	0.622	0.547	0.950	0.981	1.124	1.214	1.067	1.155	1.215	1.246	1.286	1.226	
T ₂	0.540	0.513	0.615	0.669	0.584	0.939	0.965	1.112	1.172	1.047	1.119	1.192	1.235	1.273	1.205	
T ₃	0.540	0.525	0.589	0.608	0.565	0.928	0.956	1.035	1.148	1.017	1.091	1.185	1.210	1.232	1.180	
T ₄	0.510	0.551	0.626	0.665	0.588	0.914	0.942	0.996	1.114	0.991	1.055	1.162	1.192	1.212	1.155	
Mean	0.526	0.528	0.590	0.641	0.571	0.933	0.961	1.067	1.162	1.031	1.105	1.189	1.221	1.251	1.191	
	Spacing	Treatment	Interaction		Spacing	Treatment	Interaction		Spacing	Treatment	Interaction		Spacing	Treatment	Interaction	
SEM ±	0.030	0.020	SEM ±1	0.039	0.031	0.018	SEM ±1	0.036	0.019	0.018	SEM ±1	0.035	0.019	0.018	SEM ±1	0.035
CD at 5%	NS	NS	CD 1 at 5%	NS	0.106	0.053	CD 1 at 5%	NS	0.066	0.051	CD 1 at 5%	NS	0.066	0.051	CD 1 at 5%	NS
			SEM ±2	0.045			SEM ±2	0.044			SEM ±2	0.044			SEM ±2	0.036
			CD 2 at 5%	NS			CD 2 at 5%	NS			CD 2 at 5%	NS			CD 2 at 5%	NS

CD1 means between two subplots means at same level of main plot mean. CD2 Means between two main plots means at same level of subplot mean, Main factor: Spacing: - S1- 60x30 cm, S2- 90x30 cm, S3- 1200x30 cm and S4- 120x60 cm. Sub factor: Treatments (Crop management practices): - T₁- Pruning at 40 DAS and topping at 70 DAS, T₂- Pruning at 50 DAS and topping at 80 DAS, T₃- Mepiquat chloride @ 50 ppm at 55 and 75 DAS, T₄- No pruning and no topping (Control)

Table 5: Seed cotton yield plant⁻¹ (g) of HDPS Bt cotton influenced by different spacing and treatment at 140 and 165 DAS.

Spacing	2022																			
	140 DAS					165 DAS														
Treatment	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean					
T ₁	42.64	65.96	78.00	127.84	78.61	43.47	67.71	80.10	129.09	80.09	42.64	65.96	78.00	127.84	78.61					
T ₂	40.34	63.45	75.80	124.92	76.13	41.73	65.60	73.32	125.18	76.46	40.34	63.45	75.80	124.92	76.13					
T ₃	36.22	60.60	70.24	112.40	69.87	37.86	61.24	71.74	113.12	70.99	36.22	60.60	70.24	112.40	69.87					
T ₄	25.87	41.36	55.58	102.70	56.38	26.17	40.03	57.27	105.01	57.12	25.87	41.36	55.58	102.70	56.38					
Mean	36.27	57.84	69.90	116.97	70.24	37.31	58.65	70.61	118.10	71.16	36.27	57.84	69.90	116.97	70.24					
	Spacing					Treatment					Spacing					Treatment				
SEM ±	3.692					0.89					3.245					1.04				
CD at 5%	12.775					2.59					11.230					3.03				
Interaction	SEM ±1	CD 1 at 5%	SEM ±2	CD 2 at 5%	SEM ±1	CD 1 at 5%	SEM ±2	CD 2 at 5%	SEM ±1	CD 1 at 5%	SEM ±2	CD 2 at 5%	SEM ±1	CD 1 at 5%	SEM ±2	CD 2 at 5%				
	1.77	5.18	4.00	13.52	2.08	6.07	3.71	12.37	1.77	5.18	4.00	13.52	2.08	6.07	3.71	12.37				

CD1 means between two subplots means at same level of main plot mean. CD2 Means between two main plots means at same level of subplot mean, Main factor: Spacing: - S1- 60x30 cm, S2- 90x30 cm, S3- 1200x30 cm and S4- 120x60 cm. Sub factor: Treatments (Crop management practices): - T₁- Pruning at 40 DAS and topping at 70 DAS, T₂- Pruning at 50 DAS and topping at 80 DAS, T₃- Mepiquat chloride @ 50 ppm at 55 and 75 DAS, T₄- No pruning and no topping (Control)

Table 6: Seed cotton yield ha⁻¹ (Kg) of HDPS Bt cotton influenced by different spacing and treatment

Spacing	2022															
	140 DAS					165 DAS					Total seed cotton yield ha ⁻¹ (Kg)					
Treatment	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	S1	S2	S3	S4	Mean	
T ₁	2312.39	2340.59	2163.68	1776.24	2148.22	2357.09	2402.21	2221.87	1792.92	2193.52	4669.06	4742.87	4384.73	3567.66	4341.08	
T ₂	2183.22	2252.99	2101.00	1735.00	2068.05	2258.46	2318.03	2032.92	1738.58	2087.00	4441.61	4571.02	4134.45	3473.71	4155.20	
T ₃	1949.31	2142.95	1942.48	1560.67	1898.85	2036.66	2162.37	1983.42	1571.07	1938.38	3986.49	4304.90	3925.96	3131.94	3837.32	
T ₄	1376.15	1454.40	1531.96	1410.00	1443.13	1395.67	1405.20	1578.35	1442.12	1455.34	2770.67	2859.00	3110.32	2852.00	2898.00	
Mean	1955.27	2047.73	1934.78	1620.48	1889.56	2011.97	2071.95	1954.14	1636.17	1918.56	3966.96	4119.45	3888.86	3256.33	3807.90	
	Spacing	Treatment	Interaction		Spacing	Treatment	Interaction		Spacing	Treatment	Interaction		Spacing	Treatment	Interaction	
SEM ±	83.507	40.11	SEM ±1	80.22	80.877	44.97	SEM ±1	89.95	115.497	89.22	SEM ±1	178.44	115.497	89.22	SEM ±1	178.44
CD at 5%	288.972	117.07	CD 1 at 5%	234.15	279.871	131.27	CD 1 at 5%	262.54	399.671	260.41	CD 1 at 5%	520.83	399.671	260.41	CD 1 at 5%	520.83
			SEM ±2	108.63			SEM ±2	112.29			SEM ±2	192.92			SEM ±2	192.92
			CD 2 at 5%	351.83			CD 2 at 5%	359.31			CD 2 at 5%	600.56			CD 2 at 5%	600.56

CD1 means between two subplots means at same level of main plot mean. CD2 Means between two main plots means at same level of subplot mean, Main factor: Spacing: - S1- 60x30 cm, S2- 90x30 cm, S3- 1200x30 cm and S4- 120x60 cm. Sub factor: Treatments (Crop management practices): - T₁- Pruning at 40 DAS and topping at 70 DAS, T₂- Pruning at 50 DAS and topping at 80 DAS, T₃- Mepiquat chloride @ 50 ppm at 55 and 75 DAS, T₄- No pruning and no topping (Control)

Conclusion

The treatment (canopy management practice) T₁ (pruning at 40 DAS and topping at 70 DAS) across four different spacings significantly enhanced physiological parameters, including chlorophyll index (SPAD), rate of photosynthesis and specific leaf weight etc. These improvements provided the necessary natural resources for optimal growth,

contributing to better individual plant performance. Additionally, this treatment maintained an effective source-to-sink relationship, resulting in increased yield. Based on the findings, it is concluded that, for obtaining quality and higher seed cotton yield the spacing S2 (90X30 cm) with treatment T₁ (pruning at 40 DAS and topping at 70 DAS) is more beneficial in high density planting system of

Bt cotton, which recorded higher seed cotton yield plant⁻¹ and seed cotton yield ha⁻¹ compared to all other treatment and spacing.

Author's contribution

Rajendra R. Lipane: Conducted research trial, observations, data collection, collect reviews, data analysis, interpretation of results, draft manuscript preparation, study conception and design.

Dr. R.S. Wagh: Served as scientific advisor. Contributed data or analysis tools, designing experiments, reviewed manuscript,

Dr. V.R. Awari: Reviewed the manuscript. Served as scientific advisor.

Dr. A. R. Aher: Reviewed the manuscript, Served as scientific advisor

Dr. B. M. Bhalerao: Served as scientific advisor.

Dr. D. V. Deshmukh: served as scientific advisor

Dr. V. S. Wani: Reviewed the analyzed data, served as scientific advisor

All authors reviewed the results and approved the final version of the manuscript.

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