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Sowing dates, spray scheduling, and foliar applications influence leaf biomass in soybean (*Glycine max* (L.) Merrill)

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Abstract
Soybean, known as the 'miracle bean', is cultivated mainly for its seeds rich in protein and oil. In India, soybean is traditionally grown as a rainfed crop during the '*Kharif*' or monsoon season. Nowadays, soybean is also cultivated in the *Rabi* season to achieve optimal seed yield and quality. However, high-temperature conditions adversely affect crop growth, development, and yield. Elevated temperatures during critical growth stages, such as flowering and pod formation, cause reduced biomass accumulation. Hence, to test the effect of sowing window on dry matter accumulation in leaves, an experiment was conducted during the *Rabi* to *Rabi*-summer seasons at the Experimental Research Farm, Seed Technology Research Unit, JNKVV, Jabalpur (M.P), in 2021-2022 and 2022-2023. The experiment comprised 28 treatment combinations comparing two dates of sowing with two spray schedules of seven PGR treatments (T_1 - Control (no spray), T_2 (SA @ 250 ppm, T_3 (SA @ 500 ppm, T_4 (TU @ 500 ppm, T_5 (TU @ 1000 ppm, T_6 (CCC @ 100 ppm, T_7 (IBA @ 200 ppm) were tested on soybean variety JS 20-98 using a split-split plot design with three replications. The results revealed a significant difference in dry matter accumulation in leaves at 45 DAS and 75 DAS among different sowing dates. Soybean sown in the *Rabi* season (D_1) exhibited maximum dry matter accumulation in leaves, with an increase of 24.35% at 45 DAS and 19.01% at 75 DAS over D2 (*Rabi*-summer). Foliar spray during the vegetative stage increased dry matter accumulation in leaves by 4.44% at 45 DAS and 4.38% at 75 DAS. Specifically, the application of SA @ 500 ppm (T_3) increased dry matter accumulation in leaves by 8.37% at 45 DAS, while TU @ 500 ppm (T_4) increased it by 4.65% at 75 DAS. These findings prove that foliar application of plant growth regulators during the vegetative stage helps mitigate high-temperature stress conditions by enhancing biomass production.

Keywords: *Rabi*, Leaf biomass, salicylic acid, Soybean, TU, SA, CCC, IBA

Introduction
Soybean (*Glycine max* (L.) Merrill), often referred to as the 'miracle bean', holds a significant position as one of the world's most crucial oilseed crops (Modgil *et al*., 2021) [12]. As a member of the Leguminosae family, it is cultivated primarily for its seeds, which are a rich source of protein and oil (Singh *et al*., 2007) [15]. In India, the traditional practice is to grow soybean as a rainfed crop during the '*Kharif*' or monsoon season, as it is one of the most resilient *Kharif* pulses, capable of thriving under a wide range of agro-climatic conditions (Kumar *et al*. 2008) [10]. In MP, soybean cultivation largely depends on the onset of the monsoon, and in areas such as central India, farmers sometimes miss the recommended sowing date due to delayed monsoons or persistent rainfall, which presents challenges and affects overall yield (Raza *et al*., 2021) [14]. Furthermore, crop growth and development can be significantly affected by variations in the severity and frequency of extreme weather conditions. Unpredictable weather events, such as excessive rainfall, prolonged dry spells, or sudden temperature fluctuations, can disrupt the growth cycle of soybean plants. (Raza *et al*. 2018) [13].

It has been reported that soil quality, climate conditions, and agronomic practices, such as sowing time reduce the seed yield (Ahmed *et al*., 2015) [1]. These factors are critical in determining the overall health and productivity of the plants.
Specifically, heat and drought stresses during the crop growth period are responsible for fluctuations in dry matter accumulation and seed yield competition within crop species (Ciaffi *et al*., 1996) [6]. Under favorable growing conditions, soybean tends to exhibit excessive vegetative growth, particularly in its leaves (Ainsworth *et al*., 2005) [3].
Heavy rainfall during the *Kharif* season increases soybean leaf area and reduces sunlight penetration in the canopy, causing leaf redundancy (Chen *et al*., 2017) [5]. Top canopy leaves shade lower leaves, accelerating their senescence and hindering nutrient translocation to reproductive parts (Willcott *et al*., 1984). This shading decreases photosynthetic rates and the availability of photoassimilates for seed development. This reduction in photosynthesis limits the production of carbohydrates and nutrient translocation, significantly impacting dry matter accumulation in soybean plants, and ultimately affecting seed yield (Borrás *et al*., 2004) [4].
Cytokinins help enhance dry matter accumulation by increasing photosynthetic activity, assimilate partitioning, and endosperm cell division (Dua *et al*., 2008) [10]. The foliar application of cytokinins optimizes dry matter accumulation in leaves under different environmental conditions (Islam *et al*., 2022) [8]. Based on our understanding of soybean cultivation dynamics, we hypothesized that shifting the cultivation of soybean to the *Rabi* season, along with the foliar application of plant growth regulators, could potentially enhance dry matter accumulation in soybean.

Materials and Methods

The experiment was conducted at the Experimental Farm, Seed Technology Research Unit, Jawaharlal Nehru Krishi Viswa Vidyalaya, Jabalpur, Madhya Pradesh, during the *Rabi* and *Rabi*-summer seasons of 2021-2022 and 2022-2023. The latitude and longitude of the experimental area are 23°12' N and 79°56' E, respectively. Soybean variety JS 20-98 was sown during both seasons in early January (D_1) and late January (D_2). Foliar application of plant growth regulators occurred at two stages of the soybean crop: the vegetative stage (S_1) and the flowering stage (S2). The details of the plant growth regulators used were as follows:

T_1 : Control (no spray)
T_2 : Salicylic acid (SA) @ 250 ppm
T_3 : Salicylic acid (SA) @ 500 ppm
T_4 : Thiourea (TU) @ 500 ppm
T_5 : Thiourea (TU) @ 1000 ppm
T_6 : Cycocel (CCC) @ 100 ppm
T_7 : Indole-3-butyric acid (IBA) @ 200 ppm

To estimate dry matter production, five randomly selected plants were uprooted from the field at 30 DAS, 45 DAS, and 75 DAS (days after sowing) which represent the vegetative stage, flowering stage, and seed filling stage, respectively. These plants were divided into leaves, branches, main stem, and pods, then dried in an electric oven at 80°C until reaching a constant weight. The dry weight of each plant part was measured separately using a digital balance and expressed as grams per plant.
The statistical analysis was performed using the statistical software R 4.2.2. The treatment means and their interactions were compared using least significant differences (LSD) at $p \leq 0.05$, using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Effect of Different Treatments on dry matter accumulation in Leaves (g plant^{-1})

Dry matter accumulation in soybean leaf is increasing with growth interval up to decrease thereafter:

30 DAS (Days after sowing)

Plant performance, including biomass accumulation, development, partitioning, and yield, is heavily influenced by the plant's capacity to adapt to environmental changes (Wang *et al*., 2003) [16]. Heat stress during the vegetative phase has been reported to reduce photosynthesis, leaf area, and biomass (Jumrani *et al*., 2018) [9]. The results from the pooled analysis of two consecutive years (Table 1 and 2) indicated that the dry matter accumulation in leaves ranged from 0.65 - 0.71 g plant^{-1}. Numerically maximum dry matter accumulation in leaves was observed in D_1 (0.69 g plant^{-1}) followed by D2 (0.67 plant^{-1}). Our findings align with those of Mirosavljević *et al*. (2018) [11], who observed that delayed sowing resulted in a reduction in the plant's dry weight. On the other hand, dry matter accumulation in leaves was found to be the same in S_1 and S_2 (0.68 plant^{-1}) at 30 DAS.
Among the foliar application of plant growth regulators, numerically maximum (0.69 plant^{-1}) dry matter accumulation in leaves was observed in T_2 (SA @ 250 ppm), T_6 (CCC @ 100 ppm), and T_7 (IBA @ 200 ppm), whereas T_1 (control), T_3 (SA @ 500 ppm) and T_5 (TU @ 1000 ppm) recorded minimum (0.67 plant^{-1}) dry matter accumulation in leaves. The interaction between the date of sowing and spray schedule (D x S), date of sowing and foliar spray of PGRs (D x T), spray schedule and foliar spray of PGRs (S x T) revealed non-significant differences in dry matter accumulation in leaves at 30 DAS.

Table 1: Effect of sowing dates, spray scheduled and PGRs foliar spray on dry matter accumulation in leaves of soybean at successive growth intervals

Treatments	At 30 DAS			At 45 DAS			At 75 DAS		
	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled
Main plot: Date of Sowing (D)									
D_1	0.66^{a}	0.73^{a}	0.69^{a}	3.62^{a}	4.04^{a}	3.83^{a}	4.65^{a}	5.00^{a}	4.82^{a}
D_2	0.69^{a}	0.64^{a}	0.67^{a}	2.72^{b}	3.44^{b}	3.08^{b}	3.66^{b}	4.45^{b}	4.05^{b}
Subplot: Spray Schedule (S)									
S_1	0.67^{a}	0.69^{a}	0.68^{a}	3.27^{a}	3.79^{a}	3.53^{a}	4.24^{a}	4.83^{a}	4.53^{a}
S_2	0.68^{a}	0.68^{a}	0.68^{a}	3.07^{a}	3.68^{a}	3.38^{b}	4.07^{a}	4.61^{a}	4.34^{a}
Sub-sub plot: Foliar spray of PGR (T)									
T_1	0.66^{a}	0.68^{a}	0.67^{a}	3.21^{a}	3.53^{b}	3.37^{ab}	4.04^{a}	4.57^{a}	4.30^{a}
T_2	0.68^{a}	0.69^{a}	0.69^{a}	3.14^{a}	3.68^{ab}	3.41^{ab}	4.19^{a}	4.70^{a}	4.44^{a}
T_3	0.67^{a}	0.67^{a}	0.67^{a}	3.34^{a}	3.96^{a}	3.65^{a}	4.25^{a}	4.68^{a}	4.46^{a}
T_4	0.68^{a}	0.69^{a}	0.68^{a}	3.30^{a}	3.78^{ab}	3.54^{ab}	4.23^{a}	4.76^{a}	4.50^{a}
T_5	0.68^{a}	0.67^{a}	0.67^{a}	3.14^{a}	3.78^{ab}	3.46^{ab}	4.16^{a}	4.81^{a}	4.49^{a}
T_6	0.69^{a}	0.70^{a}	0.69^{a}	2.95^{a}	3.66^{ab}	3.30^{b}	4.16^{a}	4.65^{a}	4.41^{a}
T_7	0.69^{a}	0.69^{a}	0.69^{a}	3.12^{a}	3.77^{ab}	3.45^{ab}	4.04^{a}	4.89^{a}	4.46^{a}
Interaction – Date of Sowing x Spray Schedule (D x S)									
D_1S_1	0.66^{a}	0.73^{a}	0.69^{a}	3.68^{a}	4.13^{a}	3.91^{a}	4.67^{a}	5.09^{a}	4.88^{a}
D_1S_2	0.67^{a}	0.72^{a}	0.69^{a}	3.55^{a}	3.94^{b}	3.75^{b}	4.63^{a}	4.90^{a}	4.76^{a}
D_2S_1	0.69^{a}	0.64^{b}	0.67^{a}	2.86^{b}	3.46^{c}	3.16^{c}	3.80^{b}	4.57^{ab}	4.18^{b}
D_2S_2	0.69^{a}	0.65^{b}	0.67^{a}	2.59^{b}	3.42^{c}	3.00^{c}	3.51^{b}	4.33^{b}	3.92^{b}
Interaction – Date of Sowing x Foliar spray of PGR (D x T)									
D_1T_1	0.64^{a}	0.73^{a}	0.68^{a}	3.59^{ab}	3.76^{bcd}	3.68^{ab}	4.51^{abc}	4.79^{a}	4.65^{abcd}
D_1T_2	0.67^{a}	0.73^{a}	0.70^{a}	3.52^{abc}	3.91^{bc}	3.71^{a}	4.47^{abc}	5.01^{a}	4.74^{abcd}
D_1T_3	0.64^{a}	0.72^{ab}	0.68^{a}	3.69^{ab}	4.42^{a}	4.06^{a}	4.77^{ab}	5.04^{a}	4.91^{ab}
D_1T_4	0.64^{a}	0.72^{ab}	0.68^{a}	3.89^{a}	4.10^{ab}	3.99^{a}	4.99^{a}	5.09^{a}	5.04^{a}
D_1T_5	0.67^{a}	0.71^{abc}	0.69^{a}	3.61^{ab}	4.08^{ab}	3.85^{a}	4.52^{abc}	5.06^{a}	4.79^{abc}
D_1T_6	0.69^{a}	0.73^{a}	0.71^{a}	3.48^{abc}	3.91^{abc}	3.70^{ab}	4.75^{ab}	4.77^{a}	4.76^{abc}
D_1T_7	0.69^{a}	0.72^{ab}	0.71^{a}	3.54^{abc}	4.08^{ab}	3.81^{a}	4.53^{abc}	5.21^{a}	4.87^{ab}
D_2T_1	0.68^{a}	0.64^{bcd}	0.66^{a}	2.84^{cd}	3.29^{d}	3.06^{c}	3.57^{c}	4.34^{a}	3.96^{d}
D_2T_2	0.70^{a}	0.64^{bcd}	0.67^{a}	2.75^{d}	3.45^{cd}	3.10^{c}	3.91^{bc}	4.39^{a}	4.15^{bcd}
D_2T_3	0.70^{a}	0.62^{d}	0.66^{a}	2.98^{bcd}	3.50^{cd}	3.25^{bc}	3.72^{bc}	4.32^{a}	4.02^{cd}
D_2T_4	0.71^{a}	0.66^{abcd}	0.68^{a}	2.70^{d}	3.46^{cd}	3.08^{c}	3.47^{c}	4.44^{a}	3.96^{d}
D_2T_5	0.68^{a}	0.63^{cd}	0.66^{a}	2.67^{d}	3.48^{cd}	3.08^{c}	3.80^{bc}	4.56^{a}	4.18^{bcd}
D_2T_6	0.70^{a}	0.66^{abcd}	0.68^{a}	2.42^{d}	3.41^{cd}	2.91^{c}	3.57^{c}	4.53^{a}	4.05^{cd}
D_2T_7	0.69^{a}	0.66^{abcd}	0.68^{a}	2.71^{d}	3.47^{cd}	3.09^{c}	3.55^{c}	4.56^{a}	4.05^{cd}
Interaction – Spray Schedule x Foliar spray of PGR (S x T)									
S_1T_1	0.64^{a}	0.69^{a}	0.67^{a}	3.27^{a}	3.58^{ab}	3.42^{ab}	4.38^{a}	4.67^{a}	4.52^{a}
S_1T_2	0.70^{a}	0.69^{a}	0.70^{a}	3.26^{a}	3.68^{ab}	3.47^{ab}	4.23^{a}	4.84^{a}	4.54^{a}
S_1T_3	0.65^{a}	0.67^{a}	0.66^{a}	3.48^{a}	4.05^{a}	3.76^{a}	4.35^{a}	4.86^{a}	4.60^{a}
S_1T_4	0.68^{a}	0.68^{a}	0.68^{a}	3.57^{a}	3.93^{ab}	3.75^{ab}	4.50^{a}	4.85^{a}	4.67^{a}
S_1T_5	0.69^{a}	0.66^{a}	0.68^{a}	3.23^{a}	3.75^{ab}	3.49^{ab}	4.22^{a}	4.91^{a}	4.57^{a}
S_1T_6	0.68^{a}	0.71^{a}	0.70^{a}	2.92^{a}	3.72^{ab}	3.32^{ab}	3.95^{a}	4.61^{a}	4.28^{a}
S_1T_7	0.67^{a}	0.70^{a}	0.68^{a}	3.17^{a}	3.85^{ab}	3.51^{ab}	4.05^{a}	5.06^{a}	4.55^{a}
S_2T_1	0.67^{a}	0.68^{a}	0.67^{a}	3.16^{a}	3.48^{b}	3.32^{ab}	3.71^{a}	4.47^{a}	4.09^{a}
S_2T_2	0.66^{a}	0.68^{a}	0.67^{a}	3.01^{a}	3.67^{ab}	3.34^{ab}	4.15^{a}	4.55^{a}	4.35^{a}
S_2T_3	0.69^{a}	0.68^{a}	0.68^{a}	3.20^{a}	3.88^{ab}	3.54^{ab}	4.14^{a}	4.50^{a}	4.32^{a}
S_2T_4	0.67^{a}	0.69^{a}	0.68^{a}	3.02^{a}	3.63^{ab}	3.32^{ab}	3.97^{a}	4.68^{a}	4.32^{a}
S_2T_5	0.66^{a}	0.68^{a}	0.67^{a}	3.05^{a}	3.82^{ab}	3.43^{ab}	4.11^{a}	4.71^{a}	4.41^{a}
S_2T_6	0.70^{a}	0.69^{a}	0.69^{a}	2.98^{a}	3.59^{ab}	3.29^{b}	4.38^{a}	4.69^{a}	4.53^{a}
S_2T_7	0.71^{a}	0.68^{a}	0.70^{a}	3.08^{a}	3.70^{ab}	3.39^{ab}	4.03^{a}	4.71^{a}	4.37^{a}

The values with identical letters are not significantly different at $p<0.05$ level

45 DAS

There was a significant difference in dry matter accumulation in leaves at 45 DAS regarding the date of sowing, spray schedule, and foliar application of PGR (Table 1 and 2) with a range varied from 2.92-4.23 g plant^{-1}. The date of sowing significantly affected dry matter accumulation in leaves by D1 (3.83 plant^{-1}) having maximum dry matter accumulation in leaves, followed by D2 (3.08 plant^{-1}). Our results are similar to those of Jumrani *et al.* (2018) [9], who reported that temperature stress significantly affected biomass accumulation in soybean genotypes. On the other hand, the spray S_1 (3.53 plant^{-1}) recorded maximum dry matter accumulation in leaves at 45 DAS, followed by S_2 (3.38 plant^{-1}). Our studies have shown that foliar spray of plant growth regulators at the time of the vegetative stage increased dry matter accumulation in leaves at 45 DAS, there is limited research on observing dry matter accumulation in leaves at 45 DAS on foliar spray of plant growth regulators.

Among the foliar application of plant growth regulators, T_3 – SA @ 500 ppm (3.65 plant^{-1}) recorded maximum dry matter accumulation in leaves at 45 DAS which is at par with all other treatments except T_6 (CCC @ 100 ppm, whereas T_6 recorded the minimum dry matter accumulation in leaves (3.30 plant^{-1}).

The interaction between the date of sowing and spray schedule

(D x S), date of sowing and foliar spray of PGRs (D x T), spray schedule and foliar spray of PGRs (S x T) revealed non-significant differences in dry matter accumulation in leaves at 45 DAS. That biomass production and photosynthetic activity are not significantly affected by temperature stress. However, a noticeable decrease in dry matter accumulation occurs when temperature stress happens, particularly from the flowering stage to early seed filling. This reduction is linked to a decline in leaf photosynthetic rate, stomatal conductance, and carbon isotope discrimination.

75 DAS

A significant difference in dry matter accumulation in leaves at 7 DAS was observed regarding the different dates of sowing. The range of dry matter accumulation in leaves at 75 DAS was found to be 3.60-5.37 g plant^{-1}. The maximum dry matter accumulation in leaves at 75 DAS was observed in D1 (4.82 plant^{-1}), followed by D2 (4.05 plant^{-1}). The maximum dry matter accumulation in leaves at 75 DAS was observed in D1 (4.82 plant^{-1}), followed by D2 (4.05 plant^{-1}). Our results are consistent with those, who reported that plants grown under high temperatures, from 10 days after sowing (DAS) until maturity, exhibited lower total biomass compared to control plants.

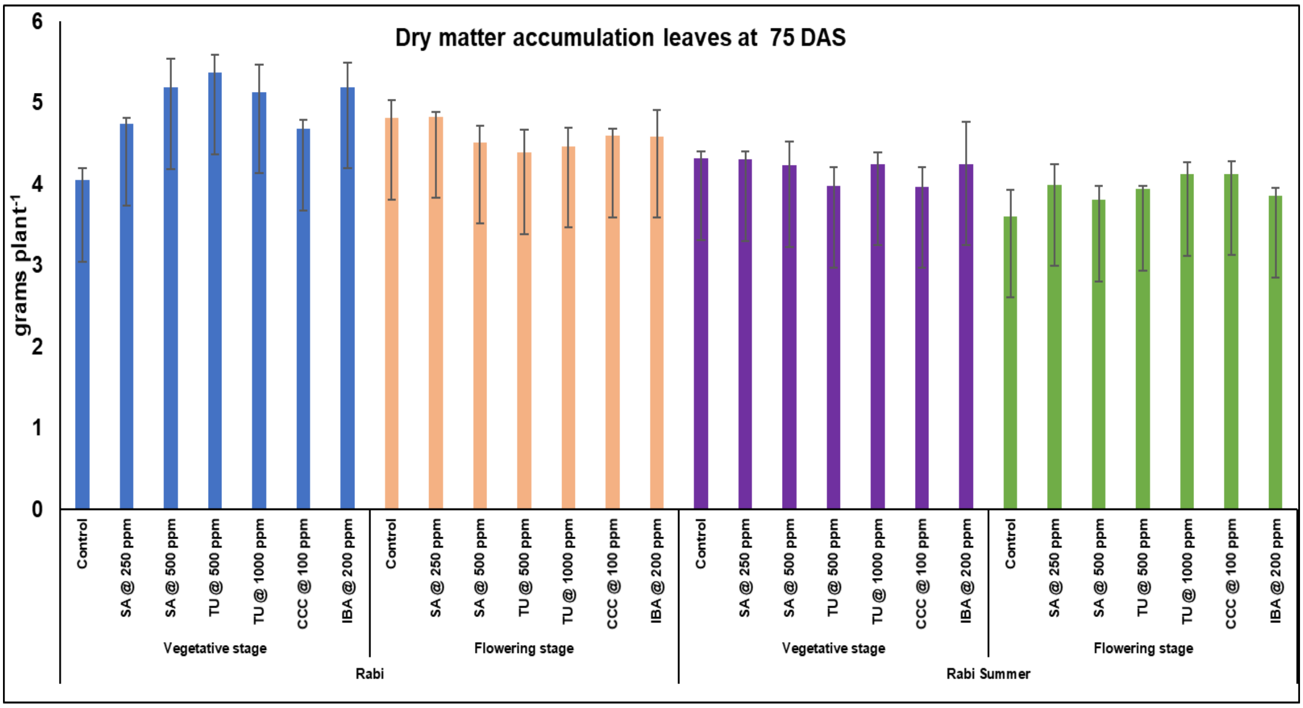
A non-significant difference was observed due to the spray schedule. S1 (4.53 plant^{-1}) exhibited the maximum dry matter accumulation in leaves at 75 DAS compared to S2 (4.34 plant^{-1}). Our studies have shown that foliar spray of plant growth regulators at the time of the vegetative stage increased dry matter accumulation in leaves at 75 DAS, there is limited research on observing dry matter accumulation in leaves at 75 DAS on foliar spray of plant growth regulators.

Similarly, the foliar spray of different plant growth regulators had no-significant effect on dry matter accumulation in leaves at 75 DAS in soybean. T_4 (TU @ 500 ppm) recorded numerically maximum (4.50 plant^{-1}) dry matter accumulation in leaves which is on par with all other treatments. The interaction between the date of sowing and spray schedule (D x S), date of sowing and foliar spray of PGRs (D x T), spray schedule and foliar spray of PGRs (S x T) revealed non-significant differences in dry matter accumulation in leaves at 75 DAS.

Table 2: Results of the two-way ANOVA and Duncan's multiple range tests for the comparative effects of PGRs on dry matter accumulation in leaves (g plant^{-1}) of soybean at successive growth intervals

	At 30 DAS			**At 45 DAS**			**At 75 DAS**		
	2021-2022	**2022-2023**	**Pooled**	**2021-2022**	**2022-2023**	**Pooled**	**2021-2022**	**2022-2023**	**Pooled**
Main plot: Date of Sowing (D)									
S.Em±	0.01	0.01	0.01	0.02	0.02	0.01	0.04	0.04	0.04
SD	0.02	0.02	0.01	0.03	0.04	0.02	0.05	0.06	0.05
CD (*p*≤5%)	0.07[a]	0.08	0.06	0.11***	0.15**	0.08***	0.23**	0.24**	0.23**
Subplot: Spray Schedule (S)									
S.Em±	0.01	0.00	0.01	0.07	0.03	0.03	0.07	0.10	0.08
SD	0.02	0.01	0.01	0.11	0.04	0.04	0.10	0.14	0.11
CD (*p*≤5%)	0.06	0.02	0.03	0.29	0.12	0.11*	0.29	0.39	0.30
Sub-sub plot: Foliar spray of PGR (T)									
S.Em±	0.02	0.01	0.01	0.10	0.07	0.07	0.15	0.13	0.11
SD	0.03	0.02	0.02	0.14	0.10	0.09	0.22	0.19	0.16
CD (*p*≤5%)	0.06	0.04	0.03	0.29	0.21**	0.19*	0.44	0.37	0.32
Interaction – Date of Sowing x Spray Schedule (D x S)									
S.Em±	0.02	0.01	0.01	0.08	0.04	0.03	0.08	0.11	0.09
SD	0.03	0.02	0.02	0.11	0.06	0.04	0.12	0.15	0.12
CD (*p*≤5%)	0.08	0.02	0.04	0.42	0.17	0.16	0.41	0.55	0.42
Interaction – Date of Sowing x Foliar spray of PGR (D x T)									
S.Em±	0.03	0.02	0.02	0.14	0.10	0.09	0.20	0.18	0.15
SD	0.04	0.03	0.02	0.19	0.14	0.12	0.29	0.25	0.22
CD (*p*≤5%)	0.09	0.05	0.04	0.41	0.29	0.27	0.62	0.53	0.46
Interaction – Spray Schedule x Foliar spray of PGR (S x T)									
S.Em±	0.03	0.02	0.02	0.15	0.10	0.09	0.21	0.20	0.17
SD	0.05	0.02	0.02	0.22	0.14	0.13	0.30	0.28	0.24
CD (*p*≤5%)	0.09	0.05	0.04	0.41	0.29	0.27	0.62	0.53	0.46

a F-values. ns: not significant F ratio ($p<0.05$); S.Em± - Standard error mean, SD – Standard deviation; CD – Critical difference;
*, ** and ** indicate significance at $p<0.05$, 0.01 and 0.001, respectively



The error bars shown in the figure represent the standard error of the mean. SA- Salicylic acid, TU – Thiourea, CCC – Cycocel and IBA - Indole-3-butyric acid

Fig 1: Effect of foliar spray of plant growth regulators on the Dry matter accumulation leaves at 75 DAS of soybean

Conclusion

Delayed sowing, particularly during high-temperature stress conditions, significantly reduces the physiological development of soybean. However, the application of foliar sprays of plant growth regulators during the vegetative stage has been found to counteract these adverse effects. Specifically, the exogenous application of plant growth regulators such as salicylic acid, thiourea, Cycocel, and indole-3-butyric acid (IBA) has shown promising results. These plant growth regulators mitigate the negative effects of high-temperature stress on plant growth and development. By enhancing the plant's resilience to environmental stress, these growth regulators contribute to an increase in the overall yield of the crop.

Competing Interest

The authors have declared that no competing interests exist.

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