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Effect of foliar-applied boron and sulphur on vegetative traits in *Calendula officinalis*

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Abstract
The present investigation was conducted to evaluate the effect of foliar-applied boron and sulphur on the vegetative growth of *Calendula officinalis* under a factorial randomized block design with three replications. Treatments included three levels each of boron (0%, 0.1%, 0.2%) and sulphur (0%, 0.1%, 0.2%). Observations on plant height, leaf area, and number of leaves were recorded at 30, 60, and 90 days after transplanting (DAT). While early-stage growth (30 and 60 DAT) showed non-significant differences in plant height, significant enhancements were observed at 90 DAT, particularly under combined application. The treatment B_1S_1 (0.1% boron + 0.1% sulphur) consistently recorded the highest values across all parameters and growth stages, indicating a synergistic effect. Notably, B_1S_1 produced the maximum plant height (28.21 cm), leaf area (60.79 cm²), and number of leaves per plant (197.10) at 90 DAT. These findings underscore the potential of moderate concentrations of foliar boron and sulphur in enhancing late-stage vegetative growth in calendula through improved leaf development and biomass accumulation.

Keywords: Calendula officinalis, vegetative growth, foliar application, Boron and Sulphur sprays

Introduction
Calendula officinalis L. is a high-value flower crop with significant economic and industrial relevance, making it an ideal candidate for dual-purpose cultivation. Its flowers serve both commercial floriculture and industrial applications, ensuring a sustainable income source for growers while fulfilling demands in pharmaceutical, cosmetic, and nutraceutical industries. *Calendula officinalis* L., commonly known as pot marigold, is an aromatic, erect, annual herb belonging to the Asteraceae family. Indigenous to Europe**,** it is widely cultivated in North America, the Balkans, Eastern Europe, Germany, and India due to its high adaptability and economic significance (Khan *et al.*, 2011) [5]. Its extensive distribution highlights its importance in floriculture, medicinal, and industrial applications, making it a valuable crop in both temperate and subtropical regions. The species thrives in temperate and subtropical climates, making it a preferred choice for both commercial floriculture and industrial applications.

Calendula officinalis L. is a pharmacologically significant species known for its rich secondary metabolite profile and extensive medicinal applications. It contains various bioactive compounds, including carotenoids, flavonoids, terpenoids, and saponins, which contribute to its diverse biological activities (Jan & John, 2017; Sapkota & Kunwar, 2024) [4, 7]. The characteristic yellow and orange pigmentation of its flowers is attributed to carotenoids, which also exhibit potent antioxidant properties. Edible calendula petals are utilized as natural food colorants, herbal teas, and dietary supplements, leveraging their antioxidant and health-promoting benefits (Sausserde & Kampuss, 2014) [8]. The phytochemical constituents of *C. officinalis* play a crucial role in its pharmacological potential, particularly in wound healing, anti-inflammatory, antioxidant, and anticancer applications (Ashwlayan *et al.*, 2018) [2]. These therapeutic effects are largely mediated by flavonoids and terpenoids, which exhibit anti-inflammatory and antimicrobial properties. Furthermore, calendula-derived cosmetic ingredients, particularly the essential oil rich in monoterpenes, sesquiterpenes, flavonoids, tannins, and sterols, have been deemed safe for cosmetic use by the Cosmetic Ingredient Review (CIR) Expert Panel (Andersen *et al.*, 2010) [1]. Given its broad-spectrum pharmacological relevance, *C. officinalis* continues to be an important candidate for furtherresearch in cosmeceuticals, nutraceuticals, and pharmaceutical formulations aimed at harnessing its therapeutic potential.

Nutrient availability plays a critical role in optimizing flower yield and quality, directly influencing farmer profitability in dual-purpose flower crops like *Calendula officinalis*. Similar to oilseeds and cereals, where Boron (B) and Sulphur (S) enhance dry matter accumulation (Pegu *et al.*, 2024; Ismail *et al.*, 2013) [6, 3], these nutrients are essential in calendula for floral biomass production, carotenoid synthesis, and secondary metabolite formation. Boron facilitates pollen viability and floral organ development, while Sulphur supports enzyme activity and essential oil synthesis, making them indispensable for both ornamental value and industrial extraction**.** The integration of balanced B and S nutrition can enhance flower size, oil yield, and bioactive compound concentration, ensuring higher economic returns. Understanding this nutrient-growth relationship is vital for precision agriculture strategies aimed at improving both floriculture and phytopharmaceutical outputs, making this a crucial area for further floriculture research.

Understanding the interactive effects of boron and sulphur on Calendula's growth parameters is essential for developing targeted nutrient management strategies for optimum yield. Given the limited research on their combined effects in flower crops, this study aims to bridge the gap and explore how optimized boron-sulphur fertilization can enhance flower productivity and phytochemical richness in Calendula.

Material and Methods

The investigation was carried out over two consecutive years, 2022-23 and 2023-24, at the Horticulture Experimental Research Farm, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh. The experimental site is situated at 21.2° N latitude, 81.6° E longitude, and lies at an altitude of 294 meters above mean sea level. The crop was initially raised in a nursery and later transplanted to the main field, where standard horticultural practices were followed for its management. Foliar applications of boron and sulphur were carried out twice—15 days after transplanting—and once at the bud initiation stage, with the aim of enhancing crop performance. The experiment was designed in a factorial randomized block design (RBD) with three replications. Powdered boric acid (H_3BO_3) served as the source of boron for foliar application, whereas a commercial liquid formulation containing 20% sulphur was used for the sulphur treatments. Details of the treatment combinations are presented in Table 1.

Table 1: Treatment combinations are presented

Treatments	Treatment combinations	Details
T_1	B_0S_0	0% B & 0% S
T_2	B_0S_1	0% B & 0.1% S
T_3	B_0S_2	0% B & 0.2% S
T_4	B_1S_0	0.1% B & 0% S
T_5	B_1S_1	0.1% B & 0.1% S
T_6	B_1S_2	0.1% B & 0.2% S
T_7	B_2S_0	0.2% B & 0% S
T_8	B_2S_1	0.2% B & 0.1% S
T_9	B_2S_2	0.2% B & 0.2% S

Plant height, leaf area, and number of leaves were recorded at 30, 60, and 90 days after transplanting to evaluate the influence of foliar-applied boron and sulphur on vegetative growth. These growth parameters were systematically measured to assess the morphological response of the plants to the different treatment combinations.

Results

The data pertaining to the study titled "Effect of Foliar-Applied Boron and Sulphur on Vegetative Traits in Calendula officinalis" are presented in Table 2. The results reveal that vegetative attributes such as plant height, leaf area, and number of leaves exhibited significant enhancement in the pooled mean values when compared to the untreated control (T_1).

Plant Height

At 30 DAT, plant height was not significantly affected by boron, sulphur, or their interaction in either year or in the pooled analysis. However, a consistent numerical trend was noted. Among boron treatments, the highest plant height was recorded with B_2 (0.2% boron), measuring 14.50 cm in 2022-23, 14.51 cm in 2023-24, and a pooled mean of 14.51 cm, followed by B_1 (0.1% boron) with a pooled mean of 14.33 cm. The lowest height was observed under B_0 (control), with a pooled mean of 13.92 cm. In the case of sulphur treatments, S_2 (0.2% sulphur) resulted in the maximum plant height (pooled mean: 14.50 cm), followed by S_1 (0.1% sulphur) with 14.44 cm. The lowest height was noted in the control (S_0), with a pooled mean of 13.82 cm.

At 60 DAT, foliar application of boron and sulphur did not significantly influence plant height in either year or in the pooled analysis. However, numerical variations were observed. Among boron treatments, B_2 (0.2% boron) recorded the maximum plant height with a pooled mean of 21.32 cm, followed closely by B_1 (0.1% boron) at 21.31 cm. The lowest height was observed under B_0 (control), with a pooled mean of 20.31 cm. Similarly, sulphur application showed no statistically significant effect, though S_1 (0.1% sulphur) recorded the highest pooled mean plant height (21.36 cm), followed by S_2 (0.2% sulphur) at 21.29 cm. The minimum height was noted under S_0 (control), with a pooled mean of 20.29 cm.

At 90 DAT, plant height was significantly influenced by the foliar application of boron, sulphur, and their interaction. Among boron treatments, B_1 (0.1% boron) recorded the highest plant height with a pooled mean of 27.23 cm, followed by B_2 (0.2% boron) at 27.11 cm. The lowest value was observed under B_0 (control), with a pooled mean of 26.32 cm, highlighting boron's positive impact on late-stage vegetative growth. Sulphur application also showed significant effects, with S_1 (0.1% sulphur) recording the highest pooled mean plant height (27.25 cm), followed by S_2 (0.2% sulphur) at 27.16 cm. The minimum height was recorded under S_0 (control), with a pooled mean of 26.26 cm. The interaction between boron and sulphur at 90 DAT was statistically significant. The combination B_1S_1 (0.1% boron + 0.1% sulphur) recorded the maximum plant height (28.21 cm), followed by B_2S_2 (0.2% boron + 0.2% sulphur) at 27.53 cm. The lowest height was observed under B_0S_0 (control), with 25.97 cm. These results highlight the synergistic role of combined boron and sulphur application in enhancing late-stage vegetative growth in calendula. The influence of boron and sulphur and interactions on plant height at 30,60 and 90 DAT is shown in Table 2 and Table 3.

Leaf Area

At 30 DAT, leaf area was significantly influenced by foliar application of boron, sulphur, and their interaction across both years and in the pooled data. Among boron treatments, B_1 (0.1% boron) recorded the highest leaf area with a pooled mean of 17.65 cm², followed by B_2 (0.2% boron) at 17.27 cm². The lowest was observed under B_0 (control), with a pooled mean of 16.11 cm². Sulphur application also had a significant effect, with S_1 (0.1% sulphur) showing the maximum pooled mean leaf area (17.77 cm²), followed by S_2 (0.2% sulphur) at 17.18 cm². The minimum was recorded under S_0 (control), at 16.07 cm².

A significant B × S interaction was observed. The treatment combination T_5 (B_1S_1) recorded the highest leaf area (pooled mean: 19.87 cm²), which was significantly superior to all other treatments. The lowest value was noted in T_1 (B_0S_0), with a pooled mean of 15.74 cm².

At 60 days after transplanting (DAT), leaf area was significantly affected by foliar application of boron, sulphur, and their interaction across both years and in the pooled analysis. Among boron levels, B_2 (0.2%) recorded the highest pooled leaf area (35.90 cm²), closely followed by B_1 (0.1%) with 35.91 cm², while the lowest was noted under B_0 (no boron) at 34.16 cm². Similarly, sulphur application showed significant influence, with S_1 (0.1%) resulting in the maximum pooled leaf area (35.95 cm²), followed by S_2 (0.2%) at 35.89 cm², and the minimum under S_0 (34.12 cm²). A significant interaction effect was observed, with the combination B_1S_1 (T_5) recording the highest pooled leaf area of 37.61 cm², while the lowest (33.19 cm²) was found under the control treatment B_0S_0 (T_1), indicating a synergistic response to combined foliar application of boron and sulphur at this stage.

At 90 days after transplanting (DAT), leaf area was significantly influenced by foliar application of boron, sulphur, and their interaction, with data indicating a pronounced improvement in leaf expansion at higher nutrient levels. Among boron treatments, B_2 (0.2%) resulted in the highest pooled leaf area (56.65 cm²), followed closely by B_1 (0.1%) at 56.42 cm², while the lowest was observed under B_0 (no boron) with 51.38 cm². Similarly, sulphur application showed a significant effect, with S_1 (0.1%) recording the highest pooled mean (56.69 cm²), followed by S_2 (0.2%) with 56.55 cm², and the minimum under S_0 (51.23 cm²). The interaction between boron and sulphur was also statistically significant, with the combination B_1S_1 (T_5) producing the highest pooled leaf area of 60.79 cm², while the lowest was recorded under B_0S_0 (T_1) at 46.88 cm². The substantial difference between these treatments, exceeding the critical difference (CD) of 1.936, highlights the synergistic effect of boron and sulphur in promoting sustained leaf development during later growth stages. The influence of boron and sulphur and interactions on plant height at 30, 60 and 90 DAT is shown in Table 4 and Table 5.

Number of leaves per plant

At 30 days after transplanting (DAT), the number of leaves per plant was significantly affected by foliar application of boron, sulphur, and their interaction. Among boron treatments, B_2 (0.2%) recorded the highest pooled mean leaf count of 27.34, followed by B_1 (0.1%) with 26.93, while the lowest was observed in the control B_0 with 25.77 leaves. Similarly, sulphur treatments S_1 (0.1%) and S_2 (0.2%) both resulted in an identical pooled mean of 27.38 leaves, significantly higher than the control S_0 which averaged 25.29 leaves. The interaction effect was also significant, with the combined treatment T_5 (B_1S_1) producing the maximum pooled leaf number of 28.38, followed by T_9 (B_2S_2) at 27.82, and the lowest under T_1 (B_0S_0) with 24.00 leaves. These differences exceeded the critical difference values, confirming the significant synergistic impact of boron and sulphur on leaf production at this growth stage.

At 60 days after transplanting, the number of leaves per plant was significantly influenced by boron, sulphur, and their interaction. Among boron treatments, B_1 (0.1% boron) recorded the highest pooled mean leaf count of 77.60, closely followed by B_2 (0.2% boron) with 77.50, while the lowest was observed in the control B_0 with 75.23 leaves. Similarly, sulphur treatment S_1 (0.1%) resulted in the maximum pooled mean of 77.63 leaves, marginally higher than S_2 (0.2%) at 77.52, with the lowest count in the control S_0 at 75.17 leaves. The combined treatment T_5 (B_1S_1) produced the highest pooled mean leaf number of 80.00, followed by T_9 (B_2S_2) with 78.81, and the lowest under T_1 (B_0S_0) at 74.17 leaves. These differences exceeded the critical difference, confirming a significant synergistic effect of boron and sulphur on leaf production at this stage.

At 90 days after transplanting, the number of leaves per plant was significantly affected by boron, sulphur, and their interaction. Among boron treatments, B_2 (0.2%) recorded the highest pooled mean leaf count of 192.41, slightly surpassing B_1 (0.1%) with 192.19, while the control B_0 had the lowest count of 187.27. Sulphur application showed a similar trend, with S_2 (0.2%) producing the highest pooled mean of 192.46 leaves, closely followed by S_1 (0.1%) at 192.35, and the lowest number in the control S_0 at 187.06. The combined treatment T_5 (B_1S_1) resulted in the maximum leaf number, averaging 197.10 leaves, followed by T_9 (B_2S_2) with 194.85, whereas T_1 (B_0S_0) recorded the lowest at 184.50. These differences were statistically significant, indicating a strong synergistic effect of foliar boron and sulphur on leaf production at 90 DAT. The influence of boron and sulphur and interactions on plant height at 30,60 and 90 DAT is shown in Table 6 and Table 7.

Table 2: Effect of Boron and Sulphur levels on plant height of *Calendula officinalis*

Treatment	Plant height (cm)								
	30DAT			60DAT			90DAT		
	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean
(Factor A) Boron									
B0	13.88	13.96	13.92	19.67	20.96	20.31	26.39	26.26	26.32
B1	14.18	14.48	14.33	20.69	21.93	21.31	27.23	27.24	27.23
B2	14.50	14.51	14.51	20.71	21.92	21.32	26.97	27.26	27.11
SEm±	0.493	0.277	0.298	0.301	0.172	0.211	0.213	0.064	0.121
CD (P=0.05)	1.477	0.831	0.895	0.903	0.515	0.634	0.639	0.192	0.364
(Factor B) Sulphur									
S0	13.81	13.83	13.82	19.64	20.94	20.29	26.31	26.22	26.26
S1	14.26	14.61	14.44	20.78	21.94	21.36	27.25	27.25	27.25
S2	14.49	14.51	14.50	20.64	21.93	21.29	27.03	27.29	27.16
SEm±	0.493	0.277	0.298	0.301	0.172	0.211	0.213	0.064	0.121
CD (P=0.05)	1.477	0.831	0.895	0.903	0.515	0.634	0.639	0.192	0.364

Table 3: Effect of Boron and Sulphur interaction levels on plant height of *Calendula officinalis*

Treatment	Plant height (cm)								
	30DAT			60DAT			90DAT		
	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean
Interaction(B×S)									
$T_1 (B_0S_0)$	13.53	13.53	13.53	19.23	20.47	19.85	26.21	25.74	25.97
$T_2 (B_0S_1)$	13.89	14.04	13.96	19.59	20.97	20.28	26.33	26.24	26.29
$T_3 (B_0S_2)$	14.23	14.33	14.28	20.18	21.44	20.81	26.62	26.80	26.71
$T_4 (B_1S_0)$	13.82	13.87	13.85	19.54	20.93	20.24	26.32	26.20	26.26
$T_5 (B_1S_1)$	14.23	15.14	14.68	21.91	22.95	22.43	28.21	28.21	28.21
$T_6 (B_1S_2)$	14.49	14.42	14.46	20.61	21.91	21.26	27.17	27.30	27.24
$T_7 (B_2S_0)$	14.07	14.09	14.08	20.15	21.41	20.78	26.39	26.71	26.55
$T_8 (B_2S_1)$	14.67	14.66	14.67	20.85	21.91	21.38	27.20	27.31	27.25
$T_9 (B_2S_2)$	14.74	14.79	14.77	21.14	22.44	21.79	27.31	27.75	27.53
SEm±	0.853	0.480	0.517	0.522	0.298	0.366	0.369	0.111	0.210
CD (P=0.05)	2.558	1.440	1.549	1.565	0.893	1.098	1.107	0.333	0.630

Table 4: Effect of Boron and Sulphur levels on leaf area of *Calendula officinalis*

Treatment	Leaf area (cm^2)								
	30DAT			60DAT			90DAT		
	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean
(Factor A) Boron									
B0	15.25	16.96	16.11	34.44	33.87	34.16	53.15	49.61	51.38
B1	17.06	18.25	17.65	36.24	35.58	35.91	57.71	55.14	56.42
B2	16.64	17.89	17.27	36.25	35.54	35.90	57.86	55.44	56.65
SEm±	0.523	0.621	0.570	0.175	0.133	0.124	0.531	0.452	0.373
CD (P=0.05)	1.569	1.863	1.710	0.526	0.398	0.372	1.592	1.354	1.118
(Factor B) Sulphur									
S0	15.23	16.91	16.07	34.40	33.84	34.12	53.13	49.33	51.23
S1	17.09	18.45	17.77	36.30	35.61	35.95	57.75	55.63	56.69
S2	16.62	17.74	17.18	36.24	35.55	35.89	57.85	55.25	56.55
SEm±	0.523	0.621	0.570	0.175	0.133	0.124	0.531	0.452	0.373
CD (P=0.05)	1.569	1.863	1.710	0.526	0.398	0.372	1.592	1.354	1.118

Table 5: Effect of Boron and Sulphur interaction levels on leaf area of *Calendula officinalis*

Treatment	Leaf area (cm^2)								
	30DAT			60DAT			90DAT		
	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean
Interaction(B×S)									
$T_1 (B_0S_0)$	14.85	16.63	15.74	33.27	33.10	33.19	47.19	46.57	46.88
$T_2 (B_0S_1)$	15.29	17.05	16.18	34.77	33.86	34.32	55.31	49.79	52.55
$T_3 (B_0S_2)$	15.60	17.21	16.41	35.28	34.65	34.97	56.96	52.49	54.73
$T_4 (B_1S_0)$	15.25	16.93	16.09	34.65	33.81	34.23	55.27	49.00	52.14
$T_5 (B_1S_1)$	19.32	20.43	19.87	37.68	37.53	37.61	60.09	61.49	60.79
$T_6 (B_1S_2)$	16.60	17.38	16.99	36.38	35.40	35.89	57.77	54.93	56.35
$T_7 (B_2S_0)$	15.57	17.16	16.37	35.27	34.60	34.94	56.92	52.41	54.66
$T_8 (B_2S_1)$	16.67	17.88	17.28	36.44	35.44	35.94	57.85	55.61	56.73
$T_9 (B_2S_2)$	17.67	18.64	18.16	37.05	36.59	36.82	58.82	58.32	58.56
SEm±	0.906	1.076	0.988	0.304	0.230	0.215	0.920	0.782	0.646
CD(P=0.05)	2.717	3.227	2.961	0.910	0.690	0.645	2.758	2.346	1.936

Table 6: Effect of Boron and Sulphur levels on number of leaves per plant in *Calendula officinalis*

Treatment	Number of leaves per plant								
	30DAT			60DAT			90DAT		
	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Pooled Mean
(Factor A) Boron									
B0	25.47	26.06	25.77	74.79	75.66	75.23	186.58	187.96	187.27
B1	26.69	27.17	26.93	77.10	78.10	77.60	191.66	192.72	192.19
B2	27.07	27.62	27.34	76.84	78.16	77.50	192.04	192.79	192.41
SEm±	0.636	0.859	0.509	0.181	0.206	0.186	0.453	0.360	0.394
CD (P=0.05)	1.905	2.574	1.526	0.542	0.619	0.558	1.358	1.078	1.181
(Factor B) Sulphur									
S0	25.02	25.55	25.29	74.76	75.58	75.17	186.26	187.86	187.06
S1	27.20	27.55	27.38	77.11	78.16	77.63	191.96	192.75	192.35
S2	27.00	27.75	27.38	76.86	78.19	77.52	192.07	192.84	192.46
SEm±	0.636	0.859	0.509	0.181	0.206	0.186	0.453	0.360	0.394
CD (P=0.05)	1.905	2.574	1.526	0.542	0.619	0.558	1.358	1.078	1.181

Table 7: Effect of Boron and Sulphur interaction levels on number of leaves per plant in *Calendula officinalis*

Treatment	Number of leaves per plant								
	30DAT			60DAT			90DAT		
	2022-23	2023-24	Pooled Mean	2022-23	2023-24	Mean	2022-23	2023-24	Pooled Mean
Interaction (B×S)									
T_1 (B_0S_0)	23.87	23.87	24.00	73.83	74.50	74.17	183.53	185.47	184.50
T_2 (B_0S_1)	25.87	25.87	26.23	75.00	75.57	75.28	186.80	187.90	187.35
T_3 (B_0S_2)	26.67	26.67	27.07	75.53	76.93	76.23	189.40	190.50	189.95
T_4 (B_1S_0)	24.67	24.67	25.16	74.99	75.53	75.26	185.97	187.83	186.90
T_5 (B_1S_1)	28.47	28.47	28.38	79.40	80.60	80.00	196.73	197.47	197.10
T_6 (B_1S_2)	26.93	26.93	27.24	76.90	78.17	77.53	192.27	192.87	192.57
T_7 (B_2S_0)	26.53	26.53	26.70	75.45	76.70	76.08	189.27	190.29	189.78
T_8 (B_2S_1)	27.27	27.27	27.52	76.93	78.30	77.62	192.33	192.90	192.61
T_9 (B_2S_2)	27.40	27.40	27.82	78.13	79.47	78.81	194.53	195.17	194.85
SEm±	1.101	1.487	0.882	0.313	0.357	0.186	0.785	0.623	0.682
CD (P=0.05)	3.300	4.459	2.643	0.940	1.072	0.558	2.352	1.867	2.046

Discussion

Effect of Boro spray

In the present investigation on Calendula officinalis, plant height was not significantly affected by boron or sulphur applications during the early growth stages (30 and 60 DAT). However, a statistically significant influence of boron, sulphur, and their interaction was observed at 90 DAT. As a short-statured herbaceous plant, Calendula displays relatively slow vegetative growth in its initial stages, which may explain the absence of early treatment effects. The significant increase in plant height at 90 DAT indicates a cumulative and delayed response to nutrient application, becoming more apparent in the later stages of development. These results are consistent with the findings of Al-Rubaye and Khudair (2020) [9] in Gazania, and similar enhancements in plant height were reported by Kurbah and Fatmi (2022) [10] in Dianthus caryophyllus (carnation) with boron application at 0.1%.

A progressive increase in leaf area of Calendula was observed with rising levels of boron at 30, 60, and 90 DAT. Although B_1 (0.1% boron) exhibited the highest leaf area at 30 DAT, the overall trend across all stages followed the order B_2 (0.2%) > B_1 (0.1%) > B_0 (control), reflecting a dose-responsive effect. The application of 0.1% boron significantly enhanced leaf expansion compared to the control, while 0.2% boron further improved this trait, highlighting the benefits of increased boron supply. These improvements may be attributed to boron's role in promoting cell enlargement, nutrient uptake, and photosynthetic efficiency, which together contribute to an increased photosynthetic surface and improved vegetative growth. Similar results were reported by Sadiq (2023) [11] in gladiolus, where higher concentrations of boron foliar spray significantly increased leaf area.

Furthermore, boron foliar application notably increased the number of leaves per plant. The highest leaf count was observed with 0.2% boron (B_2), followed by 0.1% (B_1), with both outperforming the untreated control (B_0) at 30, 60, and 90 DAT. However, at 60 DAT, B_1 and B_2 were statistically comparable, indicating a plateau in response during the mid-growth phase. This suggests that boron exerts a consistent but stage-specific influence on vegetative development. These observations are in agreement with Khan *et al.* (2012) [12] in Kinnow mandarin and Singh and Arora (1972) [13] in guava, who also reported improved vegetative traits following foliar boric acid application in the range of 0.1% to 0.4%.

Effect of Sulphur spray

In Calendula officinalis, foliar application of sulphur influenced plant height, following the trend S_2 (0.2%) > S_1 (0.1%) > S_0 (control). Although the difference between S_1 and S_2 was not statistically significant, both treatments resulted in greater plant height than the control, with a significant difference emerging only at 90 days after transplanting (DAT). This suggests that sulphur's beneficial impact on vertical growth becomes more evident during later developmental stages. Similar trends have been observed in other flowering crops, where sulphur supplementation enhanced growth and yield attributes, as reported in sunflower by Asif *et al.* (2023) [14] and Al-Bayati

(2006) [15], and in tuberose by Sharma and Mohammad (2004) [16].

Sulphur also significantly improved leaf area in Calendula, with both 0.1% (S_1) and 0.2% (S_2) concentrations outperforming the untreated control (S_0). However, the difference between S_1 and S_2 was statistically non-significant, indicating that 0.1% sulphur may be adequate for maximizing leaf expansion. The observed increase can be attributed to sulphur's essential role in chlorophyll synthesis and enzymatic functions, promoting overall vegetative growth. These findings are in agreement with Ramos *et al.* (1989) [17], who reported similar enhancements in leaf area following foliar sulphur application in spring barley.

Leaf production was also positively influenced by sulphur at all growth stages (30, 60, and 90 DAT). While both S_1 and S_2 significantly increased leaf number over the control in the early stages, S_2 demonstrated a distinct advantage by 90 DAT. The untreated control consistently recorded the lowest number of leaves across all observations. This trend highlights sulphur's contribution to chlorophyll formation, protein synthesis, and metabolic activity, which collectively enhance vegetative growth. The findings are consistent with those of Sedibe and Allemann (2012) [18], who observed improved foliar biomass in Rose geranium under varying levels of sulphur in hydroponic media, underscoring sulphur's role in promoting leaf development.

Interaction effect

The combined application of boron and sulphur has been shown to enhance plant growth and yield in various crops. In sesame, such synergy significantly increased plant height (Teja & Singh, 2022) [21], while in spring sunflower, it improved overall growth and productivity (Shekhawat & Shivay, 2009) [19]. The present study in Calendula officinalis corroborates these findings, with the combined application of boron and sulphur positively influencing plant height and vegetative growth.

Notably, the treatment T_5 (0.1% B + 0.1% S) exhibited superior performance in enhancing leaf area and leaf number compared to T_9 (0.2% B + 0.2% S). This may be attributed to a more efficient uptake and utilization of nutrients at the lower concentration, which better matched the plant's physiological needs during vegetative growth. In contrast, the higher concentration in T_9 may have approached a threshold beyond which additional nutrients offered limited benefit. These observations align with those of Mathew and George (2013) [20] in sesame, where boron and sulphur synergy significantly improved foliar traits and yield.

Research Gaps and Future Perspectives

Despite its well-documented medicinal properties, several knowledge gaps persist. Further investigations are needed to explore molecular mechanisms, pharmacokinetics, and large-scale clinical trials to validate its efficacy in treating chronic diseases, including cancer. Additionally, advancements in formulation technology can help enhance the bioavailability and therapeutic efficacy of Calendula-derived compounds. The integration of Calendula into modern pharmaceuticals, functional foods, and cosmeceuticals represents a promising avenue for future research.

Conclusion

Given its wide-ranging pharmacological benefits, Calendula officinalis remains a valuable medicinal plant with significant therapeutic and commercial potential. However, optimizing its cultivation, standardization, and clinical validation is essential to fully harness its medicinal properties. Further interdisciplinary research can bridge the existing gaps, paving the way for its expanded use in modern medicine.

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