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Influence of biostimulants on flowering behaviour of different varieties of *Calendula*

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ABSTRACT

The study entitled "Influence of Biostimulants on Flowering Behaviour of Different Varieties of *Calendula*" was conducted at the Floriculture Research Farm, CHRS, Sankara, Patan, Durg (C.G.) during 2025-26. The experiment employed a Factorial Randomized Block Design (FRBD) comprising 12 treatment combinations, which included three calendula varieties and four biostimulant treatments: irrigation, Panchagavya 4%, Neem Leaf Extract 5%, and Moringa Leaf Extract 5%, each replicated three times. The variety V3 (Fiesta Gitana Orange) demonstrated superior flowering performance, characterized by accelerated flower bud initiation, earlier blooming, extended flower stalks, and prolonged flowering duration. Among the biostimulants, Neem Leaf Extract (5%) significantly improved flowering behaviour by promoting early blooming, increasing the number of flowers per plant, extending flower stalks, and enhancing overall yield attributes. The combination of V3 with 5% Neem Leaf Extract (V3B2) yielded the highest number of flowers per plant (51.40), the longest flower stem (19.93 cm), the earliest 50% blooming (43.40 days), and the longest flowering period (48.03 days). The study concluded that foliar application of 5% Neem Leaf Extract to Fiesta Gitana Orange is an effective and environmentally sustainable biostimulant treatment for improving calendula blooming and production performance in the Chhattisgarh region.

Keywords: *Calendula*, Biostimulants, Panchagavya neem leaf extract, *Moringa* leaf extract, Flowering, Yield, Factorial RBD

Introduction

Calendula (*Calendula officinalis* L.) belongs to the order Asterales and family Asteraceae. The genus name *Calendula* is derived from the Latin word *Calendae*, which means 'first day of each month'. The genus has 29 species including *Calendula officinalis* L., *Calendula* (pot marigold) is widely used as an ornamental plant due to its bright yellow-orange flowers, long blooming period, and ease of cultivation. It is commonly grown in garden beds, borders, and pots, enhancing the aesthetic appeal of both private and public landscapes. Studies have highlighted that *Calendula* is extensively cultivated for ornamental and medicinal purposes (Arora *et al.*, 2013) [1]. The term Panchagavya represents 'Pancha' means five and 'Gavya' means produce from cows (Bunkar, 2020) [2]. Panchagavya is composed of five cow products: cow dung, ghee, milk, curd, and cow urine. Foliar application of neem leaf extract has been reported to improve plant growth and physiological performance in different horticultural crops. The extract decreases oxidative stress caused by environmental variables while increasing chlorophyll synthesis, nutrient intake, photosynthetic efficiency, and antioxidant enzyme activity. Increased plant height, branch count, leaf area, and biomass accumulation are all signs of rapid vegetative growth, which is encouraged by these physiological developments. In ornamental crops, higher flower yield and better flowering performance are naturally supported by better vegetative growth. (Verma *et al.*, 2021) [3]. *Moringa* leaf extract is also gaining popularity as a bio-stimulant. Foliar application of moringa leaf extract at a concentration of 3% significantly improved the number of branches (66%), leaves (52%), leaflets (42%), leaf chlorophyll *a* (41%) and *b* (71%), and total chlorophyll (49%) contents. (Batool *et al.*, 2020) [4]

2. Materials and method

The current study, "Influence of Biostimulants on Flowering Behaviour of Different Varieties of *Calendula*" in the Chhattisgarh plain zone, was conducted at the Floriculture Farm College of Horticulture and Research Station in Sankara, Patan, Durg (C.G.) 2025–2026. A factorial randomized block design (FRBD) was used to assess the effect of various biostimulants on the growth, flowering, and yield of different *Calendula* varieties. Twelve treatments with two factors (factor 1 – variety and factor 2 – biostimulants)

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were randomly assigned to ensure uniformity. The entire lot of seedlings was randomized and divided into 36 plots.

2.1 Treatment combination

The experiment will be conducted in a Factorial Randomized Block Design (RBD) with three replications. The details of the treatments are presented in Table 1.

Biostimulant treatments

The biostimulant treatments consisted of irrigation (control), Panchagavya (4%), neem leaf extract (5%), and *Moringa* leaf extract (5%). The first foliar spray was applied 6 days after transplanting (DAT), followed by the second spray 15 days after the first spray, and the third spray 15 days after the second spray.

Flowering parameter

The observations on flowering parameters were recorded from five randomly tagged plants in each plot. The days to first bud initiation and first flower opening were counted from the date of transplanting, while the days to 50% flowering were recorded when half of the plants in a plot had flowered. The number of flowers per plant was counted at maturity and averaged. Flower stalk length was measured in centimeters using a 1-m scale, flower diameter was measured across the maximum width of the flower head using a Vernier caliper, and flowering duration was recorded as the number of days from opening of the first flower to senescence of the last flower.

Table 1. Treatments Details

Treatment Notation	Treatment Combinations	Treatment details
T_1	$V_1 B_0$	Fiesta <i>Gitana</i> White + Irrigation
T_2	$V_1 B_1$	Fiesta <i>Gitana</i> White + Panchagavya Spray
T_3	$V_1 B_2$	Fiesta <i>Gitana</i> White + Neem Leaf Extract Spray
T_4	$V_1 B_3$	Fiesta <i>Gitana</i> White + <i>Moringa</i> Leaf Extract Spray
T_5	$V_2 B_0$	Fiesta <i>Gitana</i> Yellow + Irrigation
T_6	$V_2 B_1$	Fiesta <i>Gitana</i> Yellow + Panchagavya Spray
T_7	$V_2 B_2$	Fiesta <i>Gitana</i> Yellow + Neem Leaf Extract Spray
T_8	$V_2 B_3$	Fiesta <i>Gitana</i> Yellow + <i>Moringa</i> Leaf Extract Spray
T_9	$V_3 B_0$	Fiesta <i>Gitana</i> Orange + Irrigation
T_{10}	$V_3 B_1$	Fiesta <i>Gitana</i> Orange + Panchagavya Spray
T_{11}	$V_3 B_2$	Fiesta <i>Gitana</i> Orange + Neem Leaf Extract Spray
T_{12}	$V_3 B_3$	Fiesta <i>Gitana</i> Orange + <i>Moringa</i> Leaf Extract Spray

Result and discussion

Days to first flower bud initiation

Factor A (varieties) significantly influenced the duration until the first flower bud began to form. Lower values correspond to earlier initiation, with V_3 initiating the earliest at an average of 30.93 days, followed by V_2 at 31.16 days. V_1 took the longest, averaging 32.73 days, indicating a delay in initiation. Factor B (biostimulant treatments) also had a notable impact on the timing of the first flower bud's appearance. The earliest initiation occurred with B_2 (Neem Leaf Extract Spray 5%), averaging 31.19 days, followed by B_3 (*Moringa* Leaf Extract Spray 5%) at 31.22 days, and B_0 at 31.53 days. B_1 (Panchagavya Spray 4%) had the highest average at 32.47 days. The interaction between Factors A and B was statistically significant at the 5% level. Among all treatment combinations, V_2B_2 resulted in the earliest flower bud initiation at 29.00 days, which was statistically significant and indicated the most favorable response. This was followed by V_3B_3 and V_3B_0 , both at 30.00 days, while V_1B_1 took the longest at 34.57 days, showing delayed initiation. Singh *et al.* (2015) found earlier flower bud initiation in marigold with Panchagavya-based biodynamic management, and Trivedi *et al.* (2016) [5] noted earlier reproductive development in roses with bio-enhancer application.

Days to first flowering

Factor A (varieties) exerted the most substantial influence on the duration until the first flowering. Lower values corresponded to earlier flowering; thus, V_3 exhibited the earliest first flowering with a mean of 36.83 days, followed by V_2 at 37.28 days, whereas V_1 required the longest period at 40.22 days, indicating delayed flowering. Factor B (biostimulant treatments) also significantly affected the number of days until the first flowering. The earliest flowering occurred with B_2 (Neem Leaf Extract Spray 5%), averaging 37.32 days, followed by B_3 (*Moringa* Leaf Extract Spray 5%) at 37.96 days, and B_1

(Panchagavya Spray 4%) at 37.99 days, while B_0 (Irrigation) exhibited the highest mean of 39.17 days, suggesting delayed flowering. The interaction between Factors A and B was statistically significant at the 5% level. Among all treatment combinations, V_2B_2 demonstrated the earliest initial flowering at 34.27 days, which was statistically superior and the most favorable combination. This was followed by V_2B_1 and V_3B_1 , both at 36.33 days, with V_1B_1 showing the highest number of days to first flowering at 41.97 days, indicating later flowering. Trivedi *et al.* (2016) [5] reported enhanced flowering behavior and earlier reproductive development in roses under a bio-enhancer treatment containing Panchagavya. Patra *et al.* (2026) [6] documented improvements in flowering characteristics following the application of natural leaf extracts.

3.3 Number of flowers per plant

Among the primary effects, Factor A (varieties) did not significantly influence the number of flowers per plant. Although the variety V_3 exhibited the highest mean number of flowers at 38.18 per plant, followed by V_2 with 36.34 and V_1 with 34.73, these differences were not statistically significant. In contrast, Factor B (biostimulant treatments) had a significant impact on the number of flowers per plant. The highest mean number of flowers was recorded under treatment B_2 (Neem Leaf Extract Spray 5%), with 44.36 flowers per plant, followed by B_1 (Panchagavya Spray 4%) with 36.47 flowers, and B_3 (*Moringa* Leaf Extract Spray 5%) with 35.53 flowers per plant. The lowest mean number of flowers was observed under B_0 (Irrigation), with 29.31 flowers per plant. The interaction between Factors A and B was significant at the 5% level. Treatment 11 (V_3B_2) resulted in the highest number of flowers per plant at 51.40, which was statistically significant compared to other treatments. This was followed by Treatment 7 (V_2B_2) with 42.67 flowers per plant and Treatment 10 (V_3B_1) with 40.20 flowers per plant. Treatment 1 (V_1B_0) produced the lowest number of flowers per plant, with 31.70 flowers. Patra *et al.* (2026) [6] reported enhanced flower production using natural leaf extract.

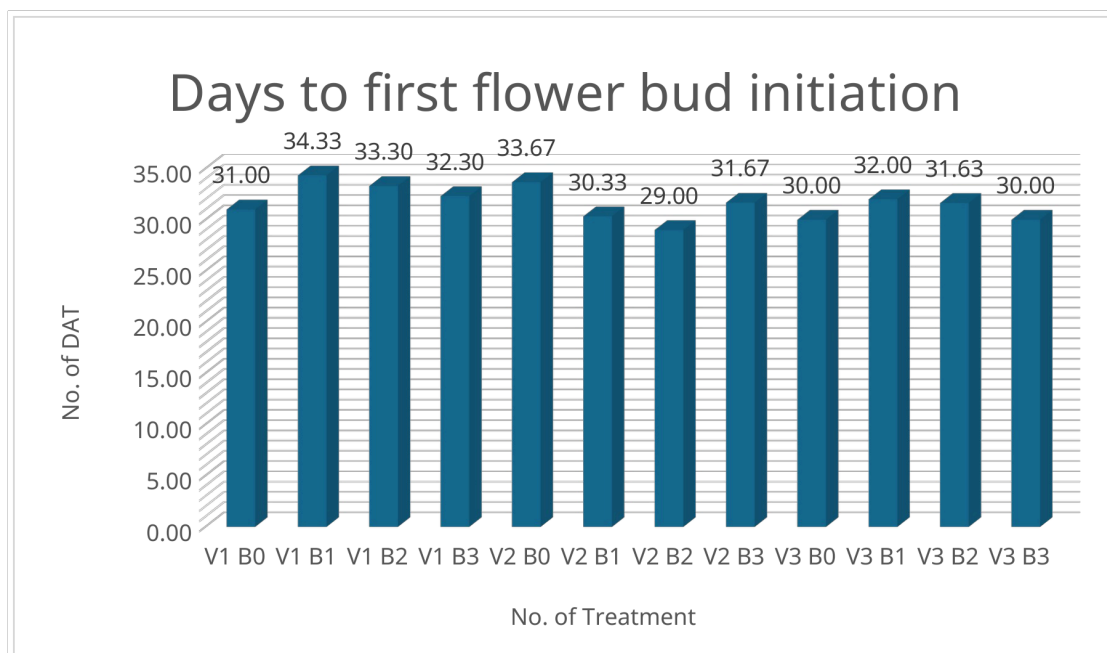


Figure 1. Days to First Flower Bud Initiation

Flower diameter (cm)

The flower diameter was influenced by the different varieties and biostimulant treatments. Among the varieties, V_2 recorded a flower diameter of 4.13 cm, followed by V_3 (4.09 cm), while the minimum flower diameter was recorded in V_1 (3.97 cm). The variation among varieties indicates differences in their inherent floral characteristics. Among the biostimulant treatments, B_2 recorded the maximum flower diameter (4.29 cm), followed by B_0 (4.16 cm) and B_3 (3.96 cm). The minimum flower diameter was observed under B_1 (3.85 cm). Thus,

B_2 was comparatively more effective in improving flower diameter. The interaction between varieties and biostimulants revealed considerable variation in flower diameter. The maximum flower diameter (4.61 cm) was recorded in V_3B_2 , followed by V_2B_2 (4.34 cm) and V_2B_0 (4.24 cm). In contrast, the minimum flower diameter (3.75 cm) was recorded in V_3B_3 , followed by V_1B_1 (3.82 cm). This indicates that the effectiveness of biostimulants differed according to the variety, with the V_3B_2 combination showing the highest flower diameter. Larger flowers were observed in roses after combined bio-enhancer therapy (Trivedi *et al.* (2016) [5]).

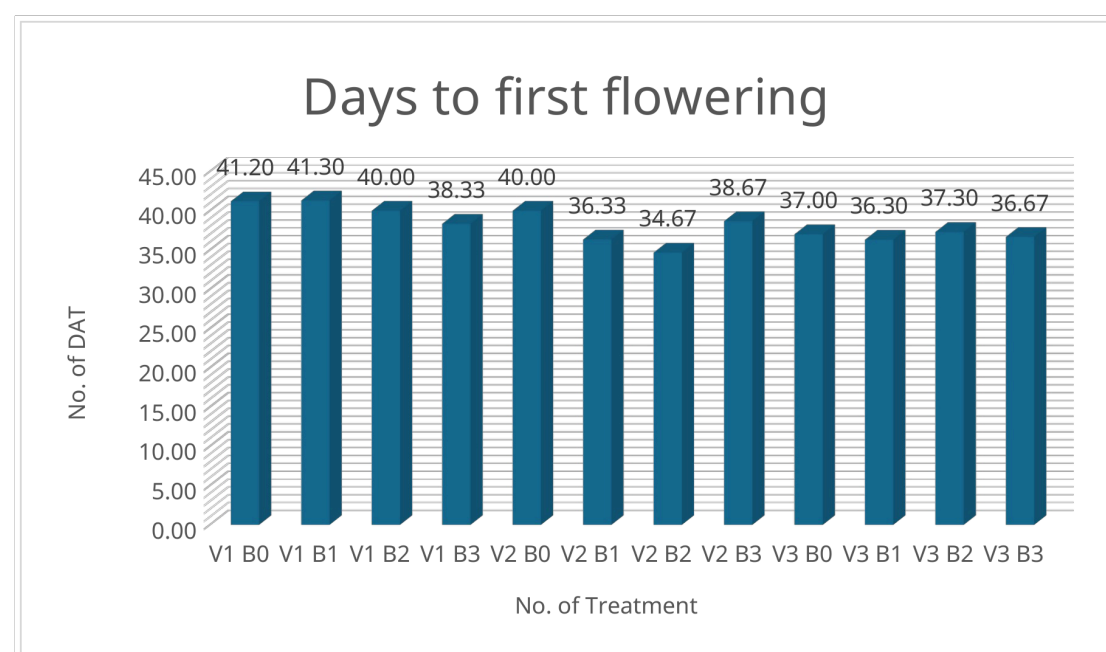


Figure 2. Days to First Flowering

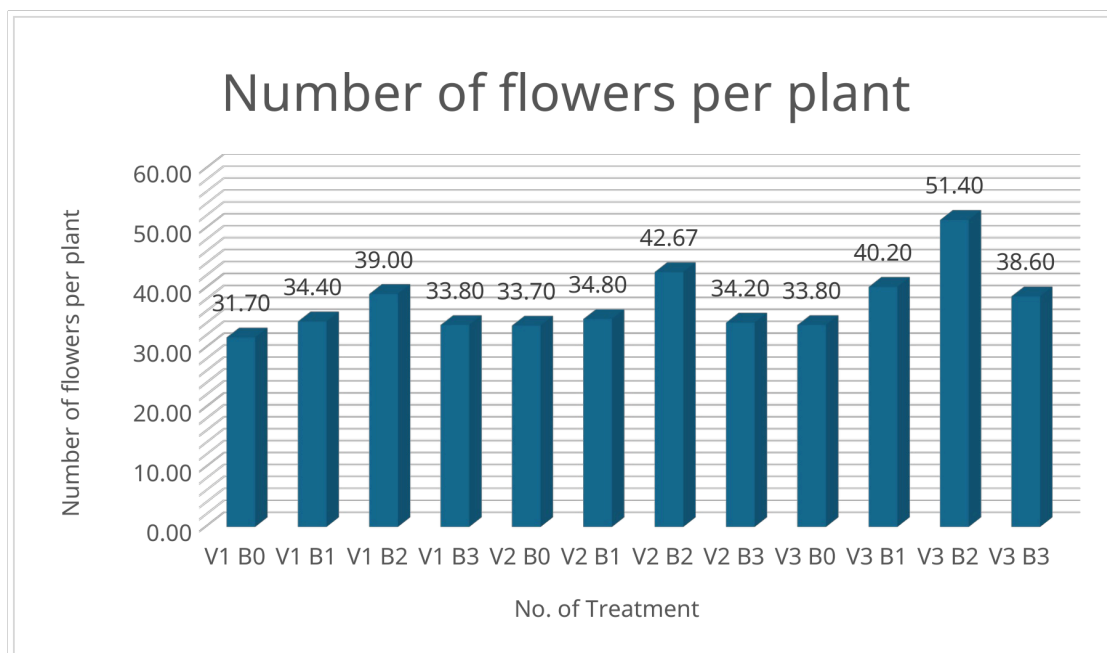


Figure 3. Number of Flowers Per Plant

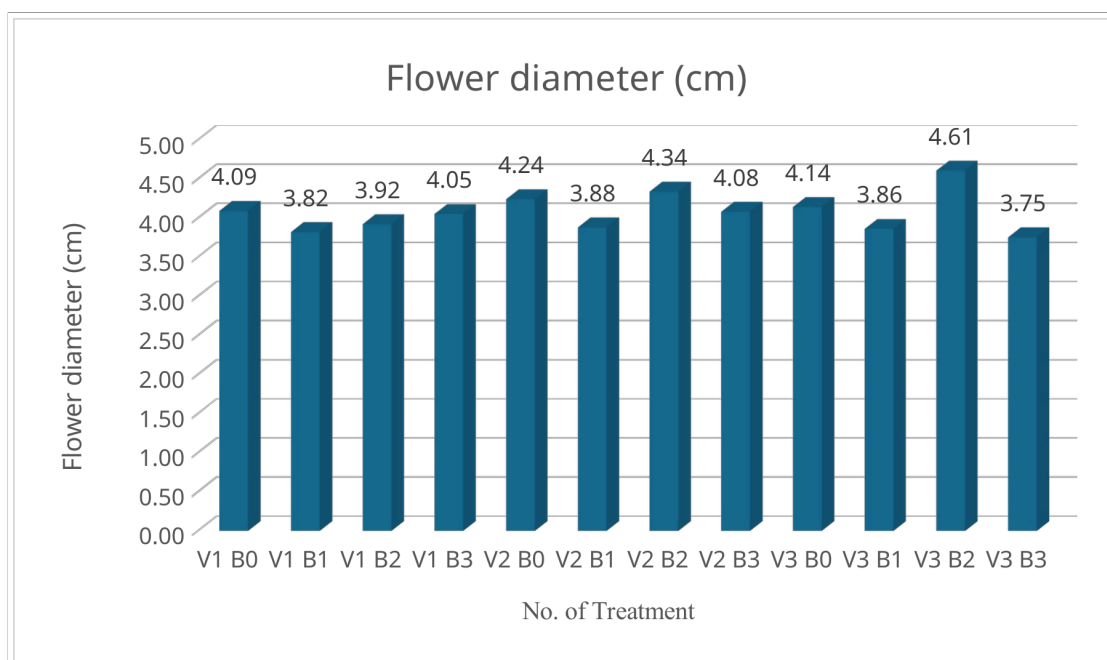


Figure 4. Flower diameter (mm)

Flower stalk length (cm)

The length of flower stalks was significantly influenced by Factor A (Varieties). Variety V_1 produced the shortest flower stems, measuring 13.83 cm, whereas V_3 exhibited the longest flower stalks at 16.06 cm, with V_2 following at 14.83 cm. The statistical significance of these differences at the 5% level underscores the superiority of V_3 in generating longer flower stalks. Factor B (Biostimulant treatments) also had a notable impact on flower stem length. The longest flower stalks were observed with B_2 (Neem Leaf Extract Spray 5%), achieving a mean length of 17.96 cm, followed by B_1 (Panchagavya Spray 4%) at 15.24 cm, and B_3 (*Moringa* Leaf Extract Spray 5%) at 13.94 cm. In contrast, B_0 (Irrigation) resulted in the shortest flower stalks. This suggests that the foliar application of neem leaf extract was most ef-

fective in promoting flower stalk elongation. The interaction between varieties and biostimulant treatments was significant at the 5% level. The combination of V_3 and B_2 (Variety 3 + Neem Leaf Extract Spray 5%) yielded the longest flower stalks at 19.93 cm, demonstrating statistical superiority over other treatments. This was followed by V_2B_2 at 18.21 cm and V_3B_1 at 16.33 cm. The shortest flower stalks, measuring 12.21 cm, were recorded under V_1B_0 (Variety 1 + Irrigation), indicating that the absence of biostimulant application led to reduced flower stalk length. Overall, the combination of V_3 and Neem Leaf Extract Spray (5%) proved to be the most effective treatment for enhancing flower stalk length in calendula. Previous studies by Patil *et al.* (2006) [7] reported improved flower stalk characteristics in tuberose with Panchagavya-based treatment, while Singh *et al.* (2007) [8] noted longer spikes with 4% Panchagavya.

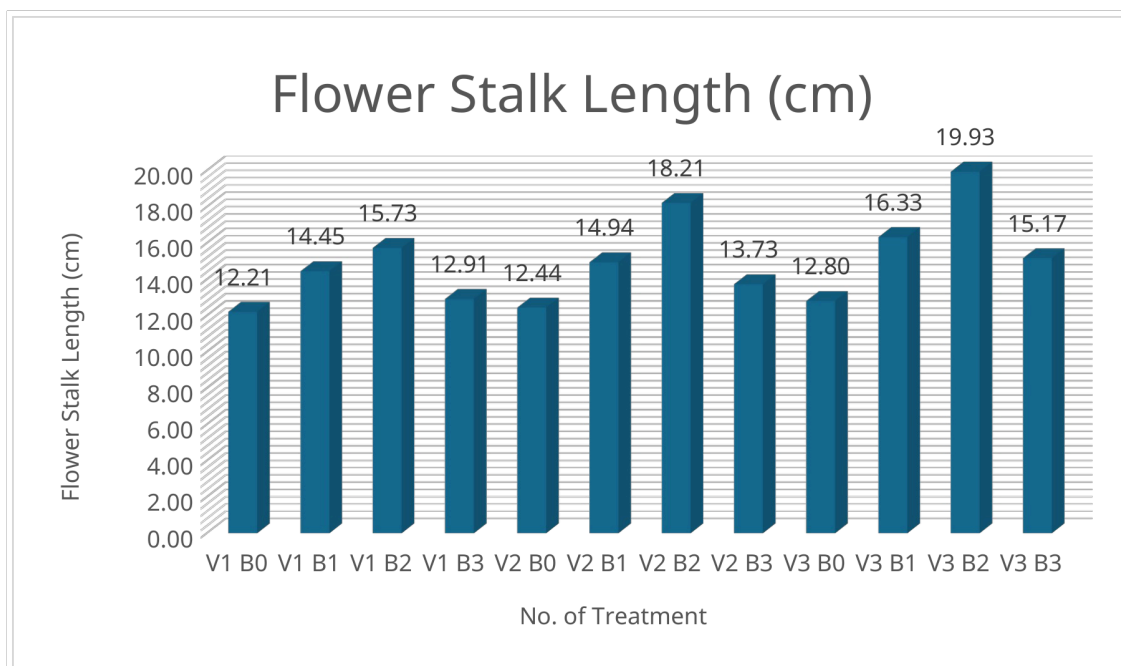


Figure 5. Flower Stalk Length (cm)

Days to 50% flowering

Factor A (varieties) had a significant effect on the number of days taken to achieve 50% flowering. Lower values indicate earlier flowering. Among the varieties, V_3 had the earliest 50% flowering (44.98 days), followed by V_2 (46.97 days), and V_1 had the longest time to achieve 50% flowering (48.36 days), showing delayed flowering. Factor B (biostimulant treatments) had a significant effect on the number of days before 50% flowering. The earliest 50% flowering was observed under B_2 (Neem Leaf Extract Spray 5%) with a mean value of 45.03 days, followed by B_1 (Panchagavya Spray 4%) (45.89 days) and B_3 (*Moringa* Leaf Extract Spray 5%) (46.30 days), while B_0 (Irrigation) had the highest mean value (49.86 days), indicating de-

layed flowering. The interaction effect between varieties and biostimulant treatments was not significant at the 5% level. Among all treatment combinations, V_3B_2 (Variety 3 + 5% Neem Leaf Extract Spray) produced the earliest 50% flowering (42.10 days) and was statistically superior to the others. This was followed by V_2B_2 (43.60 days) and V_3B_1 (44.93 days). The largest number of days to 50% flowering (56.03 days) was observed with V_1B_0 (Fiesta *Gitana* Orange + Irrigation), showing delayed flowering in the absence of biostimulant application. Overall, the combination of V_3 and Neem Leaf Extract Spray (5%) was found to be the most effective treatment for inducing earlier 50% flowering in calendula. Bellubbi *et al.* (2015) [9] reported a reduction in the time required to attain 50% flowering in gerbera following Panchagavya-based treatment.

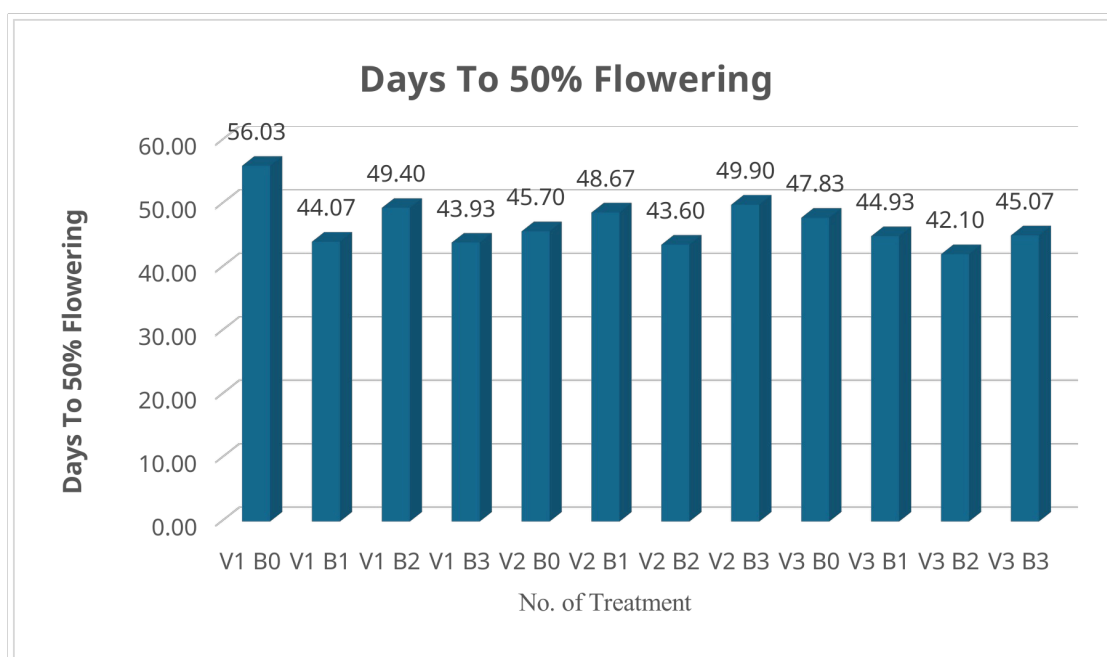


Figure 6. Days To 50% Flowering

Duration of flowering (days)

Factor A (varieties) had a significant effect on flowering duration. Higher values led to a longer flowering period. Among the varieties, V_3 had the longest flowering time (44.83 days on average), followed

by V_2 (41.69 days), while V_1 had the shortest flowering duration. This indicates that V_3 flowering occurred longer than the other varieties. Factor B (biostimulant treatments) had a significant effect on flowering time. B_1 (Panchagavya Spray 4%) had the longest flowering

length (mean = 43.56 days), followed by B_2 (Neem Leaf Extract Spray 5%) (42.92 days) and B_3 (*Moringa* Leaf Extract Spray 5%) (42.77 days). The shortest flowering duration (40.41 days) was observed under B_0 (Irrigation), showing that the use of biostimulants delayed the flowering period compared to the control. The interaction effect between varieties and biostimulant treatments was significant at the 5% level. V_3B_2 (Variety 3 + Neem Leaf Extract Spray 5%) had the longest flowering time (48.03 days) and was statistically superior to the other treatments. Flowering period (38.63 days) was observed

under V_1B_0 (Variety 1 + Irrigation), indicating that this was the control treatment. Overall, the combination of V_3 and Neem Leaf Extract Spray (5%) showed to be the most efficient treatment for increasing calendula flowering time. Trivedi *et al.* (2016) [5] reported prolong flowering in rose under bio-enhancer application containing Panchagavya. Bhalla *et al.* (2006) [10] also observed improvement in post-harvest flower behavior following Panchagavya-based treatment in carnation.

Table 2. Effect of different varieties and biostimulants on days to first bud initiation, days to 1st flowering, no. of flowers per plant, flower diameter (mm), flower stalk length (cm), days to 50% flowering, and duration of flowering.

Treatment	Days to First Bud initiation	Days to 1st Flowering	No. of flower per plant	Flower diameter (cm)	Flower stalk length (cm)	Days to 50% flowering	Duration of flowering
Varieties							
V_1	32.73	40.22	34.73	3.97	13.83	48.36	40.73
V_2	31.16	37.28	36.34	4.13	14.83	46.97	41.69
V_3	30.93	36.83	38.18	4.09	16.06	44.98	44.83
Sem±	0.28	0.36	1.63	0.03	0.20	1.05	0.52
CD at 5%	0.83	1.05	NS	0.09	0.57	3.09	1.52
Biostimulants							
B_0	31.53	39.17	29.31	4.16	12.48	49.86	40.41
B_1	32.47	37.99	36.47	3.85	15.24	45.89	43.56
B_2	31.19	37.32	44.36	4.29	17.96	45.03	42.92
B_3	31.22	37.96	35.53	3.96	13.94	46.30	42.77
Sem±	0.33	0.41	1.88	0.04	0.23	1.22	0.60
CD at 5%	0.93	1.21	5.52	0.11	0.66	3.57	1.75

Table 3. Interaction effect of different biostimulants on different varieties on days to first bud initiation, days to 1st flowering, no. of flowers per plant, flower diameter (cm), flower stalk length (cm), days to 50% flowering, and duration of flowering.

Treatment	Days to First Bud initiation	Days to 1st Flowering	No. of flower per plant	flower diameter(cm)	flower stalk length	Days to 50% flowering	Duration of flowering
$V_1 B_0$	30.97	40.97	31.70	4.09	12.21	56.03	38.63
$V_1 B_1$	34.57	41.30	34.40	3.82	14.45	44.07	41.67
$V_1 B_2$	33.17	40.20	39.00	3.92	15.73	49.40	41.20
$V_1 B_3$	32.20	38.40	33.80	4.05	12.91	43.93	41.40
$V_2 B_0$	33.63	39.77	33.70	4.24	12.44	45.70	41.77
$V_2 B_1$	30.57	36.33	34.80	3.88	14.94	48.67	43.00
$V_2 B_2$	29	34.27	42.67	4.34	18.21	43.60	39.53
$V_2 B_3$	31.57	38.73	34.20	4.08	13.73	49.90	42.47
$V_3 B_0$	30.00	36.77	33.80	4.14	12.80	47.83	40.83
$V_3 B_1$	32.27	36.33	40.20	3.86	16.33	44.93	46.00
$V_3 B_2$	31.53	37.50	51.40	4.61	19.93	42.10	48.03
$V_3 B_3$	29.90	36.73	38.60	3.75	15.17	45.07	44.43
Sem ±	0.57	0.97	3.26	0.06	0.39	2.11	1.04
CD at 5%	1.66	2.76	9.55	0.19	1.14	NS	3.04

Conclusion

The current study found that biostimulants and calendula varieties had significant effects on yield parameters, flower quality, and flowering behavior. Fiesta *Gitana* Orange (V_3) was superior to the other varieties in terms of earlier flowering, longer flowering times, more flowers per plant, and longer flower stems. Neem leaf extract spray (5%) (B_2) was the most effective biostimulant, encouraging early flower bud initiation, early blooming, maximum flower output, longer flower stalks, and superior overall flowering performance. The interaction treatment V_3B_2 (Fiesta *Gitana* Orange + Neem Leaf Extract Spray

5%) was found to be the best combination to enhance flowering and yield performance in calendula under Chhattisgarh's agro-climatic conditions. As a result, the use of 5% neem leaf extract spray with the Fiesta *Gitana* Orange variety is advised for commercial calendula production to increase flower yield and enhance flower quality.

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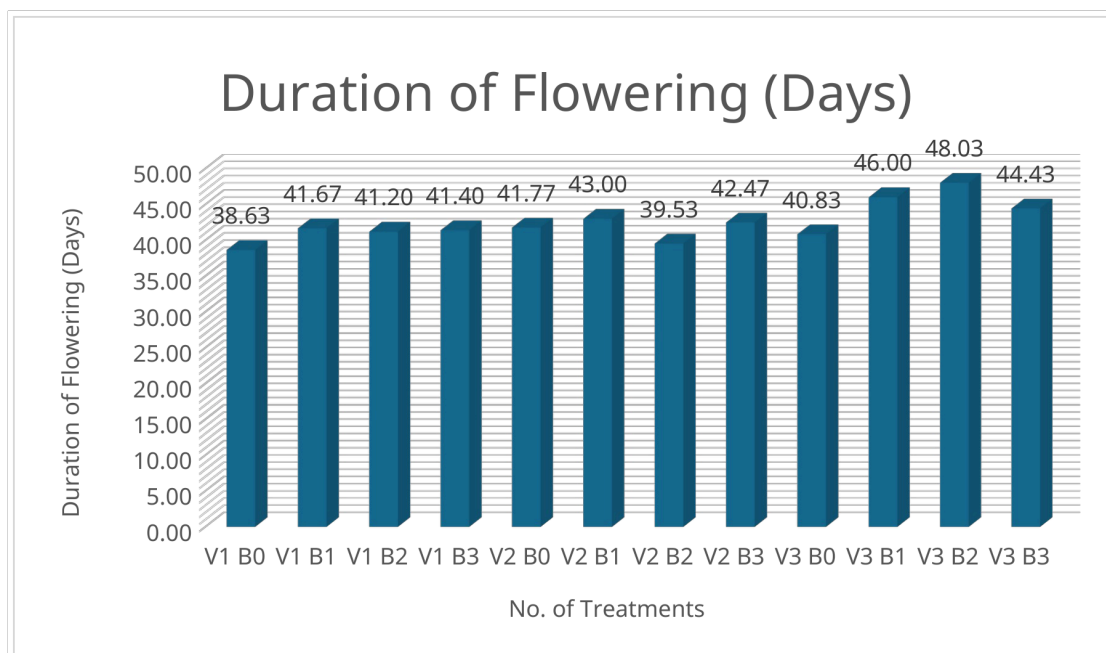


Figure 7. Duration of Flowering (Days)

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