



# Effect of Foliar Application of Biostimulants and Micronutrients on Chlorophyll and Xanthophylls Content of *African marigold* cv. Pusa Narangi Gainda

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## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

The present experiment was carried out during 2017 to 2019. The experiment was laid out in Randomized Block Design with Factorial concept (FRBD) consisting two factors and three replications. The treatment comprised with four biostimulants and three micronutrients. The result indicated that foliar application of banana pseudostemsap @ 1% with micronutrient grade-IV @ 1% in addition to recommended dose of fertilizers (200:100:100 kg/ha NPK) produced higher chlorophyll content in leaves and xanthophylls content in flower of African marigold cv. Pusa Narangi Gainda.

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**Keywords:** Biostimulants; micronutrients; African marigold cv. Pusa Narangi Gainda.

## 1. INTRODUCTION

“African marigold (*Tagetes erecta* L.) is one of the important commercial flower of India as well as of Gujarat. Marigold is being grown for its spectacular flowers, brilliant colours, delightful appearance, size, shape, forms etc. It belongs to the family Asteraceae (2n=24) and originated from Central to Southern America especially Mexico, from there it reached to Spain and became popular by the name of 'Rose of Indies'. At present, for increasing the flower production, nutrient are supplied through chemical fertilizers. Biostimulants are products of natural and organic origin that stimulate plant growth and development to achieve higher crop growth, quality of flower and yield. Seaweed extracts contain major and micro nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances” (Krishnamoorthy et al., 2022). “The liquid contained P 120 mg/100 g, K 4170 mg/100 g, Ca 66.98 mg/100 g and micronutrients like Fe 147 mg/100 g, Mn 5.84 mg/100 g, Zn 9.08 mg/100g and Cu 0.36 mg/100 g” (Yan et al., 2013). “Panchagavya is a fermented product made from five ingredients obtained from cow products such as milk, urine, dung, curd and clarified butter (Amalraj et al., 2013). Panchgavya contained macro element like total nitrogen (229 ppm), total phosphorous (209 ppm), total potassium (232 ppm), calcium (25 ppm), IAA (8.5 ppm) and GA (3.5 ppm) (Anon., 2017). While separating fibers from the banana pseudostem, the liquid available is known as sap which contains good amount of essential macro element like 119 ppm N, 50.4 ppm P, 1289 ppm K and micronutrients like 124 ppm Fe, 6.73 ppm Mn, 4.61 ppm Cu and 0.97 ppm Zn (Gundrashya, 2013) apart from growth promoting substance like, cytokinin- 137.8 mg/l and gibberellic acid- 110.2 mg/l” (Desai, 2018).

“Micronutrients are needed in very small amounts by crop plants. Their concentration in plants is generally below the 100 ppm” (Krishnamoorthy et al., 2022; Alves et al., 2019). Out of 17 essential elements, Fe, Zn, B, Cu, Mn, Mo although required in very little amounts, their

importance for the plant is no way less than those of major elements. The need of micronutrients in flower production has long been recognized in India. It is important to keep the need for micronutrient fertilizers in perspective. Towards the concern about micronutrient deficiencies, investigations are the need of the hour and need to be applied in steps. Considering the above facts, the present study was proposed.

## 2. MATERIALS AND METHODS

The field experiment was carried out twice during October 2017 to February 2019 at the Jambuvadi Farm, Department of Horticulture, Junagadh Agricultural University, Junagadh (Gujarat). The experiment was laid out in Randomized Block Design with Factorial concept (FRBD) consisting two factors with three replications. The treatment comprised with four biostimulants viz., without spray of biostimulants (B<sub>0</sub>), Seaweed extract @ 1% (B<sub>1</sub>), Panchgavya @ 3% (B<sub>2</sub>) and Banana pseudostem sap @ 1% (B<sub>3</sub>) and three treatments of micronutrients i.e. without micronutrients (F<sub>0</sub>), micronutrients grade-IV @ 0.5% (F<sub>1</sub>) and micronutrients grade-IV @ 1% (F<sub>2</sub>). Five plants from each treatment plot were randomly selected, labeled and used for recording observation. Chlorophyll content of leaf was analyzed by collecting the healthy fully matured second leaf from the centre of the plant at peak vegetative stage. Chlorophyll-a, chlorophyll-b and total chlorophyll contents of leaf tissue were determined by using Dimethyl sulfoxide (DMSO). Xanthophylls was estimated by AOAC Hot saponification method (Lawrance, 1990).

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Biostimulants on Chlorophyll Content in Leaves

Significantly maximum chlorophyll-a content in leaves (0.753, 0.790 and 0.772 mg/g) was recorded with application of banana pseudostem sap 1% (B<sub>3</sub>) during 2017-18, 2018-19 and in pooled analysis (Table 2). The maximum chlorophyll-b content was significantly increased

**Table 1. Time of applications**

Time of foliar application of biostimulants	Time of foliar application of micronutrients
1 <sup>st</sup> 30 days after transplanting	1 <sup>st</sup> 40 days after transplanting
2 <sup>nd</sup> 45 days after transplanting	2 <sup>nd</sup> 55 days after transplanting
3 <sup>rd</sup> 60 days after transplanting	3 <sup>rd</sup> 70 days after transplanting

in leaves (0.329, 0.343 and 0.336 mg/g) was recorded with application of banana pseudostem sap 1% (B<sub>3</sub>) during 2017-18, 2018-19 and in pooled analysis. The treatment was at par with panchgavya 3% (B<sub>2</sub>) during 2018-19 and in pooled analysis. The total chlorophyll content was significantly increased in leaves (1.084, 1.103 and 1.093 mg/g) with application of banana pseudostem sap 1% (B<sub>3</sub>). Maximum chlorophyll-a, b and total chlorophyll content in leaves were recorded with application of banana pseudostem sap @ 1% (B<sub>3</sub>) during the year 2017-18, 2018-19. The organic liquid generated during extracting fiber from banana pseudostem sap was used as spraying material in this experiment which contained about 124 ppm of Fe (Gundrashiya, 2013). The most well known function of Fe is in enzyme systems in which haem or haemin functions as prosthetic group. Thus Fe plays a somewhat similar role to Mg in the porphyrin structure of chlorophyll. The haem pigments constitutes only about 0.1 % of the total Fe in plant leaves. In green plants, there is good correlation between the levels of Fe supply and the chlorophyll content. Plant supplied well with Fe is high in chlorophyll. These results are in consonance with the findings in tuberose (Desai,2018) okra, cluster bean and cow pea Gundrashiya, (2013).

### 3.2 Effect of Micronutrients on Chlorophyll Content in Leaves

The maximum chlorophyll-a content significantly increased in leaves (0.673, 0.713, 0.693 mg/g) with F<sub>2</sub> treatment (micronutrient grade-IV 1%) during the year 2017-18, 2018-19 and in pooled, respectively (Table 2). This treatment was at par with micronutrient grade-IV 0.5% (F<sub>1</sub>). Significantly maximum chlorophyll-b content in leaves (0.304, 0.319 and 0.312 mg/g) was recorded in F<sub>2</sub> treatment (micronutrient grade-IV 1%) during the year 2017-18, 2018-19 and in pooled, respectively. This treatment was at par with micronutrient grade-IV 0.5% (F<sub>1</sub>). The maximum total chlorophyll content was significantly increased in leaves (0.977, 1.015 and 0.996 mg/g) with F<sub>2</sub> treatment (micronutrient grade-IV 1%) during the year 2017-18, 2018-19 and in pooled analysis. This treatment was at par with micronutrient grade-IV 0.5% (F<sub>1</sub>). Chlorophyll is a major green pigment found in green leaves and is undoubtedly determining the photosynthetic efficiency and productivity of plants. The variation in chlorophyll content due to micronutrients may be attributed to decrease in chlorophyll degradation and increased chlorophyll synthesis. Iron is involved in

chlorophyll synthesis plays a role in catalytic function in nutrient absorption, balancing other nutrients, also in biological oxidation, reduction and other metabolic processes in plants. It may also be associated with organic acid metabolism.

### 3.3 Interaction Effect of Biostimulants and Micronutrients on Chlorophyll Content in Leaves

The combined application of banana pseudostem 1% with micronutrient grade-IV 0.5% (B<sub>3</sub>F<sub>1</sub>) recorded the maximum chlorophyll-a content in leaves (0.776 mg/g) during the year 2017-18. It was at par with B<sub>3</sub>F<sub>0</sub> and B<sub>3</sub>F<sub>2</sub> during 2017-18. While, banana pseudostem 1% with micronutrient grade-IV 1% (B<sub>3</sub>F<sub>2</sub>) recorded the maximum chlorophyll-a content in leaves (0.862 and 0.796 mg/g) during the year 2018-19 and in pooled analysis (Table 2a). The results were on par with treatment of B<sub>3</sub>F<sub>1</sub>. The combined application of banana pseudostem 1% (B<sub>3</sub>F<sub>0</sub>) recorded the maximum chlorophyll-b content in leaves (0.345 mg/g) during the year 2017-18 and (0.360 and 0.351 mg/g) with combined application of banana pseudostem 1% with micronutrient grade-IV 1% (B<sub>3</sub>F<sub>2</sub>) in pooled analysis. However, it was found at par with treatment of B<sub>3</sub>F<sub>1</sub> and B<sub>3</sub>F<sub>0</sub>, B<sub>3</sub>F<sub>1</sub>, B<sub>3</sub>F<sub>2</sub>, B<sub>2</sub>F<sub>0</sub>, B<sub>0</sub>F<sub>2</sub> during 2017-18 B<sub>3</sub>F<sub>1</sub>, B<sub>3</sub>F<sub>0</sub>, B<sub>2</sub>F<sub>2</sub>, B<sub>2</sub>F<sub>1</sub>, B<sub>2</sub>F<sub>0</sub> during 2018-19. The application of banana pseudostem sap 1% with without micronutrient (B<sub>3</sub>F<sub>0</sub>) recorded the maximum total chlorophyll (1.098 mg/g) during the year 2017-18. It was found at par with treatment of B<sub>3</sub>F<sub>2</sub> and B<sub>3</sub>F<sub>1</sub>. Application of banana pseudostem 1% with micronutrient grade-IV 1% (B<sub>3</sub>F<sub>2</sub>) recorded the maximum total chlorophyll (1.134 and 1.102 mg/g) during the year 2018-19 and in pooled analysis. The results were at par with treatments B<sub>3</sub>F<sub>1</sub> and B<sub>3</sub>F<sub>0</sub> during 2018-19 and in pooled analysis, respectively. In green plants, there is good correlation between the levels of Fe supply and the chlorophyll content wherein green plants deprived of Fe soon become chlorotic in the younger plant parts. Results are in consonance with the findings of Desai (2018) in tuberose and Gundrashiya, (2013) in okra, cluster bean and cow pea. The favorable effects chlorophyll content could be due to the positive response of marigold to micronutrient application. Besides, vigorous plant growth, mainly in terms of foliage, resulted in efficient production of photosynthetic products. Another reason could be that the application of Zn and Fe regulate various metabolic activities of plants and also are involved in the auxin production.

**Table 2. Effect of biostimulants and micronutrients on chlorophyll-a content in African marigold cv. Pusa Narangi Gainda**

Treatments	Chlorophyll-a content in leaves (mg/g)			Chlorophyll-b content in leaves (mg/g)			Total Chlorophyll content in leaves (mg/g)		
	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
<b>Level of Biostimulants (B)</b>									
B <sub>0</sub> – Control	0.581	0.607	0.594	0.256	0.246	0.251	0.843	0.857	0.850
B <sub>1</sub> – Seaweed extract (1%)	0.588	0.619	0.604	0.274	0.280	0.277	0.858	0.906	0.882
B <sub>2</sub> – Panchgavya (3%)	0.671	0.654	0.663	0.294	0.319	0.306	0.965	1.000	0.982
B <sub>3</sub> – Banana pseudostem sap (1%)	0.753	0.790	0.772	0.329	0.343	0.336	1.084	1.103	1.093
<b>S.Em.±</b>	0.011	0.012	0.008	0.010	0.009	0.007	0.016	0.020	0.013
<b>C.D. at 5 %</b>	0.033	0.034	0.02	0.030	0.027	0.020	0.047	0.058	0.036
<b>Level of Micronutrients (F)</b>									
F <sub>0</sub> – Control	0.618	0.611	0.615	0.267	0.282	0.275	0.887	0.912	0.900
F <sub>1</sub> – Micronutrient Grade-IV (0.5%)	0.654	0.678	0.666	0.293	0.289	0.291	0.948	0.971	0.959
F <sub>2</sub> – Micronutrient Grade-IV (1%)	0.673	0.713	0.693	0.304	0.319	0.312	0.977	1.015	0.996
<b>S.Em.±</b>	0.010	0.010	0.007	0.009	0.008	0.006	0.014	0.017	0.011
<b>C.D. at 5 %</b>	0.029	0.030	0.020	0.026	0.024	0.017	0.041	0.051	0.032
<b>Interaction (B X F)</b>									
<b>S.Em.±</b>	0.020	0.020	0.014	0.018	0.016	0.012	0.028	0.035	0.022
<b>C.D. at 5 %</b>	0.057	0.060	0.040	0.051	0.047	0.033	0.081	0.101	0.063
<b>CV %</b>	5.21	5.28	5.25	10.51	9.34	9.93	5.1	6.19	5.69

**Table 2a. Interaction effect of biostimulants and micronutrients on chlorophyll-b content in leaves of marigold cv. Pusa Narangi Gaiinda**

Treatments	Chlorophyll-a content in leaves (mg/g)			Chlorophyll-b content in leaves (mg/g)			Total Chlorophyll content in leaves (mg/g)		
	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled	2017-18	2018-19	Pooled
B <sub>0</sub> F <sub>0</sub>	0.473	0.512	0.493	0.170	0.192	0.181	0.667	0.714	0.690
B <sub>0</sub> F <sub>1</sub>	0.608	0.628	0.618	0.290	0.242	0.266	0.895	0.874	0.885
B <sub>0</sub> F <sub>2</sub>	0.661	0.682	0.671	0.309	0.303	0.306	0.969	0.982	0.976
B <sub>1</sub> F <sub>0</sub>	0.605	0.600	0.603	0.255	0.283	0.269	0.847	0.895	0.871
B <sub>1</sub> F <sub>1</sub>	0.556	0.636	0.696	0.288	0.263	0.275	0.844	0.902	0.873
B <sub>1</sub> F <sub>2</sub>	0.602	0.621	0.612	0.279	0.294	0.286	0.883	0.920	0.901
B <sub>2</sub> F <sub>0</sub>	0.638	0.619	0.629	0.299	0.322	0.311	0.937	0.971	0.954
B <sub>2</sub> F <sub>1</sub>	0.677	0.655	0.666	0.293	0.315	0.304	0.968	1.004	0.986
B <sub>2</sub> F <sub>2</sub>	0.698	0.688	0.693	0.288	0.320	0.304	0.988	1.024	1.006
B <sub>3</sub> F <sub>0</sub>	0.755	0.714	0.735	0.345	0.333	0.339	1.098	1.070	1.084
B <sub>3</sub> F <sub>1</sub>	0.776	0.794	0.785	0.302	0.336	0.319	1.084	1.103	1.093
B <sub>3</sub> F <sub>2</sub>	0.729	0.862	0.796	0.341	0.360	0.351	1.070	1.134	1.102
B <sub>0</sub> F <sub>0</sub>	0.473	0.512	0.493	0.018	0.016	0.012	0.028	0.035	0.022
B <sub>0</sub> F <sub>1</sub>	0.608	0.628	0.618	0.051	0.047	0.033	0.081	0.101	0.063
<b>S.Em.±</b>	0.020	0.020	0.014	10.51	9.34	9.93	5.1	6.19	5.69
<b>C.D. at 5 %</b>	0.057	0.060	0.040	0.170	0.192	0.181	0.667	0.714	0.690
<b>CV %</b>	5.21	5.28	5.25	0.290	0.242	0.266	0.895	0.874	0.885

### 3.4 Effect of Biostimulants on Xanthophylls Content of Flower

Significantly maximum xanthophyll content in flower (1.593, 1.543 and 1.567 mg/g) was recorded with application of banana pseudostem sap 1% (B<sub>3</sub>). The treatment was at par with panchgavya 3% (B<sub>2</sub>). The favourable effect may be due to essential macro and micronutrients in pseudostem sap which act as growth boosters. Salunkhe, (2010) analysed the samples of banana pseudostem for its elemental composition and found that banana pseudostem contained macro elements in higher range. Beneficial effect of zinc on photosynthetic pigments might be due to its role in increasing the rates of photochemical reduction, enzyme of carbohydrates transformation, photosynthetic electron transfer as well as photosynthesis. Iron effect may be due to indirect role of iron in chlorophyll biosynthesis. Similar result also obtained by Balakrishnan et al. (2007) in marigold, EL-Naggar (2009) in carnation and Khalifa et al. (2011) in iris.

### 3.5 Effect of Micronutrients on Xanthophylls Content of Flower

Significantly maximum xanthophylls content in flower (1.350, 1.380 and 1.365 mg/g) was recorded in F<sub>2</sub> treatment (micronutrient grade-IV 1%) during the year 2017-18, 2018-19 and in pooled analysis, respectively (Table 3). This

treatment was at par with micronutrient grade-IV 0.5% (F<sub>1</sub>) during the year 2018-19, respectively. Beneficial effect of iron may be due to its indirect role in chlorophyll biosynthesis and beneficial effect of zinc on photosynthetic pigments might be due to its role in increasing the rates of photochemical reduction, enzyme of carbohydrates transformation, photosynthetic electron transfer as well as photosynthesis. Similar result also obtained by Balakrishnan et al. (2007) in marigold, EL-Naggar (2009) in carnation and Khalifa et al. (2011) in iris.

### 3.6 Interaction Effect of Biostimulants and Micronutrients on Xanthophylls Content of Flower

The combined application of banana pseudostem 1% with micronutrient grade-IV 1% (B<sub>3</sub>F<sub>2</sub>) recorded significantly maximum xanthophyll content in flower (1.700, and 1.610 mg/g) during the year 2017-18 and in pooled analysis (Table 3a). The results were at par with B<sub>3</sub>F<sub>1</sub> and B<sub>3</sub>F<sub>0</sub>. The combined application of banana pseudostem 1% with micronutrient spray (B<sub>3</sub>F<sub>0</sub>) recorded maximum xanthophylls content in flower (1.580 mg/g) during the year 2018-19. The results are at par with treatment B<sub>3</sub>F<sub>2</sub>, B<sub>3</sub>F<sub>1</sub>, B<sub>2</sub>F<sub>2</sub>, B<sub>2</sub>F<sub>1</sub> during the year 2018-19. Similar result was also reported by Balakrishnan et al. (2007) in marigold and Yadegari?? spelling (2013) in pot marigold.

**Table 3. Effect of biostimulants and micronutrients on xanthophylls content of marigold cv. Pusa Narangi Gainda**

Treatments	Xanthophylls content (mg/g)		
	2017-18	2018-19	Pooled
<b>Level of Biostimulants (B)</b>			
B <sub>0</sub> – Control	1.031	1.062	1.047
B <sub>1</sub> – Seaweed extract (1%)	1.101	1.199	1.150
B <sub>2</sub> – Panchgavya (3%)	1.296	1.493	1.394
B <sub>3</sub> – Banana pseudostem sap (1%)	1.593	1.543	1.567
<b>S.Em.±</b>	0.022	0.023	0.052
<b>C.D. at 5 %</b>	0.065	0.067	0.236
<b>Level of Micronutrients (F)</b>			
F <sub>0</sub> – Control	1.170	1.268	1.219
F <sub>1</sub> – Micronutrient Grade-IV (0.5%)	1.246	1.325	1.285
F <sub>2</sub> – Micronutrient Grade-IV (1%)	1.350	1.380	1.365
<b>S.Em.±</b>	0.019	0.020	0.014
<b>C.D. at 5 %</b>	0.056	0.058	0.039
<b>Interaction (B X F)</b>			
<b>S.Em.±</b>	0.038	0.040	0.027
<b>C.D. at 5 %</b>	0.112	0.116	0.078
<b>CV %</b>	5.25	5.16	5.21

**Table 3a. Interaction effect of biostimulants and micronutrients on xanthophylls content of marigold cv. Pusa Narangi Gainda**

Treatment combinations	Xanthophylls content (mg/g)		
	2017-18	2018-19	Pooled
B <sub>0</sub> F <sub>0</sub>	0.860	0.947	0.903
B <sub>0</sub> F <sub>1</sub>	1.100	1.087	1.093
B <sub>0</sub> F <sub>2</sub>	1.133	1.153	1.143
B <sub>1</sub> F <sub>0</sub>	1.030	1.100	1.065
B <sub>1</sub> F <sub>1</sub>	1.117	1.197	1.157
B <sub>1</sub> F <sub>2</sub>	1.157	1.300	1.228
B <sub>2</sub> F <sub>0</sub>	1.260	1.447	1.353
B <sub>2</sub> F <sub>1</sub>	1.217	1.487	1.352
B <sub>2</sub> F <sub>2</sub>	1.410	1.547	1.478
B <sub>3</sub> F <sub>0</sub>	1.530	1.580	1.555
B <sub>3</sub> F <sub>1</sub>	1.550	1.530	1.540
B <sub>3</sub> F <sub>2</sub>	1.700	1.520	1.610
<b>S.Em. ±</b>	0.038	0.040	0.027
<b>C.D. at 5%</b>	0.112	0.116	0.078
<b>CV%</b>	5.25	5.16	5.21

#### 4. SUMMARY AND CONCLUSION

Foliar application of banana pseudostem sap @ 1% at 30, 45 & 60 days after transplanting with micronutrient grade-IV @ 1% at 40, 55 & 70 days after transplanting in addition to recommended dose of fertilizers (200:100:100 kg/ha NPK) proved to be the best treatment for getting higher chlorophyll content in leaves, vegetative growth and xanthophylls content in flower of African marigold cv. Pusa Narangi Gainda.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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