Original Research Article

Effect of foliar application of biostimulants and micronutrients on chlorophyll and xanthophylls content of African marigold cv. Pusa Narangi Gainda

ABSTRACT

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The present experiment ~~entitled "Effect of foliar application of biostimulants and micronutrients on chlorophyll and xanthophylls content of African marigold cv. Pusa Narangi Gainda"~~ was carried out ~~at Jamuvadi Farm, Department of Horticulture, College of Agriculture, Junagadh Agricultural University, Junagadh,~~ during 2017 to 2019. The experiment was laid out in Randomized Block Design with Factorial concept (FRBD) consisting two factors and ~~with~~ three replications. The treatment comprised with four biostimulants and three ~~treatment of~~ 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.

Key words: Biostimulants, Micronutrients, African marigold cv. Pusa Narangi Gainda

Introduction

African marigold *(Tagetes erecta* L.) is one of the important commercial flower of India as well as of Gujarat. Marigold is ~~and~~ 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 ~~the~~ increasing the flower production, nutrient are supplied through chemical fertilizers. Biostimulants are products of natural and organic origin that stimulate~~s~~ plant~~s~~ growth and development to achieve ~~their~~ higher crop growth, quality of flower and yield ~~potential~~. Seaweed extracts contain major and micro nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances (reference???). The liquid contained ~~macronutrients like~~ P 120 mg/100g, K 4170 mg/100g, Ca 66.98 mg/100g and micronutrients like Fe 147 mg/100g, Mn 5.84 mg/100g, Zn 9.08 mg/100g and Cu 0.36 mg/100g (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 ~~Fe-~~ 124 ppm Fe, ~~Mn-~~ 6.73 ppm Mn, ~~Cu-~~ 4.61 ppm Cu and ~~Zn-~~ 0.97 ppm Zn (Gundrashiya, 2013) apart from ~~and also~~ growth promoting substance like, cytokinin- 137.8 mg/l and gibberellic acid- 110.2 mg/l ~~present~~ (Desai, 2018).

Micronutrients are needed in very small amounts by crop plants. Their concentration in plants is are generally below the 100 parts per million (ppm) level (Reference???). Out of 17 essential elements, Fe, Zn, B, Cu, Mn, Mo although required in very little amounts, but their importance for the plant is no way less than those of major elements. Due to, deficiency of these minor elements the leaves, branches and flower may not grow properly and they may even affect the flower quality as well as production. These elements also help in development of hormone, enzyme, chlorophyll and in the absorption of the 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. Application of micronutrients in the hope of increasing crop yields even through there is little evidence to suggest a deficiency exists. Towards the concern about micronutrient deficiencies, are encouraged to investigations eare the need of the hour need thoroughly and apply the nutrients in and need to be applied in stips. test strips if necessary. Considering the above facts, the present study was proposed.planned and undertaken with the objective to assess the response of biostimulants and micronutrients on chlorophyll and xanthophylls content of African marigold cv. Pusa Narangi Gainda.

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_0), Seaweed extract @ 1% (B_1), Panchgavya @ 3% (B_2) and Banana pseudostem sap @ 1% (B_3) and three treatments of micronutrients *i.e*. without micronutrients (F_0), micronutrients grade-IV @ 0.5% (F_1) and micronutrients grade-IV @ 1% (F_2). 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) (Reference???). Xanthophylls was estimated by AOAC Hot saponification method (Lawarance, 1990).

Table 1: Time of applications

Time of foliar application of biostimulants (both seasons)	**Time of foliar application of micronutrinets (both seasons)**
1st 30 days after transplanting	1st 40 days after transplanting
2nd 45 days after transplanting	2nd 55 days after transplanting
3rd 60 days after transplanting	3rd 70 days after transplanting

Result and discussion

Table 2: 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_3) during 2017-18, 2018-19 and in pooled analysis (**Table 2**). The maximum chlorophyll-b content was significantly increased in

leaves (0.329, 0.343 and 0.336 mg/g) was recorded with application of banana pseudostem sap 1% (B_3) during 2017-18, 2018-19 and in pooled analysis. The ~~Which was at par with~~ treatment was at par with ~~of~~ panchgavya 3% (B_2) 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 ~~was recorded maximum with~~ application of banana pseudostem sap 1% (B_3) ~~during 2017-18, 2018-19 and in pooled~~. ~~Significantly m~~Maximum chlorophyll-a, b and total chlorophyll content in leaves were recorded with ~~an~~ application of banana pseudostem sap @ 1% (B_3) during the year 2017-18, 2018-19 ~~and in pooled~~. 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 ~~well~~ supplied well ~~y~~ with Fe is high in chlorophyll. ~~Along with this iron is not readily mobile between different plant organs. Hence green plants deprived of Fe soon become chlorotic in the younger plant parts.~~ These r~~R~~esults are in consonance with the findings in tuberose ~~of~~ (Desai, ~~(~~2018) ~~in tuberose and Gundrashiya, (2013) in~~ okra, cluster bean and cow pea Gundrashiya, (2013).

~~Table 2:~~ Effect of micronutrients on chlorophyll content in leaves

The maximum chlorophyll-a content ~~was~~ significantly increased in leaves (0.673, 0.713, ~~and~~ 0.693 mg/g) with ~~was recorded in~~ F_2 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_1) ~~during both the year and in pooled~~. Significantly maximum chlorophyll-b content in leaves (0.304, 0.319 and 0.312 mg/g) was recorded in F_2 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_1) ~~during 2017-18 and in pooled~~. The maximum total chlorophyll content was significantly increased in leaves (0.977, 1.015 and 0.996 mg/g) with ~~was recorded in~~ F_2 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_1) ~~during 2017-18, 2018-19 and in pooled~~. Chlorophyll is a major green pigment found in green leaves and is undoubtedly determining the photosynthetic efficiency and productivity of plants. ~~The micronutrients might have increased the nutrient availability for synthesis and accumulation the chlorophyll content in leaves of marigold plant.~~ The variation in chlorophyll content due to micronutrients may be attributed to decrease in chlorophyll degradation and increased chlorophyll synthesis (Reference??). Iron is involved in chlorophyll synthesis ~~pathway. The best~~ plays a role ~~of iron is its~~in catalytic function in nutrient absorption, ~~and~~ balancing other nutrients, ~~and~~ also in biological oxidation, reduction and other metabolic processes in plants. It may also be associated with organic acid metabolism(Reference ??).

~~Table 2a:~~ 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_3F_1) recorded the maximum chlorophyll-a content in leaves (0.776 mg/g) during the year 2017-18.

It was at par with B_3F_0 and B_3F_2 during 2017-18. While, banana pseudostem 1% with micronutrient grade-IV 1% (B_3F_2) 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**), ~~respectively~~. The results were on par with ~~However, it was found at par with~~ treatment of B_3F_1, ~~during pooled~~. The combined application of banana pseudostem 1% (B_3F_0) 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_3F_2) ~~during the year 2018-19 and~~ in pooled analysis~~, respectively~~. However, it was found at par with treatment of B_3F_1 and B_3F_0 ~~during pooled, respectively. While, found at par with treatment of~~ B_3F_1, B_3F_2, B_2F_0, ~~and~~ B_0F_2 during 2017-18 ~~and during 2018-19 it was at par with treatment of~~ B_3F_1, B_3F_0, B_2F_2, B_2F_1, ~~and~~ B_2F_0 during 2018-19. The application of banana pseudostem sap 1% with without micronutrient (B_3F_0) recorded the maximum total chlorophyll (1.098 mg/g) during the year 2017-18~~, respectively~~. It was found at par with treatment of B_3F_2 and B_3F_1 ~~during 2017-18, respectively~~. Application of banana pseudostem 1% with micronutrient grade-IV 1% (B_3F_2) recorded the maximum total chlorophyll (1.134 and 1.102 mg/g) during the year 2018-19 and in pooled analysis. The results were ~~However, it was found~~ at par with treatments ~~of~~ B_3F_1 and B_3F_0 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 Plant well supply with Fe is high in chlorophyll. Along with this iron is not readily mobile between different plant organs. Hence~~ 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(reference??) .

~~Table 3:~~ Effect of biostimulants on xanthophylls content of flower

Significantly maximum xanthophyll~~s~~ content in flower (1.593, 1.543 and 1.567 mg/g) was recorded with application of banana pseudostem sap 1% (B_3). The ~~Which was~~ treatment was at par with ~~treatment of~~ panchgavya 3% (B_2) ~~during the year 2018-19 and in pooled~~. The favourable effect may be ~~It might be~~ due to ~~banana pseudostem sap contains good amount of~~ essential macro and micronutrients in peudostem sap which act as ~~well 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 ~~the~~ range ~~of 1.00 to 1.12 % N, 0.50 to 0.71 % P, 2.39 to 20.2 % K and micro nutrients in the range of 259 to 323.2 mg/kg Fe, 47.3 to 241.3 mg/kg Mn, 10.1 to 107.4 mg/kg Zn and 13.4 to 83.6 mg/kg Cu~~. 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. ~~and i~~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.

~~Table 3:~~ 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_2 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_1) during the year 2018-19, respectively. ~~Regarding the b~~Beneficial effect of iron may be due to its indirect role ~~of iron~~ 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.

~~Table 3a:~~ Interaction effect of biostimulants and micronutrients on xanthophylls content of flower

The combined application of banana pseudostem 1% with micronutrient grade-IV 1% (B_3F_2) recorded significantly maximum xanthophyll content in flower (1.700, and 1.610 mg/g) during the year 2017-18 and in pooled analysis~~, respectively~~ **(Table 3a)**. The results were ~~However, it was found~~ at par with ~~treatment of~~ B_3F_1 and B_3F_0 ~~during pooled, respectively~~. The combined application of banana pseudostem 1% with ~~without~~ micronutrient spray (B_3F_0) recorded maximum xanthophylls content in flower (1.580 mg/g) during the year 2018-19. The results are at ~~Which was at~~ par with treatment B_3F_2, B_3F_1, B_2F_2, ~~and~~ B_2F_1 during the year 2018-19. ~~It might be due to banana pseudostem sap contains good amount of essential macro and micronutrients as well as growth boosters. Salunkhe, (2010).This might be due to the indirect role of iron in chlorophyll biosynthesis. Regarding the beneficial effect of zinc on photosynthetic pigment may be due to its role in increasing the rates of photochemical reduction, chloroplast structure, photosynthetic electron transfer as well as photosynthesis.~~ Similar result was also reported by Balakrishnan *et al.* (2007) in marigold and Yadegari?? spelling (2013) in pot marigold.

Summary and conclusion

~~On the basis of result obtained in the present investigation it may be concluded that the f~~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.

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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
Level of Biostimulants (B)									
B_0 – Control	0.581	0.607	0.594	0.256	0.246	0.251	0.843	0.857	0.850
B_1 –Seaweed extract (1%)	0.588	0.619	0.604	0.274	0.280	0.277	0.858	0.906	0.882
B_2 – Panchgavya (3%)	0.671	0.654	0.663	0.294	0.319	0.306	0.965	1.000	0.982
B_3 – Banana pseudostem sap (1%)	0.753	0.790	0.772	0.329	0.343	0.336	1.084	1.103	1.093
S.Em.±	0.011	0.012	0.008	0.010	0.009	0.007	0.016	0.020	0.013
C.D. at 5 %	0.033	0.034	0.02	0.030	0.027	0.020	0.047	0.058	0.036
Level of Micronutrients (F)									
F_0 – Control	0.618	0.611	0.615	0.267	0.282	0.275	0.887	0.912	0.900
F_1 – Micronutrient Grade-IV (0.5%)	0.654	0.678	0.666	0.293	0.289	0.291	0.948	0.971	0.959
F_2 - Micronutrient Grade-IV (1%)	0.673	0.713	0.693	0.304	0.319	0.312	0.977	1.015	0.996
S.Em.±	0.010	0.010	0.007	0.009	0.008	0.006	0.014	0.017	0.011
C.D. at 5 %	0.029	0.030	0.020	0.026	0.024	0.017	0.041	0.051	0.032
Interaction (B X F)									
S.Em.±	0.020	0.020	0.014	0.018	0.016	0.012	0.028	0.035	0.022
C.D. at 5 %	0.057	0.060	0.040	0.051	0.047	0.033	0.081	0.101	0.063
CV %	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 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_0F_0	0.473	0.512	0.493	0.170	0.192	0.181	0.667	0.714	0.690
B_0F_1	0.608	0.628	0.618	0.290	0.242	0.266	0.895	0.874	0.885
B_0F_2	0.661	0.682	0.671	0.309	0.303	0.306	0.969	0.982	0.976
B_1F_0	0.605	0.600	0.603	0.255	0.283	0.269	0.847	0.895	0.871
B_1F_1	0.556	0.636	0.696	0.288	0.263	0.275	0.844	0.902	0.873
B_1F_2	0.602	0.621	0.612	0.279	0.294	0.286	0.883	0.920	0.901
B_2F_0	0.638	0.619	0.629	0.299	0.322	0.311	0.937	0.971	0.954
B_2F_1	0.677	0.655	0.666	0.293	0.315	0.304	0.968	1.004	0.986
B_2F_2	0.698	0.688	0.693	0.288	0.320	0.304	0.988	1.024	1.006
B_3F_0	0.755	0.714	0.735	0.345	0.333	0.339	1.098	1.070	1.084
B_3F_1	0.776	0.794	0.785	0.302	0.336	0.319	1.084	1.103	1.093
B_3F_2	0.729	0.862	0.796	0.341	0.360	0.351	1.070	1.134	1.102
B_0F_0	0.473	0.512	0.493	0.018	0.016	0.012	0.028	0.035	0.022
B_0F_1	0.608	0.628	0.618	0.051	0.047	0.033	0.081	0.101	0.063
S.Em.±	0.020	0.020	0.014	10.51	9.34	9.93	5.1	6.19	5.69
C.D. at 5 %	0.057	0.060	0.040	0.170	0.192	0.181	0.667	0.714	0.690
CV %	5.21	5.28	5.25	0.290	0.242	0.266	0.895	0.874	0.885

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**
Level of Biostimulants (B)			
B_0 – Control	1.031	1.062	1.047
B_1 –Seaweed extract (1%)	1.101	1.199	1.150
B_2 – Panchgavya (3%)	1.296	1.493	1.394
B_3 – Banana pseudostem sap (1%)	1.593	1.543	1.567
S.Em.±	0.022	0.023	0.052
C.D. at 5 %	0.065	0.067	0.236
Level of Micronutrients (F)			
F_0 – Control	1.170	1.268	1.219
F_1 – Micronutrient Grade-IV (0.5%)	1.246	1.325	1.285
F_2 - Micronutrient Grade-IV (1%)	1.350	1.380	1.365
S.Em.±	0.019	0.020	0.014
C.D. at 5 %	0.056	0.058	0.039
Interaction (B X F)			
S.Em.±	0.038	0.040	0.027
C.D. at 5 %	0.112	0.116	0.078
CV %	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_0F_0	0.860	0.947	0.903
B_0F_1	1.100	1.087	1.093
B_0F_2	1.133	1.153	1.143
B_1F_0	1.030	1.100	1.065
B_1F_1	1.117	1.197	1.157
B_1F_2	1.157	1.300	1.228
B_2F_0	1.260	1.447	1.353
B_2F_1	1.217	1.487	1.352
B_2F_2	1.410	1.547	1.478
B_3F_0	1.530	1.580	1.555
B_3F_1	1.550	1.530	1.540
B_3F_2	1.700	1.520	1.610
S.Em. ±	0.038	0.040	0.027
C.D. at 5%	0.112	0.116	0.078
CV%	5.25	5.16	5.21