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Influence of different spacings, SNP concentrations and sources of nitrogen on catalase activity in African marigold cv. 'Bidhan-2'

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Abstract
A factorial experiment was conducted with three different spacings, three different concentrations of sodium nitroprusside (SNP) and three different sources of nitrogenous fertilizers for two years during 2017 and 2018 at the Horticultural Research Station, Dr. Y.S.R. Horticultural University, Venkataramannagudem to find out their influence on heat stress tolerance of marigold cultivated during summer season. Statistically analysed data with respect to catalase activity revealed that spraying with SNP at 200 µM concentration recorded significant increase in catalase activity (34.03 and 7.84 µg of H_2O_2 g^{-1} min^{-1}) followed by soil application of nitrogenous fertilizer in the form of calcium nitrate (20.44 and 6.37 µg of H_2O_2 $g^{-1}min^{-1}$) respectively after the 1st and 2nd sprays. However, soil application of calcium nitrate in combination with sprayings of SNP at 200 µM concentration recorded significantly higher catalase activity (39.06 and 9.48µg of H_2O_2 g^{-1} min^{-1}) respectively at 20 and 40 days after transplanting of marigold. Spacings did not exert any significant influence on catalase activity.

Keywords: Bidhan-2, $Ca(NO_3)_2$, catalase, marigold, spacing, SNP

Introduction
African marigold (*Tagetes erecta* L.) also called as 'Herb of the Sun' or 'Mexican Marigold' belongs to the family Asteraceae and considered as native of Mexico. Today, marigold is grown for extraction of lutein, a common yellow/orange food colour (E161b). Among the commercial loose flowers cultivated in Andhra Pradesh, the area under marigold cultivation is 5,971 ha with a total production of 61,356 MT of loose flowers (DOH, 2017) [1]. In many parts of Andhra Pradesh, plant growth is ceased with a reduction in flower size and yield during summer months due to prevailing high temperatures in the surrounding atmosphere. An increase in global warming is the major constraint for better performance of crops causing abiotic stress which is considered as the primary reason for crop loss or yield reduction up to or more than 50% in average yields (Navabpour *et al.*, 2003) [2]. Oxidative stress results in the production of Reactive Oxygen Species (ROS) which are primarily responsible for membrane damage, early senescence of leaves and a significant reduction in the yield of crop plants. Detoxification of reactive oxygen species is an adequate approach to produce plants tolerant to various stresses (Wang *et al.*, 2003) [3] especially to high temperatures. Many studies were conducted by several researchers on the performance of marigold cultivars during regular season but the information available on the cultivation of marigold during summer seasonin Andhra Pradesh is very scanty. Marigold cultivar 'Siracole' is gaining popularity among the marigold growing farmers for its production during late rainy and early summer seasons in Andhra Pradesh. Marigold cv. 'Bidhan-2' is an improved selection from Siracole. It's suitability for successful cultivation during summer season in Andhra Pradesh is yet to be tested. So far no work has been carried out on this cultivar with regard to its cultivation during summer season in Andhra Pradesh. Keeping these lacunae in view, the present investigation was planned with different spacings, as plant geometry plays an important role in growth, development and yield of any crop especially during summer season. Apart from plant geometry, form of nitrogen applied to the plant affects its tolerance to damage caused by the stress created through high temperatures.

Nitrogen in the form of calcium nitrate has been reported to be beneficial during summer cultivation of crops in comparison to the amide form of urea application (Zhu *et al.*, 2000) [4]. In addition to these, spraying of sodium nitroprusside (SNP) had been reported to release nitric oxide (NO), a highly reactive and membrane permeable free radical scavenger with a broad spectrum of regulatory functions in many physiological processes and thus protecting the plants against heat stress by acting as an antioxidant directly by scavenging the reactive oxygen species generated under high temperature stress conditions. After thorough literature search, the present investigation has been initiated to study the influences of spacing, foliar application of sodium nitroprusside at different concentrations and soil application of different sources of nitrogenous fertilizers on catalase activity in alleviating the heat stress during summer cultivation of marigold cv. 'Bidhan-2' under the tropical humid coastal climatic conditions of Andhra Pradesh.

Materials and Methods

The present investigation was carried out at the Horticultural Research Station, Dr. Y.S.R. Horticultural University, Venkataramannagudem, West Godavari district of Andhra Pradesh during the period from 2016-17 to 2017-18. The location falls under 'Agro-climatic Zone-10 of East Coastal Plains and Hills' (Krishna-Godavari Zone) and experiences hot and humid summer with mild winter receiving an average annual rainfall of 900 mm.One month old rooted cuttings of African marigold cv. 'Bidhan-2' were used in the present investigation. African marigold cv. 'Bidhan-2' is a selection of cultivar 'Siracole'.

The experiment was laid out in a Randomized Block Design (RBD) with factorial concept and replicated twice with a plot size of 3.0 m x 3.2 m. A total of 27 treatment combinations comprising of three spacing's (Factor-I) *viz.*, S_1: 45 cm x 30 cm,S_2: 45 cm x 40 cm, S_3:60 cm x 30 cm; three doses of sodium nitroprusside (Factor-II)*viz.*, N_1:0 µM (water spray), N_2: 100 µM, N_3:200 µM and three sources of nitrogenous fertilizers (Factor-III) *viz.*, F_1: Urea, F_2: Calcium nitrate and F_3:Ammonium sulphate. Treatment combinations include T_1 - $S_1N_1F_1$: 45 cm x 30 cm + Water spray + Urea, T_2 - $S_1N_1F_2$: 45 cm x 30 cm + Water spray+ Calcium nitrate, T_3 - $S_1N_1F_3$:45 cm x 30 cm + Water spray + Ammonium sulphate, T_4 - $S_1N_2F_1$: 45 cm x 30 cm + 100 µM SNP + Urea, T_5 - $S_1N_2F_2$: 45 cm x 30 cm + 100 µM SNP + Calcium nitrate, T_6 - $S_1N_2F_3$: 45 cm x 30 cm + 100 µM SNP + Ammonium sulphate, T_7 - $S_1N_3F_1$:45 cm x 30 cm + 200 µM SNP + Urea, T_8 - $S_1N_3F_2$:45 cm x 30 cm + 200 µM SNP + Calcium nitrate, T_9 - $S_1N_3F_3$: 45 cm x 30 cm + 200 µM SNP + Ammonium sulphate, T_{10} - $S_2N_1F_1$: 45 cm x 40 cm + Water spray+ Urea, T_{11} - $S_2N_1F_2$:45 cm x 40 cm + Water spray+ Calcium nitrate, T_{12} - $S_2N_1F_3$: 45 cm x 40 cm + Water spray+ Ammonium sulphate, T_{13} - $S_2N_2F_1$: 45 cm x 40 cm + 100 µM SNP + Urea, T_{14} - $S_2N_2F_2$:45 cm x 40 cm + 100 µM SNP + Calcium nitrate, T_{15} - $S_2N_2F_3$: 45 cm x 40 cm + 100 µM SNP + Ammonium sulphate, T_{16} - $S_2N_3F_1$: 45 cm x 40 cm + 200 µM SNP + Urea, T_{17} - $S_2N_3F_2$:45 cm x 40 cm + 200 µM SNP + Calcium nitrate, T_{18} - $S_2N_3F_3$: 45 cm x 40 cm + 200 µM SNP + Ammonium sulphate, T_{19} - $S_3N_1F_1$: 60 cm x 30 cm + Water spray+ Urea, T_{20} - $S_3N_1F_2$:60 cm x 30 cm + Water spray+ Calcium nitrate, T_{21} - $S_3N_1F_3$:60 cm x 30 cm + Water spray+ Ammonium sulphate, T_{22} - $S_3N_2F_1$:60 cm x 30 cm + 100 µM SNP + Urea, T_{23} - $S_3N_2F_2$: 60 cm x 30 cm + 100 µM SNP + Calcium nitrate,T_{24} - $S_3N_2F_3$: 60 cm x 30 cm + 100 µM SNP + Ammonium sulphate,T_{25} - $S_3N_3F_1$:60 cm x 30 cm + 200 µM SNP + Urea, T_{26} - $S_3N_3F_2$: 60 cm x 30 cm + 200 µM SNP + Calcium nitrate andT_{27} - $S_3N_3F_3$: 60 cm x 30 cm + 200 µM SNP + Ammonium sulphate.

Just before application, fresh solutions SNP concentrations @ 100 µM and 200 µM were prepared by dissolving 0.1 mg and 0.2 mg of SNP in water by using 1000 ml volumetric flask. Sodium nitroprusside solutions of desired concentrations was sprayed at 20 and 40 days after transplanting (DAT) as per the treatment. Spraying was done during the morning hours by using a manual sprayer until the spray solution ran-off on the plants. Soil application of different nitrogenous fertilizer *viz.*, urea @ 326 kg ha^{-1}, calcium nitrate @ 968 kg ha^{-1} and ammonium sulphate @ 728 kg ha^{-1} was done in several split doses at an interval of 15 days throughout the crop growth period.

Catalase activity was estimated in leaves as per the procedure outlined by Gopalachari (1963) [5] with slight modifications as per to suit to the laboratory conditions. Fresh leaf sample of 0.5 g was macerated by using a pestle and mortar with the intermittent addition of 10 mL of phosphate buffer. The enzyme extract obtained was centrifuged at 3000 rpm for 20 minutes by using a cooling centrifuge. One mL of the supernatant enzyme extract was taken out from the test tube into 5 beakers. Five mL of 1.5% sodium perborate and 1.5 mL of phosphate buffer were added along with 10 mL of sulphuric acid to 4 beakers containing supernatant enzyme extract at 1 minute interval. The remaining 5th beaker was added with 10 mL of sulphuric acid just before adding 1 mL of enzyme extract and it was used as blank. The contents in the beaker were titrated against 0.05 N potassium permanganate till pink colour develops that persists for 30 seconds as an end point. The volume of potassium permanganate consumed was noted down and 1 mL of potassium permanganate was equal to 0.85 µg of hydrogen peroxide. Average of the differences in the blank titre value and sample titre value (X) was calculated and finally the catalase activity (µg of H_2O_2 $g^{-1}min^{-1}$) was estimated by using the following formula.

Catalase activity = (X/1 x 0.5) x 10 x 1 x 0.85

The data arrived was tabulated and subjected to statistical analysis as per the procedure explained by (Gomez and Gomez, 1984) [6] and the statistical significance was tested as per F-test at 5% level of probability. The differences among treatment means were tested by using the critical difference (CD) at the same level of probability.

Results and Discussion

The data on catalase activity was presented in Tables 1a and 1b which revealed that spacing's did not exert any significant influence on the activity of catalase enzyme after the 1st and 2nd spray of SNP which are done at 20th and 40th day after transplanting respectively. However, significant differences in catalase activity were noticed among the concentrations of SNP sprayed on marigold cv. Bidhan-2 after the 1st and 2nd spray of SNP. Spraying of 200 µM SNP recorded significantly highest CAT activity (34.03 and 7.84 µg of H_2O_2 g^{-1} min^{-1}) followed by spraying with SNP @ 100

µM (16.93 and 6.39 µg of H_2O_2 g^{-1} min^{-1}), whereas water spray (0.31 and 0.46 µg of H_2O_2 g^{-1} min^{-1}) recorded significantly lowest CAT activity in marigold cv. Bidhan-2 respectively after the 1st and 2nd spray of SNP. Significantly lower CAT activity was noticed in marigold after the 2nd spray of SNP in comparison to the 1st spray. The reason might be attributed to an increase in the temperature of atmosphere which in turn might have played a major role in decreasing the enzymatic activity. Higher CAT activity recorded at higher concentration of SNP could be attributed to the positive role of NO released by SNP thus increased the enzyme activity during heat stress period. An increase noticed in the CAT activity might be ascribed to release of NO from SNP thus stimulated the genes for their expression (Besson-Bard *et al.*, 2009 [7], Xiong *et al.*, 2010 [8]). Besson-Bard *et al.* (2009) [7] screened different plant species through a genomicmicroarray to find out their NO induced stress resistant genes. Exogenous application of SNP led to an increase in the rate of release of NO which in turn increased the activity of catalase, superoxide dismutase, peroxidase and ascorbate peroxidase in seashore mallow (Guo *et al*,. 2009) [9], mustard (Zeng *et al*., 2011) [10], wheat (Ruan *et al*,. 2002) [11] and chickpea (Sheokand *et al*., 2010) [12] thus protected the plants from oxidative damage under salt stress condition. Similar kind of observation was also reported earlier by Wu *et al.* (2011) [13] in tomato and Tian *et al.* (2015) [14] in wheat under saline water stress condition.

Different sources of nitrogenous fertilizers applied through soil also exhibited significant impact on the CAT activity at all the stages of crop growth period under examination *i.e.*, after the 1st and 2nd spray of SNP. Data revealed that soil application of $Ca(NO_3)_2$ recorded significantly highest CAT activity (20.44 and 6.37 µg of H_2O_2 g^{-1} min^{-1}) followed by $(NH_4)_2SO_4$ (17.63 and 4.77 µg of H_2O_2 g^{-1} min^{-1}), whereas soil application of urea recorded significantly lowest CAT activity (13.21 and 3.54 µg of H_2O_2 $g^{-1}min^{-1}$) respectively after the 1st and 2nd spray of SNP. Significantly lower CAT activity recorded in marigold after the 2nd spray of SNP in comparison to the 1st spray might be attributed to an increase in the mean maximum temperature of surrounding atmosphere. Soil application of $Ca(NO_3)_2$ might have created a favourable environment for better enzymatic activity in the plant system by maintaining higher amounts of water and nutrient reserves in the plant through better performance of root activity which otherwise may be lacking during heat stress period. Keen perusal of data created an impression that influence of $Ca(NO_3)_2$ was more on CAT activity than spraying of SNP. Similar kind of result was also reported by Tian *et al.* (2015) [14] in wheat under salinity stress condition and they further reported that application of $Ca(NO_3)_2$ exhibited an ameliorating effect on the antioxidative enzymes. Interaction effects between spacings and SNP concentrations, spacings and sources of nitrogenous fertilizers and spacings, SNP concentrations and sources of nitrogenous fertilizers were found non-significant during both the years of study as well in the pooled mean analysis of catalase activity.

Interaction effects between SNP concentrations and sources of nitrogenous fertilizers on CAT activity were found significant after the 1st and 2nd spray of SNP. Data on the interaction effects of catalase revealed that soil application of $Ca(NO_3)_2$ in combination with SNP @ 200 µM concentration recorded significantly highest CAT activity (39.06 and 9.48 µg of H_2O_2 g^{-1} min^{-1} after the 1st and 2nd spray of SNP respectively) followed by soil application of $(NH_4)_2SO_4$ in combination with SNP @ 200 µM concentration (36.05 and 7.81 µg of H_2O_2 g^{-1} min^{-1}) after the 1st and 2nd spray of SNP. Significantly lower activity of CAT was recognized after the 2nd spray of SNP which might be attributed to an abrupt increase in the temperature of the surrounding atmosphere of the plants. Significantly lowest CAT activity (0.22 and 0.00 µg of H_2O_2 g^{-1}) was recorded with the combined soil application of urea and water sprays respectively after the 1st and 2nd spray. Tian *et al.* (2015) [14] reported a positive effect with the combined application of SNP and $Ca(NO_3)_2$ in wheat crop which could compensate for each other's weakness to obtain a positive synergic effect.Keen perusal of the pooled mean revealed that impact of $Ca(NO_3)_2$ was found more than SNP in increasing the CAT activity in marigold. Karpets *et al.* (2011) [15] reported that induced heat resistance in plants was due to application of SNP which was further depended on calcium and ROS. Spraying with SNP might have acted as a donor of NO thus activated the protective responses necessary for the development of heat resistance in marigold cv. 'Bidhan-2' during summer cultivation. Such responses were mediated by ROS and calcium (as a universal intracellular messenger) ions due to the induction accompanied by intensification of superoxide anion radical generation. Genes responsible for release of NO to protectagainst stress agents might have bene depended not only on ROS and calcium ions but also on other signal mediators and systems particularly on the MAP kinase cascade and phosphatidic acid. Similar kind of observation was reported earlier by Karpets *et al.* (2011) [15] and Tian *et al.* (2015) [14] in wheat under salinity stress condition.

Based on the analysis of results, it could be concluded that soil application of calcium nitrate as a source of nitrogenous fertilizer coupled with sprayings of SNP @ 200 µM concentration at 20 and 40 DAT have exerted significant influence on the growth and development of marigold with their synergism by alleviating heat stress effects during summer cultivation of marigold through increased activity of catalase which is an antioxidative enzyme there by recorded an increase in the flower yield and quality.

Table 1a: Influence of Spacing's, SNP concentrations and Sources of Nitrogenous fertilizers on the activity of catalase (µg of H_2O_2 g^{-1} min^{-1}) in African marigold cv. 'Bidhan-2' after the 1st spray during summer cultivation

Spacings (S)	SNP Concentrations (N)	Sources of nitrogenous fertilizers (F)											
		First year				Second year				Pooled mean			
		F_1 Urea	F_2 $Ca(NO_3)_2$	F_3 $(NH_4)_2SO_4$	Mean	F_1 Urea	F_2 $Ca(NO_3)_2$	F_3 $(NH_4)_2SO_4$	Mean	F_1 Urea	F_2 $Ca(NO_3)_2$	F_3 $(NH_4)_2SO_4$	Mean
S_1 (45 cm x 30 cm)	N_1 0 µ M	0.27	0.57	0.30	0.38	0.19	0.39	0.24	0.28	0.23	0.48	0.27	0.33
	N_2 100 µ M	13.80	22.78	16.75	17.78	11.21	20.95	16.50	16.22	12.51	21.86	16.63	17.00
	N_3 200 µ M	22.24	39.86	31.49	31.20	31.71	38.51	40.50	36.91	26.98	39.19	36.00	34.05
	Mean	12.10	21.07	16.18	16.45	14.37	19.95	19.08	17.80	13.24	20.51	17.63	17.12
S_2 (45 cm x 40 cm)	N_1 0 µ M	0.25	0.53	0.29	0.36	0.16	0.36	0.23	0.25	0.21	0.44	0.26	0.30
	N_2 100 µ M	13.77	22.70	16.63	17.70	10.98	20.80	16.41	16.06	12.37	21.75	16.52	16.88
	N_3 200 µ M	22.46	39.50	31.91	31.29	31.56	38.31	40.30	36.72	27.01	38.90	36.11	34.01
	Mean	12.16	20.91	16.28	16.45	14.23	19.82	18.98	17.68	13.20	20.36	17.63	17.06
S_3 (60 cm x 30 cm)	N_1 0 µ M	0.26	0.53	0.29	0.36	0.18	0.38	0.24	0.26	0.22	0.45	0.26	0.31
	N_2 100 µ M	13.79	22.73	16.68	17.73	11.03	20.81	16.42	16.09	12.41	21.77	16.55	16.91
	N_3 200 µ M	22.30	39.75	31.72	31.26	31.67	38.42	40.36	36.82	26.99	39.08	36.04	34.04
	Mean	12.12	21.00	16.23	16.45	14.29	19.87	19.01	17.72	13.20	20.43	17.62	17.09
Comparing the SNP concentrations (N) and sources of nitrogenous fertilizers (F)													
N_1 0 µ M		0.26	0.54	0.29	0.36	0.17	0.38	0.24	0.26	0.22	0.46	0.26	0.31
N_2 100 µ M		13.79	22.73	16.69	17.74	11.07	20.85	16.44	16.12	12.43	21.79	16.57	16.93
N_3 200 µ M		22.33	39.70	31.71	31.25	31.65	38.41	40.39	36.82	26.99	39.06	36.05	34.03
Mean		12.13	20.99	16.23	16.45	14.30	19.88	19.02	17.73	13.21	20.44	17.63	17.09
Factor		S Em±		CD		S Em±		CD		S Em±		CD	
Spacings (S)		0.06		NS		0.19		NS		0.10		NS	
SNP Concentrations (N)		0.06		0.18		0.19		0.56		0.10		0.29	
Sources of N fertilizers (F)		0.06		0.18		0.19		0.56		0.10		0.29	
S x N		0.11		NS		0.33		NS		0.18		NS	
N x F		0.11		0.31		0.33		0.97		0.18		0.51	
S x F		0.11		NS		0.33		NS		0.18		NS	
S x N x F		0.19		NS		0.58		NS		0.30		NS	

Table 1b: Influence of Spacings, SNP concentrations and Sources of Nitrogenous fertilizers on the activity of catalase (µg of H2O2 g-1 min-1) in African marigold cv. 'Bidhan-2' after 2nd spray during summer cultivation

Spacings (S)	SNP Concentrations (N)	Sources of nitrogenous fertilizers (F)											
		First year				Second year				Pooled mean			
		F_1 Urea	F_2 $Ca(NO_3)_2$	F_3 $(NH_4)_2SO_4$	Mean	F_1 Urea	F_2 $Ca(NO_3)_2$	F_3 $(NH_4)_2SO_4$	Mean	F_1 Urea	F_2 $Ca(NO_3)_2$	F_3 $(NH_4)_2SO_4$	Mean
S_1 (45 cm x 30 cm)	N_1 0 µ M	0.30	1.19	0.58	0.69	0.10	0.74	0.34	0.39	0.20	0.97	0.46	0.54
	N_2 100 µ M	4.47	8.60	4.53	5.87	4.66	9.34	7.84	7.28	4.57	8.97	6.19	6.57
	N_3 200 µ M	5.82	9.08	6.78	7.23	6.98	10.17	9.04	8.73	6.40	9.62	7.91	7.98
	Mean	3.53	6.29	3.96	4.60	3.91	6.75	5.74	5.47	3.72	6.52	4.85	5.03
S_2 (45 cm x 40 cm)	N_1 0 µ M	0.27	1.17	0.54	0.66	0.08	0.63	0.32	0.34	0.17	0.90	0.43	0.50
	N_2 100 µ M	4.27	8.55	4.44	5.75	4.28	8.33	7.32	6.64	4.28	8.44	5.88	6.20
	N_3 200 µ M	5.61	8.72	6.43	6.92	6.52	9.98	8.90	8.47	6.07	9.35	7.67	7.69
	Mean	3.38	6.15	3.80	4.44	3.63	6.31	5.51	5.15	3.51	6.23	4.66	4.80
S_3 (60 cm x 30 cm)	N_1 0 µ M	0.29	1.18	0.56	0.68	0.09	0.66	0.33	0.36	0.19	0.92	0.45	0.52
	N_2 100 µ M	4.45	8.58	4.50	5.84	4.36	8.76	7.66	6.93	4.41	8.67	6.08	6.39
	N_3 200 µ M	5.75	8.88	6.72	7.12	6.62	10.03	9.02	8.55	6.18	9.45	7.87	7.83
	Mean	3.50	6.21	3.93	4.54	3.69	6.48	5.67	5.28	3.59	6.35	4.80	4.91
Comparing the SNP concentrations (N) and sources of nitrogenous fertilizers (F)													
N_1 0 µ M		0.00	1.18	0.56	0.58	0.00	0.68	0.33	0.33	0.00	0.93	0.44	0.46
N_2 100 µ M		4.40	8.58	4.49	5.82	4.43	8.81	7.61	6.95	4.42	8.69	6.05	6.39
N_3 200 µ M		5.73	8.89	6.64	7.09	6.71	10.06	8.99	8.58	6.22	9.48	7.81	7.84
Mean		3.37	6.22	3.90	4.50	3.71	6.51	5.64	5.29	3.54	6.37	4.77	4.89
Factor		S Em±		CD		S Em±		CD		S Em±		CD	
Spacings (S)		0.2		NS		0.16		NS		0.13		NS	
SNP Concentrations (N)		0.2		0.59		0.16		0.46		0.13		0.38	
Sources of N fertilizers (F)		0.2		0.59		0.16		0.46		0.13		0.38	
S x N		0.35		NS		0.27		NS		0.27		NS	
N x F		0.35		1.02		0.27		0.79		0.27		0.66	
S x F		0.35		NS		0.27		NS		0.27		NS	
S x N x F		0.61		NS		0.47		NS		0.39		NS	

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