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Effect of chitosan on growth and yield of African marigold (*Tagetes erecta* L.) under drought induced stress condition

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Abstract
Chitosan acts as elicitors that helps to induce the secondary metabolites, reduce the transpiration of plants which in turn reduce the water use efficiency without affecting the dry matter production of the plants. A study was conducted to investigate the effect of foliar spray of chitosan on morphological, growth and flowering characters of African marigold under induced drought conditions. The experiment with 3 levels of water stress *ie*., 100 %, 70 % and 50 % field capacity and three levels of chitosan spray *viz*., 0 (water spray), 0.2 g L^{-1} and 0.4 g L^{-1}. Among the treatments, FC_1 (70% FC) with 0.2 g L^{-1} (C_1) of chitosan improved the growth of the plants and the flowering parameters *viz.,* diameter, weight, xanthophyll content and numbers of flowers. Interaction effects of 0.4 g L^{-1} of chitosan with severe stress (FC_2) showed drastic reduction in growth and yield of the plants. Foliar application of chitosan helped to reduce the adverse effect of drought and improved the growth and yield of the plants.

Keywords: African marigold, chitosan, drought, yield, xanthophyll

Introduction

African marigold (*Tagetes erecta* L.) belongs to Asteraceae family and is usually grown as a loose flower and as a landscape plant (Riaz, 2013) [12]. As a Mexican origin, it has both ornamental and medicinal benefits. It is a herbaceous plant with pinnately divided leaves and colorful flowers of yellow and orange used as a bedding plants which attracts attention of the viewer's eye. In South Asia, flowers of *Tagetes* species can be used as a garland to decorate the religious statues and also used as offerings, decoration at weddings and other ceremonies. Environmental stress such as water deficit, salinity, heat stress and metal toxicity affects the crop production under both natural and agricultural conditions (Hayat *et al.*, 2012) [8]. Drought is the most important limiting factor in agricultural production. Under stress, plants produce reactive oxygen species (ROS) which act as an enzyme inhibitor which damages lipids and protein, thereafter decreases the photosynthesis and leads to reduction in plant growth and yield (Yang *et al.,* 2009) [14]. Soluble protein, proline, catalase are the common solutes produced during stress which protects the membrane from damage and stabilize the production of enzymes (Karimi *et al.,* 2012) [9] and helps to withstand the temporary water deficit.

Elicitors are the compounds mostly used for the production of secondary metabolites to withstand the stress condition. Chitosan is a member of polysaccharide and second most abundant non-toxic, low cost natural polymer obtained from fungal cell wall, insects and some algae produced by alkaline *N*- deacetylation of chitin. As a foliar spray it will induce the secondary metabolites and reduce the transpiration of plants (Yin *et al.,* 2011) [15]. In pepper it reduce the transpiration and resulted in 26-43% reduction in water use efficiency without affecting the dry matter production (Bitelli *et al.*, 2001) [3]. In agriculture, chitosan can be used as an effective anti-transpirant to reduce the water use efficiency of the plants.

Materials and Methods

Experimental design and treatments

Experiment was conducted as pot culture at Botanical garden, Department of floriculture and landscaping, TNAU, Coimbatore which was geographically located at 11° 02'N latitude and 76°57'E longitude at an altitude of 426.72m MSL. Each pot was filled equally with 3 Kg of pot mixture.

Factorial experiment was carried out in a randomized complete design with 3 replication. For each treatment 15 plants were used. 25 days old seedlings of African marigold were transplanted in pots and till 15 days after transplanting irrigation was given to the seedlings until saturation. After that plants are imposed to three different levels of field capacity, 100% (FC_0), 70% (FC_1) and 50% (FC_2). In the stressed plants moisture level was reduced from 100% to 70% and 50%.The pots were weighed once in two days interval. Reduction in the weight represents the transpiration and evaporation. Water was supplemented to compensate the loss. Total water loss was calculated as the differences in pots weights between the successive weights (Mohsen *et al.*, 2014) [10]. At three different stage like vegetative, bud and flowering stage, chitosan at different concentration 0.2 g L^{-1} (C_1), 0.4 g L^{-1} (C_2) was sprayed. The potted plants sprayed with water was treated as control (C_0).

Factorial experiment was carried out in a randomized complete design with 2 factors, 9 treatment combination with 3 replication.

Levels	Factor 1	Factor 2
1	FC_1: 100 %	C_1: water spray (control)
2	FC_2: 70 %	C_2: 0.2 g L^{-1}
3	FC_3: 50 %	C_3: 0.4 g L^{-1}

Observations on vegetative, flowering, yield and quality characters were recorded in all the treatments at different growth stages *viz*., 30, 45 and 60 days after transplanting. The observations were recorded on five plants per replication for each treatments. Parameters like plant height, number of branches, days to flower initiation, number of flowers, individual flower weight, flower diameter, yield per plant, root length, root – shoot ratio and xanthophyll content were recorded. Xanthophyll content was estimated by using the method given by AOAC (2006).

The data were analyzed statistically using SPSS *ver.* 16 and mean comparison were carried out by Duncan's multiple range test (DMRT) and critical difference were worked out at five percent probability level.

Results and Discussion

The results of the present study are reported in table 1 and 2. The treatment C_1 (0.2 g L^{-1}) at FC_1 recorded highest plant height and showed on par results with FC_0 C_1 for plant height at 30 DAT (18.11 and 17.62 cm), 45 DAT (27.03 and 26.73) and 60 DAT (34.40 and 33.16 cm). Plant height at 30 DAT (10.15 cm), 45 DAT (22.38 cm) and 60 DAT (28.84 cm) was reduced under severe stress (FC_2) on interaction with 0.4 g L^{-1} of chitosan.

The treatment C_1 (0.2 g L^{-1}) at FC_1 recorded highest number of branches and showed on par results with FC_0 C_1 for number of branches at 30 DAT (7.52 & 7.80 cm), 45 DAT (8.13 & 8.20 cm) and 60 DAT (8.38 & 8.59). Number of branches at 30 DAT (6.10), 45 DAT (6.53) and 60 DAT (7.40) was reduced under severe stress (FC_2) on interaction with 0.4 g L^{-1} of chitosan.

Severe reduction in plant growth was recorded under water stress in the present study. When the drought level was increased, plant growth got inhibited. In stressed plants, reduction in cell elongation occurs due to the inhibiting effect of water shortage on growth promoting hormones which might reduce the turgidity and volume of the cell and thereby lead to the reduction in plant growth (Banon *et al.*, 2006) [2].

Foliar application of chitosan improved the plant growth under mild stress. Similar results was observed in sweet pepper (Ghoname *et al.*, 2010) [6], cucumber and radish (Farouk *et al.*, 2011) [4]. Chitosan increases the availabity and uptake of water and mineral nutrients by adjusting the cell osmotic pressure as well as production of ROS which get reduced by increasing the antioxidant and enzyme activity (Guan *et al.*, 2009) [7]. Chitosan counteracts the harmful effects of water stress by increasing the key enzyme activity of nitrogen metabolism and increases the transportation of nitrogen in the functional leaves as well as the photosynthetic rate which enhance the plant growth and development (Mondal *et al*., 2012) [11].

Water stress had an effect on flowering parameters like flower diameter, individual flower weight, number of flowers per plant, yield and xanthophyll content. At 30, 45 & 60 DAT for days to flower initiation, the treatments C_1 (0.2 g L^{-1}) at FC_1 (40 days) showed on par results with FC_0 C_0 (39 days), FC_0C_1 (39 days), FC_2C_1 (39 days) and FC C_2 (39 days). The treatment FC_0C_0 recorded highest flower diameter (5.03 cm) and showed on par results with FC_1 C_1 (4.95 cm) and FC_0C_1 (4.90 cm).

At 30, 45 & 60 DAT for individual flower weight, the treatment FC_0C_0 recorded largest flower weight (4.83 g) and showed on par results with FC_1C_1 (4.68 g) and FC_0C_1 (4.62 g). The treatment C_1 (0.2 g L^{-1}) at FC_1 showed significantly highest number of flowers per plant (36) than untreated control. The treatments C_1 (0.2 g L^{-1}) at FC_1 recorded highest yield and xanthophyll content showed on par results with FC_0C_1 (156.40 & 154.08 g p^{-1}) and (1.45 & 1.43) respectively. Under 50% FC on interaction with 0.4 g L^{-1} of chitosan showed severe reduction on flower diameter (3.80 cm), individual flower weight (3.16 g), number of flowers (26), estimated yield (82.16 g/p) and Xanthophyll content (1.20).

At the flowering stage, diameter, quality and number of flowers get decreased which might due to the reduction in the availabity of assimilates for export to sink tissue thereby size and quality of flowers get affected (Fulai *et al.,* 2004) [5]. Foliar application of chitosan helps to increase the plant growth and development, thereby yield get increased. Similar result was also reported in rice (Boonlertnirun *et al.*, 2005), maize and bean (Rehim *et al.*, 2009). Turner *et al.* (2001) [13] reported that water stress leads to flower drop and poor seed set. Foliar application of chitosan alleviate the adverse effect of drought condition thereby flowering characters get improved. Chitosan spray at the earlier stage increases the number of buds thereby numbers of flowers get increased when compared to control as reported in bhendi (Mondal *et al.*, 2012) [11]. Mohsen *et al.* (2014) [10] reported that at the reproductive stage foliar spray of chitosan induce assimilates towards reproductive organs which helps in increasing the individual flower weight thereby chitosan helps to improve the flower yield in marigold.

Under water stress, singlet oxygen produced by the excited triplet state of chlorophyll which affects the photosynthetic apparatus (Foyer and Harbison, 1994). Pigments like carotenoids and xanthophyll are reported to be involved in the protection of photosynthetic apparatus from reactive oxygen species (ROS) damage. Foliar application of chitosan on stressed plants increases the xanthophyll content than the untreated control which protects the plants against the oxidative stress by declining the generation of free radicals and lipid peroxidation (Malik and Ashraf, 2012).

Table 1: Effect of chitosan on growth parameters under drought induced stress condition.

TREATMENTS		Plant height (cm) at DAT			No. of branches at DAT		
		30	45	60	30	45	60
C_0	FC_0	17.12	26.54	32.52	7.30	7.73	8.32
	FC_1	16.05	25.85	30.23	6.90	7.61	7.86
	FC_2	14.32	22.45	29.04	6.20	6.45	7.45
C_1	FC_0	17.62	26.73	33.16	7.52	8.13	8.38
	FC_1	18.11	27.03	34.40	7.80	8.20	8.59
	FC_2	16.21	26.04	32.80	6.92	7.80	8.30
C_2	FC_0	17.01	25.68	32.14	7.25	7.47	8.20
	FC_1	15.43	24.96	30.06	6.70	7.32	7.93
	FC_2	10.15	22.38	28.84	6.10	6.53	7.40
Grand mean		15.78	25.30	31.47	6.97	7.47	8.05
SEd		0.40	0.53	0.68	0.18	0.18	0.13
CD (p=0.05)		0.85	1.11	1.43	0.38	0.38	0.27

FCoCo:100% field capacity with water spray (absolute control), FC_1Co: 70% Field capacity with water spray, FC_2C0: 50% field capacity with water spray, FC_0C_1:100% field capacity with 0.2g/l of chitosan spray, FC_1C_1: 70% field capacity with 0.2g/l of chitosan spray, FC_2C_1:50% field capacity with 0.2g/l of chitosan spray, FC_0C_2: 100% field capacity with 0.4g/l of chitosan spray, FC_1C_2: 70% field capacity with 0.4g/l of chitosan spray, FC_2C_2: 50% field capacity with 0.4g/l of chitosan spray.

Table 2: Effect of chitosan on flowering and quality parameters under drought induced stress condition

Treatments		Days to flower initiation	Flower diameter (cm)	Individual flower weight (g)	No. of flowers /plant	Estimated Yield (g/p)	Xanthophyll content (mg/g)
C_0	FC_0	39.00	5.03	4.83	27.00	130.41	1.28
	FC_1	37.00	4.62	4.38	22.00	95.44	1.35
	FC_2	36.00	4.26	4.14	20.00	82.88	1.38
C_1	FC_0	39.00	4.90	4.62	34.00	154.08	1.43
	FC_1	40.00	4.95	4.68	36.00	156.40	1.45
	FC_2	39.00	4.75	4.50	32.00	144.00	1.40
C_2	FC_0	40.00	4.87	4.57	31.00	141.67	1.34
	FC_1	39.00	4.60	4.32	30.00	129.60	1.32
	FC_2	37.00	3.80	3.16	26.00	82.16	1.20
Grand mean		38.44	4.64	4.36	28.67	124.07	1.35
SEd		0.56	0.08	0.11	0.59	2.08	0.02
CD (p=0.05)		1.19	0.17	0.23	1.26	4.41	0.04

FCoCo:100% field capacity with water spray (absolute control), FC_1Co: 70% Field capacity with water spray, FC_2C0: 50% field capacity with water spray, FC_0C_1:100% field capacity with 0.2g/l of chitosan spray, FC_1C_1: 70% field capacity with 0.2g/l of chitosan spray, FC_2C_1:50% field capacity with 0.2g/l of chitosan spray, FC_0C_2: 100% field capacity with 0.4g/l of chitosan spray, FC_1C_2: 70% field capacity with 0.4g/l of chitosan spray, FC_2C_2: 50% field capacity with 0.4g/l of chitosan spray.

Conclusion

The results of this study indicated that foliar application of chitosan 0.2 g L^{-1} helps to maintain the water status of plants and reduce the production of ROS that prevents the plants from oxidative damage under mild water stress condition (70% FC). According to the results, it is concluded that chitosan can decrease the harmful effects of drought stress and can be used as a plant growth enhancer for African marigold.

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