UNDER PEER REVIEW

Effect of different levels of irrigation and Pusa hydrogel on biochemical

parameters of Marigold (Tagetes erecta l.) cv. Culcutta orange.

Abstract

The experiment entitled "Effect of different levels of irrigation and Pusa hydrogel on

biochemical parameters of Marigold (Tagetes erecta 1.) cv. Culcutta orange" was carried out at

College of Horticulture Bagalkot, University of Horticultural Sciences Bagalkot. The maximum

chlorophyll content (1.57 mg/g) was observed in plants, irrigated with 80 per cent CPE.

Significantly highest chlorophyll content (1.71 mg/g) was recorded in plants applied with 5.25

kg/ha of hydrogel. Among the interactions, the treatment combination having plants irrigated

with 80 per cent CPE along with 5.25 kg/ha hydrogel registered maximum chlorophyll content

(1.91 mg/g). Maximum proline content (11.69 µg/g fresh leaves) was observed in plant irrigated

with 60 per cent CPE. Highest proline content (12.19 µg/g fresh leaves) was recorded in plants

applied without any hydrogel. Among the interactions, the treatment combination of plants

irrigated with 60 per cent CPE along with no hydrogel registered maximum (13.28 µg/g fresh

leaves) proline content (Fig. 1).

Keywords: Pusa hydrogel, Marigold, Proline, chlorophyll, irrigation

Introduction

The issue of water management has assumed paramount importance and occupied the

centre stage of politico-economic debates in world. All biochemical and physiological processes

depend upon the availability of water. In 1980's, water-absorbing polymers or hydrogels were introduced for agricultural use. Hydrogels are cross-linked polymers with a hydrophilic group which have the capacity to absorb large quantity of water without dissolving in water. Pusa Hydrogel, a semi-synthetic super absorbent polymer, developed by the ICAR-Indian Agriculture Research Institute (IARI) has been in use since 2012, and its benefits are now being reaped across the country. Pusa hydrogels are bio-degradable and they contain labile bonds either in the polymer backbone or in the cross-links used to prepare the hydrogels. Hydrogel is a threedimensional, hydrophilic polymer with loosely cross-linked networks capable of imbibing large amounts of water or other biological fluids. It enhances the crop productivity per unit available water and nutrients, particularly in moisture stress conditions. It improves physical properties of soil, seed germination, seedling emergence rate, root growth and density that help plants to withstand prolonged moisture stress (Ekebafe et al., 2011). Water usage performance is improved by the use of super absorbents and proper irrigation scheduling. In view of the above explanation and the facts, present investigation entitled the "Effect of different levels of irrigation and Pusa hydrogel on biochemical parameters of Marigold (Tagetes erecta 1.) cv. Culcutta orange" was carried at out at College of Horticulture Bagalkot, University of Horticultural Sciences Bagalkot.

Materials and Methods

Biochemical parameters such as Chlorophyll content in leaves was analysed by collecting the healthy, fully opened and matured leaves from the centre portion of the plants at peak growth stage. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents of leaf tissue were determined by non-destructive method of chlorophyll estimation using dimethyl sulphoxide (DMSO) as suggested by **Shoaf and Lium (1976).** Fresh fully matured leaves from the plants

were brought in polyethylene bags from the field and were cut into small pieces. Known weight of sample (150 mg) was incubated in 5 ml DMSO at 60 °C for three hours in water bath. After the incubation, supernatant was collected on decanting. Then the volume of supernatant was made upto 10 ml using DMSO. The absorbance of extract was measured at 454 nm, 645 nm and 663 nm wavelength using DMSO as a blank in spectrophotometer.

Total chlorophyll =
$$\{20.2 \text{ (A645)} + 8.02 \text{ (A663)}\}\ X \ V$$

$$1000 \times W \times a$$
Total chlorophyll 'a' = $\{12.7 \text{ (A663)} + 2.69 \text{ (A645)}\ X \ V$

$$1000 \times W \times a$$
Total chlorophyll 'b' = $\{22.9 \text{ (A645)} + 4.68 \text{ (A663)}\}\ X \ V$

Where

A = Absorbance at specific wave length (645 nm and 663 nm)

V = Volume of the extract (10 ml)

W = Fresh weight of the sample (100 mg)

a = Path length of light in cuvette (1 cm)

Proline

Proline extraction and determination was performed according to Bates *et al.* (1973). 100 mg of plant tissue was homogenized in 1.2 ml 3 per cent aqueous sulfosalicylic acid and

 $1000 \times W \times a$

centrifuged at 13,000 rpm for 10 min. 500 μl of supernatant was taken in a test tube and was made up to 1 ml with distilled water and reacted with 1 ml of glacial acetic acid and 1 ml of ninhydrin (2 per cent in acetone). The mixture was incubated at 90 °C for 1 h. The samples were cooled in ice bath and 2 ml of toluene was added and vortexed for 2 min. The upper phase was aliquoted to read the absorbance at 520 nm using a spectrophotometer. Proline content (μg proline/g FW).) was quantified using the ninhydrin acid reagent method using l-proline.

Proline (
$$\mu$$
g proline/g FW) = μ g proline/mL × mL Toluene × 5

115.5 g sample

Where, 115.5 is the molecular weight of proline

Results and Discussion

Water is a vital element for the growth of plants. It is the most important factor that determines the growth and development of organisms. Drought stress is one of the major factors associated with the limitation in the growth of most plants. Plants stressed under water deficit show a decrease in photosynthesis, which is correlated to decreased growth and increased incidence of early senescence in plants. The scarcity of water has a direct effect on plants at physiological, morphological, and molecular levels. All biochemical and physiological processes depend upon the availability of water (Imadi et al., 2016).

The data on chlorophyll content as influenced by irrigation, hydrogel levels and their interaction are presented in Table 1. Significant differences were observed in total chlorophyll content due to different irrigation levels. The maximum chlorophyll content (1.57 mg/g) was observed in plant irrigated with 80 per cent CPE. Significantly highest chlorophyll content (1.71

mg/g) was recorded in plants applied with 5.25 kg/ha hydrogel. Among interactions, the treatment combination having 99 plant irrigated with 80 per cent CPE along with 5.25 kg/ha hydrogel registered maximum chlorophyll content (1.91 mg/g). The scarcity of water has a direct effect on plants at physiological, morphological, and molecular levels. All biochemical and physiological processes depend upon the availability of water (Imadi et al., 2016). The results showed that the application of hydrogel avoids the stress of humidity fluctuations and protects the durability of chlorophyll. In stress conditions the protection of durability of chlorophyll by super absorbent polymer materials were shown in sunflower (Nazarli et al., 2010) corn (Khadem et al., 2010) ginger (Kumar et al., 2018). The interaction effect of irrigation and hydrogel levels was also significant with respect to chlorophyll content. Maximum chlorophyll content (1.91 mg/g) was recorded in I2H4 irrigation scheduled at 80 per cent CPE with 5.25 kg / ha of hydrogel) which was on par with I2H3 (1.84 mg/g). Whereas, least chlorophyll content was recorded in I3H5 (0.77 mg/g) having irrigation scheduled at 60 per cent CPE with no hydrogel application. The particulars on proline content as influenced by irrigation, hydrogel levels and their interactions are presented in Table 1. Regarding irrigation intervals, it was significantly highest in irrigation I3: Irrigation 60 per cent CPE (11.69 µg/g fresh leaves) followed by I2: 80 per cent CPE (9.82µg/g fresh leaves) documented and lowest in irrigation 100 per cent CPE $(9.08 \mu g/g \text{ fresh leaves}).$

There was significant reduction trend in total chlorophyll content by reducing the schedule irrigation from 80 to 60 per cent CPE. The reduction of chlorophyll due to drought stress is related to the increase of production of free radicals in the cells. These free radicals cause peroxidation and disintegration and by reduction of chlorophyll, considerable changes are produced in the plant (Schutz and Fangmier, 2001). The results showed that the application of

hydrogel avoids the stress of humidity fluctuations and protects the durability of chlorophyll. In stress conditions the protection of durability of chlorophyll by super absorbent polymer materials were shown in sunflower (Nazarli et al., 2010) corn (Khadem et al., 2010) ginger (Kumar et al., 2018).

Similarly, maximum proline content (11.69 μ g/ g fresh leaves) was observed in plant irrigated with 60 per cent CPE. With the increase in concentration of hydrogel there was a decrease in proline content. Maximum being noted in H5 (without hydrogel) having 12.19 μ g/ g fresh leaves which was followed by H1 (10.94 μ g/ g fresh leaves) and minimum was observed in H4 (8.72 μ g/ g fresh leaves) with 5.25 kg /ha hydrogel. Essentiality of interactivity of different levels of irrigation and hydrogel on proline content showed significant differences during crop stages. Significantly higher proline content (13.28 μ g/ g fresh leaves) was recorded in I3H5: Irrigation 60 per cent CPE + without hydrogel which was followed by I3H1 (12.27 μ g/ g fresh leaves).

Significantly lowest proline content (7.63 µg/g) fresh leaves was recorded in I1H4: Irrigation 100 per cent CPE + 5.25 kg / ha of hydrogel. These results clearly confirm that higher levels of hydrogel and irrigation are very effective in conserving soil moisture thereby leading to higher productivity and improvement in the quality of marigold flowers. The results coincide with reports of previous works. Increased proline accumulation was reported in water stressed in wheat (Hamada A.M., 2000 and Manivannan *et al.*, 2007).

The higher soil moisture conservation by application of hydrogel and irrigation would mean that marigold plants suffer less degree of water stress condition, whereas the marigold plants treated without hydrogel, suffer higher level of water stress. It is well known that as soon as the plant is subjected to water stress condition the proline contents get accumulated in the plants and the higher the water stress, the higher will be proline and vice-versa. These results thus clearly confirm that higher level hydrogel and irrigation is very effective in conserving soil moisture thereby leading to higher productivity and improvement in the quality of marigold flowers. The results coincide with reports of previous works. Increased proline accumulation was reported in water stressed in wheat (Hamada.A.M., 2000 and Manivannan et al., 2007). Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Chandrashekar and Sandhyarani., 1996)

Conclusion

The experiment can be concluded that soil amended with Pusa hydrogel enhanced the biochemical parameters of marigold. Significantly, higher chlorophyll content (1.91 mg/g) was recorded in plants treated with 80 per cent CPE irrigation schedule under 5.25 kg/ha of hydrogel 111 (I2H4). Least chlorophyll and water content was noted in plant treated in irrigation 60 per cent CPE without hydrogel (I3H5). Highest proline (13.28 µg/g fresh leaves) was recorded in plants treated in irrigation 60 per cent CPE without hydrogel (I3H5) and lowest (7.63 µg/g fresh leaves) recorded in plant treated with 100 per cent CPE irrigation schedule under 5.25 Kg/ha of hydrogel (I1H4). Pusa hydrogel also reduced the number of irrigation cycles and increased the plant growth. This effect is due to the considerable absorption of water in hydrogel structure and putting the absorbed water gradually to the surrounding soil and plant root.

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Table 1. Biochemical parameters at various growth stages of marigold as influenced by different levels of irrigation and Pusa hydrogel

Treatments	Biochemical parameter									
	Total Chlorophyll (mg/g)					Proline (μg/ g fresh leaves)				
	Irrigation									
		I ₁	I_2	I_3	Mean		I ₁	I ₂	I_3	Mean
	H ₁	1.34	1.39	1.05	1.26	H ₁	9.55	11.00	12.27	10.94
Pusa Hydrogel	H ₂	1.55	1.58	1.12	1.42	H ₂	8.78	9.69	11.07	9.85
	H ₃	1.60	1.74	1.20	1.51	H ₃	7.84	8.65	11.37	9.29
	H ₄	1.84	1.91	1.37	1.71	H ₄	7.63	8.11	10.43	8.72
	H ₅	1.01	1.25	0.77	1.01	H ₅	11.60	11.68	13.28	12.19
	Mean	1.47	1.57	1.10		Mean	9.08	9.82	11.69	
ob		S.Em (±)		C.D @ 5%		S.Em (±)		C.D @ 5%		
Irrigation (I)		0.03		0.13		0.19		0.80		
Pusa Hydrogel (H)		0.02		0.06		0.16		0.47		
Interaction (I×H)		0.05		0.16		0.32		1.07		

Main plot treatments irrigation (I): I1: 100 % CPE, I2: 80 % CPE, I3: 60 % CPE CPE: Cumulative Pan Evaporation

Sub plot treatments Hydrogel (H): H_1 - 3.0 kg/ ha, H_2 -3.75 kg/ha, H_3 -4.50 kg/ha, H_4 -5.25 kg/ha, H_5 - Control DAT: Days after transplanting