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Improving the growth and flower yield of pot marigold (*Calendula officinalis* L.) under varied water stress conditions using bio inoculants

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Abstract

The experiment entitled Improving the growth and flower yield of pot marigold (*Calendula officinalis* L.) under stress conditions varied water using bio inoculants was conducted during 2020-2021 with the objectives, to evaluate the effect of bio-inoculants on growth, water relations, plant pigments, flower yield of *Calendula officinalis* grown under different water stress regimes. The experiment was conducted in a split plot design with 4 main plot and 3 subplots with 12 treatments replicated thrice. The different water stress treatments used are WS₁ (Field capacity), WS₂ (75% Field capacity), WS₃ (50% Field capacity), WS₄ (25% Field capacity) and usage of different bio inoculants viz., AMF (Arbuscular mycorrhiza fungi), AMC (Arka microbial consortium) and control (no treatment). The treatments were imposed on the calendula crop variety "Bon Bon Orange". The observations were recorded at an interval of 15, 30, 45, 60 DAP (days after planting). maximum plant height (15.56 cm) was recorded at 60 DAP in the treatments WS₂, maximum number of branches (6.32, 7.94 and 9.62) were noticed in all intervals under WS₁ stress regime and maximum branches (9.28) under AMF application, The interaction effects of water stress (WS₂) and bio inoculants have shown that the maximum number of leaves 119.8,137.67,143.27 with the treatment WS₂ x AMF at all intervals. maximum Leaf dry mass (2.98) under AMF application at 30 days interval. Maximum leaf area index was observed at 30 and 45DAP of 14.95cm²/g, 17.41 cm²/g compared to AMC and control. maximum RWC (64.9) was recorded at 45 DAP and at 60 DAP (6.3) during AMF application in comparison with control and AMC. maximum leaf electrolyte leakage observed 60 DAP (300 dSm) under WS₄ regime. maximum Chl a recorded at AMF treatment (1.504 mg) at 30 DAP. Chl a content under interaction of WS₂ X AMF showed best results (1.589mg). Maximum SPAD values (183.76,193.13,225.33,300.17s) noticed under 15,30,45,60 DAP under no waters stress condition. maximum flower production WS₂ water stress regimes at all harvest intervals (39.33, 48.67 and 56.67) were recorded. Among bio inoculants application maximum is observed with AMF inoculation observed (38.25, 47.25, and 57). Maximum flower fresh bio mass recorded at 3rd harvest and 19.62 g/plant at 4th harvest. Maximum flower dry mass observed at WS₁ stress regimes (1.61 g/plant) following WS₂ (1.91 g/plant), WS₃ (1.13), WS₄ (1.07 g/plant). The results suggest that, main plot Treatment (WS₂) and in some cases WS₁ stress regimes and Bio inoculant AMF (20 g/plant) proved to be best treatment.

Keywords: Calendula, water stress, AMF, AMC

Introduction

Calendula (*Calendula officinalis* L.) is an herbaceous plant belonging to the Asteraceae family (Compositae) with chromosome number 2n=32. In English, it is commonly known as "Pot Marigold." The name is derived from the Latin word "Calendae", which means "First Day of the Month" and it is originated in South Europe. The plant prefers bright light and well-drained rich soil. It grows best in moderately healthy soil with average drainage and a pH of 5-8, but it can be grown in a variety of soils. It can be grown as a winter seasonal in mild climates. Calendula is hardy, free-blooming annuals with lovely crowns that are grown for garden decoration and cut flowers. Calendula is a medium-sized plant with long coarse, hairy, and slightly sticky leaves, a flat capitula with broad florets, and a long stalk. The flower colour may be yellow, orange, or pale sulphur or dull white and the centre of capitulum may be dark. Their freshness brightens up any dull corner of the garden.

One of the most significant environmental stress factors is water scarcity, which is thought to pose a substantial risk to plant development and yield, particularly in arid and semi-arid places. It impacts a variety of physiological processes, including transpiration, photosynthesis, and cell elongation.

The most frequent environmental stresses that reduce plant output are lack of water or drought. Numerous metabolic mechanisms that support water acquisition and/or retention, safeguard chloroplast functions, and keep ion homeostasis in place and define a plant's capacity to survive a water deficit.

When there is a drought, the regulatory mechanisms that control net photosynthesis decrease chlorophyll content, stomatal conductance, and transpiration rates while increasing intercellular CO₂ levels (Anjum *et al.* 2011) [1]. According to the study on chrysanthemum, mild water stress led to an increase in the quantity and quality of flowers produced by plant, whereas severe water stress resulted in a decline in both (Korkmaz *et al.* 2019) [2]. The impact of water stress on the growth and yield of cut roses was the subject of another study by Korkmaz *et al.* (2019) [2]. Water stress dramatically decreased plant height, stem diameter, and flower yield, according to the study, which shows that it has a detrimental effect on the growth of cut roses.

In recent years the application of bio-inoculants *viz.*, VAM fungi have been is increasing of importance in soil fertility and phosphorus utilization efficiency of applied fertilizers. By spreading away from the root system and giving the adsorptive root system more radial root surface, soil hypha of VAM enables the root system to utilize a larger volume of soil for nutrients. Mycorrhiza is a soil born fungi, that forms symbiotic association with nearly all the plants of agricultural importance. In this symbiosis, plants supply about 10% of its photosynthetic carbon to the fungus in turn mycorrhiza mobilizes slowly diffusing mineral ions such as phosphates. Zn and Cu (Kheyrodin *et al.* 2014) [3]. In this relationship, the host plant transports 4–20% of its photosynthetic product in exchange for the mycorrhizal fungus supplying it with mineral nutrients like phosphate and nitrogen, amplifying the host plant's abiotic stress tolerance against conditions like drought, salinity, and heavy metals, and biotic stress resistance from different root pathogens.

Arka Microbial Consortium is a commercial microbial fertilizer released from ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, India. Arka microbial consortium is a mixture of various microorganisms that have been found to exhibit several beneficial properties, such as nutrient cycling, disease suppression, and improved plant growth. This consortium consists of various microorganisms, including bacteria, fungi, and other microorganisms. Arka microbial consortium has been studied extensively for its potential use in agriculture, particularly for the growth of crops. It has been found to enhance plant growth and productivity by increasing nutrient availability, improving soil structure, and suppressing plant pathogens. Several studies have demonstrated the potential of Arka microbial consortium in improving soil fertility and crop yield (Reddy *et al.* 2017) [4].

Materials and Methods

The present investigation “Improving the growth and flower yield of pot marigold (*Calendula officinalis* L.) Under varied water stress conditions using bio- inoculants” was carried out at PG Research block, College of Horticulture, Mojerla, Wanaparthy district, during Rabi, 2020-21. The experimental site College of Horticulture, Mojerla is situated at an altitude of 401m above mean sea level on 77°96' East longitudes and 16°36'North latitude and the climate is semi-arid. The experiment was conducted in a split plot design with 4 main

plot and 3 subplots with 12 treatments replicated thrice. The different water stress treatments used are WS₁ (Field capacity), WS₂ (75% Field capacity), WS₃ (50% Field capacity), WS₄ (25% Field capacity) and usage of different bio inoculants *viz.*, AMF (Arbuscular mycorrhiza fungi), AMC (Arka microbial consortium) and control (no treatment). Plant height (cm²). Branch production (Number/ plant), Number of leaves/plant (Number/plant), Leaf Area (cm²/plant), Leaf fresh weight (mg/cm²), Leaf dry mass (mg/cm²), Leaf Area Duration (LAD) (dm²/days), Leaf Area Index LAI (cm²/g), Flowering parameters, Flower production number/plot: weekly intervals, Flower fresh biomass/plot: weekly intervals, Flower dry biomass/ plot: weekly intervals, Plant water relations, Relative Water content (RWC), Electrolyte leakage (EL) mS/ppt, Plant pigments (Chlorophyll –a, b and a to b ratio) mg, SPAD readings were calculated.

Results and Discussion

Plant height (cm)

The bio-inoculants application had shown significant variation in plant height at 15, 45, and 60 DAP with maximum height in the treatment with Arbuscular Mycorrhizal fungus (AMF) –recording a plant height of 13.80 cm, 11.88 cm, 14.30 cm 13.76 cm and 15.30 cm 15.90 cm respectively. There was no significant difference in plant height among different water stress regimes at 15 and 30DAP. However, WS₁ (no water stress) regime had significantly higher plant height at 60 DAP (15.56 cm) than WS₂ (14.4 cm), WS₃ (14.35 cm), and WS₄ (14.35 cm) regimes. The interaction effects of Water stress and Bio-inoculants (WS x B) were studied at all intervals. Significant interaction effect between water stress (WS₂) and Arbuscular mycorrhizal (AMF) have shown maximum plant height of 13.80 cm, 14.30 and 15.30 cm during 15, 30 and at 60 DAP. The minimum plant height was observed in the interaction of water stress WS₄ X C and control and recorded a plant height of 9.33cm at 15 DAP and 8.07cm at 60 DAP. The possible reason for reduction in plant height at 60 DAP under stress treatments in calendula might be due reduction in cell elongation and division under water stress conditions (Abdul-Bake 1973) [5].

Number of branches

Significant variation in number of branches per plant across different water stress regimes at 30, 45 and 60DAP were recorded and maximum number of branches per plant was observed in the treatment WS₁(Irrigation up to field capacity) i.e.6.32,7.94 and 9.62 while minimum number of branches was recorded at water stress regime (WS₄ Irrigated at 75% DASM). However, there was no significant difference in number of branches per plant under different treatments at 15 days after planting. The influence of bio-inoculants had not shown significant variation in branch number. AMF treated plants had shown significantly a greater number of branches at 60 DAP with maximum branches of 9.28 when compared to AMC, control. The interaction between water stress (WS₂) and bio inoculants have shown that the maximum number of branches (6.97) and (8.03) was recorded in the treatment WS₂ x AMF at 30 DAP and 60 DAP. The minimum number of branches was observed under water stress WS₄ x Control (5.83) at 60 DAP. The possible reason for difference in resources distribution throughout the plant which may be the cause of the branch variation that occurs in Calendula as a

result of the application of stress and AMF. Similar results were earlier study by Wang *et al.* 2021 [6] investigated the effects of water stress and AMF on the growth and development of marigold (*Tagetes erecta* L.) and found that

water stress significantly reduced the number of branches in marigold, while AMF inoculation increased the number of branches under both well-watered and water stress conditions.

Table 1: Effect of water stress regimes and bio inoculants on plant height (cm) of *Calendula officinalis* at different intervals of plant growth

Factor	15DAP	30DAP	45DAP	60DAP
Water stress (WS)				
WS ₁	12.26	13.01	13.76	15.56 ^a
WS ₂	10.76	12.89	13.1	14.40 ^b
WS ₃	10.52	12.32	12.66	14.35 ^b
WS ₄	10.41	11.43	12.21	14.35 ^b
SEm±	0.50	0.49	0.44	0.24
CD at 5%	1.75	1.72	1.55	0.86
Bio inoculant (BI)				
Control	10.47 ^b	11.58	11.57 ^b	12.25 ^b
AMF	11.88 ^a	12.95	13.76 ^a	15.90 ^a
AMC	10.62 ^b	12.72	13.47 ^a	15.85 ^a
SEm±	0.32	0.58	0.60	0.39
CD at 5%	0.97	1.75	1.80	1.16
Interaction				
WS ₁ x C	9.50 ^c	12.83 ^b	12.83	12.83 ^b
WS ₁ X AMF W	11.97 ^c	12.00 ^b	15.00	13.00 ^b
WS ₁ X AMC	12.30 ^b	11.20 ^c	11.20	11.20 ^c
WS ₂ X C	12.13 ^c	12.81 ^c	12.81	12.81 ^b
WS ₂ X AMF W	13.80 ^a	14.30 ^a	13.30	15.30 ^a
WS ₂ X AMC	12.33 ^b	12.57 ^b	12.57	12.57 ^c
WS ₃ X C	10.63 ^b	12.60 ^c	12.60	12.60 ^b
WS ₃ X AMF W	13.10 ^a	9.07 ^b	9.07	9.07 ^c
WS ₃ X AMC	8.83 ^c	15.30 ^a	15.30	15.30 ^a
WS ₄ X C	9.33 ^c	8.07 ^c	8.07	8.07 ^c
WS ₄ X AMF W	13.30 ^a	14.43 ^a	14.43	14.43 ^a
WS ₄ X AMC	9.60 ^b	11.80 ^b	11.80	11.80 ^b
SEm±	0.64	1.17	1.20	0.78
CD at 5%	1.94	3.51	3.60	2.33
p-values				
WS	0.533ns	0.009	0.113ns	0.002*
BI	0.024*	0.224	0.002*	0.040*
WS x BI	0.000*	0.000*	0.047	0.006*

Table 2: Effect of water stress regimes and bio-inoculants on number of branches (per plant) of *Calendula officinalis* collected at different intervals

Factor	15 DAP	30 DAP	45 DAP	60 DAP
Water stress (WS)				
WS ₁	5.29	5.57 ^{ab}	7.14 ^{ab}	8.94 ^b
WS ₂	4.91	6.32 ^a	7.94 ^a	9.62 ^a
WS ₃	4.89	5.08 ^b	7.50 ^b	8.36 ^c
WS ₄	4.56	5.02 ^b	6.78 ^b	8.1 ^c
SEm±	0.33	0.33	0.12	0.16
CD at 5%	1.15	1.14	0.43	0.55
Bio inoculant (BI)				
Control	4.38	5.33	6.86	8.36 ^b
AMF	4.51	5.74	7.88	9.28 ^a
AMC	4.85	5.43	7.59	8.63 ^b
SEm±	0.26	0.17	0.17	0.23
CD at 5%	0.78	0.50	0.52	0.71
Interaction				
WS ₁ x C	5.13	6.93 ^b	8.27	7.77 ^c
WS ₁ X AMF W	6.77	3.67 ^c	7.77	8.27 ^a
WS ₁ X AMC	5.60	5.77 ^c	7.80	7.80 ^b
WS ₂ X C	3.47	5.23 ^b	6.47	6.47 ^c
WS ₂ X AMF W	4.73	6.97 ^a	8.03	8.03 ^b
WS ₂ X AMC	6.63	5.82 ^b	8.13	8.13 ^a
WS ₃ X C	5.53	4.40 ^b	6.87	6.87 ^c
WS ₃ X AMF W	5.13	6.90 ^a	8.10	8.10 ^a
WS ₃ X AMC	2.37	3.93 ^c	7.53	7.53 ^b

WS ₄ X C	3.37	4.73 ^c	5.83	5.83 ^c
WS ₄ X AMF W	5.40	6.40 ^a	7.60	7.60 ^a
WS ₄ X AMC	4.80	4.93 ^b	6.90	6.90 ^b
SEm±	0.52	0.33	0.35	0.47
CD at 5%	1.56	1.01	1.04	1.43
P-values				
WS	0.533	0.049*	0.003*	0.002*
BI	0.124	0.224	0.002*	0.040*
WS x BI	0.111	0.000*	0.047	0.006*

Number of leaves per plant

The number of leaves had not shown significant variation across 15,30,45,60 DAP in all water stress regimes i.e., WS₁, WS₂, WS₃ and WS₄. Bio inoculants application showed significant variation at 15, 60 DAP with maximum number of leaves with application of AMF (76.96), (103.56) application and least number of leaves found under application of AMC and control. The interaction effect of water stress and AMF can be explained by the study conducted by Porcel *et al.* (2012) [7] who found that AMF colonization increased shoot biomass and leaf area in tomato plants under water stress

conditions. Similarly, Al-Karaki (2006) [9] observed that AMF-inoculated wheat plants had a higher number of leaves compared to non-inoculated plants under water stress conditions. The interaction effect of water stress and AMF can be explained by the study conducted by Porcel *et al.* (2012) [8] who found that AMF colonization increased shoot biomass and leaf area in tomato plants under water stress conditions. Similarly, Al-Karaki (2006) [9] observed that AMF-inoculated wheat plants had a higher number of leaves compared to non-inoculated plants under water stress conditions.

Table 3: Effect of water stress regimes and bio-inoculants on number of leaves per plant of *Calendula officinalis* collected at different intervals

Factor	15 DAP	30 DAP	45 DAP	60 DAP
Water stress (WS)				
WS ₁	74.07	85.37	100.62	111.64
WS ₂	72.14	79.49	83.27	107.72
WS ₃	68.64	79.08	82.33	103.17
WS ₄	54.66	74.4	75.73	92.31
SEm±	27.39	8.77	9.39	4.11
CD at 5%	35.24	30.07	32.52	14.25
Bio inoculant (BI)				
Control	56.28 ^b	70.68	76.5	92.79 ^b
AMF	76.96 ^a	92.46	90.35	103.56 ^a
AMC	68.89 ^a	75.61	89.62	91.78 ^b
SEm±	11.65	8.32	4.31	3.93
CD at 5%	19.98	24.231	12.92	11.80
Interaction				
WS ₁ x C	72.42 ^b	60.23 ^b	81.27	97.87 ^c
WS ₁ X AMF W	65.93 ^b	100.93 ^a	96.67	105.97 ^{bc}
WS ₁ X AMC	83.87 ^b	58.21 ^b	123.93	93.84 ^c
WS ₂ X C	37.24 ^e	63.66 ^b	75.13	102.67 ^c
WS ₂ X AMF W	119.87 ^a	137.67 ^a	96.8	143.27 ^a
WS ₂ X AMC	59.37 ^c	73.93 ^b	77.87	114.53 ^b
WS ₃ X C	76.22 ^b	63.00 ^b	76.27	65.91 ^e
WS ₃ X AMF W	91.07 ^b	94.03 ^{ab}	91.87	128.57 ^{ab}
WS ₃ X AMC	67.67 ^b	85.21 ^b	78.87	115.03 ^b
WS ₄ X C	39.33 ^{de}	58.57 ^b	73.33	104.73 ^c
WS ₄ X AMF W	89.97 ^c	93.37 ^b	76.07	96.43 ^b
WS ₄ X AMC	64.67 ^b	81.27 ^b	77.86	95.77 ^c
SEm±	7.77	16.64	8.62	7.84
CD at 5%	23.31	49.91	25.84	23.60
P-values				
WS	0.378	0.85	0.363	0.067
BI	0.006*	0.185	0.065	0.007*
WS x BI	0.000*	0.049*	0.131	0.000*

Relative water content (%)

Relative water content not shown significant variation across water stress regimes at 15 and 30 and 60 DAP except at 45 DAP with significantly more results in ws1 and ws2 regimes. Relative water content is significantly more at 45, 60 DAP with 66.91, 63.32. under Bio inoculants Bio inoculants had not shown significant variation at 45, 60 DAP. AMF treated plant shown significantly more relative water content (60.9) at 45

DAP and at 60 DAP (63.32) in comparison with control and AMC.

The interaction effects of Water stress and Bio inoculants are studied at all intervals studied.

The interaction effect of water stress (WS₁. maintained near field capacity) and AMF showed more relative water content (60.49) while least relative water content was observed under water stress WS₄ (irrigated at 75% DASM) and Control also

interaction effect at 60 DAP is also more in water stress (WS₁) and AMF (51.53) and least interaction effect was observed under water stress (WS₄) and control at 60 DAP (57.94). The positive effect of AMF on RWC could be attributed to several factors. AMF can increase the root surface area and volume, which in turn enhances water and nutrient uptake efficiency. AMF can also enhance the production of plant growth-promoting hormones, such as abscisic acid (ABA), which regulates stomatal closure and reduces water loss. Additionally, AMF can induce the production of osmolytes, such as proline and soluble sugars, which protect plants from dehydration and oxidative stress (Saleem *et al.*, 2018) [10].

Table 4: Effect of water stress regimes and bio-inoculants on relative water content of *Calendula officinalis*

Factor	15DAP	30DAP	45DAP	60DAP
Water stress (WS)				
WS ₁	80.19	70.91	60.75 ^a	60.08
WS ₂	70.81	70.22	60.32 ^a	50.48
WS ₃	60.42	50.97	50.55 ^b	50.16
WS ₄	60.18	50.89	50.31 ^b	50.11
SEm±	0.64	0.51	6.35	6.58
CD at 5%	2.24	1.77	10.24	12.00
Bio inoculant (BI)				
Control	60.17	50.66	60.82 ^b	59.12 ^b
AMF	70.93	70.93	66.91 ^a	63.32 ^a
AMC	70.32	60.65	64.21 ^{ab}	57.95 ^{ab}
SEm±	0.79	0.71	5.51	4.48
CD at 5%	2.37	2.13	6.54	6.44
Interaction				
WS ₁ x C	66.06	8898	53.64 ^c	54.55 ^c
WS ₁ X AMF W	61.3	66.78	60.49 ^a	51.53 ^a
WS ₁ X AMC	70.2	77.96	56.13 ^b	56.75 ^b
WS ₂ X C	60.14	54.22	54.06 ^c	55.35 ^b
WS ₂ X AMF W	72.96	68.39	60.27 ^a	57.26 ^a
WS ₂ X AMC	69.3	69.05	55.81 ^b	53.83 ^c
WS ₃ X C	74.96	65.9	55.79 ^a	52.11 ^b
WS ₃ X AMF W	77.59	67.72	54.41 ^c	56.94 ^a
WS ₃ X AMC	66.64	64.3	55.47 ^b	51.85 ^c
WS ₄ X C	74.52	63.53	54.62 ^b	52.90 ^c
WS ₄ X AMF W	64.88	68.83	53.81 ^{ab}	54.47 ^b
WS ₄ X AMC	66.14	65.31	54.28 ^c	57.94 ^a
SEm±	1.58	1.42	4.03	5.96
CD at 5%	4.75	4.27	6.09	5.89
P-values				
WS	0.169	0.082	0.09	0.641
BI	0.307	0.111	0.034*	0.012*
WS x BI	0.282	0.104	0.001*	0.000*

Electrolyte leakage (μ Siemens g⁻¹ cm⁻¹)

Electrolyte leakage had not shown any significant variation across water stress regimes at 15, 30, 45 and 60 DAP. Bioinoculants also had not shown any significant variation at 15,30, 45DAP. However, AMF treated plants shown significantly more electrolyte leakage at 15 DAP (131.08 dSm) comparison with control and AMC.

The interaction effects of Water stress and Bio inoculants are studied at all intervals studied. The interaction of water stress (WS₄) and AMF showed that more electrolyte leakage at 60 DAP (270.07 dSm) and least interaction is seen under water stress (WS₂ -irrigated at 75% DASM)) and control at 15 DAP which is the indication of water stress regime. AMF can also improve the water-holding capacity of soil, leading to improved water availability for plants. As a result, AMF-

treated plants can maintain their cell membrane integrity and reduce electrolyte leakage under water stress conditions (Munir *et al.* 2021) [11].

SPAD readings (SPAD units)

SPAD readings had shown significantly more variation across all intervals 30,45and 60 DAP with highest SPAD values at water stress regimes of WS₂ (29.56), (33.22), (34.73). The highest significant variation was seen under WS₂ stress regime followed by WS₁, WS₃ and the least SPAD readings was seen under WS₄ stress regime (25.74).

Bio inoculants had shown significant variation with the highest SPAD values recorded under at 30, 45, 60 DAP under treatment with Arbuscular mycorrhizal Fungi (30.9, 33.47, 35.28) followed by Arka Microbial Consortium and the least is observed under control.

Table 5: Effect of water stress regimes and bio-inoculants of SPAD units readings of *Calendula officinalis* collected at different intervals

Factor	30DAP	45DAP	60DAP
Water stress (WS)			
WS ₁	26.77 ^{ab}	31.22 ^{ab}	31.31 ^{ab}
WS ₂	29.56 ^a	33.22 ^a	34.78 ^a
WS ₃	26.98 ^b	31.38 ^{ab}	32.24 ^b
WS ₄	25.74 ^b	26.01 ^b	26.97 ^c
SEm±	0.57	0.81	0.55
CD at 5%	1.98	2.82	1.90
Bio inoculant (BI)			
Control	25.86 ^b	27.52 ^b	31.21 ^b
AMF	30.91 ^a	33.47 ^a	35.28 ^a
AMC	27.28 ^b	30.98 ^a	31.98 ^b
SEm±	0.96	0.89	0.46
CD at 5%	2.88	2.66	1.39
Interaction			
WS ₁ x C	23.97 ^d	32.21 ^a	37.67 ^b
WS ₁ X AMF W	35.23 ^a	36.23 ^a	40.91 ^a
WS ₁ X AMC	30.10 ^b	31.23 ^{ab}	36.33 ^b
WS ₂ X C	22.93 ^d	29.63 ^b	32.53 ^d
WS ₂ X AMF W	35.97 ^a	33.70 ^a	35.33 ^b
WS ₂ X AMC	29.77 ^b	32.67 ^a	33.47 ^c
WS ₃ X C	23.00 ^d	30.47 ^b	31.47 ^d
WS ₃ X AMF W	31.91 ^{ab}	32.21 ^a	33.33 ^{cd}
WS ₃ X AMC	26.03 ^c	31.47 ^a	31.93 ^d
WS ₄ X C	33.53 ^a	17.77 ^c	23.17 ^f
WS ₄ X AMF W	20.5 ^d	31.73 ^a	31.53 ^d
WS ₄ X AMC	23.2	28.53	26.24
SEm±	1.92	1.78	0.93
CD at 5%	5.76	5.33	2.79
p-values			
WS	0.006*	0.003*	0.000*
BI	0.006*	0.001*	0.000*
WS x BI	0.000*	0.031*	0.038*

Flower production (number /plot)

Flower production number had shown significant variation across water stress regimes at first harvest to 3rd harvests. Significantly more flower production is noticed WS₂ water stress regimes at all harvest intervals (39.33, 48.67 and 56.67) except at 4th time harvest. Bio inoculants also shown significant variation across 1st harvest to 3rd harvest DAP with AMF inoculation high significant values observed (38.25, 47.25, 57). The interaction effects of Water stress and Bio inoculants are studied at all intervals studied and found best results under interaction of WS₁ x AMF and WS₂ x AMF values were on par from 1st harvest to 4th harvest. The

possible reason for significance variation in flower production is the primary functions of AMF are to enhance nutrient uptake by plants, particularly phosphorus, which is essential for flower development. Under water stress conditions, plant

growth and nutrient uptake are often limited, but AMF can help overcome these limitations by facilitating the absorption of nutrients and water from the soil (Fahad *et al.* 2015) [12].

Table 6: Effect of water stress regimes and bio-inoculants of Flower production (number/plot) of *Calendula officinalis*

Factor	1 harvest	2 harvest	3 harvest	4 harvest	Total harvest
Water stress (WS)					
WS ₁	39.67 ^a	45.00 ^b	57.00 ^a	62.00	219 ^a
WS ₂	39.33 ^{ab}	48.67 ^a	56.67 ^{ab}	65.00	206 ^b
WS ₃	31.67 ^c	38.67 ^c	48.33 ^c	56.67	175.3 ^c
WS ₄	29.33 ^d	37.67 ^{cd}	41.67 ^d	51.67	162.3 ^d
SEm±	0.33	0.42	0.45	0.55	0.12
CD at 5%	1.14	1.47	1.56	1.93	2.70
Bio inoculant (BI)					
Control	32.00 ^c	36.00 ^c	45.00 ^c	51.50	164.5 ^c
AMF	38.25 ^a	47.25 ^a	57.00 ^a	51.75	213.25 ^a
AMC	34.75 ^b	44.25 ^b	50.75 ^b	64.75	194 ^b
SEm±	0.28	0.39	0.46	0.49	0.33
CD at 5%	0.85	1.19	1.38	1.47	2.19
Interaction					
WS ₁ x C	36.01 ^c	42.98 ^d	50.01 ^d	62.23	190 ^d
WS ₁ X AMF W	43.78 ^a	54.12 ^a	67.89 ^a	86.90	250 ^a
WS ₁ X AMC	40.12 ^b	50.22 ^b	54.23 ^c	74.12	218 ^c
WS ₂ X C	37.89 ^c	36.67 ^e	51.98 ^d	54.02	178 ^f
WS ₂ X AMF W	42.01 ^a	52.78 ^{ab}	60.23 ^b	72.34	226 ^b
WS ₂ X AMC	39.99 ^b	47.01 ^c	59.22 ^b	69.11	214 ^c
WS ₃ X C	29.01 ^e	34.87 ^{ef}	43.01 ^f	47.90	152 ^h
WS ₃ X AMFW	36.01 ^c	42.12 ^d	52.08 ^{cd}	64.76	194 ^d
WS ₃ X AMC	30.12 ^e	40.98 ^d	50.12 ^d	59.22	179 ^{ef}
WS ₄ X C	26.27 ^f	32.23 ^f	36.23 ^h	43.19	137 ⁱ
WS ₄ X AMFW	32.23 ^d	41.12 ^d	49.43 ^e	61.08	183 ^e
WS ₄ X AMC	30.09 ^e	40.23 ^d	40.13 ^g	57.23	167 ^g
SEm±	36.01 ^c	42.98 ^d	50.01 ^d	62.23	0.85
CD at 5%	43.78 ^a	54.12 ^a	67.89 ^a	86.90	4.39
P-values					
WS	0.000*	0.001*	0.000*	0.123	0.000*
BI	0.000*	0.000*	0.000*	0.101	0.000*
WS x BI	0.032*	0.003*	0.003*	0.121	0.000*

Conclusion

In the present study on *Calendula officinalis* L. under water stress significantly reduced all plant growth parameters and flower yield. However maximum plant growth and yields were obtained under 25% water stress (WS₂) and WS₁ (near field capacity) conditions combined with application of Arbuscular mycorrhizal fungi. Thus, it can be concluded that application of arbuscular mycorrhizal fungi @ 20 g/ plant proved to be the best treatment with improvement in plant growth, water relations and flowering under both no stress and water stress conditions in *Calendula officinalis* L.

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