Evaluation of potting media for marigold under salinity stress condition

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Abstract

Salinity is a major environmental factor limiting agricultural production in the arid and semi-arid regions of the world. Capillary barrier (CB) created from substrate structuring is a technique that can potentially be used to mitigate salinity, however, little has been done to evaluate the use of structured potting media for this purpose. This study evaluated the effects of CB structured potting media on the growth and physiology of marigold under salinity stress. These configurations used the following substrates: vermiculite (V), perlite (P), sand (S), and peat moss (PM) that were stacked in a pot in three layers from top to bottom: V-P-V, V-S-V, and V-P-PM. Four salinity levels of irrigation water were applied to each of the three configurations that included (Control (Desalinated, ECi $\approx$ 0.6 dS m^{-1}), 3 dS m^{-1}, 6 dS m^{-1}, and 9 dS m^{-1}). The results showed that salinity adversely affects the vegetative, reproductive and physiological parameters of marigold. However, the best growth of marigolds was for those grown in the layered V-S-V media. The layered V-S-V media was the best media among the tested media that can mitigate salinity stress of marigold. The outcome from this study is important for farmers and nursery growers in many arid areas where saline water is the only available irrigation source.

Key words: Capillary barrier, substrates, electrical conductivity, volumetric water content, ornamentals, plant growth, salinity

Introduction

Salinity is one of the most devastating environmental factors limiting agricultural production in the world and mainly in arid and semi-arid regions. Globally, more than 45 million hectares of irrigated lands are affected by salinity, and about 1.5 million hectares are lost annually to soil salinity (Munns and Tester, 2008). Furthermore, It is estimated that globally 23 % of total cultivated area and 25 % - 30 % of irrigated agricultural lands are affected by high salinity (Shahid *et al.*, 2018). In Oman, approximately 44 % of the total area and 70 % of the agricultural area of the country are affected by salinity (Al-Rawahy *et al.*, 2010). The affected land area in Al-Suwaq (a town in Al-Batinah-North Governorate of Oman) increased from 15.8 % in 1974 to 25.1 % in 2014 (Kalbus *et al.*, 2014). Low rainfall and high temperatures together are conducive for the accumulation of salts. These conditions are predominant in Oman. Additionally, due to the agricultural expansion in Oman, mainly in the coastal area of Al-Batinah area since the 1980s, over-pumping of groundwater in the coastal area led to seawater intrusion and increased saline coastal lands (Zekri, 2008).

Salinity adversely affects plants in several ways. It can cause water deficit through osmotic effects, ion toxicity, nutritional imbalance, oxidative stress, membrane disorganization, change in metabolic processes, reduction of cell division and expansion, genotoxicity (Hasegawa *et al.*, 2000; Nemoto and Sasakuma, 2002; Munns, 2002; Zhu *et al.*, 2007; Kim *et al.*, 2008). Together, these effects reduce plant growth, development, and productivity.

Several techniques and practices are used to mitigate the problem of salinity and its effect on plants such as increasing organic matter in soil, selecting salt-tolerant crops, choosing irrigation system with uniform application, high efficiency of irrigation (drip irrigation and mini-sprinklers), more frequent irrigation with lower quantities of water (MAF and ICBA, 2012). Capillary barrier (CB) is one of the potential techniques that can be used to mitigate the effects of salinity stress on cultivated plants. CB is a composition of two soil layers having distinct differences in textural and, therefore, hydraulic characteristics. CB technique can be used to maximize crop production by improving root zone condition for cultivating crops under harsh climatic conditions such as: high temperature (Kacimov *et al.*, 2017), drought (Al-Ismaily *et al.*, 2013; Al-Maktoumi *et al.*, 2014), salinity and poor quality irrigation water (Ityel *et al.*, 2012).

Most studies on the capillary barrier (CB) were conducted on native soils and few studies on potting media. Potting media mostly contain a mixture of organic and mineral components. Cultivating in potting media is favorable for many vegetable and ornamental crop production systems because they consist of components that provide physical support to plants, enhance good drainage and root aeration, and can hold water and nutrients and easily provide them to the plants (Dumroese *et al.*, 2009).

There are four common components of potting media: peat moss, perlite, vermiculite, and sand. The texture and properties of plant growing substrates are different. Peat moss and vermiculite possess higher water holding capacity than sand and perlite, whereas perlite and sand possess a higher infiltration rate. Also, sand and perlite have macro-pores which can improve salt leaching from the root zone and provide them proper aeration (Jacques and Walden, 2005; Phogat *et al.*, 2015).

Potting media are substrates that are commonly used by growers in greenhouses and nurseries. Al-Mazrouai *et al.* (2019) found

that marigold grown in layered potting media were better than ones grown in mixed potting media. The authors found that the layered (vermiculite-perlite-vermiculite, V-P-V), (vermiculite-sand-vermiculite, V-S-V) and (vermiculite-perlite-peat moss, V-P-PM) from top to bottom were the best media for growing marigold under no-stress condition.

Many countries in arid and semi-arid climates suffer from salinity and it constitutes the main source of irrigation water (Abuelgasim and Ammad, 2019). This study was aimed to investigate the effects of layered potting media on marigold (*Tagetes erecta* L.) growth and productivity using saline irrigation water.

Materials and methods

Site: The experiment was conducted in a glasshouse located at the Agricultural Experiment Station (AES) (23°59'N, 58°16'E) at Sultan Qaboos University (SQU), Al-Khoud, Seeb, Muscat, Oman.

Treatments: Two-weeks old uniform marigold (*Tagetes erecta* L.) 'Taishan Orange' seedlings (approx. 5 cm tall) were transplanted and grown in three configurations of layered potting media. These configurations were vermiculite-perlite-vermiculite (V-P-V), vermiculite-sand-vermiculite (V-S-V) and vermiculite-perlite-peat moss (V-P-PM) based on the volume of the container (Fig. 1). Plants were subjected to four salinity treatments of irrigation water: Control (desalinated, ECi ≈ 0.6 dS m^{-1}), 3 dS m^{-1}, 6 dS m^{-1}, and 9 dS m^{-1}. Three replicates for each salinity treatment per media configuration were used for this study. Therefore, the total number of pots were 36 with one marigold seedling planted in each pot.

Irrigation: Pots were irrigated weekly at a rate of 1 L per week. Initially, all plants were irrigated with freshwater (Desalinated,
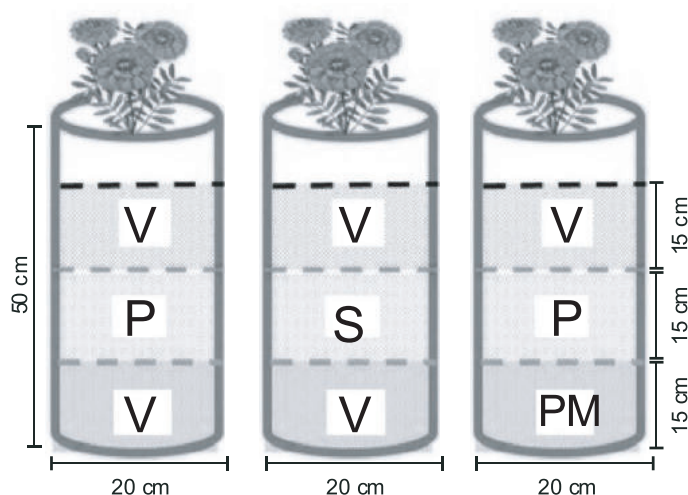

Fig. 1. The different configurations of layered media used in the experiment (V=vermiculite, *P*= Perlite, S= sand, and PM= peat moss).

ECi ≈ 0.6 dS m^{-1}) for two weeks. Then, they were subjected to one of three salinity treatments of irrigation water (ECi= 3 dS m^{-1}, 6 dS m^{-1}, and 9 dS m^{-1}) until the end of the experiment.

Moisture content measurement: Moisture content sensors (EC-5, Decagon, Pullman, WA) connected to EM50-data loggers were installed in the middle layers of two replicates per treatment. The data loggers were programmed to record the media volumetric water content (θ_v) every hour.

Plant measurements: Plant measurements included the followings: maximum plant height, canopy width, number of flowers, flower size (diameter), leaf chlorophyll content, and chlorophyll fluorescence. Root length and fresh and dry weight of shoots and roots were measured on the harvested plants. In addition, root growth pattern was observed at the end of the experiment. Chlorophyll content was measured using SPAD 502 (Minolta, Japan) device while chlorophyll fluorescence was measured using Fluor Pen FP100 (Photon Systems Instruments, Brno, Czech Republic). Three fully expanded leaves were selected randomly from three plants in each treatment to measure chlorophyll content and chlorophyll fluorescence; measurements were done every two weeks. The Electrical Conductivity of saturated Paste extract (ECe) and (pH) of the different layers of substrates in each pot (vermiculite, sand, perlite, and peat moss) were measured using electrical conductivity meter at room temperature (Oakton waterproof/pcstestr 35 pH/conductivity/TDS/salinity Tester, Oakton, USA).

Data analysis: A two-factor, completely randomized design was used for this experiment. The data were subjected to analysis of variance (ANOVA) using the PROC ANOVA of SAS version 9.4 (SAS Inst. 2018, Carey, N.C., USA). Mean separation was done using the Duncan Multiple Range Test (DMRT) at *P*=0.05.

Results

Moisture content of the media at different salinity levels: The volumetric water content (θ_v) of vermiculite-perlite-vermiculite (V-P-V) media increased with the time. The vermiculite-perlite-peat moss (V-P-PM) media also showed an increase in θ_v in the first six weeks; then it remained constant within the range of 0.10 to 0.19 m^{3}m^{-3}. Unlike V-P-V and V-P-PM, the θ_v of the vermiculite-sand-vermiculite (V-S-V) layered media was almost constant throughout the growing period (Fig. 2). In general, the V-P-PM showed the highest θ_v (0.07 m^3 m^{-3}) followed by V-P-V (0.05 m^{3}m^{-3}) and V-S-V (0.02 m^{3}m^{-3}). Moreover, as the irrigation water salinity increased, the media moisture content increased particularly in V-P-V and V-P-PM media (Table 1).

Media electrical conductivity (ECe) and pH: The electrical conductivity and pH of the substrates used in this study (sand, perlite, vermiculite, and peat moss) were tested before running the experiment using the saturated extracted method. Results indicated that all substrates (except peat moss) showed alkaline pH. The pH of vermiculite, sand, and perlite was almost the same (ranging from 7.7 to 8). Peat moss was more acidic than other substrates and had a pH of 3.5. All substrates were low in salinity (ECe < 0.5 dS m^{-1}) (Table 2).

The top layer was significantly higher in ECe than the middle and bottom layers. The ECe of the middle and bottom layer was not significantly different from each other (Fig. 3A). On the other hand, the bottom layer showed the highest acidity (pH=6.7) followed by the top layer (pH=7.3) and the middle layer (pH=7.7), respectively.

Electrical conductivity (ECe) was significantly different among media treatments particularly at ECi= 6 dS m^{-1} and 9 dS m^{-1}(Table 3). Results showed an increase in ECe as salinity level of irrigation water increased. No significant differences in ECe between the different media at control and 3 dS m^{-1} were detected. At ECi=
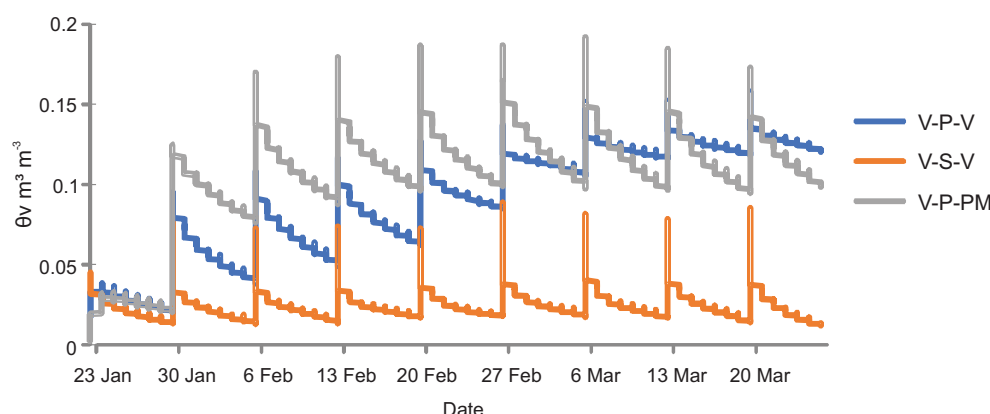
Fig. 2. Time series of volumetric water content (θv) of layered potting media vermiculite-perlite-vermiculite (V-P-V), vermiculite-sand-vermiculite (V-S-V), and vermiculite-perlite-peat moss (V-P-PM).

Table 1. Volumetric water content (θ_y) of layered potting media vermiculite-perlite-vermiculite (V-P-V), vermiculite-sand-vermiculite (V-S-V), and vermiculite-perlite-peat moss (V-P-PM) at different salinity levels

Media	Salinity Levels				Average
	Control	3 dS m^{-1}	6 dS m^{-1}	9 dS m^{-1}	
V-P-V	0.079±0.003C[b]	0.091±0.009BC[b]	0.114±0.003AB[b]	0.127±0.007A[b]	0.103±0.007[b]
V-S-V	0.018±0.008A[c]	0.022±0.003A[c]	0.025±0.002A[c]	0.035±0.004A[c]	0.025±0.003[c]
V-P-PM	0.110±0.006C[a]	0.118±0.003C[a]	0.140±0.001B[a]	0.190±0.009A[a]	0.139±0.012[a]

Different uppercase letters within the same row indicate significant difference among salinity levels and different lowercase letters within same column indicate significant difference among media at $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT).

Table 2. Electric conductivity (ECe) and pH of the substrates used in the study

Substrates	pH	ECe (dS m^{-1})*
Vermiculite	7.7±0.1 [a]	0.40±0.01[b]
Sand	7.9±0.1[a]	0.38±0.01[b]
Perlite	8.0±0.1[a]	0.33±0.00[c]
Peat moss	3.5±0.1[b]	0.47±0.01[a]

*ECe=Electrical conductivity of saturated substrates-paste extract. Different letters within the same column indicate significant difference among substrates at $P \leq 0.05$ using Duncan's Multiple Range Test

6 dS m^{-1}, V-P-V media showed the highest ECe (11.200 dS m^{-1}) followed by V-S-V (9.70 dS m^{-1}) and V-P-PM (8.31 dS m^{-1}). At ECi= 9 dS m^{-1}, V-P-V also showed highest ECe (12.68 dS m^{-1}) followed by V-S-V (10.42 dS m^{-1}) and V-P-PM (9.83 dS m^{-1}).

The three media showed no significant difference in the ECe of their top layer at all salinity levels of irrigation water. The middle layer of V-S-V medium showed the lowest ECe at all salinity levels of irrigation water, whereas there was no significant difference in the ECe of the middle layer of V-P-V and V-P-PM media at all salinity treatments. The bottom layer of the V-P-PM showed the lowest ECe at all salinity treatments, whereas, there was no significant difference between the V-P-V and V-S-V media in the ECe of the bottom layer at ECi= control, 6 dS m^{-1}, and 9 dS m^{-1}. Only at ECi= 3 dS m^{-1}, the bottom layer of the V-P-V showed less ECe (Table 3).

For pH, there was no significant change as the salinity level of irrigation water increased. The V-P-PM media showed the lowest pH among all media at all salinity levels. The V-P-V and V-S-V media were not significantly different from each other in their pH at all salinity level.

The pH of the top layers (vermiculite) of the three media was not significantly different from each other at all salinity treatments.
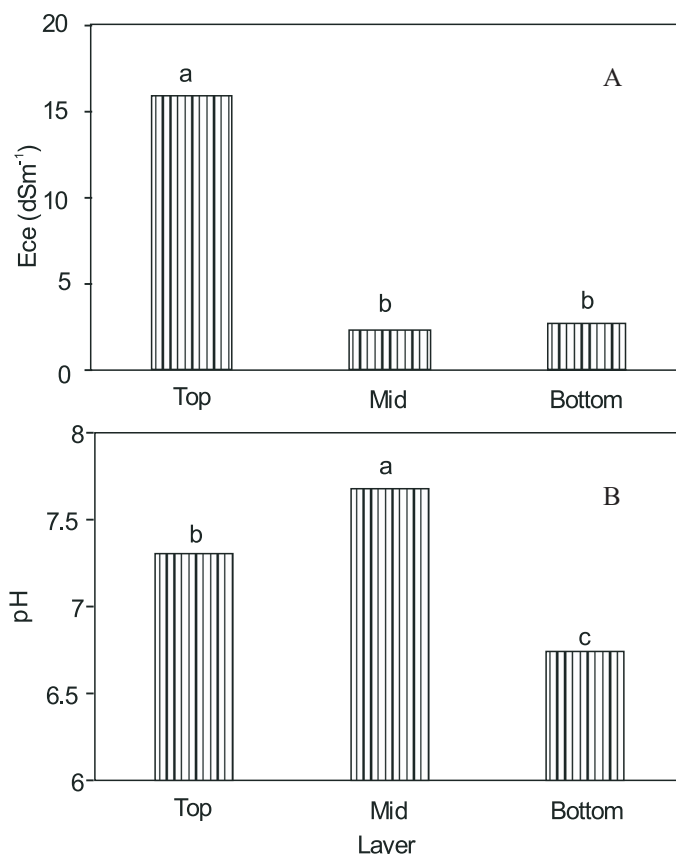

Fig. 2. Measurements of (A) electric conductivity (ECe) and (B) pH of the top, middle and bottom media layers

The pH of the middle layers (perlite) of the V-P-V and V-P-PM was not statistically different from each other at ECi= control, 3 dS m^{-1}, and 6 dS m^{-1} salinity level, however at ECi= 9 dS m^{-1}, the V-P-V middle layer showed higher pH than V-P-PM middle layer.

The bottom layer of V-P-PM resulted in the lowest pH among the bottom layers of the three media used in this study. The pH

of the bottom layer (vermiculite) of V-P-V and V-S-V was not significantly different at all salinity levels of irrigation water (ECi).

Flowering of the marigold: Salinity caused a reduction in the number and size of flowers of marigold grown in the three layered media. The different media showed no significant difference in the number and size of flowers. However, only at ECi=6 dSm^{-1} the size of flowers in the V-P-PM media (5.05 cm) was significantly smaller than flowers in the V-S-V (6.10 cm) and V-P-V (6.04 cm) media.

Vegetative growth of marigold: Salinity negatively impacted all of the vegetative parameters of marigold grown in all three media. However, the lowest impact was in marigolds which were grown in the V-S-V media which was the best media among the tested media in term of vegetative growth.

The results in Table 5 showed that at control, V-S-V medium resulted in highest shoot fresh and dry weight (106.23 g, 13.28 g), root dry weight (27.33 g), root length (73.00 cm), canopy width (28.83 cm), and leaf area (25.05 cm^2). The V-P-PM

medium showed higher root dry weight (19.65 g) than V-P-V medium (12.68 g) whereas V-P-V medium resulted in higher root length (61.67 cm) than V-P-PM medium (47.33 cm). There was no significant difference between the three media in root fresh weight and plant height.

At ECi= 3 dS m^{-1}, the V-S-V medium had the highest root length (56.67 cm) followed by V-P-V (52.00 cm) then V-P-PM medium (44.33 cm). There was no significant difference between the three media in all other vegetative parameters.

At ECi= 6 dS m^{-1}, V-S-V medium showed the highest root length (57.33 cm) and plant height (2083 cm). The V-S-V and V-P-V media resulted in highest canopy width (19.17 cm, 19.50 cm) and leaf area (17.14 cm^2, 15.59 cm^2). There was no significant difference between the three media in the other vegetative parameters.

At ECi= 9 dS m^{-1}, V-S-V also showed the highest root length (44.00 cm) and plant height (19.17 cm). The root length of marigold grown in V-P-V medium (31.00 cm) was higher than their root length when grown in V-P-PM (25.33 cm). However,

Table 3. Electrical conductivity (ECe) and pH of the top, middle and bottom layers of vermiculite-perlite-vermiculite (V-P-V), vermiculite-sand-vermiculite (V-S-V), and vermiculite-perlite-peat moss (V-P-PM) irrigated with different salinity levels.

Salinity (ECi)[*z]	Media	Top		Middle		Bottom		Average	
		ECe (dS m^{-1})[*y]	pH	ECe (dS m^{-1})	pH	ECe (dS m^{-1})	pH	ECe (dS m^{-1})	pH
Control	V-P-V	1.06±0.26 C^a	7.42±0.38 A^a	0.83±0.08 D^a	7.57±0.16 A^{ab}	1.09±0.23 B^a	7.83±0.03 A^a	0.99±0.08 C^a	7.61±0.14 A^a
	V-S-V	1.17±0.11 C^a	7.52±0.19 A^a	0.04±0.03 D^b	7.94±0.01 B^a	1.14±0.04 C^a	7.68±0.03 C^a	0.78±0.01 B^a	7.71±0.08 A^a
	V-P-PM	1.26±0.13 C^a	7.23±0.41 A^a	0.75±0.06 D^a	7.42±0.18 A^b	0.38±0.02 C^b	4.95±0.25 A^b	0.79±0.14 C^a	6.53±0.42 A^b
3 dS m^{-1}	V-P-V	15.25±0.97 B^a	7.16±0.24 A^a	2.21±0.11 C^a	7.33±0.10 A^b	2.13±0.16 B^a	7.61±0.08 A^a	6.53±2.2 BC^a	7.37±0.16 A^a
	V-S-V	12.69±0.41 B^a	7.24±0.29 A^a	0.79±0.04 C^b	8.18±0.03 AB^a	3.69±0.11 B^a	7.80B±0.10 C^a	5.72±1.79 A^a	7.74±0.10 A^a
	V-P-PM	14.64±1.12 B^a	6.96±0.35 A^a	1.91±0.22 C^a	7.42±0.33 A^b	0.88±0.00 B^c	4.88±0.46 A^b	5.81±2.22 B^a	6.42±0.44 A^b
6 dS m^{-1}	V-P-V	24.90±2.48 A^a	7.09±0.18 A^a	4.16±0.27 B^a	7.63±0.24 A^b	4.54±0.41 A^a	7.60±0.33 A^a	11.20±3.50 AB^a	7.44±0.15 A^a
	V-S-V	22.27±2.15 A^a	7.51±0.05 A^a	1.24±0.07 B^b	8.26±0.04 A^a	5.59±0.42 A^a	7.88±0.02 AB^a	9.70±3.27 A^{ab}	7.88±0.11 A^a
	V-P-PM	20.07±0.06 A^a	7.48±0.21 A^a	3.76±0.04 B^a	7.41±0.05 A^b	1.09±0.29 AB^b	4.52±0.20 A^b	8.31±2.96 AB^a	6.47±0.49 A^b
9 dS m^{-1}	V-P-V	26.8±3.67 A^a	7.48±0.06 A^a	5.45A^a±0.10	7.52±0.04 A^b	5.80±0.74 A^a	7.95±0.05 A^a	12.68±3.54 A^a	7.65±0.16 A^a
	V-S-V	23.62±4.32 A^a	7.37±0.26 A^a	1.53A^b±0.11	8.31±0.16 A^a	6.10±0.47 A^a	8.05±0.026 A^a	10.42±3.41 A^b	7.91±0.08 A^a
	V-P-PM	22.89±5.12 A^a	7.27±0.23 A^a	5.21A^a±0.07	7.16±0.01 A^c	1.40±0.14 A^b	4.18±0.06 A^b	9.83±3.35 A^b	6.20±0.51 A^b
Average	V-P-V	17.00±3.13^a	7.29±0.12^a	3.16±0.54^a	7.51±0.07^b	3.39±0.59^a	7.75±0.09^a	7.85±1.51^a	7.81±0.06^a
	V-S-V	14.93±2.78^a	7.41±0.10^a	1.00±0.13^b	8.17±0.06^a	4.13±0.60^a	7.85±0.05^a	6.69±1.37^a	7.52±0.07^a
	V-P-PM	14.71±2.69^a	7.24±0.14^a	2.90±0.52^a	7.35±0.08^b	0.94±0.12^b	4.63±0.15^b	6.19±1.33^a	6.41±0.22^b

[*z] = ECi is the electrical conductivity of irrigation water, [*y]= ECe is the electrical conductivity of saturated substrates-paste extract. Values (means ± SE) followed by capital letters within column indicate significant difference among salinity levels within the same media and superscript small letters indicate significant difference among media within salinity level at $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT).

Table 4. Number and size of marigold flowers grown in different layered media and irrigated with different salinity treatments

Media	Salinity (ECi)[*]							
	Control		3 dS m^{-1}		6 dS m^{-1}		9 dS m^{-1}	
	Flower no	Flower size (cm)	Flower no	Flower size (cm)	Flower no	Flower size (cm)	Flower no	Flower size (cm)
V-P-V	5.7±1.20 A^a	7.7±0.29 A^a	6.0±0.58 A^a	6.5±0.26 AB^a	4.0±0.00 A^a	6.0±0.24 B^a	0.7±0.33 B^a	5.5 B^a
V-S-V	7.3±1.20 A^a	7.7±0.31 A^a	5.0±0.58 B^a	6. 6±0.30 B^a	4.0±0.00 B^a	6.1±0.37 BC^a	1.7±0.33 C^a	5.5±0.31 C^a
V-P-PM	5.3±0.33 A^a	7.3±0.37 A^a	5.0±0.58 A^a	6.2±0.29AB ^{a}	2.7±0.88 B^a	5.0±0.36 B^b	1.7±0.88 B^a	5.5±0.33 B^a

[*]ECi= Electrical conductivity of irrigation water. V-P-V= vermiculite-perlite-vermiculite, V-S-V= vermiculite-sand-vermiculite, V-P-PM= vermiculite-perlite-peat moss. Values (means ± SE) followed by capital letters within same row indicate significant difference among salinity levels within the same media and superscript small letters within same column indicate significant difference among media within salinity level at $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT).

Table 5. Measurements of the shoot and root fresh and dry weights, root length, plant height and width and leaf area of marigold plants grown in three different layered media and at different salinity treatments

Salinity (ECi)*	Media	Plant height (cm)	Canopy width (cm)	Leaf area(cm²)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root length (cm)
Control	V-P-V	23.7±0.3 A[a]	26.2±1.2 A[a]	17.5±0.9 AB[b]	79.9±7.1 A[b]	9.7±1.1 A[b]	55.6±5.2 B[a]	12.7±2.2 B[c]	61.7±1.7 A[b]
	V-S-V	24.7±0.4 A[a]	28.8±0.4 A[a]	25.1±1.4 A[a]	106.2 ±10.0 A[a]	13.3±0.9 A[a]	66.7±1.6 A[a]	27.3±1.0 A[a]	73.0±1.0 A[a]
	V-P-PM	23.2±0.7 A[a]	25.7±1.9 A[a]	16.3±0.6 A[b]	66.5±2.6 A[b]	8.4±0.5 A[b]	55.5±5.1 A[a]	19.7±2.0 A[b]	47.3±3.5 A[c]
3 dS m⁻¹	V-P-V	23.a̅8±1.7 A[a]	22.0±0.6 A[a]	19.8±1.5 A[a]	55.2±2.5 B[a]	7.5±0.3 B[a]	55.2±1.1 A[a]	17.2±0.9 A[a]	52.0±0.0 B[b]
	V-S-V	23.2±1.2 A[a]	21.7±0.9 B[a]	19.9±2.0 B[a]	58.5±11.0 B[a]	7.5±1.2 B[a]	61.3±4.3 A[a]	21.9±1.5 B[a]	56.7±0.3 B[a]
	V-P-PM	21.8±1.7 AB[a]	23.3±1.8 AB[a]	17.6±2.0 A[a]	49.7±6.8 B[a]	6.6±1.1 AB[a]	54.6±11.6 A[a]	15.8±3.5 AB[a]	44.3±0.7 A[c]
6 dS m⁻¹	V-P-V	20.7±0.4 A[a]	19.5±0.5 A[a]	15.6±1.2 B[a]	38.2±2.2 C[a]	5.9±0.4 C[a]	38.9±3.2 B[a]	10.1±0.4 BC[a]	47.3±1.5 C[b]
	V-S-V	20.8±0.4 A[a]	19.2±1.0 B[a]	17.1±1.2 B[a]	43.0±1.8 B[a]	5.0±0.3 B[a]	39.6±4.9 B[a]	14.6±1.9 C[a]	57.3±2.3 B[a]
	V-P-PM	17.8±0.5 BC[b]	15.2±1.1 BC[b]	11.0±1.7 B[b]	29.9±6.5 C[a]	4.2±1.0 BC[a]	35.9±6.9 AB[a]	11.4±2.0 BC[a]	40.0±2.9 A[b]
9 dS m⁻¹	V-P-V	14.8±1.2 B[b]	14.5±0.9 B[a]	/*	1.1±0.2 D[a]	0.7±0.2 D[a]	20.7±1.6 C[a]	8.5±0.1 C[a]	31.0±0.6 D[b]
	V-S-V	19.2±0.2 A[a]	16.0±1.0 B[a]	/	3.6±0.4 C[a]	2.1±0.2 C[a]	21.7±1.0 C[a]	8.4±0.3 D[a]	44.0±1.0 C[a]
	V-P-PM	16.9±1.1 C[ab]	13.5±1.6 C[a]	/	4.0±2.4 D[a]	1.6±0.6 C[a]	20.2±1.3 B[a]	7.9±0.3 C[a]	25.3±0.3 B[c]

*ECi= Electrical conductivity of irrigation water. V-P-V= vermiculite-perlite-vermiculite, V-S-V= vermiculite-sand-vermiculite, V-P-PM= vermiculite-perlite-peat moss. Values (means ± SE) followed by capital letters within column indicate significant difference among salinity levels within the same media and superscript small letters indicate significant difference among media within salinity level at P≤0.05 using Duncan's Multiple Range Test (DMRT). *(/) = leaf area measurement was not possible because they were very small and curled.

the height of marigold grown in V-P-PM (16.90 cm) was higher than as when grown in V-P-V medium (14.83 cm).

In the V-P-PM media, the movement of marigold roots was downward till the end of the middle layer (perlite) then, they stopped downward penetration and instead they formed a net of roots in the middle layer (perlite). Marigold roots were not observed in the peat moss layer (Fig. 3-A and B) whereas, they were observed in the bottom layers (vermiculite) of the other media (V-P-V and V-S-V).

Physiological parameters of marigold: Salinity negatively affected the chlorophyll content and chlorophyll fluorescence of marigold grown in all three media. The lowest reduction in chlorophyll content and chlorophyll fluorescence was in V-S-V media followed by V-P-V and V-P-PM.

The three media did not differ significantly at the control treatment in both chlorophyll content and chlorophyll fluorescence (Table 6). At ECi= 3 dS m⁻¹, V-P-V medium showed higher chlorophyll content than V-S-V and V-P-PM media whereas, at ECi= 6 dS m⁻¹ and 9 dS m⁻¹, V-S-V resulted in higher chlorophyll content than both V-P-V and V-P-PM media. The chlorophyll fluorescence of marigold grown in V-S-V medium was almost constant at all salinity levels. At ECi= 6 dSm⁻¹, chlorophyll fluorescence of marigold grown in V-P-PM media was significantly less than chlorophyll fluorescence of marigold grown in V-S-V and V-P-V media. At ECi= 9 dSm⁻¹, V-S-V media resulted in highest chlorophyll fluorescence of marigold (0.70) followed by V-P-PM (0.67) and V-P-V (0.59) respectively.

Discussion

Moisture content, electrical conductivity (EC), and pH: Water-saving and reducing salinity impact are two main reasons for substrate layering in agriculture. The present study showed that the θ_v of V-P-PM medium was the highest, followed by

Table 6. Chlorophyll content and chlorophyll fluorescence of marigold leaves grown at different media and irrigated with a different salinity level of water

Salinity (ECi)*	Media	Chlorophyll content (SPAD value)	Chlorophyll fluorescence (Fv/Fm)
Control	V-P-V	50.85±0.70 A[a]	0.70±0.00 A[a]
	V-S-V	51.29±0.68 A[a]	0.70±0.00 A[a]
	V-P-PM	51.48±0.75 A[a]	0.71±0.00 A[a]
3 dS m⁻¹	V-P-V	50.81±0.73 A[a]	0.70±0.00 A[b]
	V-S-V	48.19±0.93 B[b]	0.71±0.00 A[a]
	V-P-PM	49.29±0.65 B[b]	0.71±0.00 A[a]
6 dS m⁻¹	V-P-V	46.68±0.81 B[b]	0.71±0.00 A[a]
	V-S-V	49.17±0.70 B[a]	0.71±0.00 A[a]
	V-P-PM	47.18±0.78 C[b]	0.68±0.01 Bb
9 dS m⁻¹	V-P-V	43.15±0.68 C[b]	0.59±0.02 B[c]
	V-S-V	47.61±0.56 B[a]	0.70±0.00 A[a]
	V-P-PM	44.17±0.71 D[b]	0.67±0.01 B[b]

*ECi= Electrical conductivity of irrigation water. V-P-V= vermiculite-perlite-vermiculite, V-S-V= vermiculite-sand-vermiculite, V-P-PM= vermiculite-perlite-peat moss. Values (means ± SE) followed by capital letters within column indicate significant difference among salinity levels within the same media and superscript small letters indicate significant difference among media within salinity level at P≤0.05 using Duncan's

V-P-V and V-S-V media. The difference between V-P-PM and V-P-V is the presence of peat moss in the bottom layer instead of vermiculite. The infiltration rate of water in the vermiculite is much higher than in the peat moss (Davis and Wilson, 2005). Peat moss can reduce the infiltration rate (Handreck *et al.*, 2002). Also, perlite possesses a higher water holding capacity than sand (Jackson, 1974). Thus, water in the V-P-PM is secured from fast infiltration. The V-P-V and V-P-PM showed an increase in θ_v with time but V-P-PM showed an increase in θ_v in the first six weeks; then it remained constant. This could be because marigold roots in the V-P-PM media did not penetrate into the peat moss layer and instead they formed a net of roots in the middle layer perlite.

Thus, the increase in the mass of the roots in the middle layer as plant grows led to an increase in water absorption from this layer too. Unlike V-P-PM media, marigold roots were observed in the bottom layer (vermiculite) of V-P-V and V-S-V media.

The ECe of the top layer was 6.5 times higher than the ECe of the middle layer and 5.5 times higher than the ECe of the bottom layer. This is because evaporation occurs higher in the top layer. Evaporation is much less in the middle and bottom layers due to the presence of a capillary barrier between the middle and top layers and between the bottom and middle layers. Capillary barrier can reduce both water evaporation and infiltration (Al-Ismaily *et al.*, 2015; Al-Saqri *et al.*, 2016).

During evaporation, salts move with water to accumulate at the soil surface and shallow sub-surface whereas water evaporates into the atmosphere (Goosen and Shayya, 1999). The top layer of three media was not significantly different in their ECe because the top layer of the three media was the same substrate (vermiculite). The ECe of the middle layer (sand) in the V-S-V media was 3 times less than the ECe of the middle layer (perlite) in both V-P-V and V-P-PM (Table 3). That means the sand is more effective in salt leaching than perlite even though both possess macro-pores, which helps in salts leaching. Sandy soils are generally low in salt because in sandy soils salt is more mobile and can be leached out more readily (Blume *et al.*, 2010; Hillel,

2000). The bottom layer (peat moss) of V-P-PM showed less ECe than the bottom layers (vermiculite) of both V-P-V (by 3.6 times) and V-S-V (by 4.3 times). Peat moss is an organic substrate which can reduce salinity stress. Wang *et al.* (2014) reported that addition of organic matter reduces salts build up.

The V-P-PM showed the lowest pH (6.41) among the tested media due to the presence of peat moss in the bottom layer of V-P-PM. The pH of the V-P-PM bottom layer (peat moss) was significantly less than the pH of the bottom layer (vermiculite) of both V-P-V and V-S-V media at all salinity levels (Table 3). Peat moss possesses acidic properties (pH= 3.5) (Table 2).

Vegetative, reproductive, and physiological parameters of marigold: All marigold vegetative, reproductive, and physiological parameters measured were negatively influenced by salinity. However, the impact of salinity was less on marigold grown in the V-S-V media than marigold grown in V-P-V and V-P-PM media. From reproductive aspects, the three media (V-P-V, V-S-V, and V-P-PM) showed no difference in the number and size of flowers. However, from vegetative aspects, at ECi= 6 dS m^{-1}, V-S-V media resulted in higher plant height (20.83 cm), leaf area (17.14 cm^2), and root length(57.33 cm) than V-P-V (20.67 cm, 15.59 cm^2, and 47.33 cm, respectively) and V-P-PM media (17.80 cm, 11.01 cm^2, and 40.00 cm respectively). At ECi= 9 dS m^{-1}, V-S-V medium resulted in significantly higher plant height (19.17 cm), canopy width (16.00 cm), and root length (44.00 cm) than V-P-V (14.83 cm, 14.05 cm, and 31.00 cm respectively) and V-P-PM media (16.90 cm, 13.59 cm, and 25.33 cm, respectively) (Table 5). From the physiological aspects, at ECi= 6 and 9 dS m^{-1} salinity level, the highest chlorophyll content was in marigold grown on V-S-V media (49.17 and 47.61 respectively). At ECi= 6 dS m^{-1}, V-S-V and V-P-V media showed the highest chlorophyll fluorescence (0.71 and 0.71 respectively) whereas, at 9 dS m^{-1}, marigold grown on V-S-V media resulted in the highest chlorophyll fluorescence (0.70) (Table 6). The better vegetative and physiological parameters of marigold grown in the layered V-S-V media could be related to the presence of sand in the middle layer, which resulted in a markedly less ECe compared to the ECe of the middle layer of the other media (Table 3). Reports indicate that sand improves drainage and has a high infiltration rate conducive to salts leaching (Blume *et al.*, 2010; Carrow *et al.*, 2000; Hillel, 2000). Gimeno *et al.* (2010) stated that seedling of citrus rootstock grown in the sand was more tolerant to salinity than seedlings grown in perlite.
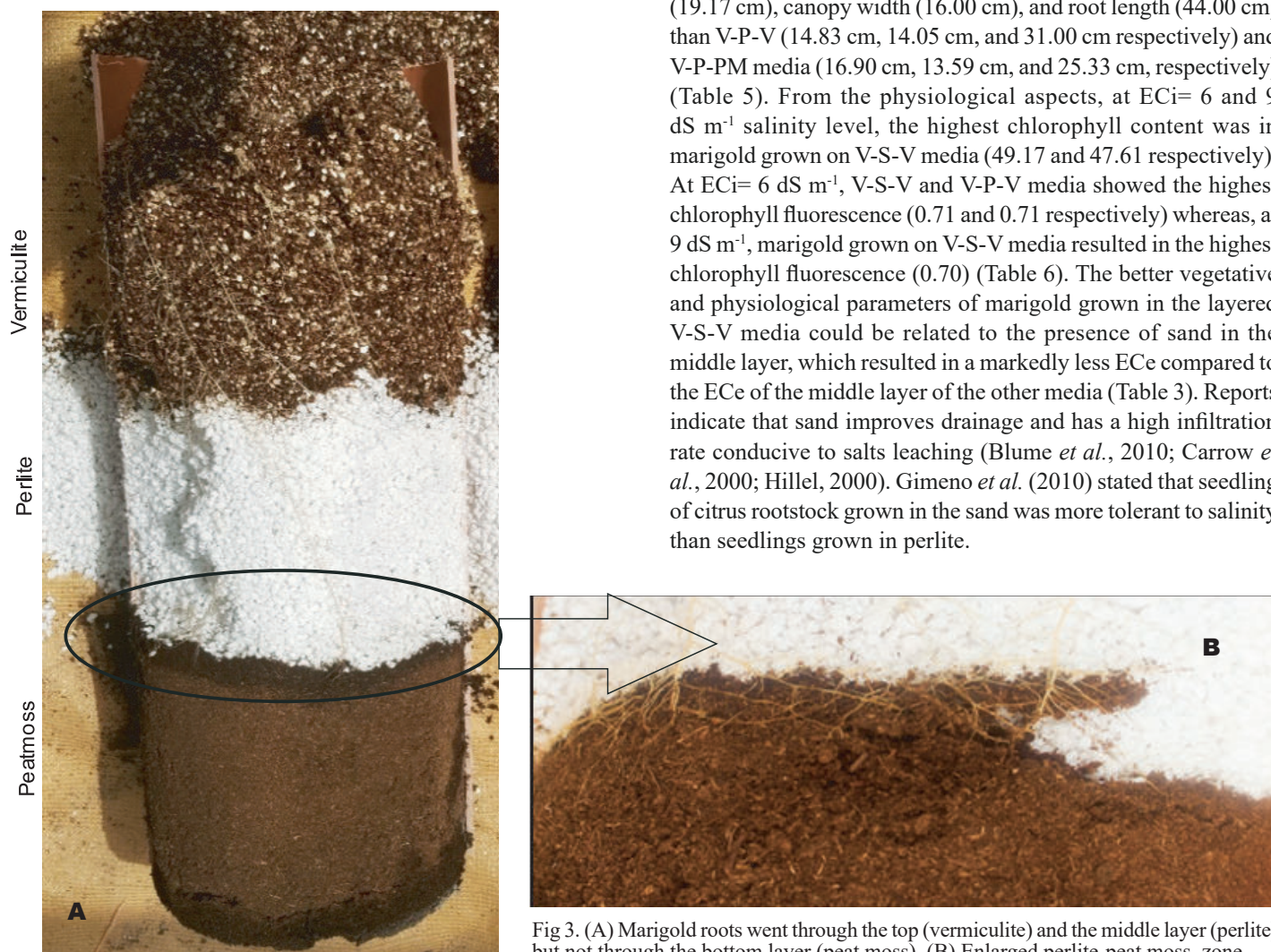

Fig 3. (A) Marigold roots went through the top (vermiculite) and the middle layer (perlite) but not through the bottom layer (peat moss). (B) Enlarged perlite-peat moss zone.

The limited growth of marigold roots in the peat moss layer of the V-P-PM media may be due to the acidic properties of the peat moss (Table 2). Marigold is moderately tolerant to salinity but highly sensitive to pH changes (Valdez-Aguilar *et al.*, 2009).

The study highlights the impact of different configurations of layered potting media in mitigating salinity stress of marigold plants. This study showed that V-S-V is the best-layered media which can mitigate salinity effects on marigold plants. Overall, the best growth of marigolds was in those grown in the layered V-S-V media which was attributed to the presence of sand in the middle layer that exhibited the lowest ECe among the middle layers of tested media. This study could potentially help farmers and nurserymen in many arid areas where saline water is the only available water for irrigation. Further studies are needed to evaluate the different layered media on mitigating salinity stress of other crops, including vegetables and other ornamental container-grown plants.

Acknowledgments

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