

Organic amendments role in reducing drought stress in *Alcea rosea* L.

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Water scarcity and dwindling natural resources due to global warming are negatively impacting ornamental plant survival. Soil fertility remains a problem in arid and semiarid regions. In this study, the effects of four media (arable soil, arable soil + cow manure, arable soil + rice hull, arable soil + wheat straw) on macronutrient content and quantitative characteristics of *Alcea rosea* L. under drought stress were investigated. Application of organic amendments mitigated the negative effects of drought in the soil and increased the available organic macronutrients. The application of organic amendments increased the total N, P, and K content in the soil and leaves of hollyhock. Total soluble sugars (by 11.9%), RWC (by 8.75%) and phenolics (by 36.4%) of hollyhock were significantly improved by the application of organic amendments at 80% FC. The amended soil (soil + cow manure) increased the activities of superoxide dismutase and ascorbate peroxidase at 80% FC. Moreover, the soil + cow manure proved to be the best supplement to improve leaf area and dry weight. In conclusion, the application of organic amendments can be successfully used as a cost-effective management method to improve soil fertility and crop production in arid and semi-arid areas.

1. Introduction

Considering the increasing population of the planet earth, stability in green space has particular importance (Wolch *et al.*, 2014). Exacerbation of environmental stressors lays the groundwork for the loss of ornamental plants. Therefore, one of the critical goals of plant producers and breeders is providing quality and stress-resistant plants for green space (Anguelovski *et al.*, 2020). Among ornamental plants, *Alcea*, commonly known as Hollyhock, is a perennial plant of the Malvaceae family, with decorative and medicinal importance. People use the flowers of this plant to produce medicinal tea due to pigments (Shehzad *et al.*, 2020). In addition, the antibacterial, anticancer (Lim, 2012), antioxidant (Ahmed *et al.*, 2016), anti-depressant, anti-inflammatory (Ahmadi *et al.*, 2012), anti-fatigue, febrifuge, mouth washing (Burt and Reinders, 2003), and blood circulation enhancer (Lim, 2012) characteristics of this plant have been proven. Regarding the climatic changes and the resistance of this plant to adverse conditions, it can be a desirable plant in the green space (Oraee

et al., 2019).

Among the environmental factors, drought is the most critical factor limiting growth in many areas (Toscano *et al.*, 2019; Bhusal *et al.*, 2020). Based on the scientists' prediction, global temperature could rise by 3 to 9°C with far-reaching effects and significantly increase the dryness of the arable lands (Haile *et al.*, 2020). Regarding the increase in global temperature by 1.5°C, plants selection with high tolerance to drought stress to ensure the survival and stability of green space is an essential strategy (Seleiman *et al.*, 2021). In such conditions, in addition to the skill and accuracy of using and consuming these water resources, recommending tolerant plants, determining the drought tolerance threshold, and increasing soil fertility has become more necessary (Banks *et al.*, 2019; Du *et al.*, 2019).

The elements required in organic matter can increase soil fertility and plant's yield in agriculture (Ukalska-Jaruga *et al.*, 2020). Organic fertilizers provide nutrient balance and increase soil nutrient availability (Verma *et al.*, 2020). These organic substances have benefits, including reducing leaching and wastage of nutrients (Quynh and Kazuto, 2018) and helping the release elements (Mupambwa and Mnkeni, 2018). They enhance root growth due to improved soil structure, increase the amount of organic matter and soil exchange capacity, and ultimately serve as a source for the growth of soil organisms and increase soil yield (Juriga *et al.*, 2018; Chew *et al.*, 2019).

Some studies documented the reduced element transfer in plants under soil drought stress (Liu *et al.*, 2018; Qi *et al.*, 2019). In water shortage conditions, organic matters can conserve water in the soil and prevent the destructive effects of drought stress on plant yield (Kaya *et al.*, 2020). Also, Khosravi Shakib *et al.* (2019) reported that the total dry weight and water use efficiency were about 3-fold higher in marigold (*Calendula officinalis* L.) grown in 30% manure compost substrate compared to the control plants.

Different plant species show a wide range of drought resistance mechanisms, including antioxidant activity and osmoregulatory adaptations (Siddique *et al.*, 2018; Khan *et al.*, 2020). There should be reconsideration of the plants type grown in arid and semi-arid regions. Also, some plants with high water requirements should be replaced with the drought-tolerant plant (De Souza Aguiar *et al.*, 2020).

Hollyhock is a low-expectation plant that grows well in natural and marginal areas that can be considered suitable for cultivation in low-input systems. This is a first work useful to develop a suitable methodology for studying the use of soil organic amendments. Also, this study was conducted to identify the drought tolerance threshold of Hollyhock and identify different growth mediums on drought tolerance of Hollyhock.

2. Materials and Methods

Plant material

The experiment was conducted in the research greenhouse of Ferdowsi University in Mashhad, Iran. Seeds were sown in August in the greenhouse at an average temperature of $20 \pm 1^\circ\text{C}$ in plug trays containing a mixture of coco peat, perlite and peat (1:1:0.5 v/v/v). Plants grew at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ combined with a photoperiod of 14 and 10 h d^{-1} . Then, all plants at the 5-6 leaf stage were transferred to pots (18 cm high and 8 cm in diameter) containing arable soil. In May, the plants were transferred to pots of size 20 with a soil mixture of four different substrates (arable soil, arable soil + manure, arable soil + rice hull, and arable soil + wheat straw).

Experimental design and treatments

The experiment consisted of two factors (organic supplements and irrigation regime). The organic supplements and irrigation regimes had four and three levels, respectively. Three sources of organic supplements were used (manure, rice hulls, and wheat straw). The pot experiment (one plant per pot) was conducted factorially in a randomised complete block experiment with three replicates under greenhouse conditions. The mixtures between the soil and cow manure, rice hull and wheat straw were prepared in a 50:50 ratio (50% w/v soil and 50% w/v organic amendments). The soil from the four treatments (control soil and the three additives) was taken to the laboratory for chemical analyses after the mixtures were prepared (as described above). The physicochemical properties of the pot samples are explained in Table 1.

Drought stress treatments

Plants were subjected to drought stress during flowering on June 5. Soil volumetric water content was measured during the flowering period using the

Table 1 - Physicochemical properties of the soils used in the experiment

Growth medium	N (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	pH	EC (ds/m)
Soil	0.065	23	235	7.15	1.93
Soil + cow manure	0.42	2214	1592	7.76	2.53
Soil + rice hull	0.23	271	343	7.26	1.77
Soil + wheat straw	0.21	117	1572	6.68	3.13

Time Domain Reflectometry (TDR) instrument (TRIKE-FM, England). Plants were exposed to drought stress for one month. Plants were irrigated through a polyethylene piping network with volumetric meters at 80, 60, and 40% FC.

Foliar nutrient analysis

Foliar nitrogen, potassium, and phosphorus were measured at the end of the experiment. Leaf samples (0.2 g) were heated to 400°C with 10 mg sulfuric acid and a catalyst mixture in a digestion apparatus, and then nitrogen content was measured using a Kjeldahl apparatus (Kjeldahl, 1998). Leaf potassium was measured using the flame photometer method (Tandon, 1998). Phosphorus content was determined by the colorimetric method (yellow molybdate vanadate) at 470 wavelengths (Rayan et al., 2001).

Determination of electrolyte leakage

Leaf electrolyte leakage was measured according to the method of Reddy et al. (2004). Tissue samples (0.2 g) from the first true leaf of 9-month-old plants were placed in 50 ml of distilled water. The samples were stored at laboratory temperature for 24 hours and the conductance of the solution was measured using a conductivity meter (Jenway model). All leaves were autoclaved for 20 minutes to measure the final leakage. Electrolyte leakage was calculated as:

$$EL\% = EC1/EC2 \times 100.$$

Total soluble sugars assay

Spectrophotometry at 620 nm was used to analyze total soluble sugars using the Anthron method (McCready et al., 1950). Total soluble sugars were extracted by homogenizing the plant material in 80% ethanol. Samples were centrifuged at 3500 rpm for 10 min. Anthrone solution (10 ml 0.15%) was added to 1 ml of the solution. The samples were then heated to 95°C and immediately transferred to an ice bath. The total sugar concentration of the samples was calculated using the standard glucose curve based on mg g⁻¹ dry weight (Ebell, 1969).

Determination of proline and relative water content

Proline content and relative water content (RWC) were determined according to the procedure described by Bates et al. (1973) and Turner (2018), respectively.

Chlorophyll determination

Chlorophyll was determined using the method of Arnon (1949). For this purpose, 0.2 g of fresh leaves were ground with 80% acetone. The resulting solution was centrifuged at 4000 rpm for 10 minutes. The optical absorbance of the supernatant was measured using a spectrophotometer (Shimadzu UV-160A) to determine the amount of chlorophyll at 645 and 663 nm.

Phenol assay

In this study, total phenol was determined using the Folin-Ciocalteu reagent (FCR) according to the method of Singleton and Rossi (1965). An amount of 4.5 ml of distilled water and 0.1 ml of Folin-Ciocalteu reagent were added to 0.1 ml of the methanolic extract. After 3 minutes, 2% sodium bicarbonate solution was transferred to 0.3 ml solution. The different concentrations of gallic acid were used to construct the standard curve and measure the absorbance using a spectrophotometer (Shimadzu UV-160A) at 760 nm. Total phenol was calculated based on mg GAE g⁻¹ dry weight.

Protein and enzyme activity assays

Bradford's method (1976) with slight modification was used to measure total protein. To assay protein, 50 mg of fresh leaf was ground in liquid nitrogen. Then 50 mg of polyvinylpyrrolidone, 495 µl of extraction buffer solution (including 40 mM hydrogen tris-chloride buffer, 2% sodium dodecyl sulfate, 20% glycerol, and 60 mM dithiothreitol), and 5 µl of phenylmethyl methanol to 200 ml of phenylmethyl methanol were added to each solution. The samples were centrifuged at 8000 rpm at 4°C for 15 minutes. Then 300 µl of the supernatant with 900 µl of acetone containing 10% trichloroacetic acid and 0.07%

dithiothreitol was added and placed at -20°C. 100 µl of adsorption buffer was added to the supernatant.

Nakano and Asada's method (1981) was used to measure the activity of ascorbate peroxidase. The reaction mixture consisted of 20 µl of enzyme extract, 770 µl of 50 mM phosphate buffer, 100 µl of 0.1 mM EDTA, 100 µl of 5 mM ascorbate, and 10 µl of 0.1 mM hydrogen peroxide. The absorption rate of the reaction was read by a spectrophotometer at 290 nm.

Superoxide dismutase activity (SOD) was measured according to the Sairam *et al.* (2002) method with slight changes. The enzymatic reaction mixture consisted of 935 µl of 50 mM phosphate buffer containing 0.1 mM EDTA, 13 mM methionine, and 75 mM nitroblue tetrazolium (NBT), 15 µl of 0.12 mM riboflavin, and 50 µl of enzymatic extract. After preparing the control and blank samples to measure enzymatic activity, the blank sample was placed in the dark for 15 minutes, and the control and enzyme extract samples were shaken for 15 minutes in a shaker at 25°C with two 20 W fluorescent lamps at 100 rpm. The absorbance was read at 560 nm using a spectrophotometer (Shimadzu UV-160A).

Aebi's method (1984) was used to measure the activity of catalase (CAT) enzyme, where 20 µl of enzymatic extract mixed with 50 mM phosphate buffer containing ten mM hydrogen peroxide and their adsorption changes were recorded at 240 nm by spectrophotometer (Shimadzu UV-160A). Glutathione reductase was measured by Sofo *et al.* (2004).

Determination of growth parameters

Leaf area of hollyhocks was determined using a Delta-T Leaf Area Meter (Device Ltd., Cambridge, UK). Plants were dried at 60°C and weighed to calculate their respective dry weights.

Statistical analysis

Statistical analysis was performed using SAS 8.1 software. Data are presented as mean ± SE of three replicates. Means were also compared using the LSD test at a probability level of 5%.

3. Results

Foliar nutrients (N, K, and P) and soil nutrients (N and K) were significantly ($P \leq 0.05$) affected by organic amendment and drought. In the desiccated soils, N content increased significantly with the application of manure amended soil. Foliar N content was 50% and 14% higher in the manure-amended and rice hull-

amended soils, respectively, than in the control at 80% FC. Finally, N foliar content was higher in soils enriched with manure in all irrigation regimes, followed by soils enriched with rice hulls at 80 and 60%, respectively FC (Fig. 1a). Similar to N content, leaf P content was also higher in soils fertilized with 80% FC. Overall, drought stress (60% FC) had no effect on P content in soils enriched with manure and rice hulls (Fig. 1b). The magnitude of the increase in K content was more significant in plants under 80% FC than in plants under 40% FC, and it was more pronounced in the amended soils than in the control. Drought stress significantly affected K content, decreasing it slightly (by 4.74%) in manure amended soils under 40% FC compared to 80% FC (Fig. 1c).

Leaf nutrient contents analyzed were similar compared with those in soils. In the unamended soils subjected to different irrigation regimes, soil N con-

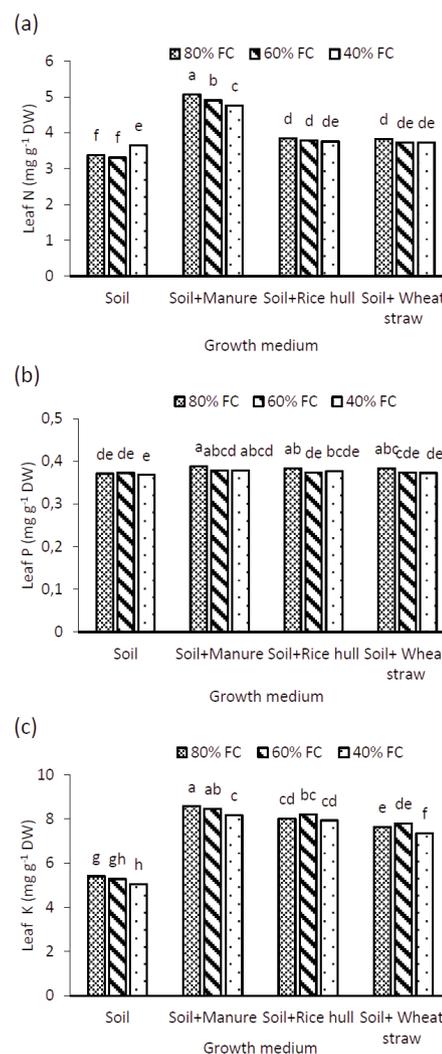


Fig. 1 - Leaf N (a), leaf P (b), and leaf K (c) of hollyhock plants. Values in columns followed by the same letter are not significantly different at the 0.05 level ($P \leq 0.05$).

tent decreased significantly. Compared to the control, organic soil amendments caused a less dramatic increase in N content. N content in soils amended with manure was higher in soils amended with 80, 60, and 40% FC than in other treatments (Fig. 2a). P contents in desiccated soils decreased progressively, and these values ($P \leq 0.01$) were significantly lower than those determined for well-watered soils. Soil P content was consistently higher in soils amended with manure and rice hulls than in control soils. Application of organic amendments to desiccated soils (40 and 60% FC) also increased K content. K accumulation was lower in soils enriched with wheat straw than in soils enriched with manure (Fig. 2b).

Organic amendment significantly affected pH index ($P \leq 0.01$), but EC was affected by organic amendment and drought stress. The pH index in soil amended with manure increased significantly (by 5.57%) compared to the control. Overall, the EC was higher in soils with 40% FC than in the corresponding soils with 80% FC. The EC was 22.9% higher in the

compared to soils with 80% FC. Conversely, 40% FC decreased RWC in unamended soils (Fig. 3a). EL % in unamended soils was higher at 40% FC than in well irrigated soils, while for rice hull and wheat straw there was no difference in this variable at 40% FC (Fig. 3b). Total soluble sugar and proline were significantly ($P \leq 0.01$) affected by the interaction between drought stress and organic amendments. In well-watered soils, soluble sugar and proline contents were consistently higher in amended than in control soils. In leaf tissues of plants treated with 40% FC, total soluble sugar content increased by 6.17% in amended soils compared with unamended soils, and this index remained unchanged in leaf tissues of soils amended with rice hull and wheat straw at 60 and 40%, respectively FC (Fig. 3c). Proline tissues were also dependent on drought stress, and a maximum increase of 177% was observed in the soils amended with slurry at 40% FC compared to the control (Fig. 3d).

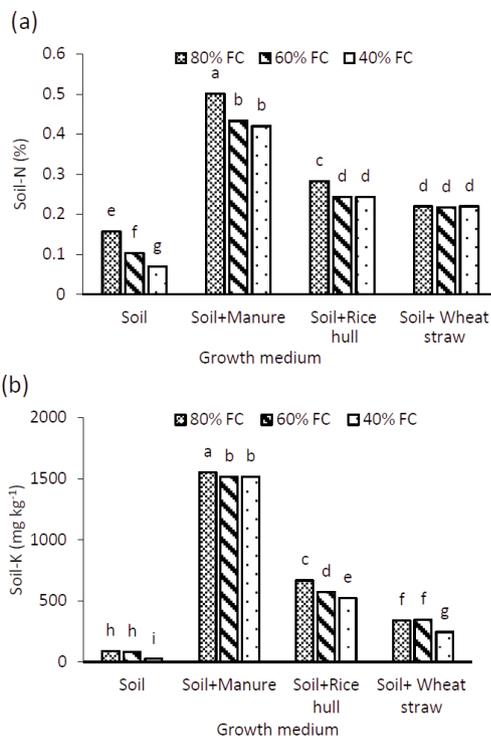


Fig. 2 - The soil-N (a) and soil-K (b). Values in columns followed by the same letter are not significantly different at the 0.05 level ($P \leq 0.05$).

manure amended soils than in the control. Organic amendments and drought stress significantly ($P \leq 0.05$) affected RWC, EL %, and phenolic content. RWC decreased slightly in amended soils with 40% FC

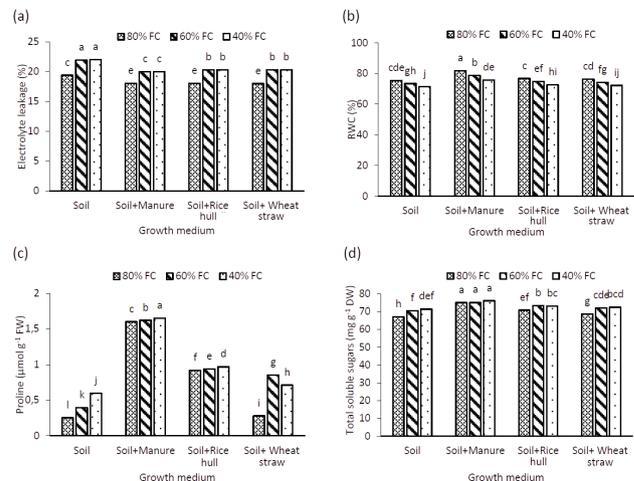


Fig. 3 - Electrolyte leakage (a), RWC (b), proline (c), and total soluble sugar (d) in hollyhock plants under different growth medium. Values in columns followed by the same letter are not significantly different at the 0.05 level ($P \leq 0.05$).

Chlorophyll a, b, and total chlorophyll were significantly ($P \leq 0.05$) affected by the interaction between drought stress and organic amendments. It was found that chlorophyll content increased in plants grown with organic amendments under drought stress. The highest chlorophyll a content was found in soils fertilized with 80% cow manure FC (Fig. 4a). Soils fertilized with cow manure showed a significant increase in chlorophyll b under drought stress (Fig. 4b). Between treatments, the highest average total chlorophyll (3.18 mg g⁻¹ FW) was measured in soils

amended with cow manure at 80% FC, while the lowest total chlorophyll (1.37 mg g⁻¹ FW) was measured in control plants at 40% FC (Fig. 4c). Changes in phenolic content of hollyhocks showed similar trends for both soils (wheat straw enriched soils), such that phenolic content increased under severe water stress, but this index in plant tissues grown in manure and pod enriched soils showed no difference under all irrigation regimes (Fig. 4d).

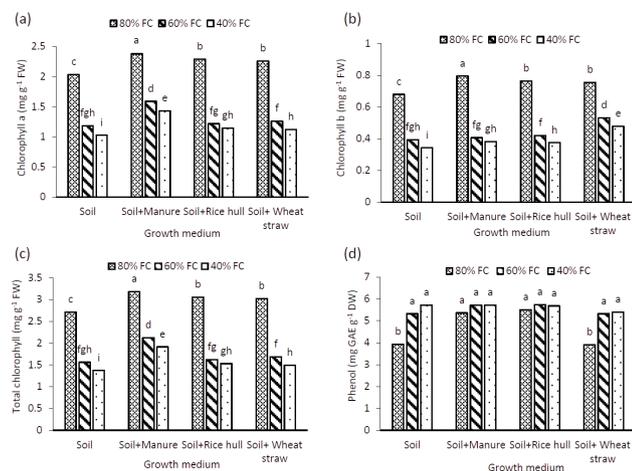


Fig. 4 - Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), phenol (d) in hollyhock plants under different medium. Values in columns followed by the same letter are not significantly different at the 0.05 level ($P \leq 0.05$).

The interaction of drought stress and organic amendments significantly affected antioxidant activity ($P \leq 0.01$). Glutathione reductase levels were higher in rice-treated soils than in other treated soils. Drought stress significantly increased this index in amended soils. However, glutathione reductase in leaves of hollyhocks grown in manure amended soils (40% FC) increased significantly by 28% compared to control plants (80% FC). There was no significant difference between glutathione reductase in manure and wheat straw -amended soils at 40% FC (Fig. 5a). In addition, significant improvement of SOD and APX was observed under severe drought stress. The maximum value of SOD was measured in the amended and unamended soils in 40% FC treatments (Fig. 5c). The activity of APX was lower in the unamended soils than in the corresponding soils in amended soils, while there was no significant difference between soil irrigation regimes. Overall, APX activity was higher in manure amended soils at 60 and 40% FC than in the corresponding soils at 80% FC (Fig. 5d). No differences were observed in CAT values between manure-

amended and non-manure-amended soils at 40% FC, while a slight increase was measured in manure-amended soils (Fig. 5d).

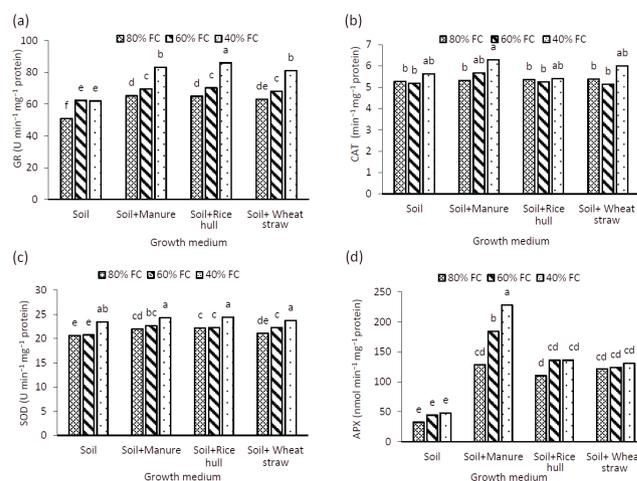


Fig. 5 - GR (a), CAT (b), SOD (c), and APX (d) in hollyhock plants under different medium. Values in columns followed by the same letter are not significantly different at the 0.05 level ($P \leq 0.05$).

Leaf area and dry weight of hollyhock ($P \leq 0.05$) were significantly affected by drought and amended soils. Leaf area at 40% FC was generally lower than that of the control throughout the evaluation period. Leaf area at 80% FC was higher in manure amended soils than in nonamended soils (Fig. 6a). In well-watered soils, dry weight was consistently higher in amended soils than in control soils. In general, this index was lower in soils at 60 and 40% FC than at 80% FC. Drought (40% FC) had a negative effect on dry weight, especially in the unworked soils. At 80% FC, this index was lower in soils with rice hull admixture than on wheat straw medium (Fig. 6b).

4. Discussion and Conclusions

The results of a recent experiment show that a suitable nutrient medium reduces the negative effects of drought stress. The results show that organic amendments in soils improved macronutrients in leaves and soil. Our results are in agreement with those of Banik *et al.* (2006), who showed an increase in nitrogen, phosphorus, and potassium in rice (*Oryza sativa* L.) after the application of various organic matter such as animal and poultry manure and rice. The researchers observed the highest phosphorus and potassium content in rice grain

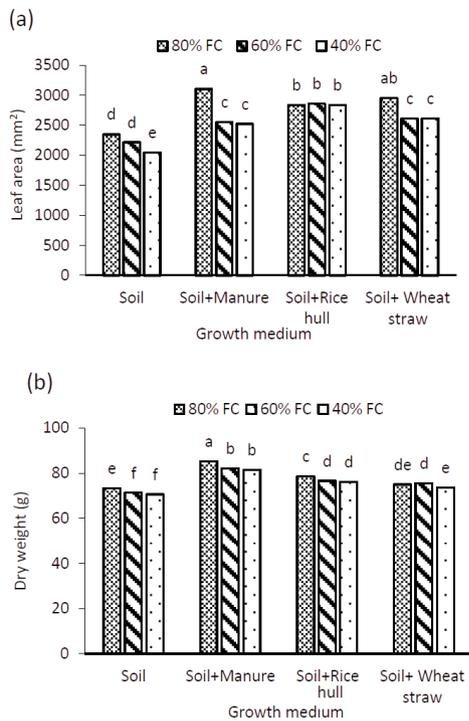


Fig. 6 - Leaf area (a), and dry weight (b) in hollyhock plants under different medium. Values in columns followed by the same letter are not significantly different at the 0.05 level ($P \leq 0.05$).

and rice branches, respectively, under cow manure.

He and Dijkstra (2014) found that nitrogen and phosphorus concentrations decreased under drought stress. A significant decrease in nitrogen, phosphorus and potassium uptake in maize (*Zea mays* L.) branches was found to be twofold under drought stress conditions (Alizadeh, 2010). In the studies of Ghazi (2017), increasing drought stress from 50% to 100% FC reduced nitrogen, phosphorus and potassium uptake in maize leaves. Under drought stress, the average potassium concentration in the roots of treated *Apocynum* plants (*Apocynum venetum* L.) decreased by 40%, but in the leaves of this plant, the amount of potassium remained constant and increased in the stem. The result suggests that the plant stores large amounts of potassium to maintain osmotic adjustments (Cui et al., 2018).

Leaf potassium content decreased under severe drought stress because nutrient transport and uptake depend on soil moisture. Decreased element transfer in plants under drought stress conditions has been documented in many studies (Ahangar et al., 2016; Li et al., 2021). Hollyhock plants were able to maintain potassium content in leaves up to 60% FC, which was consistent with the results of Qi et al. (2019), which

showed that the accumulation of potassium in the plant was higher under non-stress conditions than under stress conditions, in addition, further reduction of irrigation under severe stress reduced the amount of potassium in the leaves of plants. The reduction in transpiration rates and changes in membrane transporters under drought conditions due to water deficit, which lead to reduced mineral nutrition in plants, showed that phosphorus uptake in rice increases with increasing moisture content (Roy, 2018). The highest phosphorus and potassium uptake occurred due to mineralization of fertilizer elements in the soil. Application of nitrogen and phosphorus fertilizers in tortoiseshell bamboo (*Phyllostachys edulis*) increased soil phosphorus, nitrogen, and leaf phosphorus (Wu et al., 2018). There is a positive correlation between soil macroelements and leaf elements (Table 2). Numerous studies have shown that organic fertilizer increases soil pH (Liu et al., 2010; Han et al., 2016). Organic fertilizers increase calcium carbonate, which is believed to increase buffering and thereby improve soil pH (Whalen, 2000). Electrical conductivity can serve as a critical indicator of nutrient and water uptake (Dhaliwal et al., 2019). Application of poultry, cattle, and goat manure significantly increased soil electrical conductivity, and the potential for manure-induced soil salinity was relatively high for poultry and goat manure (Azeez and Van Averbeke, 2012). The increased electrical conductivity of soil is due to the release of salts from fertilizers. As a result of the decomposition of organic matter in the soil, the ions obtained from the decomposition entered the soil solution and consequently increased the salinity of the soil, which was consistent with Roy and Kashem (2014).

An increase in drought stress resulted in an increase in electrolyte loss, such that electrolyte loss increased in leaves of maize (Mozdzen et al., 2021) and tomato (Ors et al., 2021) compared to the control under drought conditions. In addition, nitrogen uptake occurs by mass uptake and potassium and phosphorus uptake occurs by diffusion (Li Bot et al., 2021). In the absence of water, the uptake of nitrogen and phosphorus is reduced. In addition, the presence of potassium under drought stress conditions maintains turgor pressure and osmotic adjustments of cells (Singh et al., 2021). In turtle shell bamboo, phosphorus and nitrogen addition to plants under drought stress decreased malondialdehyde and reduced electrolyte loss compared to the control (Wu et al., 2018). In the current experiment, due to

Table 2 - Correlation coefficients among different parameters of hollyhocks

	Soil N	Soil P	Soil K	Leaf N	Leaf P	Leaf K	Chlorophyll	EL	RWC	Proline	Total soluble sugars	GR	CAT	APX	SOD	Leaf area	Dry weight
Soil N		0.82 **	0.90 **	0.92 **	0.55 **	0.74 **	0.47 **	-0.31 **	0.71 **	0.89 **	0.71 **	0.84 **	0.06 NS	0.75 **	0.30*	0.89 **	0.95 **
Soil P			0.65 **	0.97 **	0.52 **	0.66 **	0.46 **	-0.25 *	0.57 **	0.81 **	0.57 **	0.81 **	0.17 NS	0.59 **	0.49 **	0.71 **	0.82 **
Soil K				0.82 **	0.45 **	0.78 **	0.41 **	-0.25 *	0.67 **	0.94 **	0.72 **	0.81 **	0.02 NS	0.72 **	0.28 *	0.79 **	0.56 **
Leaf N					0.47 **	0.67 **	0.47 **	-0.42 **	0.77 **	0.87 **	0.71 **	0.71 **	0.02 NS	0.66 **	0.31 **	0.79 **	0.89 **
Leaf P						0.68 **	0.42 **	-0.70 **	0.64 **	0.42 **	0.36 **	0.49 **	0.19 NS	0.52 **	0.25 *	0.76 **	0.61 **
Leaf K							0.31 **	-0.42 **	0.54 **	0.71 **	0.61 **	0.86 **	0.01 NS	0.87 **	0.37 **	0.91 **	0.82 **
Chlorophyll								-0.52 **	0.81 **	0.19 NS	0.15 NS	0.08 NS	0.36 *	0.06 NS	-0.36 **	0.57 **	0.51 **
EL									-0.73 **	-0.18 NS	0.16 NS	-0.20 NS	-0.24 *	-0.34 **	0.26 *	0.61 **	0.48 **
RWC										0.56 **	0.31 **	0.43 **	0.22 NS	0.37 **	0.05 NS	0.76 **	0.78 **
Proline											0.83 **	0.86 **	-0.06 NS	0.72 **	0.40 **	0.71 **	0.86 **
Total soluble sugars												0.78 **	-0.13 NS	0.70 **	0.58 **	0.55 **	0.65 **
GR													-0.02 NS	0.86 **	0.54**	0.74 **	0.80 **
CAT														-0.02 NS	-0.1 NS	0.20 NS	0.12 NS
APX															0.60 **	0.74 **	0.73 **
SOD																0.26 *	0.31 **
Leaf area																	0.89 **
Dry weight																	

EL= electrolyte leakage, RWC= relative water content, GR= glutathione reductase, CAT= catalase, APX= ascorbate peroxidase, SOD= superoxide dismutase, * P≤0.05; ** P≤0.01; NS= not significant.

the increase of nitrogen, potassium, and phosphorus in the soil and the increase of these elements in the leaves of the irrigated plants by 80% FC, the rate of electrolyte loss in these plants decreased compared to other substrates. Researchers showed that K⁺ efflux could play an essential role in anabolic reactions by stimulating catabolic processes and saving “metabolic” energy for adaptation and repair needs in plants (Demidchik *et al.*, 2014).

In our study, an increase in drought stress also decreased the amount of chlorophyll in leaves, which is consistent with the opinions of others (Khayatnezhd and Gholami, 2021). In peach plants, the use of phosphorus increased chlorophyll content, but reducing phosphorus decreased protein and chlorophyll content (Dutt *et al.*, 2013). The use of nitrogen- and phosphorus-containing fertilizers effectively increased the chlorophyll and carotenoid content of apples because an increase in nitrogen promoted the formation of photosynthetic pigments by thylakoid and stomatal proteins, which also increased the formation of chloroplasts in growing leaves (Jahan *et al.*, 2020; Siddiqui *et al.*, 2021). The biochemical and biosynthetic properties of photosynthetic pigments require phosphorus as well as nitrogen (da Silva Tavares *et al.*, 2020).

In the recent experiment, there was a positive

correlation between nitrogen and potassium with chlorophyll (Table 2). The amended soil has increased the leaf elements by increasing the number of elements in the soils under 80% FC. In hyssop (*Hyssopus officinalis*), the effects of drought stress and potassium fertilizer on chlorophyll content were significant, such that the amount of total chlorophyll increased with increasing potassium and irrigation regime (Lopo de Sa *et al.*, 2014). In plants under higher drought stress, nutrient deficiencies and reduced energy absorption of sunlight lead to damage to the photosynthesis and chlorophyll systems because nutrients play an essential role in the electron transfer system and carbon metabolism (Xu *et al.*, 2020; Ma *et al.*, 2021), which is consistent with the study results because the lowest chlorophyll in drought treatment of 40% FC was recorded in the soil substrate.

With increasing drought stress from 80 to 40% FC, the plant’s RWC decreased. However, Organic fertilizers reduced the adverse effects of drought stress on the RWC. There have been numerous reports of changes in relative water content and osmotic adjustments in leaves occurring under drought stress, and that these variations were different depending on cultivar, species, duration, and intensity of stress (Kizilgeci *et al.*, 2020; Zhu *et al.*, 2020). The main rea-

son for the improvement in drought stress relief in the presence of potassium is due to the osmotic adjustments and the preservation of the RWC (Ibrahim et al., 2020). Fahri et al. (2021) reported an increase in the RWC of two oilseeds with potassium fertilizers under drought stress. Similar responses to potassium fertilizers were reported in corn (Khadem et al., 2010) under drought stress. Also, in the study, a positive and significant correlation was recorded between leaf nitrogen and potassium content with the RWC of leaves (Table 2), and the lowest leaves relative water content was recorded in soil-substrate plants with the lowest amount of nutrition.

Drought stress leads to biochemical and physiological changes that lead to osmotic changes and decreased turgor pressure in the cell. These changes degrade nitrate assimilation by reducing nitrate reductase activity (Zhang et al., 2009). Plants use osmotic adjustments to reduce the effects of drought stress due to nitrate assimilation, in which proline accumulation plays a vital role in osmotic adjustment (Ozturk et al., 2021). Zhang et al. (2014) showed that the accumulation of osmotic compounds such as proline, betaine, and potassium ion in two maize cultivars was higher in more resistant cultivars than in more sensitive cultivars under drought stress. Moreover, potassium maintains the turgor pressure by reducing the leaf water potential (Da Silva et al., 2021). Potassium application causes proline accumulation by maintaining osmotic adjustments (Aksu and Altay, 2020). In the present experiment, the proline increased with increasing drought stress. Leaf analysis showed that the potassium in leaves of plants in animal manure culture is higher than other substrates, which increases the accumulation of proline in osmotic adjustments. Although its exact mechanism is unknown, it seems to be due to the role of potassium in amino acid metabolism or the effect of potassium on the proline cycle (Zhu et al., 2019). Drought stress not only increases the stomatal resistance of plants but also causes the accumulation of osmotic substances (Zahoor et al., 2017).

By increasing water uptake and reducing water loss, plants under drought stress prevent further damage (closing stomatal and smaller leaves) (Hill et al., 2020). When photosynthesis is not responsive to the plant, carbohydrates break down, maintaining osmotic adjustment in the cell. The sucrose significantly decreased under drought stress (Yang et al., 2019). Nutrients play an important role in drought stress on total soluble sugars as an osmotic adjust-

ment and mitigate drought stress. The potassium accumulation in the vacuole and sucrose maintains the turgor and osmotic pressure and effectively increases water uptake in plants (Sardans and Peñuelas, 2021). In addition, drought stress conditions prevent chloroplast damage by maintaining high cellular pH (Cakmak, 2005). Drought stress treatment on apple (*Malus domestica* Borkh.) plants showed that plant sucrose decreased at the beginning of stress but increased over time. Glucose and fructose also had an increasing trend at all times. The process of reducing sucrose reduces vegetative growth in plants under drought stress. The leaf nitrogen decreased with decreasing the amount of applied irrigation water, and with re-irrigation, the nitrogen increased. Phosphorus levels also showed a downward trend in plant parts under drought stress (Jie et al., 2010).

In a recent experiment, plants in soil + manure substrates kept the total soluble sugars constant in irrigation at 40% FC compared to 80% FC by absorbing nutrients from the soil. The correlation between the total soluble sugars with nitrogen and potassium proves these results (Table 2). In addition, in the treatment of 40% FC, plants used the total soluble sugars increase for osmotic adjustment and as a system to resist drought stress.

In the present experiment, antioxidant activity was increased under drought conditions. Several studies reported that the antioxidant enzymes activities increased under stress (Hasan et al., 2021; Sepahvand et al., 2021). Our results were in line with Safari et al. (2021) and Babaei et al. (2021), who showed that drought stress increased ascorbate peroxidase activity in impatiens and marigold, respectively.

Applying nitrogen and phosphorus fertilizers significantly increased the activity of the peroxidase enzyme under drought-stressed plants when compared to the non-application of these two elements (Wu et al., 2018). Using vermicompost organic fertilizer under drought stress conditions in lettuce (*Lactuca sativa* L.) increased the activity of superoxide dismutase and catalase enzymes as an enzyme system, reducing the adverse effects of stress. The correlation between growth traits and the activity of these enzymes under stress showed that the vermicompost application increased plant growth and drought tolerance. The results also showed that the nitrogen and organic matter in vermicompost soil was higher, which led to increased plant growth

(Kiran, 2019).

In plants treated with 80% FC in soil substrate + animal manure with higher leaf elements, enzymatic antioxidant activity increased. Moreover, enzyme activity were positively correlated with elements such as nitrogen and potassium in the leaves (Table 2). The plant needs potassium to maintain enzyme activity and protein synthesis because protein structure need high k^+ in cytocele (Blevins, 1985).

A reduction in leaf area and dry weight under drought stress is attributed to photosynthesis and carbon assimilation. In the present experiment, the plant leaf area decreased with increasing drought stress, and the dry leaf weight decreased with decreasing leaf area, consistent with the results of Khaleghnezhad *et al.* (2021). In the low moisture conditions, the plants reduce the leaf area to decrease the level of respiration. In a recent experiment, plant leaves fell under severe drought stress. To this aim, the leaf area was reduced relative to sufficient moisture conditions, and this may be a positive adaptation to acclimatization to dehydration (Isa *et al.*, 2021). Chekanaia *et al.* (2018) showed that the application of nitrogen (ammonium phosphate) and phosphorus (superphosphate) in beans doubled the dry weight and plant yield in both treatments compared to the control. Lubis *et al.* (2021) stated that dry weight of rice and plant height that received elements increased under manure medium. Potassium reduces the negative effect of drought stress on leaf area index and plant's dry weight by increasing the ability of photosynthesis, the rate of CO_2 stabilizing with interference in osmosis, the activity of the enzyme Rubisco, and improving the synthesis and transport of dry matter (Wang *et al.*, 2013). The data from the present experiment show that potassium increased plant resistance to drought stress, for which researchers published many reports on the subject (Aksu and Altay, 2020; Aqaei *et al.*, 2020).

In the present experiment, the negative correlation between plant dry weight and electrolyte leakage (Table 2) indicates that dry weight plants decreased in severe drought stress with increasing electrolyte leakage, which was consistent with the opinion of Khan *et al.* (2021). They showed that the plants dry weight decreased with increasing electrolyte leakage due to the increase of malondialdehyde in severe drought stress. Also, the results showed a positive correlation between chlorophyll content and plant dry weight (Table 2). Xiao *et al.* (2008) showed that chlorophyll depletion occurred under drought stress due to

chlorophyll decomposition.

This is the first work aimed at developing an appropriate methodology to study the use of organic soil amendments for plants such as hollyhock. The application of organic amendments also efficiently improved the uptake of N, P and K from the soil under water stress. Drought stress negatively affected plant traits, while soil amendments in planting beds improved plant performance and promoted plant resistance to drought stress. Based on the results of this study, soil with cow manure is suggested as an efficient soil amendment that is statistically better than rice husks and wheat straw for minimizing water requirements and improving plant tolerance to drought in a potted hollyhock. Due to the availability of cow manure compared to other substrates, the use of this material under potting soil conditions is recommended as these amounts cannot be used in the field. Further studies should be conducted on different methods to optimize the use of organic additives in the field as much as possible.

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