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JOURNAL OF WATER AND LAND DEVELOPMENT

e-ISSN 2083-4535

Polish Academy of Sciences (PAN) Institute of Technology and Life Sciences – National Research Institute (ITP – PIB)

JOURNAL OF WATER AND LAND DEVELOPMENT
DOI: 10.24425/jwld.2023.143755
2023, No. 56 (I–III): 142–152

Effect of proline and humic acid application in salinity stress mitigation on some vegetables in hydroponics

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RECEIVED 14.06.2022 ACCEPTED 10.08.2022 AVAILABLE ONLINE 13.03.2023

Abstract: The research aimed to find suitable solutions to reduce the salinity stress of irrigation water for some types of vegetables in hydroponics under two drip and mist irrigation systems. The different concentrations of NaCl for irrigation water, are 500, 1000, 2000, 3000, and 4000 ppm used. Proline (30 mg·dm^{-3}), humic acid (300 mg·dm^{-3}) and compared without any from them were used to study their effect on the yield, and water use efficiency. The results indicated that the highest spinach and courgette yield (4.657 and 5.153 kg·m^{-2}) was observed for the DP500 treatment, and the lowest yield (0.348 and 0.634 kg·m^{-2}) was observed for the SW4000 treatment, respectively. The use of humic acid led to an increased yield on average by about 16.8 and 19.3% for spinach, and 39.4 and 51.7% for courgette, under drip and mist irrigation, respectively. Using proline led to an increased average yield of about 32.9 and 33% for spinach, and 51.8 and 58.4% for courgette, under drip and mist irrigation, respectively. The highest water use efficiency (*WUE*) of spinach and courgette (43.1 and 51.5 kg·m^{-3}) was observed for the DP500 treatment, and the lowest (3.2 and 6.3 kg·m^{-3}) was observed for the SW4000 treatment. According to our study, the use of proline and humic acid could compensate for the adverse effects of salinity under mist spraying more than drip irrigation.

Keywords: courgette, drip irrigation, mist irrigation, spinach, yield

INTRODUCTION

Salinity stress is one of the most important and damaging abiotic stresses that globally affect plant growth and yield. Increased salinity levels in the root medium damage the plants during vegetative and reproductive stages and therefore reduce biomass and crop yield [Wani, Gosal 2011]. Under salt stress conditions, more Na^+ accumulated in the shoot and root of both cultivars while K^+ content decreased [Saidimoradi *et al.* 2019]. In addition, Atzori *et al.* [2019] pointed out that the use of seawater in soilless culture is an interesting option to limit freshwater withdrawal for food production, with any additional negative input into soils like salt accumulation. In The Netherlands, the paper was recently published on the usage of irrigation water up to a salinity level of 5–7 dS·m^{-1} for crop production of some varieties of the tested crops without any yield loss [De Vos *et al.* 2016]. Only recently, the effects of salt stress induced by different solutions made of NaCl and seawater have been evaluated on red lettuce [Sakamoto *et al.* 2014].

Using proline and humic acid might represent an option to reduce irrigation water salinity stress in coastal regions to pursue the relevant goal of increasing sustainable food production through resources not used for conventional agriculture, i.e. seawater and brackish waters as complementary irrigation waters [Atzori *et al.* 2017]. Humic acid is having an important role in the promotion of plant growth as biostimulation. It can induce alteration in plant primary and secondary metabolism linked to abiotic stress tolerance, which leads to improved plant growth and increased resistance against abiotic stress in addition to exogenous application of humic acid that increased shoot and root dry weight, plant growth, and improved plant tolerance against stress [Canellas *et al.* 2015; Canellas, Olivares 2014; Cimrin *et al.* 2010; Rose *et al.* 2014]. Saidimoradi *et al.* [2019] showed that humic acid reduced Na^+ and increased K^+ accumulation under salinity treatment. Salinity stress increased leaf necrotic area, the activity of antioxidant enzymes, hydrogen peroxide, lipid peroxidation, proline, and total soluble carbohydrates, while the supplementation of nutrient solution with humic acid recovered these traits and increased the salt tolerance index. Leaf relative water content, membrane stability index, chlorophyll content, total biomass, and yield were also negatively affected by salt stress; however, humic acid mitigated the adverse effects of salinity on these traits.

Some researchers demonstrate that the growing season, irrigation system, and nutrient solution concentration have an interactive effect on potted geranium production. During the spring season, the growing medium electrical conductivity (*EC*) increases much more rapidly with sub-irrigation than with drip-irrigation systems, especially at 2 dS·m^{-1}. So at higher temperatures, less concentrated fertiliser solutions should be used to maintain the *EC* of the growing medium at the desired level to avoid plant growth and quality reductions. The effect of the growing season was more pronounced than the effects of irrigation system and nutrient solution concentration [Rouphael *et al.* 2008]. An irrigation system can significantly impact the effect of irrigation water salinity on crop performance. Comparing the sub-irrigation and drip-irrigation systems using saline and non-saline nutrient solutions can be useful for developing optimal management strategies in semiarid regions which are characterised by the shortage of good quality water [Rouphael *et al.* 2006].

Spinach (*Spinacia oleracea*) has a large nutritional value and is a rich source of vitamins A, C, E, and K, as well as a source of folate, fibre, magnesium, and several important antioxidants [Abul-Soud, Mancy 2015]. Spinach is a moderately salt-sensitive leafy vegetable. Furthermore, spinach showed little growth impairment within a 17-day period after the addition of 100 mM NaCl to hydroponic cultures and in solution, cultures have shown that on an osmotic basis spinach is less sensitive to NaCl salt [Shannon, Grieve 1998; Speer, Kaiser 1991]. However, irrigation with saline water with an electrical conductivity of irrigation (EC_i) of 4 dS·m^{-1} on sandy soils in Israel resulted in no yield reduction and a harvestable product of superior quality [Pasternak, De Malach 1994]. The use of seawater in the hydroponic spinach cultivation determined positive effects on growth parameters, with an increased relative growth rate assessed for both seawater treatments compared to the control. Leaf morphological adaptations were assessed only in 20% of seawater-treated plants (i.e. reduced leaf water content, leaf area, and specific leaf area; increased specific leaf weight and leaf succulence), whereas the moderate seawater treatment did not lead to any difference compared to the control [Caparrotta *et al.* 2019].

Courgette (*Cucurbita pepo* L.) is a rich source of nutrients, vitamins, natural antioxidants, and healthful minerals [Rana 2017]. It is highly rated for economic value. Courgette's highest yield is at *EC* of 4.9 dS·m^{-1} and it is classified as a moderately salt-tolerant crop [Tanji, Kielen 2002]. The effect of *EC* of nutrient solution on courgette growth in hydroponic culture, where there was the concentration of the nutrient solution in the hydroponic sub-layer, is the cultivating factor that can be controlled and which has impacts on the plant growth and fruit quality. In courgette plants, the *EC* of 2.2 dS·m^{-1} resulted in further elongation of the leaf blades and petiole lengths of both leaves than the corresponding ones irrigated with the nutrient solution with increased salinity, with a *EC* of 4.4 dS·m^{-1}. The *EC* of 4.4 dS·m^{-1} caused an increase in the total soluble solid components of the fruits, a parameter that defines the preference of the courgette [Liopa-Tsakalidi *et al.* 2010].

The recycling of excess irrigation water applied to greenhouse crops is feasible since soilless growing systems are becoming increasingly popular among Mediterranean growers therefor. Recycling of the excess irrigation water that drains out of the root zone is possible in closed-cycle soilless growing systems and can considerably improve the water use efficiency in greenhouse crops consequently, to enable growers to adopt cropping systems with recycling of the excess irrigation water, henceforth termed drainage solution, efficient technologies have to be developed to minimise salt accumulation [Savvas, Passam 2002].

The control of nutrient solution was proposed with the adjustment of water level, the concentration of nutrients, and pH where, it is at a constant water level, the decrease in salt concentration is related to a decrease in *EC*, which can be used for monitoring the nutrient levels in the solution [Nielsen 1984].

It was the effect of two levels of *EC* at 2.2 dS·m^{-1} and 4.4 dS·m^{-1} in two nutrient solutions studied in the hydroponic culture of courgette *Cucurbita pepo* var. The results demonstrate that growing courgette can be adopted using the two conductivity levels. The results also indicate that the effect of conductivity of 4.4 dS·m^{-1} on total soluble solid components of courgette was significant and more pronounced than the effect of 2.2 dS·m^{-1} [Liopa-Tsakalidi *et al.* 2015].

The objective of this paper was to study the effect of using proline and humic acid at different concentrations of water salinity under drip and mist irrigation systems on the quality, yield, water use efficiency (*WUE*), and energy productivity of some vegetables like spinach and courgette in hydroponics.

MATERIALS AND METHODS

EXPERIMENTAL SITE

The field experiments were carried out in a plastic greenhouse (dimensions: length of 12 m, width of 4 m, and height in the range of 2–3 m). The greenhouse is situated at Tractors and Farm Machinery Research and Test Station, Alexandria Governorate (latitude 31°24' N, and longitude 29°98' E) during one season in 2021–2022 from November 20 to the middle of March. Weather data for the experimental site was taken from El-Nouzha airport station, Alexandria Governorate, Egypt, including daily temperature observations (°C), relative humidity (%), and wind speed (km·h^{-1}) at 2 m height. Weather data inside the greenhouse were measured using the environment meter apparatus (EM9300SD). The average air temperature in the experiment site ranged from 10 to 23°C, whereas the optimum temperature in the greenhouse for spinach, and courgette crops growth ranged from 21 to 28°C as mentioned by [Zaki *et al.* 2010]. Therefore, the daily mean air temperature in the greenhouse was 20–30°C during the growth season. Relative humidity in the study site ranged from 55.8 to 88.3% on average during the growing season. The optimum range of relative humidity for spinach and courgette crops is ranged from 60 to 70% which conforms to Zaki *et al.* [2010]. Therefore, the daily mean relative humidity inside the greenhouse was 30–70% during the growth season. The max wind speed ranged between 9 and 61 km·h^{-1} with an average of 23.3 km·h^{-1} during the growing season.

EXPERIMENTAL DESCRIPTION

The field experiments were carried out for some types of vegetables like spinach (*Spinacia oleracea*), and courgette (*Cucurbita pepo* L.) using proline and humic acid in a closed hydroponic system. A hydroponic system was established under two systems of drip irrigation and mist spraying at different concentrations of irrigation water salinity in the nutrient solution, which are 500, 1000, 2000, 3000, and 4000 ppm of NaCl. Proline (L. proline extra pure; $C_5H_9NO_2$, molecular weight (MW) – 115.13, concentrate – 30 mg·dm^{-3}) and humic acid (potassium humate, humate 75%, K_2O 10%, concentrate – 300 mg·dm^{-3}) were used to mitigate water salinity stress; they were added once every two weeks at the mentioned rate during irrigation. In hydroponics, the Hoagland nutrient solution was used and replaced every 15 days. The experiment was divided into five salinity levels of NaCl for the nutrient solution and each solution salinity level was divided into two irrigation systems; each irrigation system used three materials (proline, humic acid, and without any of them). Each material is considered a treatment. The experimental treatments were as shown in Table 1. The treatment was divided into four replicates. Each replicate was specified as a square tube of a scale 0.1 (width) × 0.1 (depth) × 3 m (length), and each square tube contains a number of buckets for spinach and courgette plants, 15 and 6 buckets respectively. The bucket scale for spinach was 0.15 (diameter) × 0.16 m (depth) but for the courgette plant, it was 0.16 (diameter) × 0.25 m (depth). Each bucket was planted with one seedling. Growth media in the bucket were sand, peat moss, and perlite at a rate of 3:1:1. The growth media were disinfected and sterilised before starting cultivation. Distances between buckets were 20 and 50 cm for spinach and courgette plants respectively. The drip irrigation system used lateral lines containing online emitters of 4 dm^3·h^{-1} discharge, where each bucket used one emitter. The mist spray irrigation system used lateral lines containing a mist sprayer of 39.6 dm^3·h^{-1} discharge at low pressure of 2 psi (1 psi = 6 894.75729 Pa), where each treatment used five sprayers to cover the area completely. Drip and mist spraying irrigation systems powered by solar photovoltaics with direct current (DC) pump were used to study the effect on energy productivity. Components of the solar pumping system were the solar panel (0.40 × 0.55 m), the charging unit (12 V – 10 A) that delivered a signal to charge battery, the sealed lead acid battery (12 V – 2.2 A), and DC pump (12 V – 15 W), flow – 3.1 dm^3·min^{-1} and pressure – 0.55 MPa, as shown in Figure 1. Data analysis was carried out based on a triple factorial analysis using the Minitab software package (ver. 16). The mean values of the treatments were compared using the least significant difference (*LSD*) test at a significance level of 0.05.

YIELD AND PLANT CHARACTERISTICS

The harvest was in the growing season (2021/2022) at the optimum stage of physiological maturity. Harvesting was done about 55, and 120 days after transplanting the vegetables used in the study, such as spinach and courgette for one season, respectively. The fruit yield (grams per plant) was determined based on all fruits harvested from each treatment. The yield was determined for each bucket and each represented treatment. The crops were hand-harvested and weighted using a sensitive scale of ±0.01 g with a capacity of 2.0 kg and adjusted to yield in kg·m^{-2}. Plants' height, leaf number (*n*) and total leaf area (*TLA*) were determined.

1. **Fresh and dry weight.** Fully expanded leaves were collected at the end of the experiment and immediately after harvest; leaf samples were frozen in liquid nitrogen and were stored at −80°C until further analyses. At the end of the experiment from each treatment and each crop from used vegetables, such as red beet, spinach, and courgette, four plants per treatment were randomly assigned to measure vegetative characteristics at the end of the season. To measure the dry mass of leaves, petioles, crowns, and roots, samples were placed in an air-circulating oven (UFP800, Memmert, Germany) at 65°C for 72 h.
2. **Salt tolerance index (*STI*).** *STI* is the ratio of the plant dry weight (*DW*) of the control treatment and the plant dry weight of the salt treatments [El Goumi *et al.* 2014; Saidimoradi *et al.* 2019] as shown from the following relation:

$$STI\% = \frac{\text{Total } DW \text{ salt stress}}{\text{Total } DW \text{ control}} 100 \tag{1}$$

3. **Plant water content (*PWC*).** *PWC* was estimated gravimetrically on the basis of [Santos *et al.* 2013] method using Equation (3), as follows:

Table 1. The experimental treatment solutions

Salinity levels of NaCl for nutrient solution	Irrigation system					
	drip (D)			mist spray (S)		
	used material to mitigate salinity stress					
	without (W)	humic (H)	proline (P)	without (W)	humic (H)	proline (P)
500	DW500	DH500	DP500	SW500	SH500	SP500
1000	DW1000	DH1000	DP1000	SW1000	SH1000	SP1000
2000	DW2000	DH2000	DP2000	SW2000	SH2000	SP2000
3000	DW3000	DH3000	DP3000	SW3000	SH3000	SP3000
4000	DW4000	DH4000	DP4000	SW4000	SH4000	SP4000

Source: own study.

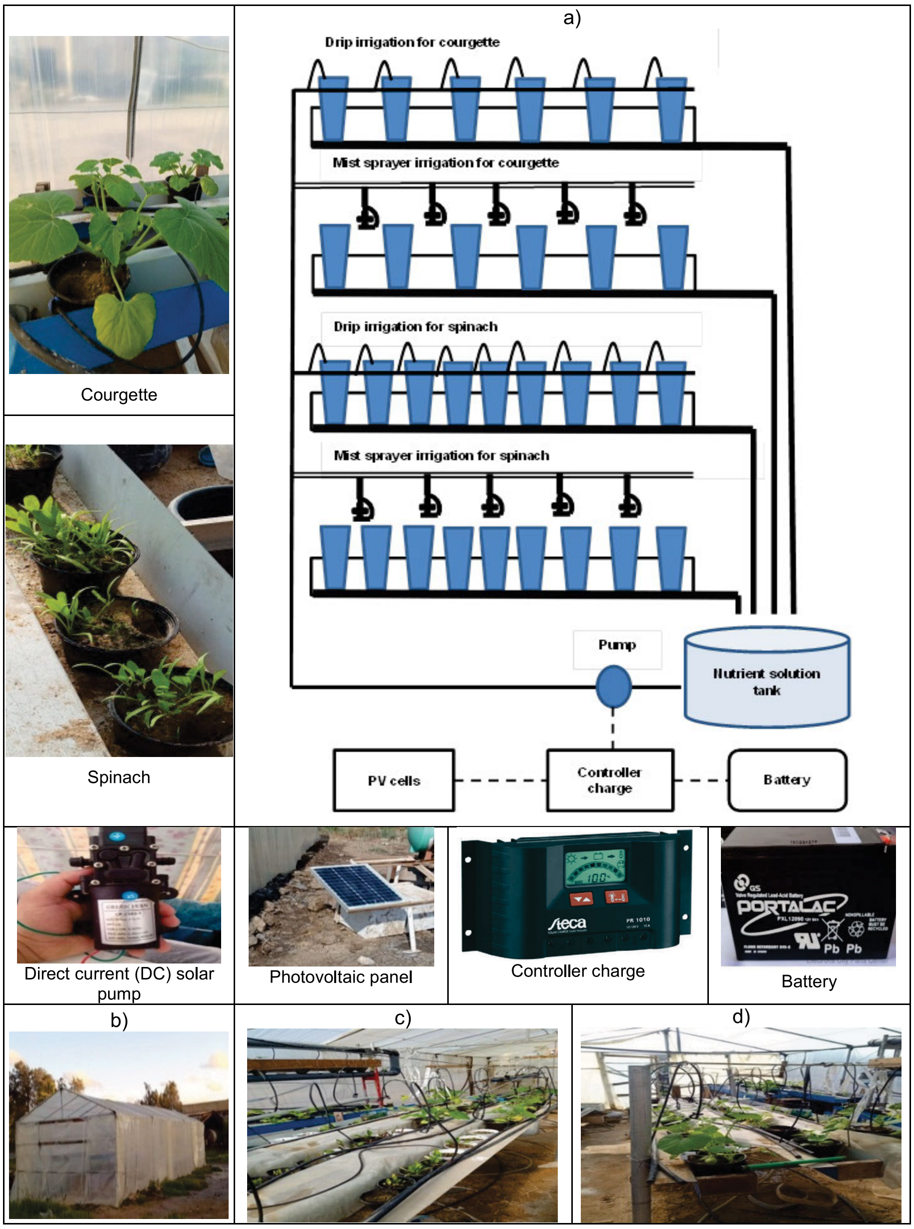


Fig. 1. Layout of hydroponics system in greenhouse powered by photovoltaic (PV) solar energy for cultivated spinach and courgette in buckets; a) diagram of experiment components, b) outside greenhouse, c) and d) inside greenhouse; source: own study

$$PWC\% = \frac{FW - DW}{FW} 100 \qquad (2)$$

where: FW = sample fresh weight, DW = sample dry weight.

4. **Water use efficiency (*WUE*)**. *WUE* g·dm^{-3} (kg·m^{-3}) was calculated according to JAMES [1988] as follows:

$$WUE = \frac{1000Y}{Wa} \qquad (3)$$

where: Y = total crop yield (kg·m^{-2}), Wa = total applied water (dm^3·m^{-2}).

Total applied water was calculated to assist the relations and equations from references [ISMAIL 2002; KHALIL 1998].

5. **Solar energy productivity.** Solar energy requirement for irrigation relies on several parameters including the type of irrigation system (mist or drip), pump head, and volumetric flow rate. Equation (4) shows solar energy as a function of some important parameters [KELLEY *et al.* 2010]:

$$Es = \frac{r \cdot g \cdot Q \cdot h \cdot t \cdot n}{3600 E_p \cdot E_{pv}} \qquad (4)$$

where: Es = solar energy requirement during the growth season (kWh·m^{-2}), r = water density (1000 kg·m^{-3}), g = the gravitational acceleration (9.81 m·s^{-2}), Q = the volumetric flow rate (m^3·h^{-1}), h = pumping head (m), t = daily operating time (h), n = a number of days during the season, E_p = DC-pump efficiency (90%), E_{pv} = PV cell efficiency (74%).

The solar energy productivity for pumping irrigation water during the growing season was calculated as follows:

$$SEP = \frac{Y}{Es} \qquad (5)$$

where: SEP = solar energy productivity (kg·kWh^{-1}), Y = total crop yield (kg·m^{-2}).

RESULTS AND DISCUSSION

CHARACTERISTICS OF PLANTS

Implementation of salt stress decreased almost all vegetative traits physiological responses, also salinity stress decreased salt tolerance index and plant water content while the use of proline and humic acid recovered these traits and increased salt tolerance index and plant water content, and thus led to a decrease in spinach and courgette yield and water use efficiency as shown in Tables 2 and 3.

Height of plants

The use of humic acid led to an increase in spinach and courgette height of plants (*H*) of about 12 and 16%, while the use of proline led to an increase of about 19 and 23%, respectively. For spinach,

Table 2. Growth and productivity parameter of spinach under using proline and humic acid with different concentrations of NaCl under drip and mist irrigation systems

Treatment	*H*	*n*	*TLA* (cm^2)	*FW*	*DW*	*STI*	*PWC*	Yield ($kg \cdot m^{-2}$)	*WUE* ($kg \cdot m^{-3}$)
DW500	33.4	13.0	1283.9	187.3	15.2	76.7	91.85	3.371	31.2
DH500	37.3	14.0	1540.3	220.5	17.0	85.7	92.26	3.969	36.8
DP500	39.5	13.8	1696.7	258.7	18.0	90.9	93.03	4.657	43.1
DW1000	30.8	11.8	829.7	143.0	11.3	57.1	92.08	2.574	23.8
DH1000	32.4	13.0	1031.7	166.7	12.7	64.0	92.36	3.001	27.8
DP1000	34.1	13.8	1169.3	188.4	13.7	69.3	92.69	3.391	31.4
DW2000	22.8	8.3	400.5	89.5	8.3	42.3	90.43	1.611	14.9
DH2000	25.5	9.3	500.0	104.3	9.3	47.3	90.85	1.877	17.4
DP2000	26.9	9.8	562.0	117.9	10.1	51.3	91.20	2.123	19.7
DW3000	16.8	5.5	187.9	55.1	4.3	21.7	92.17	0.991	9.2
DH3000	18.7	6.3	238.3	64.1	4.8	24.3	92.47	1.154	10.7
DP3000	19.9	6.5	263.5	72.4	5.0	25.3	93.06	1.304	12.1
DW4000	12.4	3.8	73.6	23.0	3.6	18.1	84.38	0.414	3.8
DH4000	13.8	4.4	94.0	26.8	4.0	20.3	84.97	0.483	4.5
DP4000	14.7	4.5	98.6	30.3	4.2	21.1	86.17	0.545	5.0
SW500	32.5	12.0	982.1	170.5	13.5	68.4	92.06	3.026	28.4
SH500	35.8	13.3	1205.0	206.0	15.1	76.4	92.66	3.707	34.3
SP500	39.3	14.0	1436.5	227.0	16.4	82.9	92.76	4.087	37.8
SW1000	27.6	10.5	671.1	134.5	10.4	52.2	92.31	2.421	22.4
SH1000	30.0	11.5	823.2	159.5	11.6	58.3	92.73	2.871	26.6
SP1000	31.0	12.5	942.4	178.1	12.6	63.2	92.95	3.206	29.7
SW2000	20.1	7.5	324.3	81.4	6.5	32.7	91.83	1.465	13.6
SH2000	22.5	8.5	410.7	96.3	7.3	36.6	92.30	1.733	16.0
SP2000	23.8	9.0	463.6	107.7	7.5	38.0	92.86	1.938	17.9
SW3000	14.5	4.5	137.9	37.1	3.6	18.2	90.24	0.667	6.2
SH3000	16.2	5.3	182.3	43.9	4.0	20.3	90.78	0.789	7.3
SP3000	17.2	5.5	201.5	49.2	4.2	21.1	91.36	0.886	8.2
SW4000	10.8	3.3	53.4	19.3	3.4	17.1	82.39	0.348	3.2
SH4000	12.0	3.5	66.6	22.9	3.8	19.1	83.38	0.412	3.8
SP4000	12.7	4.0	76.3	25.6	3.9	19.9	84.39	0.462	4.3

Explanations: D, S, W, H, P = as in Tab. 1, *H* = height of plants, *n* = number of leaves, *TLA* = total leaf area, *FW* = fresh weight, *DW* = dry weight, *STI* = salt tolerance index, *PWC* = plant water content, *WUE* = water use efficiency.
Source: own study.

Table 3. Growth and productivity parameter of courgette under using proline and humic acid with different concentrations of NaCl under drip and mist irrigation systems

Treatment	*H*	*n*	*TLA* (cm^2)	*FW*	*DW*	*STI*	*PWC*	Yield ($kg·m^{-2}$)	*WUE* ($kg·m^{-3}$)
DW500	37.3	32.0	2962.4	1557.5	141.0	76.5	90.94	3.167	31.7
DH500	38.3	35.6	3974.2	2024.8	157.6	85.5	92.21	4.590	45.9
DP500	41.1	38.3	5258.0	2180.5	169.9	92.1	92.20	5.153	51.5
DW1000	31.0	30.0	2576.3	1460.0	133.5	72.4	90.85	2.677	26.8
DH1000	32.8	30.5	3272.3	1546.2	149.2	80.9	90.30	3.782	37.8
DP1000	34.3	32.0	4671.0	1535.4	161.9	87.8	89.42	4.452	44.5
DW2000	30.5	29.5	1559.5	1042.5	123.0	66.7	87.95	2.014	20.1
DH2000	34.8	33.5	2164.3	1152.4	137.5	74.5	87.82	2.994	29.9
DP2000	34.8	34.8	3192.8	1190.1	149.2	80.9	87.22	3.241	32.4
DW3000	24.0	24.0	1459.6	362.5	64.3	34.9	82.03	1.355	13.6
DH3000	24.5	25.5	1795.9	418.3	71.8	39.0	82.44	1.889	18.9
DP3000	26.8	26.6	1765.5	425.2	74.8	40.6	82.34	1.915	19.2
DW4000	21.3	17.3	895.6	150.0	39.0	21.1	73.19	0.677	6.8
DH4000	22.4	19.5	957.6	169.5	43.7	23.7	73.12	0.833	8.3
DP4000	24.3	21.6	1598.3	175.2	45.4	24.6	73.56	0.865	8.7
SW500	35.5	30.8	2601.6	1392.5	133.5	72.4	90.41	2.976	29.8
SH500	37.3	32.8	3790.7	1502.3	149.2	80.9	90.04	4.675	46.8
SP500	41.2	34.6	4366.9	1521.5	161.9	87.8	89.34	4.383	43.8
SW1000	31.8	28.3	2658.9	1385.0	128.3	69.5	90.74	2.800	28.0
SH1000	33.3	30.8	3053.4	1527.0	143.4	77.7	90.61	4.191	41.9
SP1000	36.5	31.3	3908.6	1582.5	155.6	84.3	90.16	4.483	44.8
SW2000	25.8	24.3	1863.0	912.5	82.3	44.6	90.97	1.700	17.0
SH2000	28.2	26.5	2510.2	1008.2	92.0	49.9	90.86	2.632	26.3
SP2000	30.6	28.7	3442.5	1042.4	95.7	51.9	90.80	2.870	28.7
SW3000	20.8	22.0	1310.1	320.0	53.5	29.0	83.10	1.003	10.0
SH3000	22.0	21.5	1215.6	360.3	59.9	32.5	83.11	1.532	15.3
SP3000	25.5	23.0	1956.2	375.9	62.3	33.8	83.38	1.632	16.3
SW4000	19.8	19.3	780.9	124.8	31.8	17.2	74.54	0.634	6.3
SH4000	22.9	21.4	810.6	140.3	35.5	19.3	74.57	0.916	9.2
SP4000	23.6	22.8	1372.0	151.8	37.0	20.0	75.60	0.971	9.7

Explanations: D, S, W, H, P = as in Tab. 1, *H*, *n*, *TLA*, *FW*, *DW*, *STI*, *PWC*, *WUE* as in Tab. 2.
Source: own study.

the highest length (39.5 cm) was observed using the DP500 treatment, and the lowest length (10.8 cm) – for the SW4000 treatment. For the courgette, the highest length (41.2 cm) was observed using the SP500 treatment, and the lowest length (19.8 cm) – for the SW4000 treatment. It can be noted there were no significant differences ($p > 0.01$) in the lengths of spinach and courgette plants, as a result of the binary and triple interaction of the materials used to mitigate salinity stress, like proline and humic acid application for salinity stress mitigation in hydroponics with salinity levels of NaCl for the nutrient solution in drip and mist irrigation systems. The statistical analysis showed that there were significant effects ($p < 0.01$) due to irrigation systems, salinity levels, and each factor alone on plant height.

Number of leaves

For spinach, the most number of leaves (*n*) (14 leaves per plant) was observed for both the DH500 and SP500 treatments, and the minimum number of leaves (3.25 leaves per plant) was observed for the SW4000 treatment. For courgette, the most number of leaves (38.25 leaves per plant) was observed for the DP500 treatment, and the minimum number of leaves (17.25 leaves per plant) – for the DW4000 treatment. It can be noted there were no significant differences ($p > 0.01$) in the number of spinach and courgette leaves as a result of the binary and triple interaction of the materials used to mitigate salinity stress, like proline and humic acid application for salinity stress mitigation in hydroponics with NaCl salinity levels for a nutrient solution in drip and mist irrigation systems. The statistical analysis showed that there were significant effects ($p < 0.01$) due to irrigation systems, salinity levels, and each factor alone on the number of leaves per plant for both spinach and courgette.

Total leaf area

The total leaf area (*TLA*) represents one of the important physiological factors indicating the effect of irrigation water salinity in hydroponics on spinach and courgette plants. For spinach, the highest total leaf area per plant (1696.7 cm^2) was observed for the DP500 treatment, and the lowest total leaf area (53.4 cm^2) – for the SW4000 treatment. For the courgette, the highest total leaf area (5258 cm^2) was observed for the SP500 treatment, and the lowest total leaf area (780 cm^2) – for the SW4000 treatment. It can be noted there were no significant differences ($p > 0.01$) in the total leaves area per plant of spinach and courgette as a result of the binary and triple interaction of the materials used to mitigate salinity stress, such as proline and humic acid application for salinity stress mitigation in hydroponics with NaCl salinity levels for the nutrient solution in drip and mist irrigation systems. The statistical analysis showed that there were significant effects ($p < 0.01$) due to irrigation systems, salinity levels, and each factor alone on the total leaf area for both spinach and courgette.

Fresh weight

Fresh weight (*FW*) includes water, which is less reliable when trying to judge how specific plant management or system management is impacting the plant. Salinity decreased the fresh weight of spinach and courgette from 187.3 to 23 g, and from 170.5 to 19.3 g, for drip and mist irrigation, respectively for salinity levels from 500 to 4000 ppm of NaCl salts as shown in Tables 2 and 3. For spinach, the highest fresh weight (258.7 g) was observed for the DP500 treatment, and the lowest fresh weight (19.3 g) – for the SW4000 treatment. The use of humic acid led to an increase of the fresh weight of spinach by about 16–18 and 18–21%, while the use of proline – by about 31–38 and 32–33%, for drip and mist irrigation, respectively. For the courgette, the highest fresh weight (2180.5 g) was observed for the DP500 treatment, and the lowest fresh weight (124.8 g) – for the SW4000 treatment. The use of humic acid led to increasing the fresh weight of the courgette by about 6–30 and 8–13%, while the use of proline – by about 5–40 and 9–22%, for drip and mist irrigation, respectively. It can be noted there was no significant difference ($p > 0.01$) in the fresh weight as a result of the triple and binary interaction between the materials used to mitigate salinity stress in irrigation systems for spinach. Also, there was no significant difference ($p > 0.01$) in the binary interaction between the used materials to mitigate salinity stress in irrigation systems for courgette. The statistical analysis showed that there were significant effects ($p < 0.01$) due to the rest of the single factors on fresh weight and also, the binary and triple interactions for both spinach and courgette.

Dry weight

Dry weight (*DW*) refers to all constituents of plants except water and is a more reliable option to analyse weight. Salinity decreased the dry weight of spinach, from 15.2 to 3.6 g, from 13.5 to 3.4 g, and also courgette, from 141 to 39 g, and from 133.5 to 31.8 g, for drip and mist irrigation, respectively for salinity levels from 500 up to 4000 ppm of NaCl salts. For spinach, the highest dry weight (18 g) was observed for the DP500 treatment, and the lowest dry weight (3.4 g) – for the SW4000 treatment. Using humic acid led to an increase of the dry weight of spinach by about 12 and 12%, while using proline led to an increase of about 16–21 and 16–21%, for drip and mist irrigation, respectively. For the courgette, the highest dry weight (169.9 g) was observed for the DP500 treatment, and the lowest dry weight (31.8 g) – for the SW4000 treatment. The use of humic acid led to an increase of the dry weight of the courgette by about 12 and 12%, while the use of proline led to an increase by about 16–21 and 16–21%, for drip and mist irrigation, respectively. It can be noted there were no significant differences ($p > 0.01$) in the *DW* of spinach and courgette as a result of the triple interaction between the indicated factors in the study. Also, there was no significant difference ($p > 0.01$) in the binary interaction between the used materials to mitigate salinity stress in irrigation systems for spinach and courgette. The statistical analysis showed that there was a significant effect ($p < 0.01$) of the rest individual factors also on dry weight, the binary interactions between salinity levels, and the materials used to mitigate salinity for spinach and courgette.

Salt tolerance index

The salt tolerance index (*STI*%) is a guide to the ability of plants to grow and complete their life cycle on a substrate that contains high concentrations of soluble salt. Salinity decreased the salt tolerance index of spinach and courgette by 76 and 72%, and by 75 and 76%, for drip and mist irrigation, respectively for salinity levels from 500 up to 4000 ppm of NaCl, which is confirmed by Saidimoradi *et al.* [2019]. In comparison, humic acid and proline application improved the growth of the shoot under salinity, which contributed to an increase in the *STI* [Cimrin *et al.* 2010]. For spinach, the highest *STI* (91%) was observed for the DP500 treatment, and the lowest *STI* (17%) – for the SW4000 treatment. The use of humic acid led to an increase of *STI* of spinach by about 12%, while the use of proline – by about 16–21% for drip and mist irrigation, respectively. For courgette, the highest *STI* (92%) was observed for the DP500 treatment, and the lowest *STI* (17.2%) – for the SW4000 treatment. Using humic acid led to an increase in the *STI* of the courgette by about 12%, while the use of proline led to an increase of about 16–21%, for drip and mist irrigation, respectively. It can be noted there were no significant differences ($p > 0.01$) in the *STI* of spinach and courgette as a result of the binary and triple interaction of the materials used to mitigate salinity stress, like proline and humic acid application for salinity stress mitigation in hydroponics, with salinity levels of NaCl for the nutrient solution in drip and mist irrigation systems. The statistical analysis showed that there were significant effects ($p < 0.01$) due to irrigation systems, salinity levels, and each factor alone on the salt tolerance index for both spinach and courgette.

Plant water content

Salinity decreased the water content of spinach from 91.85 to 84.38%, and from 92 to 82.4%, as well as courgette – from 91 to 73%, and from 90 to 75%, for drip and mist irrigation, respectively for salinity levels from 500 up to 4000 ppm of NaCl. The highest plant water content (*PWC*) of spinach (93%) was observed for the DP500 treatment, and the lowest (82.4%) – for the SW4000 treatment. The highest *PWC* of courgette (92.21%) was observed for the DH500 treatment, and the lowest (73.12%) –

for the DH4000 treatment. It can be noted there were no significant differences ($p > 0.01$) in the *PWC* of spinach and courgette plants as a result of the triple and binary interactions between the indicated factors in the study. Also, there was no significant difference ($p > 0.01$) in the irrigation systems factor of spinach and courgette plants, and the materials used to mitigate the salinity stress of courgette. The statistical analysis showed that there was a significant effect ($p < 0.01$) of the single factors alone on water content, as well as the binary interactions between salinity levels and irrigation systems for spinach and courgette.

Spinach and courgette yield

The results showed that increasing the salinity concentration of irrigation water from 500 up to 4000 ppm led to a decrease in spinach and courgette yield (from 23.6 up to 87.7%; from 20 up to 88.5%, and from 15.5 up to 87.6%; from 5.9 up to 78.7%, for drip and mist irrigation, respectively, as shown in Figure 2). The highest spinach yield (4.657 kg·m^{-2}) was observed for the DP500 treatment, and the lowest spinach yield (0.348 kg·m^{-2}) – for the SW4000 treatment, as shown in Table 2. The highest courgette yield (5.153 kg·m^{-2}) was observed for the DP500 treatment, and the lowest courgette yield (0.634 kg·m^{-2}) – for the SW4000 treatment, as shown in Table 3. The results showed that the use of humic acid led to an increase in spinach yield by 17.8, 16.6, 16.5, 16.5, and 16.5% under drip irrigation, and by 22.5, 18.6, 18.3, 18.3, and 18.5% under mist irrigation for salinity levels of 500, 1000, 2000, 3000, and 4000 ppm, respectively, as shown in Figure 3a.

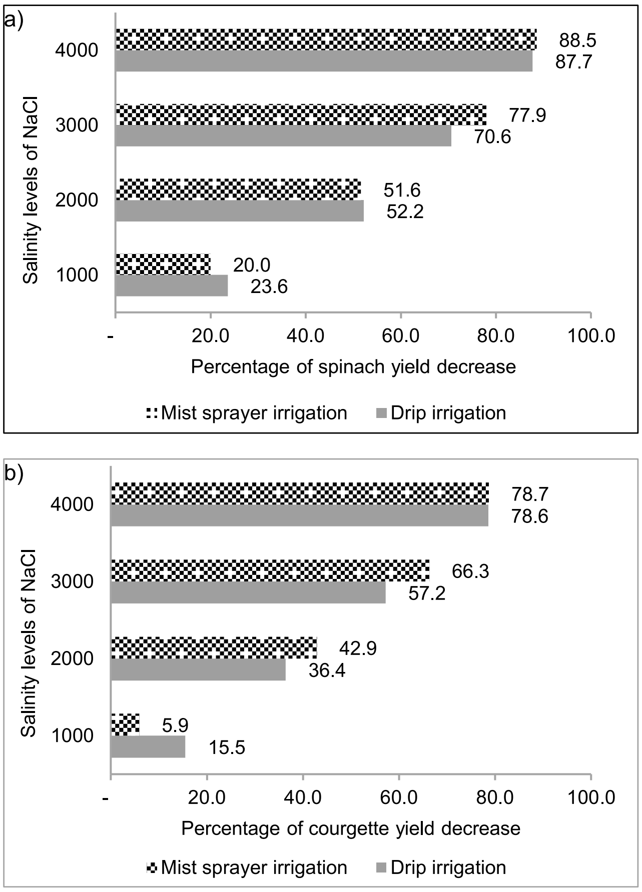


Fig. 2. Decrease percentage in yield with different concentrations of NaCl under drip and mist irrigation systems: a) spinach, b) courgette; source: own study

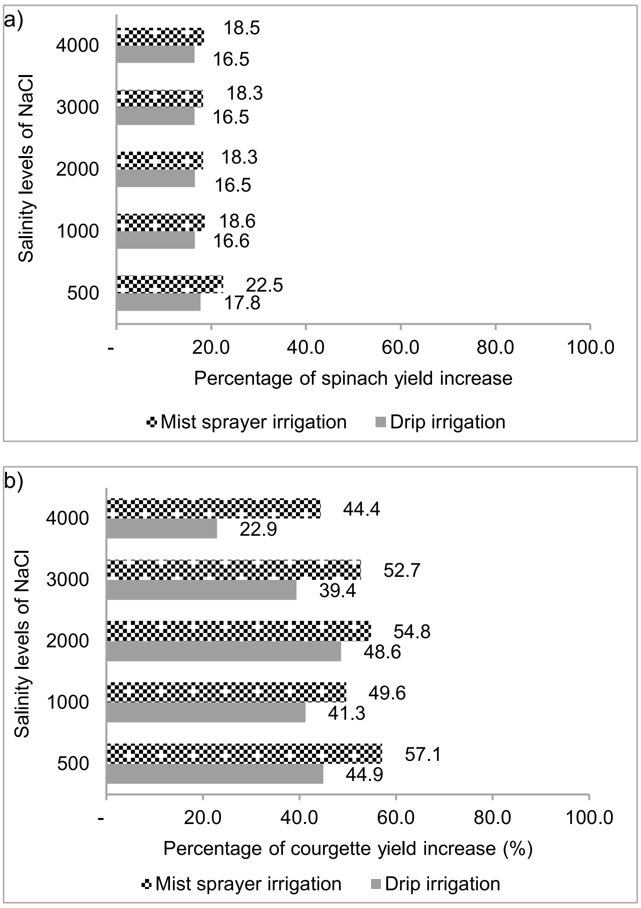
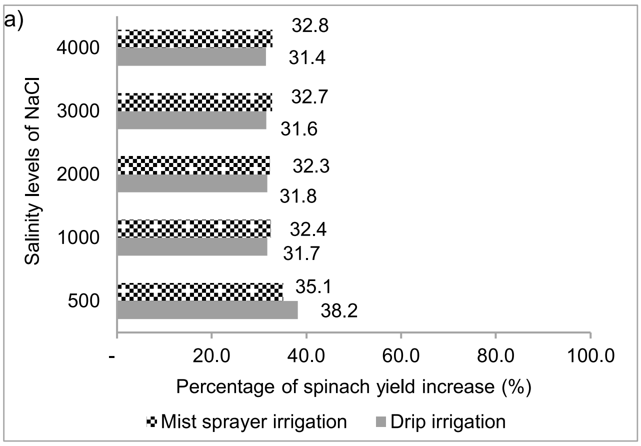


Fig. 3. Increase percentage in yield due to using humic acid with different concentrations of NaCl under drip and mist irrigation systems; a) spinach, b) courgette; source: own study

Also, the use of humic acid led to an increase in courgette yield by 44.9, 41.3, 48.6, 39.4, and 22.9% under drip irrigation, and by 57.1, 49.6, 54.8, 52.7, and 44.4% under mist irrigation for salinity levels 500, 1000, 2000, 3000, and 4000 ppm, respectively, as shown in Figure 3b. Results demonstrated that the use of proline led to an increase in spinach yield by 38.2, 31.7, 31.8, 31.6, and 31.4% under drip irrigation and by 35.1, 32.4, 32.3, 32.7, and 32.8% under mist irrigation for salinity levels 500, 1000, 2000, 3000, and 4000 ppm, respectively, as shown in Figure 4a. Also, the use of proline led to an increase in courgette yield by 62.7, 66.3, 60.9, 41.3, and 27.8% under drip irrigation, and by 47.3, 60.1, 68.8, 62.7, and 53.1% under mist irrigation for salinity levels 500, 1000, 2000, 3000 and 4000 ppm, respectively, as shown in Figure 4b. The results also showed that the humic and proline spraying system is better than the addition of drip irrigation. The use of proline was better than that of humic acid. It can be noted, there was no significant difference ($p > 0.01$) in the spinach and courgette yield as a result of the triple interaction between the indicated factors in the study. Also, there was no significant difference ($p > 0.01$) in the binary interaction between the materials used to mitigate salinity stress and irrigation systems. The statistical analysis showed that there was a significant effect ($p < 0.01$) of the single factors alone on spinach, as well as courgette yield, the binary interactions between salinity levels and irrigation systems, salinity levels, and the used materials to mitigate salinity for spinach and courgette.



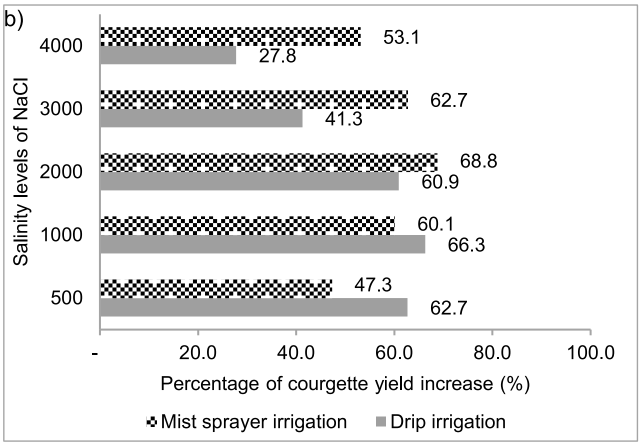


Fig. 4. Increase percentage in yield due to using proline with different concentrations of NaCl under drip and mist irrigation systems: a) spinach, b) courgette; source: own study

Water use efficiency

The highest water use efficiency (*WUE*) of spinach (43.1 kg·m^{-3}) was observed for the DP500 treatment, and the lowest *WUE* (3.2 kg·m^{-3}) – for the SW4000 treatment, as shown in Table 2. The highest *WUE* of courgette (51.5 kg·m^{-3}) was observed for the DP500 treatment, and the lowest *WUE* (6.3 kg·m^{-3}) – for the SW4000 treatment, as shown in Table 3. The results showed that the use of humic acid led to an increase in *WUE* of spinach from 16 to 18% under drip irrigation, and from 18 to 21% under mist irrigation, respectively, as shown in Table 2. Also, the use of humic acid led to an increase in *WUE* of courgette from 23 to 49% under drip irrigation, and from 44 to 57% under mist irrigation, respectively, as shown in Table 3. Using proline led to an increase in *WUE* of spinach from 31 to 38% under drip irrigation, and from 32 to 33% under mist irrigation, respectively, as shown in Table 2. Also, the use of proline led to an increase in *WUE* of courgette from 28 to 66% under drip irrigation, and from 47 to 69% under mist irrigation, respectively, as shown in Table 3. It can be noted, there were no significant differences ($p > 0.01$) in the *WUE* of spinach as a result of the triple interaction between the indicated factors in the study, while there were significant effects ($p < 0.01$) for the rest of the single factors, the binary, and triple interactions. Also, there were significant effects ($p < 0.01$) for the *WUE* of courgette for all of the factors.

SOLAR ENERGY PRODUCTIVITY

The highest energy productivity of spinach (69.8 kg·kWh^{-1}) was observed for the DP500 treatment, and the lowest (0.7 kg·kWh^{-1}) – for the SW4000 treatment. Also, the highest energy productivity of courgette (45.1 kg·kWh^{-1}) was observed for the DP500 treatment, and the lowest (0.7 kg·kWh^{-1}) – for the SW4000 treatment, as shown in Table 4. The energy productivity of mist irrigation was lower than that of drip. The results showed that the use of humic acid led to an increase in energy productivity of spinach by 16–18% under drip irrigation, and by 18–23% under mist irrigation for salinity levels of 500, 1000, 2000, 3000, and 4000 ppm,

Table 4. Solar energy productivity (*SEP*) under using proline and humic acid with different concentrations of NaCl under drip and mist irrigation systems

Treatment	*SEP* (kg·kWh^{-1})	
	spinach	courgette
DW500	50.5	27.7
DH500	59.5	40.1
DP500	69.8	45.1
DW1000	28.9	17.6
DH1000	33.7	24.8
DP1000	38.1	29.2
DW2000	12.1	8.8
DH2000	14.1	13.1
DP2000	15.9	14.2
DW3000	5.6	4.4
DH3000	6.5	6.2
DP3000	7.3	6.3
DW4000	1.6	1.5
DH4000	1.8	1.8
DP4000	2.0	1.9
SW500	24.2	13.9
SH500	29.6	21.8
SP500	32.7	20.4
SW1000	14.5	9.8
SH1000	17.2	14.7
SP1000	19.2	15.7
SW2000	5.9	4.0
SH2000	6.9	6.1
SP2000	7.7	6.7
SW3000	2.0	1.8
SH3000	2.4	2.7
SP3000	2.7	2.9
SW4000	0.7	0.7
SH4000	0.8	1.1
SP4000	0.9	1.1

Explanations: D, S, W, H, P = as in Tab. 1.
Source: own study

respectively. Also, the use of humic acid led to an increase in energy productivity of courgette by 23–49% under drip irrigation, and by 44–57% under mist irrigation for salinity levels of 500, 1000, 2000, 3000, and 4000 ppm, respectively, as shown in Table 4. Using proline led to an increase in energy productivity of spinach by 31–38% under drip irrigation, and by 32–35% under mist irrigation for salinity levels of 500, 1000, 2000, 3000, and 4000 ppm, respectively. The use of proline led to an increase in energy productivity of courgette by 28–66% under drip irrigation, and by 47–69% under mist irrigation for salinity levels of 500, 1000, 2000, 3000, and 4000 ppm, respectively, as shown in Table 4.

CONCLUSIONS

The field experiments were carried out in a plastic greenhouse aiming to study the effect of using proline and humic acid with different concentrations of irrigation water salinity in the nutrient solution under drip and mist irrigation systems on plants, to characterise the productivity of some vegetables and water use efficiency. It was found that salt stress reduced almost all vegetative traits' physiological responses, like plant height, the number of leaves, total leaf area, fresh and dry weight. Salinity stress decreased the salt tolerance index and plant water content while the use of proline and humic acid led to the restoration of these traits and increased the salt tolerance index and plant water content. The results showed that increasing the salinity concentration of irrigation water from 500 up to 4000 ppm led to a decrease in spinach and courgette yield and water use efficiency by about 80%. The highest spinach and courgette yield (4.7, 5.2 $kg \cdot m^{-2}$) were observed for the DP500 treatment, and the lowest (0.3, 0.6 $kg \cdot m^{-2}$) – for the SW4000 treatment, respectively. The highest *WUE* of spinach and courgette (43.1, 51.5 $kg \cdot m^{-3}$) was observed for the DP500 treatment, and the lowest (3.2, 6.3 $kg \cdot m^{-3}$) – for the SW4000 treatment. Using proline led to an increased average yield of about 32.9, 33% for spinach, and 51.8, 58.4% for courgette, under drip and mist irrigation, respectively. The use of the humic acid led to a yield increase on average by about 16.8, 19.3% for spinach, and 39.4, 51.7% for courgette, under drip and mist irrigation, respectively. The humic and proline spraying system irrigation is better than the addition of drip irrigation. The use of proline was better than that of humic acid. The highest energy productivity of spinach and courgette (69.8, 45.1 $kg \cdot kWh^{-1}$) was observed for the DP500 treatment, and the lowest (0.7, 0.7 $kg \cdot kWh^{-1}$) – for the SW4000 treatment. The results concluded that the use of humic acid led to an increase in energy productivity of spinach and courgette by 16–18, and 23–49% under drip irrigation, and by 18–23, and 44–57% under mist irrigation, respectively. Also, it was found that the use of proline led to an increase in energy productivity of spinach and courgette by 31–38, and 28–66% under drip irrigation, and by 32–35, and 47–69% under mist irrigation, respectively. The statistical analysis showed a highly significant effect as a result of the application of proline and humic acid with different salinity levels on spinach and courgette yield and water use efficiency. According to our study, spinach and courgette plants, that were fed with proline and humic acid, were more able to offset the negative effects of salinity under spray mist irrigation than with drip irrigation.

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