

Improving Herb and Volatile Oil Yield, As Well as Salt Resistance Index of Sweet Basil Plants (*Ocimum Basilicum*, L.) Grown Under Saline Water Stress by Spraying with Nano-Micronutrient

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ABSTRACT:

A pot experiment was carried out at net greenhouse at Horticulture Department roof, Faculty of Agriculture, Zagazig University, Egypt, during the two consecutive seasons of 2022/2023 and 2023/2024 to study the effect of different saline water irrigation levels (0.0, 1000, 2000 and 3000 ppm), different nano-micronutrients (Nm) rates as (0.0, 250 and 500 ppm) and their combinations on herb yield as well as volatile oil production of sweet basil (*Ocimum basilicum* L.) plant. 25 cm diameter plastic pots filled with 5 kg clay soil were used in this experiment. The current study was set up in a split-plot design with three replicates. The main plots were occupied by saline water irrigation levels and the sub plots were entitled to SE concentrations. The results showed a significant decrease in most studied yield parameters (fresh and dry herb yield per plant), salt resistance index and volatile oil production (volatile oil percentage and volatile oil yield /plant) with increasing the levels of salinity, but an opposite impact occurred with increasing the SE concentrations. However, for the combination among the levels of saline water and 500 ppm nano-micronutrient, the combination treatment (1000 ppm salinity level + 500 ppm Nm) showed the highest increment in the herb yield and volatile oil production of sweet basil plant under Sharkia Governorate conditions. **Conclusion:** It is preferable to spray *Ocimum basilicum* plants with nano-micronutrients at 500 ppm under the saline water irrigation conditions to enhance the basil herb yield, volatile oil production, as well as salt resistance index under Sharkia Governorate conditions.

Key words: *Ocimum basilicum*, Saline water irrigation, nano-micronutrients, yield, volatile oil

INTRODUCTION:

The annual plant known as sweet basil, or basil (*Ocimum basilicum* L.), is indigenous to tropical parts of Southeast Asia and Africa. It belongs to a family called Lamiaceae, which has roughly 30 species with a wide range of morphological and chemical traits (Vina and Murillo, 2003 and Telci *et al.*, 2006). Sweet basil adds taste and perfume to dishes and has a pleasing, alluring aroma. In addition to being used to make fragrant soaps and aromatherapy goods, basil oil is also utilized as a diet aid. Sweet basil contains metal salts (Mn, Cu, Mg, and K), antioxidant vitamins, beta-carotene, and antibiotics that can help prevent some types of cancer (Marotti *et al.*, 1996). Additionally, it helps treat a number of illnesses, including boiled persistent diarrhoea, kids' vomiting, intestinal colic, tooth pain, asthma, headaches, kidney stones, colds, and coughs (Ozcan and Chalchat, 2002 and Sajjadi, 2006).

Salinity is one of the main abiotic factors that negatively affect plant yield globally (Koca *et al.*, 2007). Osmotic balance, net photosynthetic rate, hydraulic conductivity, intercellular CO₂ concentrations, and nutrient absorption are all negatively impacted by rising salt concentrations in soil. The capacity of plants to develop is being adversely affected by all of these processes (Al-Karaki *et al.*, 2001). Additionally, saline subsurface water—a secondary water source—is being used more and more to irrigate large, fragrant, and therapeutic plants (Hoss, 1981). Furthermore, the plant's ability to absorb water in the root zone is impacted by salinity stress since it lowers the soil's water potential (Sabir *et al.*, 2009). Furthermore, Ibrahim *et al.* (2019) noted that while the development of basil and its photosynthetic pigments significantly decreased as irrigation water

concentrations increased in salt, the reverse effect was observed when the proline content of the sweet basil herb increased.

Nanotechnology researches and investigates several elements of particles smaller than 100 nanometers, including their design, production, application in primary habitats, and impact on target locations (Mejias *et al.*, 2021). According to Andrews *et al.* (2019), nano-materials differ from the same bulk or conventional materials in that they are extremely sensitive due to their greater surface area to volume and their decreased size produced some substantial changes in their physicochemical features. Janmohammadi and Sabaghnia (2023) pointed out that applying a foliar spray of nano Zn + Fe produced the greatest lateral expansion of the canopy and the greatest number of safflower capitula; this foliar treatment enhanced the canopy's lateral growth by 23% and 18% over the control. Plants cultivated with NPK fertilizer and sprayed with nano Zn + Fe had the maximum seed production. Overall, soil conditions have a significant impact on the beneficial effects of Zn + Fe nanoparticles; soils that have previously received appropriate organic and chemical fertilizers showed the highest nanoparticle efficiency.

The goal of the current study was to determine if nano-micronutrients foliar spray may lessen the negative effects of salty water irrigation on *Ocimum basilicum* herb yield, the salt resistance index and volatile oil production.

MATERIALS AND METHODS:

Experiment establishment:

A pot experiment was carried out on the roof of the Horticulture Department Building in a net greenhouse at Zagazig University's Faculty of Agriculture in Egypt during the winters of 2022–2023 and 2023–2024. The purpose of this study was to find out how different irrigation levels of salt water (0.0, 1000, 2000, and 3000 ppm) and rates of nano-micronutrients (0.0, 250, and 500 ppm) and their combinations affected the sweet basil plant. The experimental unit included the six pots. Pots with a diameter of 25 cm were filled with 5 kg of clay soil, which contained 41.82% clay, 25.73% silt, and 27.35% sand. Next, a typical nutrient solution with different concentrations of salty water (0.0, 1000, 2000, and 3000 ppm) with tap water in between was used to water the soil once a week. According to Chapman and Pratt (1978), Table 1 displays the pH, cation and anion concentrations, electrical conductivity (EC), and saline water irrigation salts of the soil used to fill pots.

Table 1. Electrical conductivity (EC), pH, and the concentration of cations and anions of the salt extract and in the soil used in the pot experiment

Parameter	EC (mmhos/ cm)	pH	Cations (meq/l)				Anions (meq/l)		
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	HCO ₃ ⁻	SO ₄ ⁻	Cl ⁻
Soil clay	0.84	7.82	13.10	5.45	2.45	18.65	3.00	2.72	4.98
Salt extract at (5:1)	158.4	7.63	11.32	9.84	1612.64	1.98	7.60	79.29	1582.48

Plant materials and Planting:

The sweet basil seedlings came from Mustafa Abo-Eisa Nursery in Belbas District, Sharkia Governorate, Egypt. On November 2, seedlings from both seasons were planted in experimental pots, one seedling per pot. By dissolving known weights of the saltwater's natural salt crust in tap water, four levels of artificial seawater were created. Plants were irrigated once a week with different ratios of salt water and tap water during the week to maintain soil moisture levels between 65 and 70 percent of field capacity.

Treatments and application:

We bought the nano-micronutrients from Modern Agricide Company (MAC) under the product name Magro NanoMix. In addition to 4% citric acid, they contain the following minerals: Fe (6%), Zn (6%), B (2%), Mn (5%), Cu (1%), and Mo (0.1%). However, five foliar sprays of different rates of nano-micronutrients were applied to sweet basil plants at 25, 55, 85, 125 and 155 days from the date of planting. Twelve distinct treatments interacted with the rates of nano-micronutrients and the saline water irrigation level. When necessary, every recommended agricultural technique for growing sweet basil plants was used.

Experimental Design:

This experiment was conducted using a split-plot design. The main plots were irrigated with four different concentrations of saline water. In the subplots, there were four rates of nano-micronutrient application. The experiment included combinations of varying concentrations of saline water irrigation and varying rates of nano-micronutrient. There were three duplicates of each therapy.

Data recorded:**Herb yield components:**

Sweet basil plants were harvested twice a season in both seasons by trimming the aerial portions of each plant five centimeters above the soil's surface. In both seasons, the two cuts were made on January 31 and April 30. The fresh and dry weights of the herbs per plant (g) were noted at the time of harvest.

Salt resistance index:

salt resistance index SRI (%), as a real indicator for salinity tolerance was calculated from the equation mentioned before by **Wu and Huff (1983)**: $SRI (\%) = \text{Mean herb fresh weight of the salt treated plants} / \text{mean herb fresh weight of control one} \times 100$.

Volatile oil production:

After the two cuts the volatile oil from dried herb of sweet basil plants was isolated by hydro distillation for 3 hr., in order to extract the volatile oil according to **Guenther (1961)** and the volatile oil yield per plant (ml) was calculated.

Statistical Analysis:

All collected data were analyzed with analysis of variance (ANOVA) procedure according to **Gomez and Gomez (1984)**. The Statistix version 9 computer program was used to compare the means (**Analytical software, 2008**). Difference between means was compared by using least significant differences (LSD).

RESULTS AND DISCUSSION:**Herb yield components:**

Results recorded in Tables 2, 3 and 4 reveals that all determined herb yield traits were gradually decreased as irrigation water salinity concentration increased. The minimum values of these parameters (herb fresh and dry weights as well as total herb fresh and dry yields/ plant) were detected with the highest concentration of saline water irrigation. On the other side, the maximum values of these parameters were belonged to control treatment. It is worth to mention that in some cases there were no significant difference between control treatment and the lowest level (1000 ppm) of saline water irrigation. These observations hold true at both cuts during both seasons. The suppression of meristematic activity and internal differentiation may be the cause of the decrease in the herb yield per plant. Salinity may have produced a loss in plant water content by increasing osmotic pressure, which in turn may have contributed to the fall in fresh weight of herb (**Munns et al., 2006**). These results are in accordance with those found by **Cheruth et al. (2008)** on periwinkle and **Elhindi et al. (2017)**, **Shehata and Nosir (2019)** and **Abdelhamed et al. (2025)** on sweet basil plants and **Janmohammadi and Sabaghnia (2023)** on safflower plants.

Results listed in Tables 2, 3 and 4 spraying sweet basil plants with nano-micronutrients at any rate enhanced all recorded plant growth traits (herb fresh and dry weights as well as total herb fresh and dry yields/ plant). Moreover, there was a positive relationship between nano-micronutrients rate and values of these traits. While, the lowest values of these traits were observed with control treatment. This result was demonstrated at both cuts during both seasons.

Table 2. Impact of saline water irrigation concentration (S), Nano-micronutrients concentration (N) and their combinations (S×N) on fresh weight of herb per plant (g) of sweet basil during 2023/2024 and 2024/2025 seasons

Saline water	Nano-micronutrients concentration (ppm)
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concentration (ppm)	0.0	250	500	Mean(S)	0.0	250	500	Mean(S)
First season								
	First cut				Second cut			
Control	27.77	34.57	38.67	33.67	41.73	44.97	51.10	45.93
1000	26.73	33.43	34.33	31.50	43.53	42.80	45.87	44.07
2000	24.53	30.43	28.50	27.82	36.60	39.90	42.27	39.59
3000	16.10	24.70	28.93	23.24	34.57	38.70	41.13	38.13
Mean (N)	23.78	30.78	32.61		39.11	41.59	45.09	
L.S.D. at 5%	(S)= 1.63	(N)= 1.31	(SN)= 2.69		(S)= 1.97	(N)= 0.50	(SN)= 2.13	
Second season								
	First cut				Second cut			
Control	34.03	38.50	52.40	41.64	37.97	42.73	45.40	42.03
1000	25.20	40.67	47.47	37.78	35.70	36.33	43.80	38.61
2000	25.00	30.43	41.30	32.44	33.43	37.10	41.60	37.38
3000	21.63	25.33	31.33	26.10	30.53	31.77	40.53	34.28
Mean (N)	26.47	33.73	43.13		34.41	36.98	42.83	
L.S.D. at 5%	(S)= 0.99	(N)= 1.04	(SN)= 1.96		(S)= 0.82	(N)= 0.45	(SN)= 1.10	

Table 3. Impact of saline water irrigation concentration (S), Nano-micronutrients concentration (N) and their combinations (S×N) on dry weight of herb per plant (g) of sweet basil during 2023/2024 and 2024/2025 seasons

Saline water concentration (ppm)	Nano-micronutrients concentration (ppm)							
	0.0	250	500	Mean(S)	0.0	250	500	Mean(S)
First season								
	First cut				Second cut			
Control	5.33	6.37	7.27	6.32	6.87	7.77	11.30	8.64
1000	4.90	6.10	6.80	5.93	9.10	8.43	10.13	9.22
2000	4.47	5.77	4.70	4.98	6.87	7.40	7.80	7.36
3000	2.67	4.40	5.30	4.12	6.00	6.07	7.30	6.46
Mean (N)	4.34	5.66	6.02		7.21	7.42	9.13	
L.S.D. at 5%	(S)= 0.29	(N)= 0.20	(SN)= 0.43		(S)= 0.41	(N)= 0.36	(SN)= 0.72	
Second season								
	First cut				Second cut			
Control	5.70	6.57	11.37	7.88	7.57	8.67	10.37	8.87
1000	5.23	7.13	8.23	6.87	6.27	6.97	8.00	7.08
2000	4.13	5.17	6.33	5.21	5.07	5.73	6.67	5.82

3000	3.80	4.30	5.17	4.42	4.67	5.23	5.97	5.29
Mean (N)	4.72	5.79	7.78		5.89	6.65	7.75	
L.S.D. at 5%	(S)= 0.41	(N)= 0.43	(SN)= 0.82		(S)= 0.40	(N)= 0.20	(SN)= 0.52	

Table 4. Impact of saline water irrigation concentration (S), Nano-micronutrients concentration (N) and their combinations (S×N) on total fresh and dry herb weight per plant (g) of sweet basil during 2023/2024 and 2024/2025 seasons

Saline water concentration (ppm)	Nano-micronutrients concentration (ppm)							
	0.0	250	500	Mean(S)	0.0	250	500	Mean(S)
Total fresh herb weight per plant (g)								
	First season				Second season			
Control	69.50	79.53	89.77	79.60	72.00	81.23	97.80	83.68
1000	70.27	76.23	80.20	75.57	60.90	77.00	91.27	76.39
2000	61.13	70.33	70.77	67.41	58.43	67.53	82.90	69.62
3000	50.67	63.40	70.07	61.38	52.17	57.10	71.87	60.38
Mean (N)	62.89	72.38	77.70		60.88	70.72	85.96	
L.S.D. at 5%	(S)= 2.90	(N)= 1.38	(SN)= 3.67		(S)= 1.70	(N)= 1.21	(SN)= 2.60	
Total dry herb weight per plant (g)								
	First season				Second season			
Control	12.20	14.13	18.57	14.97	13.27	15.23	21.73	16.74
1000	14.00	14.53	16.93	15.16	11.50	14.10	16.23	13.94
2000	11.33	13.17	12.50	12.33	9.20	10.90	13.00	11.03
3000	8.67	10.47	12.60	10.58	8.47	9.53	11.13	9.71
Mean (N)	11.55	13.08	15.15		10.61	12.44	15.53	
L.S.D. at 5%	(S)= 0.62	(N)= 0.41	(SN)= 0.91		(S)= 0.68	(N)= 0.51	(SN)= 1.08	

These findings could be explained by the fact that nano-fertilizers facilitate plants' uptake of nutrients, accelerating photosynthesis and dry matter creation while simultaneously enhancing vegetative development (Hediat, 2012). Said and Noaman (2021) reported that iron, manganese, and zinc nanoparticle foliar application outperformed all other treatments in this regard and recorded the greatest mean values of all yield parameters, oil yield, and seed yield in both seasons.

Concerning the influence of the combination between different concentrations of saline water irrigation and various rates of nano-micronutrients, it was found that the combination between the highest rate of nano-micronutrients (500 ppm) and the lowest saline water irrigation concentration (0.0 ppm) gained the maximum values of all investigated herb yield parameters (Tables 2, 3, 4, 5 and 6). On the contrary, the maximum values of these characters were obtained when plants were irrigated with the highest level of saline water and sprayed with tap water instead of nano-micronutrients. This result was proved at both cuts during both seasons. It is clear that treating plants with nano-micronutrients especially at the highest rate (500 ppm) could reduce the harmful effect of saline water irrigation on herb yield.

In agriculture, nano fertilizers are crucial instruments for improving crop growth, yield, and quality characteristics while increasing nutrient usage efficiency and lowering fertilizer waste and cultivation costs. In precision agriculture, nano-fertilizers are highly successful in precisely managing nutrients by matching the crop's growth stage and potentially supplying nutrients for the duration of the crop's growth. Crop development is boosted by nano-fertilizers up to optimal concentrations; higher concentrations may impede crop growth because of stress (Singh, 2017).

Salt resistance index:

It is evident from data in Table 5 that sweet basil's salt resistance index (SRI %) was changed considerably in response to saline water irrigation concentration. As saline water irrigation level increased, salt resistance index decreased. In addition, the increases in this connection were about 14.35 and 16.22 % for the control plants and were about 8.73 and 6.10 % at 1000 ppm in the 1st and 2nd seasons, respectively. In the same connection, a substantial and obvious decrease in *Schizonepeta tenuifolia* plant height and total dry weight of plants was observed at 25, 50, 75 and 100 mM NaCl compared to control (Zhou *et al.*, 2018). Likewise, salt resistance index (SRI%) of common sage was significantly decreased with 1000 and 2000 ppm levels of salinity compared with control (Hegazy *et al.*, 2021).

Table 5. Impact of saline water irrigation concentration (S), Nano-micronutrients concentration (N) and their combinations (S×N) on salt resistance index (%) of sweet basil during 2023/2024 and 2024/2025 seasons

Saline water concentration (ppm)	Nano-micronutrients concentration (ppm)							
	0.0	250	500	Mean(S)	0.0	250	500	Mean(S)
Salt resistance index (%)								
	First season				Second season			
Control	100.00	114.44	129.16	114.53	100.00	112.82	135.83	116.22
1000	101.10	109.69	115.39	108.73	84.58	106.95	126.76	106.10
2000	87.96	101.20	101.82	96.99	81.16	93.80	115.14	96.70
3000	72.90	91.22	100.81	88.31	72.45	79.30	99.81	83.86
Mean (N)	90.49	104.14	111.80		84.55	98.22	119.39	
L.S.D. at 5%	(S)= 2.54	(N)= 2.14	(SN)= 4.31		(S)= 2.21	(N)= 1.56	(SN)= 3.36	

Table 5 shows that, in both seasons, using of nano-micronutrients considerably raised the *Ocimum basilicum* salt resistance index (%) as compared to the control. As nano-micronutrients were used at 250 and 500 ppm, respectively, the salt resistance index (%) also rose. Consulting the available literature revealed that there was no information on how foliar spray with nano-micronutrients affect plant's salt resistance index.

Data recorded in Table 5 show that, sweet basil salt resistance index (%) was increased as a result of treating plants with nano-micronutrients combined with most of saline water levels compared to control basil plants or those treated with saline water at 1000-3000 ppm during both seasons. The maximum salt resistance index values (129.16 and 135.83 %) were detected when plants were treated with 500 ppm nano-micronutrients and irrigated with the lowest concentration (0.0 ppm) of saline water irrigation. This stimulating effect of spraying plants with nano-micronutrients on plant growth may be attributed to the fact that nano-micronutrients are a well-known important fertilizer as well as stimulant which has positive effects on plant growth and significantly mitigates the injuries caused by a biotic stresses (Singh, 2017).

Volatile oil production:

Volatile oil of sweet basil

Data of both seasons listed in Table 6 show that, volatile oil percentage and volatile oil yield/ plant were significantly decreased by saline water irrigation levels increased in the two cuts during both seasons compared to the lowest level (1000 ppm) and control. However, the decrease in this connection were about 11.90, 10.72 and 38.18, 48.28 % for the salinity level at 3000 ppm in the first and second cuts during the first and second seasons, respectively. However, this result may be due to salt-induced water stress reduction of chloroplast stoma volume and regeneration of reactive oxygen species in playing an important role in the inhibition of photosynthesis seen in salt stressed plants (Price and Hendry, 1991 and Allen, 1995). In this connection, it was found that increasing of salinity stress decreased almost essential oil amount in Chamomile (Razmjoo *et al.*, 2008). There are report of an increase in essential oil percentage due to lower levels of salinity was also found thyme (Ezz El-Din *et al.*, 2009). Also, Keramati *et al.* (2016) showed that *O. basilicum* essential oil content and essential oil yield decreased significantly with salinity increase. However, there was a slight increase (3% compared to the control) in essential oil content when basil plants were subjected to moderate salinity stress (3 dS m⁻¹ NaCl). Polanski *et al.* (2018) indicated that drought stress motivated a significant reduction in all of the growth parameters and essential oil yield and percent of peppermint plant.

Data presented in Table 6 indicate that, using nano-micronutrients at 500 ppm under irrigated with saline water at 1000 ppm resulted in significant increase in volatile oil percentage and volatile oil yield/plant compared to the highest rate of 500 ppm combined with the same level of salinity. Concerning volatile oil production of sweet basil, it was found that the highest levels of salinity (2000 and 3000 ppm) combined without nano-micronutrients application gave the minimum values in this regard compared to the other combination treatments in the two cuts during both seasons. However, El-Metwally *et al.* (2018) demonstrated that using nano-fertilizer with concentration 30 ppm give the highest value of total soluble sugars, total carbohydrate, total proteins and oil percentages content in peanut seeds compared with other treatments.

Table 6. Impact of saline water irrigation concentration (S), Nano-micronutrients concentration (N) and their combinations (S×N) on volatile oil percentage and yield per plant (ml) of sweet basil during 2023/2024 and 2024/2025 seasons

Saline water concentration (ppm)	Nano-micronutrients concentration (ppm)							
	0.0	250	500	Mean(S)	0.0	250	500	Mean(S)
Volatile oil (%)								
	First season				Second season			
Control	0.703	0.730	0.760	0.731	0.667	0.687	0.717	0.690
1000	0.703	0.757	0.763	0.741	0.713	0.680	0.743	0.712
2000	0.667	0.740	0.797	0.734	0.640	0.667	0.710	0.672
3000	0.630	0.657	0.647	0.644	0.607	0.613	0.627	0.616
Mean (N)	0.676	0.721	0.742		0.657	0.662	0.699	
L.S.D. at 5%	(S)=0.024	(N)=0.017	(SN)=0.037		(S)=0.016	(N)=0.010	(SN)=0.023	
Volatile oil yield per plant (ml)								
	First season				Second season			
Control	0.086	0.103	0.141	0.110	0.088	0.105	0.156	0.116
1000	0.098	0.110	0.130	0.113	0.082	0.096	0.121	0.100
2000	0.075	0.097	0.099	0.091	0.059	0.073	0.092	0.075
3000	0.054	0.069	0.082	0.068	0.051	0.059	0.070	0.060
Mean (N)	0.078	0.095	0.113		0.070	0.083	0.110	
L.S.D. at 5%	(S)=0.006	(N)=0.003	(SN)=0.008		(S)=0.004	(N)=0.004	(SN)=0.007	

Data listed in Tables 6 reveal that, all combination between saline water irrigation levels (2000 and 3000 ppm) and nano-micronutrients treatments significantly decreased volatile oil production of sweet basil plants in both cuts in both seasons. Moreover, the control plants which sprayed without nano-micronutrients resulted in the highest values of volatile oil %, volatile oil yield/plant of sweet basil compared to the other interaction treatments under study. Also, as mentioned above, nano-micronutrients enhanced volatile oil of sweet basil herb, in turn; they together under salinity conditions might maximize their effects leading to more volatile oil yield per plant. Nano agronomic technologies for the cultivation of medicinal plants have the potential to completely transform sustainable practices in the near future. This is because, with the aid of newly developed instruments, the usefulness of these plants—including the potency and quality of beneficial bioactive ingredients they contain—is realized by minimizing their detrimental effects on the environment. It promotes more advantageous methods of honor cultivation and enhances health security (**Nicolétis et al., 2019**)

CONCLUSION:

From above mentioned results, it is preferable to spray *Ocimum basilicum* plants with nano-micronutrients at 500 ppm under the saline water irrigation conditions to enhance the basil herb yield, volatile oil production, as well as salt resistance index under Sharkia Governorate conditions.

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