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Impact of Seed Pre-Soaking in Saline and Salicylic Acid on Germination and Nutritional Quality of Radish Microgreens

Tarek HA Hussein[1]*, Tahany AA Aly[1], Yasser A ELGabry[2], Usama AA El-Behairy[3]
1. Regional Central for Food and Feed, Agricultural Research Center, Giza 12619, Egypt
2. Agronomy Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68-Hadayek Shubra 11241, Cairo, Egypt
3. Horticulture Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68-Hadayek Shubra 11241, Cairo, Egypt

*Corresponding author: tarekhussein.1968.th@gmail.com

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Abstract: This study was conducted to determine the impact of sodium chloride (2000 ppm) and salicylic acid (SA) concentrations (0, 50, 100, 150, and 200 ppm) on the nutritional value and germination of radish microgreens. The experiment was based on a completely random design with three replicates. Results indicated that applying SA at 50 and 100 ppm mitigates the negative effects of salinity, even better than higher concentrations, on the nutritional value and bioactive contents of radish microgreens where SA at 50 ppm lowered the sodium contents from 1.75 to 1.10 ppm in addition to increasing the moisture content. Applying SA at 50 and 100 ppm caused a noticeable increase in carbohydrates, proteins, fats, fibers and energy under unstressed condition. In addition, SA at 50 ppm caused a significant increase in oxalic and malic acid contents under both normal and salinity conditions while SA at 100 ppm significantly enhanced the levels of total phenols, flavonoids and antioxidants under salinity conditions. This practical study highlights the significant role of salicylic acid, at 50 and 100 ppm, in increasing the levels of phytochemicals and minerals, hence improving the nutritional value of microgreen radishes.

1 Introduction

Radish is a root vegetable crop and a member of the cabbage family. It belongs to the Cruciferous family. It is an edible root vegetable and is considered part of the human diet; it is grown and consumed all over the world. It has a high nutritional value and is used in Greek, Arabic, and Indian folk medicine. Radish contains carbohydrates, dietary fiber and proteins, in addition to containing many water-soluble minerals (Banihani 2017). Furthermore, radish was found to contain unique bioactive compounds (Malik et al 2010) which have potential health-promoting and beneficial impacts on the human body.

Salinity is one of the most dangerous vital stresses and is a main problem around the world (Khan and Qaiser 2006); it slows down plant growth and productivity. By highlighting osmotic and ionic imbalances, salinity stress also adversely affects plant morphology. This, in turn, causes progressive alterations in the biochemical responses of plants.

Microgreens, young tender leafy vegetables, are produced 7–14 days after germination. They have an excellent nutritional profile and are currently considered one of the most interesting innovations (Zhang et al 2021; Sharma et al 2022). Microgreens are rich in phenolic compounds and a good source of macroelements e.g. K and Ca, as well as microelements. It has been demonstrated that microgreens have high quantities of phenolics and vitamins, as well as zinc and iron (Janovská et al 2010, Xiao et al 2019, Zhang et al 2021, Sharma et al 2022).

Salicylic acid (SA) is a naturally active phenolic compound; humans have used it for thousands of years as a pain reliever (Mitchell and Broadhead 1967, Klessig et al 2018). Recent studies have shown that salicylic acid plays an important physiological and biochemical role in plant growth and flowering; it is also considered a multifactorial factor to withstand abiotic stresses and harsh environmental conditions (Nadarajah et al 2021, Es-sbihi et al 2021, Chen and Pang 2023, Kaya et al 2023). In fact, SA is an internal regulator of plant growth (Zhou et al 2021); its external application on plants can enhance salinity tolerance (Abdelhameed et al 2021, Kaya et al 2023). External use, in an appropriate concentration, can improve and increase the activity and capacity of total antioxidant enzymes (Safari et al 2018, Nasırcılar et al 2020, Parveen et al 2021). In addition, it also acts as a signal for stimulating the response of plants to various stressful conditions, such as salinity (Mohammad et al 2019, Roshdy et al 2021, Jamshidi et al 2023). This study aims to increase the nutritional quality of radish microgreens as a functional food and reduce the negative impact of salinity.

2 Materials and Methods

This study was conducted at the Agricultural Experiments Center of the Agricultural Research Center. Chemical analyses were evaluated in the laboratories of the Regional Center for Food and Feed (RCFF) of the Agricultural Research Center (ARC), Giza, Egypt.

2.1 Seeds of Radish

Dry radish (*Raphanussativus*) seeds were obtained from the seed department of the Agricultural Research Center in Giza. Sodium chloride and salicylic acid were obtained from El Gomhoria Chemical Company, Cairo, Egypt.

To assess how varying NaCl salinity concentrations affects radish germination, a factorial experiment with three replicates of salinity concentrations (1000, 2000, 3000 and 4000 ppm) was carried out using a fully randomized design. The study used a fully randomized design and found that the 2000 ppm NaCl concentration was the most preferable for root length, plant weight, and germination percentage. Therefore, 2000 ppm was chosen as the optimal NaCl concentration (Hussein et al 2019, Elgebaly et al 2022).

2.2 Sample Preparation

Before planting, radish seeds were washed and sterilized by soaking in 2% calcium hypochlorite for 20 minutes. They were then rinsed three times with distilled water and allowed to air-dry on a towel of tissue paper. According to Abdallah (2008) and Elgebaly et al (2022), the glass beaker technique of soaking (12 hours) was employed, utilizing tap water, NaCl salinity at concentrations of 2000 ppm and various concentrations of salicylic acid (0, 50, 100, 150, and 200 ppm).

2.3 Production of microgreens from radish seeds

The radish seeds were planted at the Agricultural Research Centre of the Agricultural Experiments Department in November 2022, with an average temperature of 15-20°C and humidity of 70–90. After soaking the seeds, it was planted using a completely random design with three replicates. Then tap water (control)or different concentrations of salicylic acid were sprayed in addition to spraying NaCl solution (2000 ppm); spraying was continued every four days. The plants were harvested after 17 days when the true leaves started to appear. Finally, the microgreens were dried in an oven at 55±2ºC for 48 hours and then ground into soft powder for chemical analyses (Hussein et al 2019). Ten treatments were prepared, which were tap water and sodium chloride (2000 ppm) treatments as controls in addition to four salicylic acid (50, 100, 150, 200 ppm) treatments and four salicylic acid (same concentrations) plus sodium chloride (2000 ppm) treatments. Each treatment was implemented in three replicates.

2.4 Proximate Composition

2.4.1 Moisture, proteins, crude fats, crude fiber and ash contents

The moisture, proteins, crude fats, crude fiber and ash contents of the dried samples were determined according to AOAC (2012).

2.4.2 Total Carbohydrates and energy value

The percentage of total carbohydrates in dried samples was calculated by subtraction, as reported by Sumati and Rajagopal (1996).

Carbohydrates = 100 - % proteins - % fats - %Ash - % moisture

While the energy value was calculated using the equation described by Chinma and Igyor (2007) as follows:

Energy value = [(9 × fat) + (4 × carbohydrates) + (4 × protein)]

2.4.3 Mineral contents

Atomic absorption spectrometry (Analyst 3300, Perkin Elmer, Inc., Massachusetts, USA) was used to assess the mineral contents, namely potassium (K), magnesium (Mg), iron (Fe) and zinc (Zn). Using the ICP (Optima 2000, DV Perken Elmer Inc., Massachusetts, USA), calcium (Ca) and manganese (Mn) were measured according to the method described in AOAC (2012).

2.4.4 Total antioxidant and organic acids

The total antioxidant was assessed using the phosphomolybdenum test method (Prieto et al 1999). The absorbance of the solution at 695 nm (A695 nm) was then measured using a UV-visible spectrophotometer. Aluminum chloride was used to determine the total flavonoid content using the method of Kelly et al (2014). The total phenol content using the Folin-Ciocalteu reagent method, using gallic acid as a standard; absorption at 765 nm was measured.

Oxalate was determined, briefly, by adding 3M sulfuric acid to samples, stirring, filtering, and titrating against 0.05 M potassium permanganate (Day and Underwood 1986). The contents of citric acid and L-malic acid were measured according to Alamo et al (1993).

2.4.5 Statistical analysis

Using a completely random design, the data were statistically analyzed for bidirectional variance and two-way ANOVA. The method outlined by Snedecor and Cochran (1980) was utilized to compare significant differences between the treatment methods using a least significant difference (LSD) at a significance level of 0.05.

3 Results and Discussion

Data in (**Table 1)** show a considerable increase in carbohydrates, proteins, fats, fibers and energy in radish microgreens with the use of tap water and application of salicylic acid at 50 and 100 ppm compared to the control. An additional accumulation of protein (33.5%) was detected in the treatment of tap water and salicylic acid at a concentration of 150 ppm. The moisture content was generally increased by the application of salicylic acid under either normal or salinity conditions. Previous reports indicated also that salicylic acid enhanced protein and energy contents (Fahad and Bano 2012, Jini and Joseph 2017, Wakeda et al 2023). In salt-stressed condition, the SA treatment at 50 ppm still raised the carbohydrate, fiber and energy contents of the radish microgreens compared to the control of the unstressed condition.

Results showed that some radish nutritional components are significantly affected. **Table 2** showed that salinity significantly increased radish Ca, Na and Cl from 4.22, 1.20 and 3.68 ppm to 5.10, 1.75 and 4.46 ppm respectively but decreased K content from 24.04 to 21.99 ppm. However, the application of SA at 50 ppm decreased the Na content to 1.10 ppm indicating that SA application could lower the sodium uptake and accumulation. The application of salicylic acid (SA) at 100 ppm under salinity resulted in a significant increase in the contents of Cu, Zn, Mn and Mg. Moreover, the obtained results demonstrated that the total element content increased with salicylic acid applications and was most effective at a salinity of 2000 ppm (Tavangar et al 2021, Jamshidi et al 2023, Wakeda et al 2023). The reaction between salicylic acid and saline water concentrations was also demonstrated. Though their concentration varies, radish microgreens are rich in nutrients (Xiao et al 2016).

Data in **Table 3** show the impact of soaking radish seeds for 12 hours in saline solution (0 and 2000 ppm) and salicylic acid (SA) at different concentrations on organic acid contents, including citric acid, oxalic acid, and malic acid. The results show SA at 50 ppm caused a significant increase in oxalic and malic acid contents under both normal and salinity conditions compared to the control without salinity while citric acid decreased under both conditions. The highest level of oxalic acid was observed when SA was applied at 100 ppm and 2000 ppm NaCl. The study reflected an increase of citric and malic acids in plants at low salicylic acid concentrations and 2000 ppm NaCl water salinity. The improvement in citric acid and malic acid was more noticeable and had noteworthy impacts. According to Koo et al (2020) and Abdelhameed et al (2021), increasing organic acids (citric and malic acid) is a powerful salt tolerance technique that shields the plant from the negative effects of salinity and SA is crucial for controlling the seed germination as well as the plant growth and development.

Table 1. Effects of soaking radish seeds, in 2000 ppm NaClsalinity and salicylic acid (SA) at different concentrations on the proximate analysis and energy value of radish microgreens (age of 17 days)

	SA (ppm)	Moisture %	Carbohy-drate %	Protein %	Fat %	Fiber %	Ash %	Energy (Kcal/g100)
TW	0	7.83 ±0.152f	52.97 ±0.149h	31.4±0.9f	4.23 ±0.146d	12.5 ±0.19e	15.2 ±0.19b	383.5±4fg
	50	8.73±0.154cd	58.57±0.153a	32.2 ±1.1d	4.43 ±0.152cd	15.6± 0.19a	11.1 ±0.18f	408.2±4a
	100	8.73 ±0.157ed	56.47±0.146c	33.3 ±1.0b	4.53 ±0.154c	14.2 ±0.18bc	10.9 ± 0.18f	402.4±3ab
	150	8.53 ±0.150d	51.17±0.152i	33.5 ±1.1a	3.73 ±0.146e	12.0 ±0.19f	14.0 ±0.2d	382.0±3fg
	200	7.83 ±0.151f	54.27 ±0.157f	32.8 ±0.9c	4.23 ±0.150d	14.5 ±0.2b	14.5±0.19c	394.3±4cd
Mean		**8.33^{B}**	**54.69^{B}**	**32.64^{A}**	**4.23^{B}**	**13.76^{A}**	**13.14^{B}**	**394.08^{A}**
NaCl 2000 ppm	0	8.23±0.151e	56.97 ±0.150b	32.0 ±1.0e	5.23 ±0.156a	14.5 ±0.17b	12.5 ±0.2e	399.2±4bc
	50	8.93 ±0.148bc	54.67 ±0.149e	29.7 ±1.0g	3.93 ±0.158e	14.0 ±0.19c	14.7 ± 0.21c	391.6±4de
	100	9.03 ±0.151b	53.97 ±0.146g	27.8 ±0.9h	5.23±0.153a	13.4 ±0.19d	16.9 ±0.2a	378.5±3g
	150	9.33 ±0.149a	54.87±0.156e	29.7 ±1.0g	4.93 ±0.149b	13.5 ± 0.18d	14.5 ±0.21c	384.3±4fg
	200	8.53 ±0.154d	55.27 ±0.152d	26.1 ± 0.8i	4.83±0.147b	11.9±0.2f	15.2 ±0.19b	386.8±4ef
Mean		**8.81^{A}**	**55.15^{A}**	**29.06^{B}**	**4.83^{A}**	**13.46^{B}**	**14.76^{A}**	**388.08^{B}**
Mean	0	8.03^{b}	54.97^{c}	31.7^{a}	4.73^{a}	13.5^{c}	13.85^{c}	391.35^{b}
	50	8.83^{a}	56.62^{a}	30.95^{b}	4.18^{c}	14.8^{a}	12.9^{d}	399.9^{a}
	100	8.88^{a}	55.22^{b}	30.55^{c}	4.88^{a}	13.8^{b}	13.9^{c}	390.45^{d}
	150	8.93^{a}	53.02^{e}	31.6a	4.33^{c}	12.75^{e}	14.25^{b}	383.15^{c}
	200	8.18^{b}	54.77^{d}	29.45^{d}	4.53^{b}	13.2^{d}	14.85^{a}	390.55^{b}
LSD 0.05	Water salinity	0.11635	0.11635	0.076169	0.11635	0.152337	0.152337	3.04675
	S A.	0.18397	0.18397	0.120433	0.183965	0.24087	0.240866	4.8173
	WS × SA	0.2602	0.26017	0.170318	0.260165	0.340636	0.34064	6.81273

TW = tap water SA = salicylic acid and ± standard deviation (SD). Means with different letters within the same column are significantly different at ($p \leq 0.05$) according to Duncan's multiple range test

The obtained results demonstrated that the total content of organic acid was increased with salicylic acid applications; this rise was more effective at a water salinity of 2000 ppm.

Results in **Table 4** indicated that the concentrations of flavonoids, phenols and total antioxidants in microgreen radish were significantly decreased under salinity conditions from 2016, 3032 and 5698 ppm to 1822, 1428 and 4442 ppm respectively but application of SA at 100 ppm under salinity elevated them to 2612, 5028 and 10515 ppm respectively. Salicylic acid treatment in the presence of salt had a significant antioxidant action (Rabab and Reda 2019, Zlatić, et al 2019, Zhang et al 2021) and led to a great increase in the concentration of phenolic compounds (Eraslan et al 2007, Parveen et al 2021) while the negative consequences of salt stress result from the direct physiological action of (SA) (Alsahli et al 2019, Naz et al 2022).

4 Conclusion

This study presents the efficacy of salicylic acid (SA) application in revealing the negative effects of salinity on sodium accumulation and chemical contents in radish microgreens in addition to enhancing the contents of some bioactive compounds e.g. flavonoids, phenols, oxalic acid malic acid. The current results also imply that applying lower concentrations of salicylic acid (SA) is more beneficial than the higher concentrations. Moreover, SA can effectively alter plant development responses and improve the nutritional value of the plants. Finally, this study recommends using salicylic acid in combination with other methods to enhance and boost the nutritional content of microgreen radish. Microgreen radish is a novel source of super, functional, or full food.

Table 2. Effect of soaking radish seeds, in 2000 ppm NaCl salinity and salicylic acid (SA) at different concentrations on radish microgreens up to the age of 17 days on minerals content (mg/g)

	SA (ppm)	Ca	K	Na	P	Cu	Fe	Mn	Zn	Cl	Mg
TW	0	4.22±0.019g	24.04±0.051a	1.20±0.017e	0.14±0.019ab	2.35±0.019f	57.52±1.09g	100.21±1.11c	49.21±1.09h	3.68±0.007h	3.25±0.019c
	50	4.32±0.021f	22.93±0.059b	1.25±0.019d	0.11±0.017bc	3.22±0.018e	75.31±1.08f	105.21±1.09b	50.63±1.1gh	3.55±0.01i	3.85±0.017a
	100	4.50±0.018e	21.54±0.058f	1.24±0.021d	0.15±0.020a	3.35 ±0.02d	90.57±1.13e	109.06±1.1a	54.41±1.08f	4.01±0.008g	3.84±0.018a
	150	4.58±0.018d	19.96±0.059h	1.32±0.020c	0.14±0.018ab	3.62±0.017c	92.46±1.09d	110.63±1.11a	62.22±1.11c	4.31±0.009	3.20±0.02d
	200	4.86±0.019c	18.84±0.057i	1.61±0.019b	0.11±0.019bc	3.70±0.021b	99.22±1.11c	79.21±1.08g	50.62±1.1gh	4.62±0.01c	3.66±0.015b
Mean		**4.496^{B}**	**21.47^{B}**	**1.324^{B}**	**0.13^{A}**	**3.248^{B}**	**83.02^{B}**	**100.86^{A}**	**53.418^{B}**	**4.034^{B}**	**3.56^{A}**
NaCl 2000 ppm	0	5.10±0.018b	21.99±0.058c	1.75±0.02a	0.14 ±0.02ab	3.22±0.019e	100.01±1.08c	82.00±1.12f	52.41±1.08g	4.46±0.009e	1.89±0.019h
	50	5.12±0.02b	21.85±0.057d	1.10 ±0.02f	0.10 ±0.017c	3.70±0.018b	103.22±1.09b	95.61±1.09e	58.24±1.09e	4.48±0.009d	2.15±0.018g
	100	5.10±0.019b	21.93±0.061cd	1.32 ±0.019	0.12±0.018abc	3.76±0.021a	99.62±1.13c	95.78±1.13de	60.31±1.1d	5.22±0.008b	2.55±0.017f
	150	5.22±0.02a	20.24±0.060g	1.30±0.02c	0.13±0.017abc	3.69±0.019b	110.21±1.1a	95.00±1.11e	68.41±1.11a	5.21±0.009b	2.60±0.02e
	200	5.20±0.019a	21.75±0.059e	1.24±0.018d	0.15±0.019a	3.72±0.02b	111.06±1.1a	97.61±1.12d	65.62±1.09b	5.40±0.01a	2.54±0.019f
Mean		**5.148^{A}**	**21.56^{A}**	**1.342^{A}**	**0.128^{A}**	**3.618^{A}**	**104.82^{A}**	**93.2^{B}**	**60.998^{A}**	**4.954^{A}**	**2.346^{B}**
Mean	0	4.66^{e}	23.02^{a}	1.475^{a}	0.14^{a}	2.785^{e}	78.765^{a}	91.105^{c}	50.81^{d}	4.07^{d}	2.57^{e}
	50	4.72^{d}	22.39^{b}	1.175^{e}	0.105^{b}	3.46^{d}	89.265^{d}	100.41^{b}	54.435^{c}	4.015^{e}	3.00^{c}
	100	4.80^{c}	21.74^{c}	1.28^{d}	0.135^{a}	3.555^{c}	95.095^{c}	102.42^{a}	57.36^{b}	4.615^{c}	3.20^{a}
	150	4.90^{b}	20.10^{e}	1.31^{c}	0.135^{a}	3.655^{b}	101.36^{b}	102.82^{a}	65.315a	4.76^{b}	2.90^{d}
	200	5.03^{a}	20.30^{d}	1.425^{b}	0.13^{a}	3.71^{a}	105.14^{a}	88.41^{d}	58.12^{b}	5.01^{a}	3.10^{b}
LSD 0.05	Water salinity	0.0152	0.04398	0.0152	0.0152	0.01523	0.83786	0.8378	0.83786	0.00762	0.01523
	S A.	0.02409	0.0695	0.02409	0.0241	0.0241	1.32477	1.3248	1.3247	0.01204	0.02409
	WS×SA	0.0341	0.0983	0.03406	0.03406	0.0341	1.8735	1.8735	1.87350	0.01703	0.03406

TW=tap water SA=salicylic acid and ± standard deviation (SD). Means with different letters are significantly different at ($p \leq 0.05$).

Table 3. Effect of soaking radish seeds, in 2000 ppm NaCl salinity, tap water and salicylic acid (SA) at different concentrations on radish microgreens up to the age of 17 days on organic acids (ppm).

	Organic acid / SA (ppm)	Maleic acid	Oxalic acid	Citric acid
TW	0	**2.35**±0.04d	**14.69**±0.03i	**2.40** ±0.03f
	50	**2.60**±0.03b	**25.76**±0.04d	**1.74** ±0.04g
	100	**2.04**±0.04f	**15.72**±0.02g	**2.47**±0.02e
	150	**2.05**±0.02f	**40.73**±0.03b	**2.92**±0.03c
	200	**1.20**±0.03h	**23.20**±0.03e	**0.20** ±0.03h
Mean		**2.048**^{B}	**24.02**^{B}	**1.946**^{B}
NaCl 2000 ppm	0	**2.22**±0.03e	**51.38**±0.03a	**4.49**±0.03b
	50	**2.60**±0.04b	**31.45**±0.04c	**0.06**±0.04i
	100	**2.77**±0.02a	**12.06**±0.02j	**4.56**±0.02a
	150	**1.96**±0.03g	**21.18**±0.03f	**2.88**±0.03c
	200	**2.44**±0.03c	**14.90**±0.03h	**2.80**±0.03d
Mean		**2.398**^{A}	**26.194**^{A}	**2.958**^{A}
Mean	0	2.285^{c}	33.035^{a}	3.445^{b}
	50	2.60^{a}	28.605^{C}	0.90^{e}
	100	2.405^{b}	13.89^{e}	3.515^{a}
	150	2.005^{d}	30.955^{b}	2.90^{c}
	200	1.82^{e}	19.05^{d}	1.50^{d}
LSD 0.05	Water salinity	0.030467	0.0512801	0.039162
	S A.	0.04817	0.065311	0.0419833
	WS× SA	0.06813	0.083720	0.051761

TW = tap water, SA = salicylic acid. Means with different letters within the same column are significantly different at ($p \leq 0.05$) according to Duncan's multiple range test

Table 4. Effect of soaking radish seeds, in 2000 ppm NaCl salinity and salicylic acid (SA) at different concentrations on radish microgreens up to the age of 17 days on antioxidant compounds (ppm)

R	Antioxidant / SA (ppm)	Total Flavonoids	Total Phenols	Total Antioxidant
TW	0	2016 ±11d	3032 ±12c	5698 ±12d
	50	2278 ±12b	1536 ±11g	3395 ±10g
	100	1437 ±12h	1673 ±11f	3382 ±11g
	150	2163 ±11c	1531 ±10g	2722 ±11h
	200	513 ±10j	2550 ±12e	5635 ±12e
Mean		1685^{B}	2064.4^{B}	4166.4^{B}
NaCl 2000 ppm	0	1822 ±11f	1428 ±12h	4442 ±11f
	50	1508 ±10g	1269 ±10i	2385 ±12i
	100	2612 ±12a	5028 ±11a	10515 ±12a
	150	1002 ±11i	3242 ±11b	5854 ±11c
	200	1992 ±12e	2979 ±12d	9110 ±10b
Mean		1787.2^{A}	2789.2^{A}	6461.2^{A}
Mean	0	1919^{b}	2230^{d}	5070^{c}
	50	1893^{c}	1402.5^{e}	2890^{e}
	100	2024.5^{a}	3350.5^{a}	7372.5^{a}
	150	1582.5^{d}	2386.5^{c}	4288^{d}
	200	1261.5^{e}	2764.5^{b}	6948.5^{b}
LSD 0.05	Water salinity	9.140234	10.35749	11.47215
	S A.	14.45198	14.927541	15.72183
	WS× SA	20.43818	19.821632	22.34271

TW = tap water, SA = salicylic acid. Means with different letters within the same column are significantly different at ($p \leq 0.05$) according to Duncan's multiple range test

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