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Synergistic Interactions of Arbuscular Mycorrhizal Fungi and Salicylic Acid Alleviate Adverse Effects of Water Salinity on Growth and Productivity of Watermelon *via* Enhanced Physiological and Biochemical Responses



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> ALINITY is one of the serious abiotic stresses adversely affecting the productivity of most Scrops. Arbuscular mycorrhizal fungi and salicylic acid treatments are known to ameliorate salinity stress, but their combined effect has never been examined on watermelon. Therefore, to investigate the synergetic effects of them on vegetative growth, nutrient content, physiological and biochemical characteristics, and fruit yield and quality of watermelon cv. Aswan F1 grown under saline water conditions, a split split-plot design with three replications was conducted in the North Sinai Governorate, Egypt, during the two successive growing seasons of 2020 and 2021. The main factor included irrigation water salinity regimes at three levels: 1600, 4000 and 5000 ppm. Subfactors included arbuscular mycorrhizal fungi at two levels (noninoculated and inoculated), and salicylic acid foliar spraying at four concentrations (0, 1, 2 and 4 mM) in subplots. The results revealed that saline water increasing led to evident reductions in vegetative growth parameters and fruit yield. Mycorrhizal inoculation or foliar application of salicylic acid improved the growth and productivity of watermelon plants under salinity conditions by maintaining a higher leaf relative water content and membrane stability index, enhancing chlorophyll content, and inducing the accumulation of proline and the activity of antioxidant enzymes. In addition, this study affirmed the synergistic effects of mycorrhizal inoculation and salicylic acid spraying on ameliorating the deleterious effects of saline-water irrigation on the growth and productivity of watermelon plants via generating simulative impacts on all physiological and biochemical attributes.

Keywords: Salt stress, Antioxidant enzymes, Membrane permeability, Proline.

# **Introduction**

Water scarcity and the inadequacy of good quality water have become one of the major constraints for agricultural sustainability in the world. Recently, Egypt is severely suffering from a lack of fresh-irrigation water particularly after the building of the Grand Ethiopian Renaissance Dam. Such a situation forced the farmers to utilize low quality water as semisaline underground water making it inevitable for irrigation to compensate for rapidly increasing water requirements for irrigation, especially in arid and semi-arid regions. Therefore, the use of saline water for agriculture requires more detailed studies and using new innovative technologies and techniques to alleviate the detrimental effects of salt stress on the growth and productivity of the crops should be investigated.

Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) is one of the main vegetable crops in the gourd family, Cucurbitaceae, widely cultivated in Egypt. The watermelon cultivated area in Egypt was 45029 ha with a total production of 1491108 tons and an average

Corresponding author: Sabry M. Youssef, E-mail: sabrysoliman@hotmail.com, Tel. 01024218053 (Received 03/04/2023, accepted 17/04/2023) DOI: 10.21608/EJOH.2023.203697.1237 ©2023 National Information and Documentation Centre (NIDOC) yield of 33.11 tons ha<sup>-1</sup> in 2020 (FAOSTAT 2022). Salinity is a dilemma for the growth and productivity of watermelon since it is considered a moderately sensitive plant to salinity, where salinity values of 2.5, 3.5 and 4.5 dSm<sup>-1</sup> reduce the production by 10, 25 and 50%, respectively, (Amacher et al. 2000, Ali et al. 2015).

High salt accumulations in soils or water irrigation cause both osmotic and ionic stresses producing different changes in plant metabolism and generating reactive oxygen species (ROS) as reported by Mittler (2002) and Youssef et al. (2018) which further lead to deleterious effects at morphological, physiological, biochemical, cellular and molecular levels, eventually causing reductions in plant growth and productivity (Ashraf and Foolad 2007, Kapoor & Evelin 2014, Youssef et al. 2018, Abdel Motaleb et al. 2020 and Bijalwan et al. 2021). However, plants develop several biochemical and molecular mechanisms to mitigate the deleterious effects of salinity and protect the cells against oxidative damage. Among these mechanisms, plants undergo osmotic adjustments under stress by excluding Na<sup>+</sup> and Cl<sup>-</sup> ions which cause ion toxicity and accumulating K<sup>+</sup> and Ca<sup>2+</sup> that enable plants to withstand salt stress (Youssef et al., 2018). Moreover, plants also accumulate several compatible solutes such as proline, betaine, trehalose, ectoine and polyols in the cytosol (Hosseinifard et al., 2022), and/or activate an array of enzymatic and non-enzymatic antioxidant defenses in response to salinity stress (Sachdev et al., 2021). These mechanisms may be enhanced by several strategies such as inoculation with arbuscular mycorrhizal fungi (AMF) or/and foliar application of salicylic acid (SA).

Arbuscular mycorrhizal fungi (AMF) are important soil microorganisms that are colonized by more than 80% of terrestrial plants and establish symbiotic relationships with the roots of plants, in which the plant obtains water and mineral nutrients from the mycorrhizal and the fungus acquires carbohydrates from plants (Bijalwan et al., 2021). AMF plays an important role in stimulating plant uptake of water and nutrients such as phosphorus and increasing the plant root surface area (Ren et al., 2019), increasing plant tolerance to adverse conditions especially salt stress (Bijalwan et al., 2021) via improving chlorophyll content, photosynthetic efficiency and capacity, hormone homeostasis and antioxidant activity (Youssef et al. 2018, Bijalwan et al. 2021 and Wu et al., 2021) and thereby enhancing the

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plant growth, survival rate, yield, and fruit quality under biotic and abiotic stressful conditions.

Salicylic acid, a monohydroxy-benzoic acid, is one of the strong candidates for salinity ameliorators (Khan et al. 2014, Youssef et al. 2018, Naeem et al. 2020 and Desire & Arslan 2021) that have recently been recognized as a naturally occurring plant hormone that can be involved in diverse plant physiological and biochemical processes including plant growth, flower induction, photosynthesis, thermogenesis, nutrient uptake, membrane permeability, ethylene biosynthesis, stomatal movements and enzyme activity (Hayat et al., 2010, and Ram et al., 2014). Youssef et al. (2018) found that salicylic acid attenuated the adverse effects of salinity on growth and yield and enhanced peroxidase isozyme expression more competently than the other osmoprotectants, proline and glycine betaine, in cucumber plants. In addition, exogenous application of salicylic acid induces the antioxidant enzymes activity and subsequently augments plant resistance to NaCl toxicity (He and Zhu 2008). The ameliorative effects of foliar application of salicylic acid have been reported in inducing salt tolerance in some cucurbits such as cucumber (Yildirim et al., 2008, Dong et al., 2011 and Youssef et al., 2018), summer squash (Elwan and El-Shatoury 2014), melon (Nasrabadi and Saberali 2020) and watermelon (Avyub et al., 2015 and Ribeiro et al., 2020).

However, no empirical research exists addressing the synergistic interactions between inoculation with arbuscular mycorrhizal fungi (AMF) and foliar application of salicylic acid (SA) in attenuating the harmful effects of salt stress. Therefore, the objective of this work was to study the effect of AMF inoculation alone or in combination with foliar application of SA on the growth, some physiological and biochemical attributes, yield, and fruit quality of watermelon plant under different levels of irrigation water salinity.

# Materials and Methods

#### Plant Material and Experimental Site

This experiment was carried out to study the ameliorating effects of arbuscular mycorrhizal fungi inoculation or/and foliar application of salicylic acid on growth, some physiological and biochemical parameters, and productivity of grafted watermelon plants (*Citrullus lanatus* (Thunb.) Matsum & Nakai) cv. Aswan F, grown

under salt stress during the two successive growing seasons of 2020 and 2021 at Balouza Research Station (Latitude 31°01′44′′N, Longitude 32°35′26′′E), Desert Research Center (DRC), North Sinai Governorate, Egypt.

#### Irrigation Water and Soil Analyses

The chemical analysis of three levels of irrigation water is shown in Table 1. Also, representative samples of the experimental soil were collected from the surface layer (0-30 cm) and analyzed for chemical properties as shown in Table 2. The analyses were conducted at the Laboratory of the Soil Fertility Department, Desert Research Center according to Richards (1954) and Jackson (1967), respectively.

#### Experimental Design and Growth Conditions

The experiment was arranged in a split splitplot design with three replications in both seasons. Every replicate included twenty-four treatments resulted from the combinations of three salinity treatments, two arbuscular mycorrhizal fungi treatments and four foliar applications of salicylic acid. The main plots were devoted to the salinity treatments, while the sub-plots were occupied with the mycorrhizal treatments and the salicylic acid levels were allotted in the sub-sub plots.

#### TABLE 1. Chemical analysis of the water resources.

Grafted watermelon seedlings cv. Aswan F, onto Star rootstock were used in the two tested seasons. The grafting process was carried out in a private nursery for vegetables in Berqash area, Giza Governorate, by using the tongue approach grafting method. Seedlings of grafted watermelon were transplanted after the formation of 2 -4 true leaves. Thirty plants for each treatment (ten plants per replicate) were transplanted on the first week of April in both seasons of the study. The experimental plot area was 60 m<sup>2</sup> contained one row with a length of 30 m and a width of 2 m. The distance between plants was 1.0 m apart. The agricultural management practices (soil preparation for cultivation, fertilization, irrigation, weed control and disease and pest control) were performed according to recommendations of the Egyptian Ministry of Agriculture for watermelon production.

# Experimental Treatments

# Salinity Treatments

The application of salinity treatments was started directly after transplanting the watermelon seedlings. Three levels of salinity were used, 1600 ppm (Al-Salam Conduit water), 4000 ppm underground well 1, and 5000 ppm underground well 2). Al-Salam Conduit water (1600 ppm) was used as the check treatment.

Irrigation Water	Soluble cations (meq/100g)					Soluble (meq/	anions 100g)	pН	EC	SAR	ESP	
	Ca <sup>2+</sup>	$Mg^{2+}$	$Na^+$	$\mathbf{K}^{+}$	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> -	Cl	SO <sub>4</sub> <sup>2-</sup>		usm		
Al-Salam Conduit	2.10	1.99	15.66	0.85	0.0	3.98	13.91	2.61	7.45	2.60	10.95	15.81
Well 1 (4000 ppm)	4.67	6.58	40.89	10.19	0.0	5.97	38.93	7.28	7.49	6.22	17.17	24.31
Well 2 (5000 ppm)	10.94	17.92	50.69	12.23	0.0	11.99	72.28	7.23	7.91	7.96	13.34	18.61

Data analyzed by the Laboratory of the Soil Fertility Department in Desert Research Center.

TABLE 2.	Chemical	analysis o	f the ex	perimental	soil.
		•/			

Soluble cations (meq/100g)				Soluble anions (meq/100g)				рН	EC	SAR	ESP
Ca <sup>2+</sup>	$Mg^{2+}$	Na <sup>+</sup>	$\mathbf{K}^{+}$	CO <sub>3</sub> <sup>2-</sup>	HCO <sub>3</sub> -	Cŀ	SO <sub>4</sub> <sup>2-</sup>		dSm <sup>-2</sup>		
10.15	4.21	2.39	1.68	0.0	2.99	8.36	21.26	8.14	3.26	3.30	3.48

Data analyzed by the Laboratory of the Soil Fertility Department in Desert Research Center.

#### Arbuscular Mycorrhizal Fungi Treatments

These treatments included inoculated and non-inoculated watermelon seedlings with mycorrhizal fungi (*Glomus Gigaspora*), which was obtained from the Microbiology Department, Faculty of Agriculture, Ain Shams University. Mycorrhizal inocula were placed directly on the roots of watermelon seedlings before transplanting by soaking for 15-30 minutes. Mycorrhizal inocula contained 200-300 spores/cm<sup>3</sup>. Successful inoculation can be detected about three weeks after the transplanting date.

#### Foliar Spraying of Salicylic Acid Treatments

Four concentrations of extra pure salicylic acid (HOC, H, COOH, MW 138.122) of 0, 1, 2 and 4 mM were used. The SA was initially dissolved in ethanol and then compensated with distilled water. The control treatment was only prepared with distilled water. The pH of the solutions was set to 6.5-7 and a few drops of surfactant agent Tween-20 was added. The watermelon plants in each plot were sprayed with salicylic acid four times during the growing period of the watermelon plants. The first application was carried out after 15 days from the transplanting date. After that, three other subsequent foliar applications were made in a 10-day interval at 25, 35 and 45 days, respectively. Sprayings were applied during the early morning using a Knapsack power sprayer with one nozzle. The volume of the spraved solution was enough to cover completely the whole plant's foliage each time.

# Data Recorded

#### Vegetative Growth Parameters

A random and representative sample of three plants from each experimental plot was taken at 65 days after transplanting to measure the vegetative growth parameters. Plant length was measured from the beginning of the soil surface to the plant stem apex. The number of lateral branches and the number of leaves per plant were counted. Leaf area was calculated as the relation between unit area and leaf fresh weight using the following equation (Koller, 1972):

 $\label{eq:least} \begin{array}{c} \text{Disk area}{\times} \text{ No.disks}{\times} \text{ fresh weight of leaves} \\ \text{Leaf area}{=} \end{array}$ 

# Fresh weight of disks

Leaf area index was calculated as a ratio of plant foliage area to soil area. The three plants were removed with a shovel, to prevent damage to the plant root system. The excess soil attached to the roots was carefully removed. The vegetative growth and root fresh weights were recorded.

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Then they were dried in an electric drying oven at 70°C until a constant weight to record the vegetative growth and root dry weights.

### Mineral Composition of Leaves

Fully expanded leaves of plant samples were used to determine some macro- and micro-nutrients. Dried leaf samples were grinded to a fine powder material using a high-speed stainless-steel grinder, then 0.1 g of the fine powdered leaves samples were wet digested using a mixture of sulphuric acid  $(H_2SO_4, 98\%)$  and hydrogen peroxide  $(H_2O_2, 30\%)$ as described by Allen (1974). The nitrogen content was assayed by the modified micro-Kjeldahl method described by Peach and Tracey (1956). Phosphorus content was spectrophotometrically measured according to Watanabe and Olsen (1965). Sodium and potassium percents were measured by flame photometer (Irri 1976). The chloride content was determined according to Jackson and Thomson (1960).

#### Physiological and Biochemical Measurements

Fresh fully expanded leaves from three plants per experimental plot (the fourth leaf from the plant top) from the plant samples were collected to measure the following physiological and biochemical measurements:

#### Leaf Relative Water Content (LRWC)

Fresh weight (FW) of leaf samples was immediately recorded, and then they were soaked for 4 hours in distilled water at room temperature under a constant light and saturated humidity to record turgid weight (TW). At the end of the imbibition period, the samples were then dried for 24 hours at 80°C to determine dry weight (DW). Relative water content (RWC) was calculated using the following formula (Kaya et al. 2003):

$$RWC (\%) = \frac{(FW-DW)}{(TW-DW)} \times 100$$

# Leaf Membrane Stability Index (LMSI)

Leaf membrane stability index was determined by the method of Sairam et al. (1997). Leaf disks were cut using a stainless-steel-cork borer, about 200 mg leaf disks were immersed in two sets of test tubes containing 10 ml of distilled water. One set was kept at 40°C in a water bath for 30 min and then the electrical conductivity (EC-1) was measured using an electrical conductivity meter. The second set was incubated at 100°C for 15 min and electrical conductivity (EC-2) was measured. MSI was calculated according to the following formula:

$$MSI(\%) = (1 - EC1/EC2) \times 100$$

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#### Leaf Chlorophyll Quantification

Chlorophyll (*a*, *b* and total) content of fresh fully expanded leaves were measured after the extraction with 80% acetone. The absorbance of the extract was read at 663 and 645 nm for Chlorophyll *a* (*Chl a*) and *b* (*Chl b*), respectively, the chlorophyll a and b quantification were calculated as previously described by Lichtenthaler and Wellburn (1983) as follows:

Chlorophyll a =  $12.7 \times DO_{663}$  -  $2.69 \times DO_{645}$ 

Chlorophyll b = 22.9 x  $DO_{645}$  - 4.69 x  $DO_{663}$ 

#### Determination of Leaf Proline

For proline determination, fresh leaf samples (1 g) were grinded and homogenized with one volume of 100 ml of 3% sulfosalicylic acid (w/v). After centrifugation at 16,000  $\times$ g for 10 min, the supernatants were collected and used for the analysis of proline by the ninhydrin reagent method, as previously described by López-Orenes et al. (2013).

# Determination of Antioxidant Enzyme Activity Determination of Catalase Activity

The catalase (CAT, EC 1.11.1.6) assay was carried out according to the method of Montavon et al. (2007). Fresh leaf tissue (100 mg) was homogenized in 50 Tris-HCl buffer (pH 8.5), which contained 2 mM EDTA and 5% of soluble polyvinylpyrrolidone. Thereafter, the homogenate was centrifuged at 12000 rpm for 20 min at 4 °C. The enzymatic extract (100 µl) was added to 800 µl of the reaction mixture (50 mM Tris-HCl buffer pH 6.8 containing 5 mM H<sub>2</sub>O<sub>2</sub>). Then, the reaction was stopped with 100 µl of 20% titanium tetrachloride after 10 min at 20°C. The reaction absorbance was determined at 415 nm. One unit of CAT activity corresponds to the amount of enzyme required to reduce 1 µmol hydrogen peroxide  $(H_2O_2)$  per minute.

#### Determination of Peroxidase Activity

Leaf tissue was grinded and homogenized in 100 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1 % polyvinyl pyrrolidone (PVP, w/v) at 4 °C. The ratio of extraction was 4 ml buffer for each 1 g of plant material. The extract was centrifuged for 15 min at 4 °C at 11,000 xg. The activity of peroxidase (POD, EC 1.11.1.7) was assayed by the method of Hammerschmidt et al. (1982). The mixture (2.9 ml) consisted of 0.25 % (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6 containing 10 mM hydrogen peroxide  $H_2O_2$ ). A volume of 100 µl of the crude enzyme extract was added to initiate the reaction which was measured spectrophotometrically at 470 nm per minute. Protein concentration was determined according to the method of Bradford (1976). Enzyme activities were expressed as  $\Delta$  OD = 0.01 POD activity expressed as unit min<sup>-1</sup>mg<sup>-1</sup> protein.

#### Yield and Its Attributes

At the harvesting stage, three fruits from each experimental plot were randomly taken to estimate fruit weight and diameter, flesh and rind thickness, and total yield per plant.

# Fruit Quality Parameters

The total soluble solids (TSS) were recorded in watermelon fruits using a hand refractometer. Lycopene content was measured by a spectrophotometer according to the procedure of Sadler et al. (1990). Total sugar was determined in each fruit sample according to the Shaffer and Somogyi method which was described by Ranganna (1986). Titratable acidity was also determined according to A.O.A.C. (2012).

#### Statistical Analysis

Data obtained were subjected to a statistical analysis of variance procedure of two-way ANOVA using the package of the CoStat program (version 6.303, CoHort Software, USA) based on a split split-plot design. Significant differences among treatment means were estimated based on the LSD test at P $\leq$ 0.05 level of probability using the same software.

# **Results**

#### Vegetative growth

Data shown in Figures 1 and 2 clearly show that increasing saline-water concentrations caused negative impacts on all vegetative growth parameters expressed as plant length, number of branches and leaves per plant, leaf area, leaf area index, and fresh and dry weights of shoots and roots where water salinity at a level of 5000 ppm recorded the lowest significant values compared to the check treatment (1600 ppm) which recorded the highest significant values in both growing seasons.

Generally, arbuscular mycorrhiza fungi inoculation had a significant effect on all the above-mentioned characteristics, where the highest values were recorded by the inoculated watermelon plants under all saline-water treatments. Similarly, foliar application of salicylic acid had a significant effect on all vegetative growth parameters where the best values were recorded with the foliar spraying at

a concentration of 4 mM. In contrast, the lowest values were obtained from the check treatment in both experimental seasons.

The obtained results affirmed that the salinity ameliorative treatments (mycorrhiza inoculation along with foliar spraying of salicylic acid) compared to the check treatment, non-inoculated and unsprayed plants, enhanced all the abovementioned plant vegetative growth parameters affected by salt stress. AMF inoculation combined with SA spraying at a concentration of 4 mM was significantly the best combination in mitigating the salt stress impacts at the lowest level of salinity (1600 ppm), but it was moderately neutralizing the destructive effects at the highest level of irrigation water salinity (5000 ppm) for all growth parameters. Similar trends were detected in both growing seasons.

#### Mineral Composition in Leaves

Data on the influence of arbuscular mycorrhiza fungi or salicylic acid and their interaction on N, P, K, Na and Cl contents under saline-water concentrations are presented in Figure 3. As regard to the impact of salinity, the results indicated that progressive salt stress levels decreased N, P and K contents in watermelon leaves and resulted in an increase in Na and Cl concentrations. Salinity treatment at a level of 5000 ppm recorded lower values of N, P and K and the maximum values of Na and Cl when compared to the check treatment (1600 ppm). The differences among the three salinity levels treatments were significant during the two tested seasons.

As for the effect of mycorrhizal inoculation under different salt-water treatments, it was found that there were significant gradual increments in N, P and K contents in the leaves of the inoculated watermelon plants, while Na and Cl contents were significantly decreased during the two growing seasons.

Referring to the effect of salicylic acid application on mineral contents in watermelon leaves, the maximum contents of N, P and K were recorded by spraying SA at a rate of 4 mM followed, in descending order, by spraying at 2 mM compared to the check plants (spraying at 0.0 mM) which recorded the lowest values of N, P and K. At the same time, gave the highest significant values of Na and Cl concentrations when compared to the other treatments during the two seasons.

Mycorrhiza and salicylic acid applications positively reduced the harmful effects of salt stress on mineral contents of watermelon plants compared to the non-inoculated unsprayed plants. Generally, at the lowest saline water level (1600 ppm) mycorrhiza inoculation combined with salicylic acid spraying at 2 mM or 4 mM enhanced the concentrations of N, P and K and reduced Na and Cl concentrations. The enhancement of mineral nutrient levels (N, P and K) as well as the diminishment of Na and Cl in the inoculated plants with AMF and sprayed with salicylic acid would lead to increased photosynthesitic rate which leads to an increase in vegetative growth attributes under all treatments. In this regard, the increments of nutrient contents in the leaves are well coupled with the increase of chlorophyll content in the leaves and closely related to enhance vegetative growth characteristics.

# Physiological and biochemical measurements Leaf Relative Water Content (LRWC) and Leaf Membrane Stability Index (LMSI)

Leaf relative water content and the leaf membrane stability index were negatively affected by increasing irrigation water salinity and recorded the lowest values under the highest saline-water level (5000 ppm) compared to the check plants (1600 ppm) in both studied seasons (Figure 4). However, AMF inoculation positively improved both LRWC and LMSI. Also, foliar application of SA at 4 mM enhanced both parameters. Additionally, the achieved results indicated that the synergistic effects of mycorrhizal inoculation and salicylic acid application alleviated the negative effects of water salinity on both parameters in both growing seasons.

#### Leaf Chlorophyll Content

The chlorophyll content of the leaves plays an important role in determining plant photosynthetic potential under salt stress. Data presented in Figure 5 demonstrated that increasing salinewater level from 1600 to 500 ppm significantly decreased the chlorophyll content of the leaves. The obtained data also revealed that inoculation with AMF counterbalanced the damaging effects of salinity on chlorophyll content. Furthermore, foliar application of salicylic acid at the highest rate (4 mM) positively mitigated the deleterious effects of salinity and induced stimulative effects on chlorophyll content. These enhancements were more pronounced with the interactive combination of AMF inoculation and SA application.



Fig. 1. Effect of mycorrhizal inoculation and/or foliar application of salicylic acid on some vegetative growth parameters of watermelon cv. Aswan F1 plants grown under different water salinity levels in 2020 and 2021seasons. Vertical bars indicate the LSD value at p≤ 0.05.



Fig. 2. Effect of mycorrhizal inoculation and/or foliar application of salicylic acid on shoot and root fresh and dry weights of watermelon cv. Aswan F1 plants grown under different water salinity levels in 2020 and 2021seasons. Vertical bars indicate the LSD value at p≤ 0.05.



Fig. 3. Effect of mycorrhizal inoculation and/or foliar application of salicylic acid on leaf mineral composition of watermelon cv. Aswan F1 plants grown under different water salinity levels in 2020 and 2021seasons. Vertical bars indicate the LSD value at p≤ 0.05.



Fig. 4. Effect of mycorrhizal inoculation and/or foliar application of salicylic acid on leaf relative water content and membrane stability index of watermelon cv. Aswan F1 plants grown under different water salinity levels in 2020 and 2021seasons. Vertical bars indicate the LSD value at p≤ 0.05.



Fig. 5. Effect of mycorrhizal inoculation and/or foliar application of salicylic acid on some biochemical parameters of watermelon cv. Aswan F1 plants grown under different water salinity levels in 2020 and 2021seasons. Vertical bars indicate the LSD value at p≤ 0.05.

#### Proline content

As known, proline is one of the non-toxic compatible organic solutes that are used by plants as an osmoprotectant that maintains the osmoregulation under salt stress. Figure 5 reveals that increasing salinity levels increased proline accumulation in watermelon leaves. As for the effect of mycorrhizal inoculation, it was found that there were gradual increments in leaf proline content with AMF application in both seasons. Similarly, foliar application of salicylic acid maintained the highest values of proline content in the leaves of watermelon plants compared to the check plants which recorded the lowest values. The application of mycorrhiza and salicylic acid together attenuated the damaging effects of salinity by recording the highest values of proline content under the highest level of irrigation water salinity (5000 ppm).

# Antioxidant Enzyme Activity

It is known that salinity stress induces reactive oxygen species (ROS), which have a major deleterious effect on plants. To combat the salinity stress, plants have both enzymatic and nonenzymatic mechanisms for scavenging these ROS. Thus, the corresponding antioxidant synthesis like catalase and peroxidase enzymes can be considered a plant response to mitigate the oxidative stress. Catalase and peroxidase enzymes activities were determined in the watermelon leaves in this study. Data of the antioxidant enzymes activities in Figure 5 reveal significant increments in the studied antioxidant enzyme activities (catalase and peroxidase) with increasing salinity level. Data from the same Figure also show that mycorrhizal inoculated plants had a higher accumulation of antioxidative enzymes and consequently improved plant growth under all levels of water salinity. Also, the obtained results confirmed that foliar application of salicylic acid increased the activities of catalase and peroxidase at all levels of water salinity compared to the unsprayed plants. The application of both mycorrhiza and salicylic acid alleviated the damaging effects of salinity stress. The combined treatments recorded the highest values of catalase and peroxidase activity under the highest level of water salinity (5000 ppm) in comparison with the check plants which received water salinity of 1600 ppm.

#### Yield and Its Components

It is clear from Figure 6 that there were sharp decreases in the fruit yield parameters with increasing water salinity levels. The decrements reached 25.86 and 23.24% in fruit weight, 14.60

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and 15.76% in fruit diameter, 10.51 and 8.99% in flesh thickness and 39.21 and 37.17% in total vield per plant under the highest water salinity level (5000 ppm) in the 1st and 2nd seasons, respectively, compared to the check treatment (water salinity level of 1600 ppm) which recorded the best values in both seasons. AMF inoculation exhibited a pronounced enhancement of yield attributes, since the obtained results indicated that the highest values were recorded with the inoculated watermelon plants during both seasons compared to the non-inoculated plants under all treatments. As for the impact of salicylic acid treatments, the highest values of fruit yield and yield components were obtained from SA foliar sprayed at 4 mM compared to the other treatments. The check treatment recorded the lowest values of fruit yield and yield components in both seasons. The interactive impact between antisalinity applications (AMF with SA) positively minimized the detrimental effect of salt stress and improved the fruit yield parameters under different irrigation water salinity levels. Hence, the highest values were significantly observed with AMF inoculation combined with SA spraying at a level of 4 mM, compared with the non-inoculated unsprayed plants at all water salinity levels.

# Fruit Quality Parameters

Concerning the characteristics of the chemical quality of watermelon strawberry fruits, it was found that TSS, total sugars, lycopene content and titratable acidity increased significantly with increasing the water salinity level in both growing seasons. Treating with AMF significantly affected the quality of watermelon fruits, where the inoculated plants recorded the highest values of TSS, total sugars, and lycopene content compared with non-inoculated plants in the two tested seasons as shown in Figure (7). Salicylic acid foliar spraying at a rate of 4 mM gave the highest values of total soluble solids, total sugars, and lycopene content in the two studied seasons compared to the other treatments whereas the check treatments recorded the lowest values. The salinity attenuative treatments led to a significant mitigation in fruit quality characteristics under salt stress conditions. The best values were observed by water salinity at a level of 5000 ppm with the AMF inoculated plants combined with SA at 4 mM compared to the non-inoculated unsprayed plants. On the contrary, AMF inoculation or/and spraying of salicylic acid reduced titratable acidity values in watermelon fruits under all treatments.



Fig. 6. Effect of mycorrhizal inoculation and/or foliar application of salicylic acid on yield and its attributes of watermelon cv. Aswan F1 plants grown under different water salinity levels in 2020 and 2021seasons. Vertical bars indicate the LSD value at p≤ 0.05.



Fig. 7. Effect of mycorrhizal inoculation and/or foliar application of salicylic acid on some fruit quality characteristics of watermelon cv. Aswan F1 plants grown under different water salinity levels in 2020 and 2021 seasons. Vertical bars indicate the LSD value at p≤ 0.05.

#### **Discussion**

Salinity, soil salinity and/or irrigation-water salinity have been recognized as one of the most abiotic stresses that suppresses the growth and productivity of vegetable crops including watermelon which is a moderately sensitive crop to salinity (Amacher et al. 2000 and Ali et al. 2015). For mitigation of the deleterious effects of salinity stress, plant cells adapt numerous mechanisms to protect the cells against oxidative damage. These mechanisms may be enhanced by several eco-friendly strategies such as inoculation with arbuscular mycorrhizal fungi (AMF) or/and foliar application of salicylic acid (SA) which are known to help as ameliorators of salinity stress that can enhance salinity tolerance in the plants, but their combined effect has never been examined on watermelon.

In this study, the obtained results clearly demonstrated that increasing water salinity levels reduced plant growth parameters (Fig. 1 and 2) and yield components (Fig. 6) and reached their lowest values at the highest water salinity level (5000 ppm). These results of this study are consistent with the results of Ayyub et al. (2015), Silva et al. (2017), Yanyan et al. (2018), Ye et al. (2019), Bezerra et al. (2020), Gomes et al. (2020), Junior et al. (2020), Ramezan et al. (2020), Ribeiro et al. (2020), Silva et al. (2020) and Cova et al. (2021) who found that plant growth and productivity of watermelon were negatively affected by salt stress. These reductions in growth parameters and productivity could be attributed to the deleterious effects of salinity on various physiological and biochemical processes such as water absorption, photosynthesis, stomatal conductance, protein synthesis, antioxidant metabolism, osmolytes accumulation, mineral nutrients homeostasis, and hormonal signaling (Huang et al. 2011, Kere et al., 2016, Hegazi et al. 2017, Youssef et al. 2018, Abdel Motaleb et al. 2020, Bijalwan et al. 2021 and Roshdy et al. 2021). In this study, the reductions in vegetative growth characteristics and yield attributes are well coupled with the reductions in leaf contents of N, P and K and the promotions of Na and Cl conents (Fig. 3), decreasing the relative water content and leaf membrane stability index of the leaves (Fig. 4) and decreasing chlorophyll content (Fig. 5). Arbuscular mycorrhiza fungi inoculation enhanced all plant growth characteristics and yield components under all water salinity treatments. These results agree with those of Omirou et al. (2013), Miceli et al. (2016), Mo et al. (2016), Ren et al. (2019), Ye et al. (2019) and Wu et al. (2021) who stated that AMF treatment can exhibit excellent effects on growth and productivity of watermelon plants. In addition, inoculation with arbuscular mycorrhizal fungi has been demonstrated to tackle the detrimental effects of salinity in some other vegetable crops like pepper (Kaya et al. 2009, Abdel Latef 2011, Abdel Latef and Chaoxing 2014, Beltrano et al. 2013, Hegazi et al. 2017), tomato (Hajiboland et al. 2010, Abdel Latef & Chaoxing 2011 and Damaiyanti et al. 2015), and green bean (Abdel Motaleb et al. 2020). AMF colonization may alleviate the impacts of salt stress in watermelon plants through improving soil structure (Miller and Jastrow 2000), enhancing the absorption and transport of water and nutrients (Augé 2003, Ruiz-Lozano 2003, Rouphael et al. 2010, Ruiz-Lozano et al. 2012, Youssef et al. 2017, Ren et al. 2019) through enlarging the effective root area and penetration of substrate and activation and excretion of various enzymes by infected mycorrhizal fungi roots and/ or hyphae (Smith and Read 2008, Mo et al. 2016), promoting the photosynthesis and synthesis of chlorophyll (Youssef et al. 2017, Abdel Motaleb et al. 2020) which could supply assimilates for fruit productivity and contribute to the higher vield. as well as AMF alleviated the injury of Fusarium wilt and inhibited the soil borne pathogens (Ren et al. 2015) and thereby enhancing the plant growth and yield. In addition, such stimulatory effects were found to be correlated with the increase in the contents of endogenous hormones particularly auxins and gibberellins (Abd Allah et al. 2015) which promote the growth of plant organs. Moreover, the obtained results revealed that foliar spraying with salicylic acid at a rate of 4 mM improved all vegetative growth parameters and yield attributes under all water salinity treatments. Similar results were reported the alleviating effects of salicylic acid for some cucurbit plants under salt stress such as watermelon (Ayyub et al. 2015, Ribeiro et al. 2020), cucumber (Yildirim et al. 2008, Youssef et al. 2018), summer squash (Elwan and El-Shatoury 2014), and melon (Nasrabadi and Saberali 2020). Also, other studies conveyed similar growth promotion in various vegetable crops with salicylic acid spraying including pepper (Kaya et al. 2009, Abdul Qados 2015, Ahmed et al. 2020), mungbean (Ghassemi-Golezani et al. 2015, Lotfi et al. 2020), common bean (Osman and Salim 2016), tomato (Naeem et al. 2020, Rao et al. 2021), strawberry (Roshdy et al., 2021), and eggplant (Sousa et al., 2022). These stimulatory effects could be attributed to the positive effect of salicylic acid upon the endogenous phytohormones especially the growth promoters, i.e., auxins, gibberellins and cytokinins (Shakirova 2007, Mady 2014) which improve cell division and cell enlargement (Hayat et al., 2005) and consequently enhanced the vegetative growth parameters and thereby consequently enhanced yield. Also, the results showed that addition of mycorrhizal fungi combined with application of salicylic acid alleviated the harmful impacts of water salinity in watermelon crop.

The current study indicated that increasing irrigation water salinity caused significant decreases in N, P, and K contents in watermelon leaves, and significant increases in Na and Cl contents (Fig. 3). Several investigators reported similar results (Ayyub et al. 2015, Yanyan et al. 2018, Ye et al. 2019, Ramezan et al. 2020, Bijalwan et al. 2021) who stated that the higher level of salinity led to a decrease in the root system formation and reduced nutrient absorption, which led also to a decrease in their concentration in the plant. In addition, it is well documented that salt stress adversely affects N acquisition and utilization by influencing different stages of N metabolism, such as NO<sub>3</sub><sup>-</sup> uptake and reduction and protein synthesis (Evelin et al. 2009). Ye et al. (2019) revealed that salinity reduced the availability of phosphate in the soil resulting in a reduction of phosphate uptake and accumulation in plants. Furthermore, the possible reason for potassium decreasing with salinity increasing could be that elevated concentrations of sodium in the soils reduced the uptake of potassium, through the antagonism between these two ions as well as potassium ion leakage when calcium substitutes for sodium in the cell membranes (Pardo and Quintero 2002). A higher Na<sup>+</sup>/K<sup>+</sup> ratio resulting from salinity interferes with the cytoplasm ionic balance, and consequently inhibits various metabolic pathways (Hajiboland 2013). When the plants of watermelon were inoculated with mycorrhiza under different water salinity treatments, gradual increments in N, P and K contents in the leaves were reported, while Na and Cl contents were decreased. Similar findings were reported by Omirou et al. (2013), Ye et al. (2019), Bijalwan et al. (2021) who found that there were significant increases in nutrient percent with mycorrhizal inoculated plants compared to that of their check, since AMF increases the plant root surface area (Ren et al. 2019) and establishes

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symbiotic relationships with the roots of plants (Birhane et al. 2018). Moreover, it is well known that mycorrhizal inoculated plants have two pathways of nutrient acquisition, the direct one through the root epidermis and its root hairs, (the only uptake route of non-inoculated mycorrhizal plants), and the mycorrhizal pathway through the fungal mycelium that provides nutrients to the root cortex through the arbuscules (Smith and Read 2008). Furthermore, the fungal hyphae are much thinner than roots (Bago et al. 1998), allowing them to explore small cracks in the micrometer range that are inaccessible to roots. In addition, AMF inoculation enhances the permeability of the root cells, leading to increases in plant tolerance to adverse conditions especially salt stress (Abdel Motaleb et al. 2020), and thereby enhancing the nutrient contents in watermelon plants. On the contrary, many investigators revealed that mycorrhizal-inoculated plants had lower levels of Na (Abdel Latef and Chaoxing 2011, Evelin et al. 2012), suggesting that AMF have a buffering effect on Na uptake when the concentration of Na is within the permissible limit (Allen and Cunningham 1983). This also points out the possibility of a regulatory mechanism in the plant to modify Na concentration (Evelin et al. 2009. Kapoor et al. 2013). In the same manner. foliar spraying of salicylic acid at a rate of 4 mM improved N, P and K contents and decreased Na and Cl contents in the leaves under all water salinity treatments. Similarly, several researchers found that salicylic acid application increased the concentrations of N, P and K of many vegetable crops and decreased the concentrations of Na and Cl (Ayyub et al. 2015, Cheng et al. 2018, Abdel Motaleb et al. 2020, Roshdy et al. 2021). Such enhancements in macro-nutrients (N, P and K) accumulation and reductions in Na and Cl contents resulted from salicylic acid application under salt stress may result in high chlorophyll content, low membrane injury and high-water content, inducing salt stress adaptation and improving growth and productivity of the plants. Also, the obtained results demonstrated that mycorrhiza and salicylic acid applications together positively diminish the detrimental effects of salt stress on the mineral contents of watermelon plants compared to the non-inoculated unsprayed watermelon plants. The enhanced mineral nutrient levels (N, P and K) in the AMF inoculated and salicylic acid sprayed plants would lead to increased photosynthesis which leads to increases of the vegetative growth attributes under all water salinity treatments. In this regard, the increased nutrient contents in the leaves are well coupled with the increased chlorophyll content in the leaves (Fig. 5) and closely related to the increased vegetative growth characteristics (Fig. 1 and 2).

Water status is considered one of the main reasons for growth reductions in plants under salt stress. It is well-known that salt stress decreases the water potential of leaves and subsequently, diminishes the absorption of water and nutrients (Romero-Aranda et al. 2001). These declines in water absorption induced by salt stress may cause a reduction in the relative water content of leaves (Bayuelo-Jiménez et al. 2012). Similar damaging effects of salt stress on leaf relative water content have been previously reported for cucumber plants (Yildirim et al. 2008, Youssef et al. 2018). These reductions in water status are linked with the reductions in vegetative growth parameters under salinity conditions (Figs. 1 and 2). AMF treatment positively enhanced LRWC and significantly decreased the detrimental effect of salinity in mycorrhizal-inoculated plants compared to uninoculated plants. These results are in harmony with the results of Colla et al. (2008) who reported water status enhancement of Zucchini plants colonized by Glomus intraradices under salinity stress. Also, Sousa et al. (2016), Chen et al. (2017), Yanyan et al. (2018), Ye et al. (2019), Ramezan et al. (2020), Bijalwan et al. (2021) reported similar results. These improvements in LRWC in mycorrhizal-inoculated plants under salinity stress could be attributed to (a) a higher hydraulic conductivity of mycorrhizal roots at low water potential (Garg & Manchanda 2009 and Kaya et al. 2009) through improving membrane integrity and stability, (b) altered morphology of the root system induced by mycorrhizal fungi (Kothari et al. 1990), (c) higher stomatal conductance, which increases the demand for transpiration (Colla et al. 2008, Sheng et al. 2008), or/ and (d) the fungi make the host plant to acquire nutrients and thereby improve the photosynthetic rate as well as water osmotic homeostasis (Sheng et al. 2008 and Abdel Latef & Chaoxing 2014) and all those enhanced processes that resulted from mycorrhizal inoculation may make the plant able to absorb more water. Moreover, salicylic acid application alleviated the negative effects of the salt stress on leaf relative water content of salt stressed watermelon plants which may be attributed to the effect of SA application on increased leaf diffusive resistance and lower transpiration in the plants (Yildirim et al. 2008). Enhancing leaf

water content resulted in the accumulation of different osmolytes such as sugars, sugar alcohols and proline (Szepesi et al. 2005 and Youssef et al. 2018) which is related to the enhancement of plant tolerance by contributing to cellular osmotic adjustment, ROS scavenging, protection of membrane integrity, maintaining nutrients homeostasis and ion compartmentalization, and enzymes/protein stabilization (Khan et al. 2012). One more time, the obtained results showed the synergetic effect of AMF inoculation and SA foliar application to ameliorate the damaging impacts of salinity on leaf water status.

Membrane stability index is one of the indirect indicators to predict cell membrane damage caused by salt stress and consequently induced significant increases in electrolyte leakage (Yildirim et al. 2008, Abdul Qados 2015). The present study demonstrated that salinity reduced the values of the membrane stability index in watermelon leaves (Fig. 4). Similar results were obtained by Sousa et al. (2016), Chen et al. (2017), Yanyan et al. (2018), Ye et al. (2019), Ramezan et al. (2020), Bijalwan et al. (2021). Also, the obtained results pointed out that AMF treatment positively improved LMSI and significantly decreased the harmful effect of salinity in mycorrhizalinoculated plants. These results agree with the former studies of Kaya et al. (2009) and Abdel Motaleb et al. (2020) who demonstrated higher relative membrane permeability when treated with AMF under salinity conditions. The higher stability of cellular membrane and the integrity of membrane proteins by arbuscular mycorrhizal fungal inoculation may be attributed to enhanced P uptake and increased antioxidant production (Evelin et al. 2009). Additionally, exogenous application of salicylic acid ameliorated the injurious impacts of salt stress on the membrane stability index. In this regard, salicylic acid lowered the electrolyte leakage in salt stressed cucumber plants (Yildirim et al., 2008). Earlier studies demonstrated that salicylic acid could attenuate the membrane damaging in the stressed plants, indicating salicylic acid facilitated the maintenance membrane functions (Stevens et al., 2006, Gunes et al., 2007). Recently, AMF inoculation along with SA spraying had the synergistic effects to attenuate the detrimental effect of salinity on the leaf membrane stability index.

This study also revealed that chlorophyll contents in the leaves were gradually decreased

with the increasing-water salinity level (Fig. 5). These results are consistent with those of Ayyub et al. (2015), Sousa et al. (2016), Chen et al. (2017), Yanyan et al. (2018), Ye et al. (2019), Ramezan et al. (2020), Silva et al. (2020), Bijalwan et al. (2021). These reductions in chlorophyll content under salinity stress could be ascribed to stomata closure (Paranychianakis and Chartzoulakis 2005), reduction in water potential (Fig. 4), reduction in N absorption (Fig. 3), which is an essential component of the chlorophyll molecule (Kaya et al. 2009), suppress the activity of specific enzymes required for the biosynthesis of chlorophyll (Sheng et al. 2008), increase the degradation of chlorophyll due to the elevated chlorophyllase enzyme activity, or damage to the photosynthetic apparatus (Sevengor et al. 2011, Rahdari et al. 2012), or/and decrease uptake of magnesium needed for chlorophyll biosynthesis (Sheng et al. 2008, Abdel Latef and Chaoxing 2011). Moreover, the breakdown of the ultrastructure of chloroplasts in salt stressed plants may be due to the direct Na<sup>+</sup> toxicity or salt-induced oxidative damage (Mittler 2002). These reductions may reduce carbon fixation that eventually supplies energy and substrates for metabolic pathways (Yadav et al. 2011), causing a reduction in plant growth. In this concern, the reductions of chlorophyll contents under salinity stress (Fig. 5) are in good accordance with the reductions of plant growth parameters (Figures 1 and 2). Inoculation with AMF counterbalanced the damaging effects of salinity on chlorophyll content. These results are substantiated with the findings of several research works which indicated that mycorrhizal symbiosis attenuated the negative impacts on chlorophyll content under saline conditions for many vegetable crops (Kaya et al. 2009, Hajiboland et al. 2010, Abdel Latef and Chaoxing 2011, 2014, Abdel Motaleb et al. 2020). These improvements in chlorophyll content in the leaves of mycorrhizal inoculated plants under different water salinity levels might be attributed to one or more of the following, an increase in stomatal conductance, photosynthestic rate, transpiration, carbon assimilation and enhancement of plant growth (Sheng et al. 2008, Arumugam et al. 2010), the presence of large and more numerous bundle sheath chloroplasts in the mycorrhizal-inoculated leaves (Arumugam et al. 2010) or/and the counterbalance of antagonistic effects of Na on Mg uptake, needed for chlorophyll biosynthesis, by AMF inoculation (Giri et al. 2003, Zuccarini 2007, Talaat and Shawky 2014).

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Likewise, foliar applications of salicylic acid ameliorated the deleterious effects of saline water on chlorophyll content. High stressed plants treated with SA showed significantly higher levels of total chlorophyll compared to unsprayed plants. The obtained results agree with those of Avvub et al. (2015) who found that salicylic acid enhanced the chlorophyll content of watermelon plants under salinity stress. This enhancement in chlorophyll content may be attributed to the antioxidant scavenging effect of salicylic acid which protected chloroplasts and prevented chlorophyll degradation by the toxic reactive oxygen radicals (Mady, 2014, Abu-Muriefah and Abdul Qados 2015, Youssef et al. 2018) under salt stress in this study. The mycorrhizal inoculation combined with salicylic acid spraving plants showed high values of chlorophyll contents under all water salinity treatments. These increases in chlorophyll contents resulted from the synergistic effects between AMF inoculation and salicylic acid application. The increment of chlorophyll content in leaves under different treatments was coupled with the increased vegetative growth attributes (Fig. 1 and 2).

As known, proline is one of the non-toxic compatible organic solutes that is used by plants as an osmoprotectant that maintains the osmoregulation under salt stress (Hayat et al. 2010, Youssef et al. 2018). The present study demonstrated that increasing water salinity levels increased proline accumulation in watermelon leaves (Fig. 5). Sousa et al. (2016), Chen et al. (2017), Yanyan et al. (2018), Ye et al. (2019), Ramezan et al. (2020), Silva et al. (2020), Bijalwan et al. (2021) reported similar results for watermelon. Many other plant species accumulated proline under salinity stress (Abdel Latef 2011, 2013, Hegazi et al. 2017, Roshdy et al. 2021), since proline acts as a reservoir of energy and nitrogen for utilization during salt stress, and plays key roles in scavenging free radicals, stabilizing of cellular proteins and membranes, buffering cellular redox potential, and reducing enzyme denaturation under salinity stress (Kaur and Asthir 2015). As for the effect of mycorrhiza inoculation, it was found that there were gradual increments in leaf proline content with AMF application. Similar results were reported by Mo et al. (2016), Ye et al. (2019), Abou El-Goud (2020), Bidabadi and Mehralian (2020), Bijalwan et al. (2021), Wu et al. (2021). Also, in many plant species, several authors have reported that proline content increased in mycorrhizal plants than in nonmycorrhizal plants at various salinity levels (Evelin et al. 2009). A higher proline content in mycorrhizal inoculated plants might be attributed to the increase in the expression of gene encoding P5CS involved in proline biosynthesis, the higher activity of glutamate dehydrogenase enzyme that is responsible for synthesizing glutamate which is the precursor of proline and/or inactivation of the proline dehydrogenase enzyme that catalyzes the degradation of proline (Abo-Doma et al. 2016). Similarly, foliar application of salicylic acid increased proline content in the leaves of watermelon plants. Many studies conveyed similar results in various vegetable crops with salicylic acid spraying (Roshdy et al. 2021). Applications of mycorrhiza combined with salicylic acid alleviated the damaging effects of salinity since AMF inoculation and SA spraying plants recorded the highest values of proline content under the highest level of water salinity (5000 ppm).

It is well documented that salinity induces the production of reactive oxygen species (ROS), which have a major damaging effect in plants. To combat the salinity stress, plants have both enzymatic and nonenzymatic mechanisms for scavenging these ROS generated by salinity stress. Thus, the corresponding antioxidant synthesis like catalase and peroxidase enzymes can be considered a plant response to mitigate the oxidative stress (Evelin et al. 2009). Catalase and peroxidase activities were determined in the watermelon leaves in this study. Data of the antioxidant enzyme activity in Figure 5 reveal significant increments in the studied antioxidant enzyme activities (catalase and peroxidase) with increasing water salinity level. Similar results were obtained by several works (Sousa et al. 2016, Chen et al. 2017, Yanyan et al. 2018, Ye et al. 2019, Ramezan et al. 2020, Silva et al. 2020, Bijalwan et al. 2021). Mycorrhizal inoculated plants had a higher accumulation of antioxidative enzymes which consequently improved plant growth under all levels of water salinity. Several studies suggested that mycorrhizal symbiosis helps plants to alleviate salt stress by enhancing the activities of antioxidant enzymes in various vegetable crops (Hajiboland et al. 2010, Hayat et al. 2010, Abdel Latef and Chaoxing 2011, 2014, Evelin and Kapoor 2013, Hegazi et al. 2017, Youssef et al. 2018, Abdel Motaleb et al. 2020). In the same way, foliar application of salicylic acid increased the activities of catalase and peroxidase enzymes at all levels of salinity

compared to the unsprayed plants. Alleviation of salinity stress in many plants was associated with the induction of an antioxidant enzyme system by the application of salicylic acid, which improved the plant performance as well as protected against various damages caused by salt stress (Roshdy et al. 2021). The ameliorative treatments (AMF + SA) recorded the highest values of catalase and peroxidase activities under the highest level of water salinity.

Concerning the characteristics of the chemical quality of watermelon fruits, the obtained results demonstrated that increasing water salinity levels resulted in gradual increments in total soluble solids (TSS), total sugars, lycopene content and titratable acidity. The results are in good accordance with several studies which showed that higher salinity levels reduced water uptake in the salt stressed plants and thus improved fruit quality by increasing the soluble solids, sugar content and acidity (Sousa et al. 2016, Simpson et al. 2018, Ramezan et al. 2020, Cova et al. 2021). Moreover, the increase in total soluble solids and total sugars of watermelon fruits due to salt stress may reflect an osmotic adjustment obtained by enhanced synthesis of soluble organic compounds in the plant tissue (Simpson et al. 2018). When watermelon plants were inoculated with mycorrhiza or/and sprayed with salicylic acid separately or in combination increased total soluble solids, sugars and lycopene content but decreased titratable acidity in the watermelon fruits. These results agree with Miceli et al. (2016), Mo et al. (2016), Abou El-Goud (2020) who found that mycorrhizal colonization significantly gave better performance in fruit quality through improving photosynthesis efficiency which may have contributed to an increase in total soluble solids and total sugars in watermelon fruits.

#### **Conclusion**

Based on the results, it could be concluded that water salinity has significant negative effects on vegetative growth, yield and yield components of the watermelon crop. Findings revealed that mycorrhizal fungi had a positive effect on the studied traits. Also, spraying with salicylic acid enhanced vegetative growth parameters and productivity under all water salinity levels. In addition, the current study affirmed the synergistic effects of mycorrhizal inoculation and salicylic spraying on ameliorating the deleterious effects of irrigation water salinity on the growth and productivity of watermelon plants *via* generating

stimulative impacts on all physiological and biochemical attributes.

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# Conflict of interest

The authors declare that they have no competing interests.

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تأثير التفاعلات التآزرية للميكوريزا وحمض السالسيليك علي تخفيف الآثار الضارة للملوحة على نمو وإنتاجية نباتات البطيخ من خلال تعزيز الاستجابات الفسيولوجية والبيوكيميائية

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أجريت تجريتان حقليتان فى محطة بحوث بالوظه التابعة لمركز بحوث الصحراء بمحافظة شمال سيناء، جمهورية مصر العربية، خلال موسمى الزراعة المتتاليين ٢٠٢٠ و٢٠١ وذلك لدراسة تأثير العدوي بفطر الميكوريزا (شتلات معداه وشتلات غير معداه) والرش الورقي بحمض السالسيليك (٠، ٢، ٢٠ ٤ ملليمول/ لتر) علي تخفيف الأثار السلبية للإجهاد الملحي لمياه الرى (١٦٠٠، ٢٠٠٠، ٢٠٠٠ جزء في المليون) علي النمو الخضرى والثمرى وبعض الصفات الفسيولوجية والبيوكيماوية لنباتات البطيخ هجين أسوان. أظهرت النتائج المتحصل عليها أن المستوى العالي من ملوحة مياه الرى (٢٠٠٠، جزء في المليون) كان له تأثير سلبي على صفات النمو الخضرى وبعض الصفات الفسيولوجية والبيوكيماوية لنباتات البطيخ هجين أسوان. أظهرت والجاف للمتحصل عليها أن المستوى العالي من ملوحة مياه الرى (٢٠٠٠ جزء في المليون) كان له تأثير والجاف للمجموع الخضري والجزري) والمحصول ومكوناته (وزن وقطر الثمرة، سمك اللحم والمحصول والجاف للمجموع الخضري والجزري) والمحصول ومكوناته (وزن وقطر الثمرة، سمك اللحم والمحصول ومحتوى الكلوروفيل) والتركيب المعدني للأوراق (٢٠٣ ولا). في حين كان له تأثير ومحتوى الكلوروفيل) والتركيب المعدني للأوراق (٢٠٩ ولا). في حين كان له تأثير إيجابي على جودة الثمار (السكريات الكلية، مجموع المواد الصلبة الذائبة ومحتوى الليكوبين) وسجل أعلى القيم لمحتوى الأوراق من ومحتوى والكلوريد والبرولين ونشاط إنزيمات الكتاليز والبيروكسيديز مقارنة بمعاملة المقارنة (ملوحة مياه (السكريات الكلية، مجموع المواد الصلبة الذائبة ومحتوى الليكوبين) وسجل أعلى القيم لمحتوى الأوراق من ومحتوى والكلوريد والبرولين ونشاط إنزيمات الكتاليز والبيروكسيديز مقارنة بمعاملة المقارنة (ملوحة مياه الرى ٢٠٠٠ جزء في المليون) خلال الموسمين. أوضحت النتائج المتحصل عليها ايضا أن التلقيح بالميكوريزا أو الرش بحمض السالسيليك بتركيز ٤ مليمول/لتر قد خفف من الأثار الضارة للإجهاد الملحي على مو وإنتاجية نبات البطيخ وسجل الفضل القيم المعنوية لجودة الثمار والصفات الفسيولوجية والبيوكميانية في كلا الموسمين.

الكلمات الدالة: البطيخ ، الملوحة ، فطر الميكوريزا ، حمض السالسيليك.