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Alleviation the adverse effect of the salinity on cotton plant by using azolla extract

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Abstract

A pot experiment was conducted in two seasons 2020 and 2021 at wire green house of Plant Physiology Department, Cotton Research Institute, Agricultural Research Center, Giza, Egypt, to evaluate the effect of using azolla extract (AE) at concentration of 30% on cotton plants Giza 95 cultivar under different salinity water irrigation (SWI). The experiment design was split-plot, which the main plots were devoted to SWI treatments (0, 8000 and 12000 ppm), while AE at concentration of 30% applications were randomly occupied the subplots including control (C), azolla seed soaking (ASS), azolla foliar application (AFA) and azolla combination application (ACA) of ASS+AS treatments. The results noticed that AE treatments improved the plant growth characters, chemical constituents of cotton leaves (total chlorophyll, carotenoids, total soluble sugars, total phenols, total free amino acids, proline and total soluble proteins), in addition to increase the enzymes activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GR), total antioxidant capacity and yield characters, while fiber properties did not affected when compared with untreated plants. Cotton plants treated with ACA gave the maximum values of seed cotton yield/plant about 39.09 and 27.45%, number of bolls/plant about 24.74 and 25.66% and boll weight about 11.40 and 10.52% in both seasons, respectively, compared to untreated plants. The plants treated with SWI (8000 and 12000ppm) had reduced pigments content, growth and yield than plants under normal condition (0ppm). AE treatments by ACA gave the best results in leaves chemical constituents, enzymes activities, growth, yield characters, and improving salinity tolerance in cotton plants under different SWI concentration.

Keywords: Cotton, Salinity, Azolla extract, chemical constituents, growth, yield components, fiber properties.

1. Introduction

Most of cotton planted areas in Egypt are irrigated by wastewater and groundwater, which contain a high salinity that cause to limited plants growth because of lack of water, and nutrients in the environment. Although, cotton plant is classified as salt tolerant crop, yet it is sensitive at germination stage [1]. Salinity stress delays and reduces germination and emergence rates, decreases cotton shoot growth and may finally lead to reduced seed cotton yield and fiber quality [2]. Salinity is the major abiotic stresses in irrigation water that is affecting the morphological, physiological and biochemical processes of plants which can be observed in the form of plant growth, productivity and quality of the crops especially in arid and semi-arid regions [3]. Plants grown under salinity conditions are stressed in water deficit, phytotoxicity of Na^+ and Cl^- ions and reduction in nutrient uptake [4]. Salt stress decreases crop production by inhibiting the photosynthesis and damage of photosynthetic apparatus of plants. It also inhibits the photochemical activities and decreases the activity of enzymes in the calvin cycle [5]. Therefore, to adapt salt stress, plants rely on signals and pathways that re-establish cellular ionic, osmotic, and reactive oxygen species (ROS) homeostasis, which ROS accumulate by high levels in plant leaves under stress conditions, it mutant and cannot be properly detoxified and disrupts normal metabolism through oxidative damage to the lipids, protein and nucleic acids. So that tolerant plants can regulate a better antioxidant system for effective removal of ROS [6].

The using of biofertilizer and organic fertilizer succeeded to minimize the amount of applied chemical fertilizer and reduce the production costs and environmental pollution [7]. Biofertilizers are a

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nutritious, renewable and eco-friendly substance such as azolla. Azolla is belongs to the family of Azollaceae, a heterosporus, free floating, fast growing aquatic fern and is wide spread in fresh water habitat. Azolla is rich source of in proteins (30%), a combination of essential amino acids, vitamins (vitamin A, vitamin B12, β-Carotene), growth promoter intermediaries (10%) and minerals (15%) like Ca, P, K, Fe, Cu, Mg etc. But very low carbohydrate and oil content [8]. Azolla association is the favorite biofertilizer of crops, because of azolla is good nitrogen fixing at high rates and low cost. Due the positive effect of N_2-fixing azolla on plant growth and yield of crops to synthesize and secrete phytohormone, like substances as (thiamin, riboflavin pyridoxine, indol acetic acid, gibberellins, cytokines, abscic acid) and vitamins, antibiotics and amino acids content, that enhance the plant growth and overcome the adverse effect of salinity on plants [9]. AE can supplement the nitrogen requirement of plant and replacing about 30-50 % of plant requirement of mineral nitrogen [10]. As well as, [11] noted that spraying of AE significantly increased leaves chemical constituents, growth and yield components of cotton. Similarly, [5] explained that bio-fertilizers spray application significantly increased the plant nutrients content and had a positive effect on plant growth, oxidation behavior and activity of antioxidant enzymes in plant affected by salt stress, Also they explained that AE is characterized by its bio-stimulators contents that enhance the plant growth and materials are proved to overcome the adverse effect of salinity.

The aim of this study was to evaluate the effective application of AE (30%) on chemical constituent, enzymes activities, growth, yield and fiber properties of cotton under different SWI conditions.

1. Materials and Method

1.1. Plant material

Cotton (*Gossypium barbadense* L. cv Giza 95) seeds were obtained from Cotton Research Institute, Agricultural Research Center, Giza, Egypt.

1.2. Preparation of azolla extract

Azolla leaves (*Azollacaroliniana*) were obtained from Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.About one Kg of azolla was boiled in 1 liter distilled water for about 30-45 minutes, after filtration, filtrate was considered as 100% raw azolla extract, from which concentration was made 30% by means of distilled water. Azolla biofertilizer was kept refrigerated prior to use [8].

2. Methods

2.1. Experimental design and treatments

A pot experiment was conducted in two successive seasons 2020 and 2021 at wire green house of Plant Physiology Department, Cotton Research Institute, Agricultural Research Center, Giza, Egypt. This experiment was carried out to evaluate the effect of using AE (30%) on growth, yield, chemical composition and enzymes activities of cotton plants under different SWI concentration (0, 8000 and 12000 ppm), which seeds of cotton plants were soaked in AE at concentration of 30% beside the untreated seeds (soaking in tap water)for 6 hours.

The experimental design was split–plot with four replications. The main plots were devoted to the irrigation by SWI concentration (0, 8000, and 12000 ppm), while AE applications were randomly occupied the subplots as follow: control (C), azolla seed soaking (ASS) before sown (ASS), azolla foliar application (AFA) at flower stage and azolla combination application (ACA) of ASS+AFA treatments. Seeds of cultivar Giza 95 were sown on 24th of April in the first season and on the 18th April in the second season and plants were thinned to two plants per pot (40-cm in diameter containing 16 kg of soil). The standard agricultural practices were followed throughout the two growing seasons, which each pot before sowing received 2.2 g calcium superphosphate (15.5% P_2O_5) and 1.1 g potassium sulfate (48% K_2O). After 30 days planting each pot received 2.0 g urea (46% N). All pots were irrigated by tap water until 45 days (squaring stage). Then irrigation started with SWI concentrations (0 ppm, 8000 ppm and 12000 ppm) followed by tap water alternately during the whole season, which control treatments (0ppm) irrigated by tap water only. The soil analysis was conducted according to [12]. The soil chemical properties of the experimental soil are presented in Table (1) during two seasons.

Table 1: Chemical properties of experimental soil

	2020	2021		2020	2021
pH	7.84	7.95	Soluble anions (meq/l)		
E.C. (dsm^{-1})	1.50	1.58	CO_3^{2-}	--	--
Available minerals (mg/Kg soil)			HCO_3^-	3.54	3.67
N	43.93	44.24	Cl^-	5.29	5.62
P	9.20	9.53	SO_4^{2-}	6.32	6.84
K	477.5	485.2	Soluble cations (meq/l)		
Cu	8.12	8.46	Ca^{2+}	5.93	6.22
Fe	34.42	35.31	Mg^{2+}	2.98	3.18
Mn	8.91	9.04	Na^+	5.95	6.17
Zn	11.34	11.52	K^+	0.36	0.39

2.2. Growth characters

Plant samples (whole plant) were taken after 10 days from sprayed with AE 30% at flowering stage (75 days from sowing) during the experimental period. In this stage, 4 plants were taken from each treatment (2 pots). The growth characters of plants were recorded for this experiment as follows: plant height (cm), number of fruiting branches/plant, plant dry weight (g), leaf area (cm^2) which is determined by leaf area meter Model L1 – 3100. In addition to, the growth parameter of root shoot ratio were calculated on the basis of formulae described by [13]. Relative water content was determined according to the method of [14].

2.3. Chemical analysis

Cotton samples of 4th upper leaf/plant were taken randomly after 10 days from sprayed with AE 30% at flowering stage to determine the chemical analysis as follows:

– **Total chlorophyll and carotenoids contents**

Total chlorophyll (mg/g, FW) estimated by the spectrophotometric method recommended by [15] and carotenoids of [16]. Leaf samples (0.3g from each replicate were homogenized in 50ml 80% (v/v) acetone and centrifuged at 10,000 × g for 10min. The absorbance of each acetone extract was measured at 665, 649, and 440nm using a UV-visible spectrophotometer.

– **Total soluble sugars content**

Total soluble sugars were determined in ethanol extract of leaves by the phenol-sulfuric acid method according to [17]. A stranded curve was prepared using different concentration (10 to 100mg/ml) of pure glucose.

– **Total free amino acids content**

Total free amino acids were determined in ethanol extract of cotton leaves by ninhydrin method according to [18].

– **Total phenols content**

Total phenols were determined in ethanol of leaves using Folin-Ciocalteau method according to [19]. One milliliter of sample was mixed with 1ml of Folin and Ciocalten's phenol reagent, after 3min, 1ml of saturated Na_2CO_3 (14%) was added to the mixture and completed to 10ml by adding distilled water. The reaction was kept in the dark for 90min, after which its absorbance was read at 725nm. A calibration curve was constructed with different concentrations of gallic acid (0.01–1mM) as standard.

– **Free proline content**

It was determined using the method [20] described by [21] as a physiological indicator of plant status under SWI stress treatments. The results are expressed (free proline content) as µmoles of proline/g of fresh weight.

– **Total soluble proteins content**

Total soluble proteins were estimated according to the method of Lowry- Folin as described by [22]. The results are expressed (total soluble protein) as mg/g of fresh weight.

– **Total antioxidant capacity**

Total antioxidant capacity was determined in ethanol extract of cotton leaves using the phosphomolybdenum method of [23] as described by [24]. The results are expressed as the increase in absorbance ($O.D_{695}$).

– **Crude enzyme extract**

Crude enzyme extract was prepared for assay of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and glutathione reductase (GR) activities according to [25], as described by [26].

– **Catalase activity**

The activity of CAT was measured according to the method of [27]. Differences in the absorbance at 240 nm were measured every 30 sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance (U/mg protein).

– **Peroxidase activity**

The activity of POD was assayed according to the method of [28]. Differences in absorbance at 470 nm were measured every 30 sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance (U/mg protein).

– **Superoxide dismutase and Glutathione reductase activity**

The activity of SOD was assayed according to the method of [29] and the activity of GR was assayed according to the method of [30].

3.4. Yield and its components

At harvest stage, samples from four pots were taken. Yield and its components, including, number of open bolls/plant, boll weight (g), lint percentage, seed index (g) and seed cotton yield/plant.

3.5. Fiber quality

Samples of lint cotton under different treatments were tested at the laboratories of the Cotton Technology Research Division, Cotton Research Institute in Giza to determine fiber properties, under controlled conditions of 65% ± 2 of relative humidity and 21° ± 2 C ° temperatures. Fiber length, fiber strength and micronaire reading were determined on digital Fibrograph instrument 630, Pressley instrument and micronaire instrument 675 respectively, according to [31] at the C.R.I. laboratories. Analysis of variance of the obtained data of each season was performed.

3.6. Statistical Analysis

The measured variables were analyzed by ANOVA using M Stat-C statistical package [32]. Mean comparisons were done using least significant differences (L.S.D) method at 5% level ($P \leq 0.05$) of probability to compare differences between the means [33].

3. Results and discussion

3.1. Growth characters

Results in Table (2) illustrated that SWI and AE applications had significant effect in growth characters, while the interaction between them did not significantly affect growth characters of cotton plant in the two growing seasons.

Increasing SWI concentration (from 0 ppm to 8000 or 12000 ppm) reduced significantly in growth characters (plant height, number of fruiting branches/plant, plant dry weight, root shoot ratio, leave area and relative water content %) of plants. Cotton plants treated with SWI at concentration of 0 ppm recorded the maximum mean of plant height (54.22 and 58.67 cm), no. of fruiting branches/plant (6.67 and 6.55), plant dry weight (16.11 and 17.14 g), leave area (316.70 and 309.05 cm^2) and relative water content (62.25 and 59.95 %) in both seasons, respectively. Whereas, cotton plants treated with SWI at concentration of 12000 ppm obtained the lowest means of plant height (34.35 and 38.72 cm), no. of fruiting branches/plant (3.74 and 3.15), plant dry weight (6.95 and 8.43 g), leave area (165.82 and 158.80 cm^2) and relative water content (44.75 and 45.10 %) in both seasons, respectively. Generally, increasing SWI concentration gradually decreased all growth characters of whole treatments. The decline in dry weight in response to increase salinity may be attributed to a combination of osmotic and specific ion effects of Cl^- and Na^+. The reduction in seedling growth under saline conditions may either be due to decrease in the availability for water or increase in sodium chloride toxicity, associated with increasing salinity [6]. Also, [4] reported that the reduction in dry weight of cotton tissues reach to 60% under salt stress conditions. High salinity levels led to reducing leaf area due to turgor pressure resulting from salt stress which can cause inhibition of cell division and expansion [1].

As for the effect of AE applications, the data stated that the AE applications (ASS, AFA and ACA) were significantly increased growth characters of plant height, number of fruiting branches/plant, plant dry weight, leave area and relative water content %, while root shoot ratio was not affected in both seasons.

Table 2 Effect of salinity water concentrations, azolla extract treatments and the interaction between them on growth characters of cotton during 2020 and 2021 seasons

Treatments		**Plant height (cm)**		**No. of fruiting branches/ plant**		**Plant dry Weight (g)**		**Root shoot ratio (g/g)**		**Leave area (cm^2)**		**Relative water content %**	
Salinity water concentration (A)	**Azolla extract application (B)**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**	**2020**	**2021**
0 ppm	**Control**	50.00	54.50	5.80	6.00	14.25	15.95	0.836	0.897	277.22	284.32	59.06	55.65
	Soaking	53.70	56.20	6.60	6.30	15.62	16.70	0.814	0.899	306.82	300.98	61.43	58.99
	Spraying	55.00	61.40	7.00	6.80	16.87	17.74	0.831	0.909	333.06	322.23	63.78	62.08
	Soaking+ Spraying	58.20	62.60	7.30	7.10	17.72	18.19	0.827	0.938	349.89	328.55	64.97	63.31
Mean		**54.22**	**58.67**	**6.67**	**6.55**	**16.11**	**17.14**	**0.827**	**0.911**	**316.70**	**309.05**	**62.25**	**59.95**
8000 ppm	**Control**	39.10	43.40	3.80	4.00	9.09	11.33	0.881	0.830	218.68	214.25	49.62	48.04
	Soaking	42.50	46.50	4.40	4.60	10.16	12.00	0.864	0.880	231.46	226.88	52.00	51.05
	Spraying	45.20	49.30	4.70	4.90	10.94	12.69	0.950	0.876	240.88	240.10	55.59	54.73
	Soaking+ Spraying	47.00	51.10	5.00	5.30	11.35	13.23	0.951	0.869	252.99	242.39	57.16	58.21
Mean		**43.45**	**47.57**	**4.47**	**4.70**	**10.38**	**12.31**	**0.911**	**0.863**	**235.92**	**230.85**	**53.50**	**52.97**
12000 ppm	**Control**	31.30	35.70	2.80	2.50	5.68	7.32	0.956	0.908	132.55	141.30	40.93	40.09
	Soaking	33.70	37.50	3.40	3.00	6.93	8.09	0.937	0.961	170.23	156.81	43.20	44.33
	Spraying	34.90	39.80	3.70	3.30	7.36	8.86	0.918	0.942	178.31	163.70	46.82	47.13
	Soaking+ Spraying	37.50	41.90	4.00	3.80	7.84	9.48	0.902	0.936	182.34	173.47	48.14	49.01
Mean		**34.35**	**38.72**	**3.47**	**3.15**	**6.95**	**8.43**	**0.928**	**0.936**	**165.82**	**158.80**	**44.75**	**45.10**
General mean of azolla extract application (B)	**Control**	40.13	44.53	4.13	4.16	9.67	11.53	0.891	0.878	209.43	213.26	49.83	47.86
	Soaking	43.30	46.73	4.80	4.63	10.90	12.26	0.872	0.913	236.13	228.16	52.20	51.40
	Spraying	45.03	50.16	5.13	5.00	11.72	13.09	0.900	0.909	250.70	242.10	55.27	54.60
	Soaking+ Spraying	47.56	51.86	5.43	5.40	12.30	13.63	0.893	0.914	261.66	248.06	56.70	56.83
LSD at 0.05 of	**A**	1.19	0.67	0.13	0.19	0.62	0.71	0.017	N.S	13.96	7.06	0.78	1.34
	B	0.87	0.86	0.21	0.13	0.58	0.34	N.S	N.S	9.26	5.31	0.86	0.94
	A x B	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	16.04	N.S	N.S	N.S

ACA treatments recorded an increase in plant height by 18.51 and 16.46 %, in number of fruiting branches/plant by 31.47 and 29.80 %, in plant dry weight by 27.19 and 18.21 %, in leave area by 24.93 and 16.31 % and in relative water content by 13.78 and 18.74 % in both seasons, respectively, as compare with untreated cotton plants, that obtained the lowest values in both seasons. These results in line with [8] who found that that ASS in lower concentrations of the AE showed high germination rate (20%), while higher concentrations inhibited the germination (50%). This behavior may be due to the presence of some natural growth promoting hormones, trace elements, vitamins and amino acid in lower concentrations that used to improve the germination percentage, root shoot length, fresh, dry weight and growth of plant. As well as, [11] mentioned that the positive effect of azolla on cotton plant growth due to increase in nutrients availability to plan, and also its function as photo-stimulators that improve plant growth through contents of phytohormones. Also, [10] revealed that azolla biofertilizer greatly improved fruit weight and numbers.

The interaction between SWI and AE treatments did not reach to the level of significance 0.5% concerning the all growth characters; except leave area which affected significantly in season 2020. ACA treatments gave the best results as compared to the other interactions under different SWI concentrations.

3.2. Chemical constituents of cotton leaves

Data presented in Table (3) revealed that AE and SWI applications and their interaction had significantly affected on chemical constituents (total chlorophyll, carotenoid, total soluble sugars, total phenols, total free amino acids, free proline and total soluble proteins) of cotton leaves.

Cotton plants treated with SWI at concentration of 0 ppm gave the highest content of total chlorophyll and carotenoid (7.77 and 1.123 mg/g, FW, respectively), while cotton plants treated with SWI at concentration of 12000 ppm gave the lowest content of total chlorophyll and carotenoid (4.52 and 0.664 mg/g, FW, respectively. Significant reduce were found in total chlorophyll and carotenoid with increasing SWI concentration (i.e. increasing salinity level from 0 ppm to 8000 or 12000 ppm). On the other hand, the cotton leaves content of total soluble sugars, total phenols, total free amino acids, free proline and total soluble proteins raised by increasing SWI concentration. The lowest concentration of SWI (0 ppm) produced the minimum contents of total soluble sugars, total phenols, total free amino acids, total soluble proteins and free proline (22.79, 19.61, 14.83, 11.18 mg/g, FW and 4.77 µmol/g, FW, respectively). Conversely, the highest concentration of SWI (12000 ppm) recorded the maximum contents of total soluble sugars, total phenols, total free amino acids, total soluble proteins and free proline (31.85, 26.24, 20.41, 25.63 mg/g, FW and 31.20 µmol/g, FW, respectively). The harmful effect of salinity stress on chlorophyll biosynthesis may be due to the formation of proteolytic enzymes like chlorophyllase, which it led to chlorophyll damage. Also, salinity decrease of ALA (5-aminolinolic acid) synthesis, which it is a precursor of protochlorophyllide that led to limit of chlorophyll synthesis [1 and 6]. On the other hand, the significantly accumulation of chemical constituents contents of total soluble sugars, total phenols, total free amino acids, free proline and total soluble proteins in cotton plants exposed to SWI may be related to their roles as cellular osmoprotectant that stabilizes cellular membranes, maintains turgor, buffer cellular redox potential and free radical scavengers. The same observation was obtained by [1 and 34] who found that salinity induced disturbances of the metabolic process leading to increase organic molecules such as soluble sugars and total phenols may act as osmoprotect of plants under salinity stress.

In regard, Applications of AE (ASS, AFA and ACA) remarkably increased the chemical constituents of total chlorophyll, carotenoids, total soluble sugars, total phenols, total free amino acids, free proline and total soluble proteins contents of cotton leaves as compare to untreated plants. The application of ACA recorded an increase in total chlorophyll content by 36.98 %, in carotenoids by 32.34 %, in total soluble sugars by 16.44 %, in total phenols by 21.28 %, in total free amino acids by 18.41 %, in free proline by 20.62 % and in total soluble proteins by 25.29 %, respectively, compared to untreated plants that recorded the lowest one. The increasing in all chemical constituent might be related to AE enhanced the net CO_2 assimilation rate, so that it increased pigments contents, carbohydrate biosynthesis, total phenols and total amino acids contents in cotton leaves. The obtained results also supported by the suggestion of [7] reported that all treatments of azolla led to CO_2 evolution, dehydrogenase and nitrogenase activity that increase the soil fertility, improved plant quality, that positively turn on protein content and yield production. Also, [11] noted that the increase in cotton pigments caused by azolla may be related to its biostimulates contents which effects on pigments formation. Likewise, [10] concluded that all biochemical parameters significantly increased by using biofertilizer AE (20%) as compared to control plants under stress conditions.

Table 3 Effect of salinity water concentrations, azolla extract treatments and the interaction between them on total chlorophyll, carotenoid, total soluble sugars, total phenols, total free amino acids, free proline and total soluble proteins contents in leaves of cotton plant.

Treatments		Total Chl (mg/g FW)	Carotenoids (mg/g FW)	Total soluble sugars (mg/g FW)	Total phenols (mg/g FW)	Total free amino acids (mg/g FW)	Free proline (μmol/g FW)	Total soluble proteins (mg/g FW)
Salinity water concentration (A)	Azolla extract application (B)							
0 ppm	Control	6.93	0.885	21.34	17.49	13.43	3.62	9.84
	Soaking	7.60	1.175	22.78	19.85	14.26	4.98	11.03
	Spraying	8.12	1.191	23.25	19.93	15.69	4.82	11.72
	Soaking + Spraying	8.45	1.242	23.82	21.18	15.95	5.69	12.14
Mean		7.77	1.123	22.79	19.61	14.83	4.77	11.18
8000 ppm	Control	4.93	0.654	24.04	20.51	16.12	16.75	16.42
	Soaking	5.11	0.778	26.86	21.92	17.65	18.63	17.91
	Spraying	5.53	0.795	27.46	22.43	17.94	19.24	18.59
	Soaking + Spraying	6.75	0.854	28.85	23.86	18.73	19.83	20.27
Mean		5.58	0.770	26.80	22.18	17.61	18.61	18.28
12000 ppm	Control	3.48	0.585	29.25	22.75	18.05	28.52	22.15
	Soaking	4.32	0.675	30.84	26.62	20.75	30.76	25.43
	Spraying	4.48	0.682	33.12	26.95	21.18	32.08	26.68
	Soaking + Spraying	5.80	0.715	34.21	28.65	21.66	33.45	28.28
Mean		4.52	0.664	31.85	26.24	20.41	31.20	25.63
General mean of azolla extract application (B)	Control	5.11	0.708	24.87	20.25	15.86	16.29	16.13
	Soaking	5.67	0.876	26.82	22.79	17.55	18.12	18.12
	Spraying	6.04	0.889	27.94	23.10	18.27	18.71	18.99
	Soaking + Spraying	7.00	0.937	28.96	24.56	18.78	19.65	20.21
LSD at 0.05 of	A	0.139	0.042	0.078	0.075	0.125	0.058	0.110
	B	0.205	0.054	0.099	0.083	0.098	0.120	0.160
	A x B	0.355	0.095	0.172	0.144	0.170	0.209	0.277

The results of analysis of variance revealed that the all chemical constituents were statistically affected by the interaction between AE and SWI treatments. The highest values for all chemical constituents were obtained by ACA treatments under different SWI concentrations, whereas the untreated plants gave the lowest values under different SWI concentrations.

The data in Table (4) showed the effect of SWI and AE treatments and their interaction on enzymes activities and total antioxidant capacity.

The finding illustrated that increasing SWI concentration increased significantly total antioxidant capacity and activity of CAT, POX, SOD and GR enzymes. The treatment of SWI at concentration of 12000 ppm gave the highest values of total antioxidant capacity and activity of CAT, POX, SOD and GR enzymes (1.724 O.D$_{695nm}$, 1.504, 1.385, 1.305 and 1.287 U/mg, protein, respectively). On the other hand, Plants treated with SWI at concentration of 0 ppm obtained the lowest values of values of total antioxidant capacity and activity of CAT, POX, SOD and GR enzymes (0.892 O.D$_{695nm}$, 0.359, 0.861, 0.385 and 0.308 U/mg, protein, respectively). That might due to salinity conditions enhanced production of ROS, so that antioxidant enzymes actives are increasing under salinity conditions to scavengers ROS assalinity tolerance mechanism in plant [6].

Concerning the major effect of AE treatments, the results mentioned that the AE treatments significantly increased total antioxidant capacity and enzyme activities of CAT, POX, SOD and GR in cotton plants as compared with untreated plants. Treatments of ACA increased total antioxidant capacity about 21.74 %, CAT activity about 79.31 %, POD activity about 35.30 %, SOD activity about 97.90 % and GR activity about 114.43 %, respectively, as compare to untreated plants that recorded the minimum values. AE applications caused significant and positive effect on enzymes activities that may be related to its important role in maximize the effect of valuable antioxidant properties by providing plant with its valuable contents of vitamins, beta carotene, growth promoters and minerals which in turns help plant fruits to have many advantages. The same observation was obtained by [10].

Table 4 Effect of salinity water concentrations, azolla extract treatments and the interaction between them on CAT, POX, SOD and GR activities and total antioxidant capacity in leaves of cotton plant.

Treatments		Total antioxidant capacity ($O.D_{695\ nm}$)	Catalase activity (U/mg protein)	Peroxidase activity (U/mg protein)	Superoxide dismutase (U/mg protein)	Glutathione reductase (U/mg protein)
Salinity water concentration (A)	Azolla extract Application (B)					
0 ppm	Control	0.794	0.241	0.804	0.306	0.207
	Soaking	0.829	0.342	0.835	0.362	0.308
	Spraying	0.915	0.405	0.897	0.425	0.325
	Soaking + Spraying	1.030	0.446	0.908	0.447	0.391
Mean		0.892	0.359	0.861	0.385	0.308
8000 ppm	Control	1.240	0.604	0.936	0.480	0.504
	Soaking	1.450	0.701	0.972	0.526	0.582
	Spraying	1.513	0.783	1.180	0.644	0.606
	Soaking + Spraying	1.552	0.909	1.244	0.739	0.658
Mean		1.439	0.749	1.083	0.597	0.588
12000 ppm	Control	1.581	0.982	1.046	0.787	0.703
	Soaking	1.706	1.350	1.293	1.109	0.884
	Spraying	1.788	1.764	1.582	1.400	1.580
	Soaking + Spraying	1.819	1.921	1.620	1.924	1.982
Mean		1.724	1.504	1.385	1.305	1.287
General mean of azolla extract application (B)	Control	1.205	0.609	0.929	0.524	0.471
	Soaking	1.328	0.798	1.033	0.666	0.591
	Spraying	1.405	0.984	1.220	0.823	0.837
	Soaking + Spraying	1.467	1.092	1.257	1.037	1.010
LSD at 0.05 of	A	0.008	0.019	0.012	0.012	0.003
	B	0.011	0.018	0.008	0.013	0.006
	A x B	0.019	0.031	0.015	0.021	0.010

The interaction between SWI and AE applications affected significantly on total antioxidant capacity and enzymes activities of CAT, POX, SOD and GR in cotton leaves. In general, the best enzymes activities recorded for cotton plants treated by ACA under different SWI concentrations, whereas the lowest values for those enzymes activities exhibited for untreated cotton plants under different SWI concentrations.

3.3. Yield, yield components and fiber properties

The data in Table (5) represented that yield and yield components affected by SWI and ZE treatments and their interaction.

With regard the effect of SWI treatments, the finding clearly stated that increasing SWI concentration had significantly negative effect yield (seed cotton yield/plant) and yield components (number of open bolls/plant, boll weight, seed index), while it was significantly increased lint % in both seasons of cotton plant. The maximum means of seed cotton yield/plant (24.42 and 27.60 g/plant), number of open bolls/plant (13.60 and 15.12), boll weight (1.79 and 1.81 g) and seed index (9.93 and 10.07 g) for both seasons, respectively, were observed when cotton plants treated with SWI at concentration of 0 ppm. On the other hand, the minimum means of seed cotton yield/plant (6.37 and 7.15 g/plant), number of open bolls/plant (4.55 and 4.92), boll weight (1.39 and 1.44 g) and seed index (7.94 and 8.07 g) for both seasons, respectively, were recorded when cotton plants treated with SWI at concentration of 12000 ppm. The reduction in seed cotton yield might be due to the reduction in its components, number of open bolls/plant, boll weight and seed index [2]. In general, SWI especially at higher concentration exerts harmful effects on plant metabolites required to form more sound bolls with higher seeds. Also, salinity decreased the yield characters especially number of open bolls/plant [1 and 6].

Considering AE applications, the data demonstrated that, AE applications significantly increased effect yield (seed cotton yield/plant) and its components (number of open bolls/plant, boll weight, seed index), whereas lint % did not affected in both seasons of cotton plant. Cotton plants treated with ACA treatments gave the highest values of seed cotton yield/plant about 39.09 and 27.45 %, number of open bolls/plant about 24.74 and 25.66 %, boll weight about 11.40 and 10.52 % and seed index about 5.74 and 4.80 % in both seasons, respectively, compared to untreated plants that yielded the lowest one. That might attributed to the positive effect of AE applications especially ACA treatment, which using

AE due to increased significantly yield and its components under salt stress condition [8]. Also, AE treatments significantly increased the plant contents of nutrients and had a positive effect on growth and yield productivity, which it increased oxidation behavior and activity of antioxidant enzymes in plants under salt conditions [10]. Moreover, [11] found that azolla treatments increased cotton yield and its components as compared to control plants.

As for interaction between two studied applications, the data revealed that number of open bolls/plant and boll weight increased significantly in the two seasons, also seed index and seed cotton yield/plant increased significantly in the season 2021. However, lint % did not affect by the interaction between SWI and AE applications in both seasons. The highest values of these parameters were indicated with ACA treatments under different SWI concentrations, whereas the control plants exhibited the lowest one. These results in line with those found by [8] and [10]

Data in Table (6) showed that SWI and AE treatments and the interaction between them insignificantly effected on fiber properties (fiber length, micronaire reading and fiber strength) in both seasons that may be due to less effect by environmental factors.

4. Conclusion

AE applications on cotton plant decreased the negative effect of SWI conditions. ACA treatment recorded the best results of cotton plant productivity under different SWI levels by improving plants tolerance, increasing osmolality constitutions, enzymes activities and plant productivity. Also, AE acted as biofertilizer, which it is good nitrogen fixing, low cost and reduces the environmental pollution from the wide using of chemical fertilizers. However, azolla needs to more study for reaching the most effective treatments on cotton productivity thus to have the condition led to recommendation.

Table 5 Effect of salinity water concentrations, azolla extract treatments and the interaction between them on yield and its components of cotton during 2020 and 2021 seasons

Treatments		No. of open bolls/plant		Boll weight (g)		Seed index (g)		Lint %		Seed cotton yield/plant (g)	
Salinity water concentration (A)	Azolla extract application (B)	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
0 ppm	Control	12.83	13.41	1.64	1.68	9.63	9.46	37.32	37.47	21.04	22.52
	Soaking	13.75	14.72	1.77	1.75	9.88	9.50	37.58	37.70	24.34	25.76
	Spraying	13.96	15.53	1.84	1.84	9.96	9.74	37.76	37.81	25.69	28.57
	Soaking+ Spraying	14.00	16.95	1.92	1.90	10.26	10.13	37.84	37.96	26.88	32.25
Mean		13.60	15.12	1.79	1.81	9.93	10.07	37.62	37.73	24.42	27.60
8000 ppm	Control	7.36	9.42	1.49	1.49	8.74	9.13	38.13	38.37	10.96	14.03
	Soaking	8.92	10.06	1.52	1.56	8.83	9.20	38.25	38.54	13.55	15.69
	Spraying	9.68	10.88	1.59	1.60	8.91	9.29	38.47	38.96	15.39	17.41
	Soaking+ Spraying	10.04	11.34	1.62	1.64	9.24	9.41	38.80	39.03	16.26	18.59
Mean		8.95	10.38	1.55	1.58	8.92	9.25	38.41	38.72	13.97	16.45
12000 ppm	Control	3.32	4.21	1.35	1.31	7.75	7.92	39.16	39.49	4.48	5.51
	Soaking	4.84	4.87	1.38	1.38	7.89	8.00	39.34	39.77	6.68	6.72
	Spraying	4.97	5.09	1.41	1.48	8.03	8.12	39.92	40.29	7.01	7.53
	Soaking+ Spraying	5.21	5.76	1.45	1.50	8.12	8.27	40.22	40.57	7.55	8.64
Mean		4.55	4.92	1.39	1.44	7.94	8.07	39.66	40.03	6.37	7.15
General mean of azolla extract application (B)	Control	7.80	9.00	1.49	1.52	8.70	8.94	38.20	38.44	12.10	14.13
	Soaking	9.14	9.83	1.55	1.60	8.86	9.03	38.39	38.67	14.80	16.37
	Spraying	9.46	10.43	1.61	1.65	8.95	9.19	38.71	39.02	15.96	18.01
	Soaking+ Spraying	9.73	11.31	1.66	1.68	9.20	9.37	38.95	39.18	16.83	19.76
LSD at 0.05 of	A	0.37	0.49	0.01	0.04	0.14	0.07	0.51	1.18	0.90	0.69
	B	0.21	0.32	0.03	0.02	0.07	0.07	N.S	N.S	0.71	0.72
	A x B	0.37	0.55	0.05	0.04	N.S	0.13	N.S	N.S	N.S	1.24

Table 6 Effect of salinity water concentrations, azolla extract treatments and the interaction between them on fiber properties of cotton during 2020 and 2021 seasons

Treatments		Fiber length (mm)		Micronaire reading		Fiber strength	
Salinity water concentration (A)	Azolla extract application (B)	2020	2021	2020	2021	2020	2021
0 ppm	Control	30.80	30.90	4.50	4.60	9.60	9.60
	Soaking	31.50	31.70	4.50	4.70	9.70	9.80
	Spraying	31.80	31.90	4.60	4.70	9.80	9.80
	Soaking + Spraying	32.00	32.20	4.60	4.70	9.90	9.90
Mean		31.52	31.67	4.55	4.67	9.75	9.77
8000 ppm	Control	30.40	30.60	4.40	4.50	9.40	9.50
	Soaking	30.80	30.90	4.50	4.60	9.50	9.60
	Spraying	31.00	31.10	4.50	4.60	9.50	9.60
	Soaking + Spraying	31.20	31.30	4.60	4.70	9.70	9.70
Mean		30.85	30.97	4.50	4.60	9.52	9.60
12000 ppm	Control	30.10	30.40	4.40	4.50	9.20	9.30
	Soaking	30.30	30.60	4.40	4.50	9.40	9.40
	Spraying	30.80	30.90	4.50	4.60	9.40	9.60
	Soaking + Spraying	31.00	31.20	4.50	4.60	9.50	9.60
Mean		30.55	30.77	4.45	4.55	9.37	4.47
General mean of azolla extract application (B)	Control	30.43	30.63	4.43	4.53	9.40	9.46
	Soaking	30.86	31.06	4.46	4.60	9.53	9.60
	Spraying	31.20	31.30	4.53	4.63	9.56	9.66
	Soaking + Spraying	31.40	31.56	4.56	4.66	9.70	9.73
LSD at 0.05 of	A	N.S	N.S	N.S	N.S	N.S	N.S
	B	N.S	N.S	N.S	N.S	N.S	N.S
	A x B	N.S	N.S	N.S	N.S	N.S	N.S

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