Egypt. J. Bot. **Vol. 61**, No. 1, pp. 271-282 (2021)

Egyptian Journal of Botany
http://ejbo.journals.ekb.eg/

The Effect of Ascorbate Salts on Mushroom (*Pleurotus ostreatus*) Grown under Salinity Stress

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THIS STUDY aimed to investigate the impact of the mixture of two ascorbate salts (1:2, K-ascorbate: Ca-ascorbate, w/w) on the growth, biochemical and physiological activities and nutritive value of *Pleurotus ostreatus* fruiting bodies grown under salt stress. Sets of rice straw inoculated with *P. ostreatus* spawns were sprayed with NaCl (50mM or 100mM) and/or ascorbate mixture solution. Salinity stress reduced the fungal growth (fresh and dry weights), water content %, polysaccharides, total carbohydrate, protein, individual and total fatty acids content. On the other hand, salt stress had a negative impact on *P. ostreatus* accumulated defence molecules such as soluble sugars, total amino acids, antioxidant activity and total phenolics. Interestingly, the application of ascorbate salt mixture significantly improved the growth and activity of mushroom under stressed and nonstressed conditions. It enhanced the metabolism of carbohydrate, protein and lipid, activated mineral uptake and improved antioxidant defense system of stressed *P. ostreatus*. Together, applying ascorbate salt mixture is an environmentally friendly approach to mitigate the impact of salinity stress on *P. ostreatus*.

Keywords: Ascorbate salt mixture, Flavor amino acids, Mushroom, Nutritive value, Primary metabolism, Salinity stress.

Introduction

Saline water is recently applied as an alternative source of fresh water in agriculture (Cominelli et al., 2009; Aeschbach-Hertig & Gleeson, 2012). However, salinity can retard the growth through, for example, inhibition of cell biological activity and/or altering of cell membrane integrity (Chokshi et al., 2017). Muthangya et al. (2013) cultivated mushroom on salinized waste and they found that salinity inhibited its mycelial vegetative growth. Similar results were reported by Boumaaza et al. (2015), where they found that salinity reduced spore germination and growth of mushroom. In addition to the reduction in mushroom growth, salinity also reduces the absorption of some minerals, mainly calcium ions (Ramesh et al., 2004) and accretion the level of reactive oxygen species (ROS). Consequently, ROS affect lipids, proteins and nucleic acids metabolism (Rahdari et al., 2012; Naudts et al., 2014; Sinha et al., 2015; Casasole et al., 2017; Versieren et al., 2017; Zinta et al., 2018) through alteration of β-oxidation of fatty acids and activity of the mitochondrial electron transport chain (Hamed & Abd Elgawad, 2017). Thus, scope of research worldwide should focus on developing efficient and ecofriendly approach to mitigate the impact of salinity stress.

Mushroom does not compete with crops on agricultural soil and it is easily produced (Isokauppila, 2017). It is widely used as a foodstuff because it is rich in proteins, carbohydrates, vitamins and minerals and low in fat content (Boda et al., 2012; Singh, 2017). It is also a medicinal fungus because mushroom extracts moderate many physiological reactions of human metabolism that keep the state of good health and make the body capable of decreasing disease risk (Wani et al., 2010). Several reports indicted

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Received 11/10/ 2020; Accepted 6/12/ 2020
DOI: 10.21608/ejbo.2020.45896.1565
Edited by: Prof. Dr.: Hamada Abdelgawad, Department of Biology, University of Antwerp, Belgium.
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various biological activities such as antifungal, antibacterial, antioxidant, antiviral activities, antitumor, immunomodulating, cardiovascular, hepatoprotective, hypocholesterolemic, antiparasitic and antidiabetic activities (Wani et al., 2010; Singh, 2017). These various biological activities of mushroom are explained by its constituents like glucans (Du et al., 2019), mannitol (Zhou et al., 2016), trehalose (Barros et al., 2007), antioxidant (Boda et al., 2012).

Ascorbate is an antioxidant, and it is widely applied as an alleviating agent for salinity stress (Cai et al., 2016). For example, Georgiou et al. (2003) studied the impact of ascorbic acid on the sclerotial differentiation of *Sclerotium rolfsii* grown under oxidative stress, and authors proved that exogenous reduced lipid damage (lipid peroxidation) and the degree of sclerotial differentiation in the stressed strain. Moreover, they attributed such impact due to sinking its lipid peroxidation before differentiation and this may cause sclerotial strain to reduce oxidative stress. Ascorbate was also used to alleviate Cd, Pb, Zn, Ni, and Cu stress impact on *Paecilomyces marquandii* growth (Słaba et al., 2013). In more details, this study indicated that ascorbic acid (1mM) endangered fungal growth and banned the alteration in the fatty acid composition and saturation but failed to ease lipid peroxidation. Another mechanism is the aptitude impact of ascorbate to decrease the stress-induced levels of ROS and the additionally induced anti-oxidant defense systems (Horemans et al., 2000; Al-Hakimi & Hamada, 2011). Ascorbate also increases the activity of catalase, peroxidase and superoxide dismutase enzymes which are minimizing enzymes responsible for controlling the oxidative damage effects of salinity (Munir & Aftab, 2011).

In addition to the positive impact of ascorbate, some essential minerals also showed stimulating impact on fungal growth and wall biosynthesis. Kaluzewicz et al. (2014) and Ahmed et al. (2016) pointed to the beneficial role of Ca for fungal growth. Also potassium has a crucial role in fungal growth and increased the nutritive value of mushroom (Roy et al., 2015).

In a previous study, Hamed & AbdElgawad (2017) investigated the positive impact of ascorbate salt mixture 1:2 (K-ascorbate: Ca-ascorbate, w/w) on the growth and anabolic reactions, enzyme activity (especially antioxidant enzymes) and mineral uptake of kidney bean. They found that ascorbate salt mixture enhanced the growth of kidney bean, activated its antioxidant enzymatic system, improved the metabolism of carbohydrate and protein, and upgraded the photosynthetic activity.

The aim of this work was to explore the possibility of using this ascorbate salt mixture to improve the growth of *Pleurotus ostreatus* mushroom under normal and salt stress conditions.

Materials and Methods

Mushroom strain

The spawns (the mycelia which propagated vegetatively on sorghum grains) of the mushroom *Pleurotus ostreatus* (NRRL 2366) were obtained from the Mushroom Production Department, Agriculture Research Center (ARC), Giza, Egypt.

Experimental setup

The mixture of ascorbate salt was prepared by mixing 0.5mM potassium ascorbate with 1.0mM calcium ascorbate to reach 1:2 ratio (Hamed & AbdElgawad, 2017).

Three replicates of six sets of rice straw were prepared. Each set is represented by a plastic tray (36X25X14cm) filled with 1k of sterilized rice straw, and inoculated with 2% mushroom spawns. The trays were daily sprayed to get moisture content of 80% as suitable humidity for mushroom fruiting (Ahmed et al., 2016). The first set was sprayed with distilled water. The second set was sprayed with 50mM NaCl. The third set was sprayed with 100mM NaCl. The fourth set was sprayed with ascorbate salt mixture. The fifth set was sprayed with ascorbate salt mixture containing 50mM NaCl. Finally, the sixth set was sprayed with ascorbate salt mixture containing 100mM NaCl. Each set was covered with a transparent thick plastic sheath in humid conditions. After 30 days, the mycelia appeared and the plastic sheath was removed. Then, the mycelia were regularly sprayed one time daily by the specific solution for the set. At the end of the experiment (45 days), the fruiting bodies were collected and used for different analyses and estimations.

Measurements and estimations

Growth criteria

The collected fruiting bodies were weighted to calculate its fresh weight (F Wt) in grams. To calculate the dry weight, the collected bodies were dried at 70°C till constant weight (D Wt), then water content percentage (WC %) was calculated as:

WC%= [(F Wt -D Wt)/F Wt]X100

Ashing was done by the ignition of fruiting bodied at 550°C by using Muffle furnace Model No STM-6-12 (Afify et al., 2017).

Sugar profile

Total soluble sugars, insoluble polysaccharides and total carbohydrate fractions were estimated by the method published by Naguib (1964). For the estimation of glucose, galactose, mannitol and trehalose, samples (100mg) were extracted in 50mL of water at 100°C for 2hrs. by high-performance anion chromatography using NaOH as a mobile phase according to the protocol of Zhou et al. (2012). While, the β-glucan content was determined according to the method of Perez- Vendrell et al. (1995) where the samples were hydrolyzed by lichenase then the yield was measured by reversed phase HPLC using water as a mobile phase.

Amino acid profile

Total amino acids (TAA) were estimated by reaction with ninhydrin reagent (Lee & Takahashi, 1966) by reaction with 1% ninhydrin in 0.5M citrate buffer (pH 5.5). Individual amino acids were analyzed by using the RP-HPLC according to the protocol described by Sun et al. (2017). The mobile phase was A: 40mM NaH_2PO_4 (pH 7.8) and B: CAN: MeOH: H_2O (45:45:10). An FLD detector was set at 340nm excitation, 450nm emission, with PTM gain 10, and changed the signal to 266nm excitation, 305nm emission, with PTM gain 9 at 15.1min..

Fatty acid profile

Fatty acids were saponified, esterified and finally estimated as total and individually by the GC-HPLC (Farag et al., 1978). Plant lipids were saponified by alcoholic sodium hydroxide, and then extracted with petroleum ether. The aqueous layer (containing the potassium salt of fatty acid) was acidified and extracted by petroleum ether then methylated with diazomethane. These methyl esters were analyzed with a Pye Unicam gas chromatograph Model 104 equipped with a dual flame ionization detector and a dual (FID) channel recorder.

The amino acids and fatty acids analyses were done in labs of the National Organization for Drug Control and Research (NODCAR), Giza, Egypt.

Protein and lipid

Crude protein was measured by using the method of Lowry et al. (1951). Determination was carried out in the samples which were extracted in 85% aqueous ethanol. NaOH and sodium potassium tartrate mixture and Folin reagent were applied for its measuring.

Total lipids were extracted by using chloroform-methanol mixture (1:1 v/v) then measured by using sulphuric acid and phosphovanillin reagent (Burnes & Blackstock, 1973).

Antioxidant activity

The antioxidant activity was assessed by different protocols. Firstly, by measuring [0.004% methanol extraction of 1, 1- diphenyl-2-picryl-hydrazyl (DPPH radical scavenging activity)] using the method of Braca et al. (2001) where catechin was used as the standard free radical scavenger. Secondly, by measuring hydroxyl radical scavenging by applying thiobarbituric acid reactive species (TBARS) as the degrading agent as described by Halliwell et al. (1987). In the third protocol, superoxide anion radical scavenging activity was estimated by xanthine/ xanthine oxidase system as described by Jung et al. (2006).

Total phenols

Total phenols were measured by Ferreira et al. (2007) method in which gallic acid was used as a standard. The reaction mixture was 10mL containing 1mL of the methanolic extracts, 1mL of Folin Ciocalteu's phenol reagent, 1mL of saturated sodium carbonate and 7mL distilled water. Measurement was carried out spectrophotometrically at 725nm.

Mineral analysis

Minerals were estimated according to the protocol published by Watson & Isaac (1990). The ash was dissolved in 2NHCl then injected

to be estimated by Perkin Elmer 3030 Atomic absorption at Occupational Health Department, National Institute of Occupational Safety and Health (NIOSH), Cairo, Egypt.

Statistical analysis

Data were analyzed by one-way ANOVA, using SPSS 19.1 statistical software (SPSS Inc., Chicago, IL, USA), and significant differences between the means were determined by using the Duncan test ($P< 0.05$) ($n= 3$).

Results

Growth analysis

The impact of salinity induced a reduction in both fresh and dry weights. This inhibition was more distinct in fresh weight (59.5% and 83.6% for 50 and 100mM, respectively), more than in dry weight (14.5% and 44.8% for 50 and 100mM, respectively) (Table 1). This inhibition in the fungal growth was also indicated in the reductions of its ash. Overall, this growth inhibition was dosage-dependent and the reduction of water content percent is coincident with this attribution. On the other hand, the application of ascorbate mixture improved this negative effect. The mixture increased fungal water content from 80.3% to 85% and from 68.7% to 73%, at 50 and 100mM, respectively.

Sugar profile

The salinity stress significantly affected the carbohydrate metabolism of mushroom cells (Table 2). This effect varied according to the carbohydrate type, where there was an increase in the total soluble sugars, especially glucose, galactose, mannitol and trehalose, while there was a decrease in the polysaccharides (especially β-glucan) and total carbohydrates levels. Such accumulation is a deceitful increment because there is no corresponding increment in polysaccharide or in total carbohydrate. On the other hand, the application of ascorbate salt mixture improved the metabolism of carbohydrates in both normal and stressed conditions.

Amino acid profile

At amino acid level, salinity stress positively increased free amino acids, particularly proline. In this work, the pattern of the response of all amino acids, except proline, was similar, and the increment was concentration-dependent (Table 3). Interestingly, essential amino acids (EAA) (Val, Met, Phe, Ile, Leu, Lys, Thr, and Trp) significantly increased. Moreover, some of measured amino acids are known as flavor amino acids and they are responsible for the specific taste of mushroom (FAA; Glu and Asp), while other amino acids are responsible for the sweet taste of mushroom (sweet amino acids i.e., SAA; Ser, Gly, Ala and Pro). In this investigation both FAA and SAA were increased due to the application of salinity stress. The antioxidant impact of amino acids varied and differed from one amino acid to another. In this study, the contents of hydrophobic amino acids (HAA; Ala, Pro, Cys, Val, Met, Phe, Ile and Leu) with high antioxidant activity (Zhuang et al., 2009) were increased. Contrary, the increment of amino acids was not accompanied by more protein biosynthesis. Application of ascorbate mixture activated the anabolism of all investigated amino acid and their assimilation to protein. This result can be integrated with the improvement of the growth criteria, under normal and stressed conditions.

TABLE 1. Effect of ascorbate mixture on fresh weight (F Wt), dry weight (D Wt), water content percentage (WC) % and ash content of *Pleurotus ostreatus* grown under salinity stress

Spraying Solution	F Wt	D Wt	WC %	Ash
Water	206.844±4.61^{c}	19.275±0.24^{c}	90.667±0.33^{e}	10.216±0.13^{d}
50mM NaCl	83.874±1.63^{ab}	16.484±0.05^{b}	80.333±0.34^{c}	7.737±0.03^{b}
100mM NaCl	33.951±0.28^{a}	10.637±0.07^{a}	68.667±0.31^{a}	4.637±0.04^{a}
Ascorbate mixture	351.730±51.45^{d}	22.477±0.21^{d}	91.333±0.33^{e}	11.913±0.11^{e}
50mM NaCl + ascorbate mixture	128.461±4.75^{b}	19.214±0.06^{c}	85.000±0.57^{d}	10.184±0.03^{d}
100mM NaCl + ascorbate mixture	60.719±1.01^{ab}	16.386±0.25^{b}	73.000±0.58^{b}	8.685±0.13^{c}

- Weights are expressed as g/kg substrate dry weight.
- Data are mean of three replicates.
- Means followed by different letter in the same column are significantly different at 0.05% level of significance according to Duncan's method.

TABLE 2. Effect of ascorbate mixture on carbohydrate content (mg/ g F Wt) of *Pleurotus ostreatus* grown under salinity stress

Spraying solution	Glucose	Galactose	Mannitol	Trehalose	TSS	β-glucan	Polysaccharides	Total carbohydrate
Water	75.79±0.96^{a}	10.52± 0.13^{a}	31.55±0.68^{a}	14.50±0.23^{a}	205.45±0.83^{ab}	37.55±0.47^{e}	367.68±2.18^{e}	573.13±4.83^{e}
50mM NaCl	86.48±0.12^{c}	12.34±0.02^{c}	36.00±0.04^{a}	16.55±0.35^{b}	217.97±0.57^{ab}	20.56±0.01^{b}	203.45±0.88^{b}	421.42±0.33^{b}
100mM NaCl	115.30±0.52^{f}	16.64±0.21^{f}	48.24±0.07^{a}	23.25±0.15^{d}	252.24±0.66^{b}	18.19±0.06^{a}	104.21±0.33^{a}	356.4±1.20^{a}
ascorbate mixture	90.03±0.51^{d}	13.64±0.18^{e}	37.69±0.30^{a}	17.80±0.19^{c}	229.19±0.88^{ab}	49.67±0.31	419.41±0.66^{f}	648.60±1.33^{d}
50mM NaCl + ascorbate mixture	79.26±0.16^{b}	11.31±0.02^{b}	33.36±0.29^{a}	15.18±0.30^{a}	189.18±0.33^{a}	23.65±0.10^{c}	262.32±0.88^{d}	451.50±0.88^{b}
100mM NaCl + ascorbate mixture	95.45±0.41^{e}	13.06±0.037^{d}	38.22±0.01^{a}	18.15±0.06^{c}	237.42±0.88^{ab}	28.56±0.18^{d}	209.82±0.33^{c}	447.24±0.58^{b}

- Data are mean of three replicates.
- Means followed by different letter in the same column are significantly different at 0.05% level of significance according to Duncan's method.

Fatty acid profile

The impact of salinity on fatty acids and lipid metabolism (Table 4) showed an inhibition. Such inhibition is coincident with the retardation that occurred in both carbohydrate and protein biosynthesis. The values of fatty acids composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), between the untreated and ascorbate treated mushrooms were varied. Generally, the decrease in levels of individual fatty acids and total lipids depends on NaCl concentration. Ascorbate mixture activated lipid metabolism of both stressed and nonstressed samples.

Biological activity

Free radical-scavenging potency and phenolics content were represented in Table 5. This potency was measured as DPPH radical scavenging, hydroxyl radical scavenging and superoxide anion radical scavenging. Results showed that salinity stimulated the activity of this potency system and caused accumulation of phenolics constituent. Ascorbate salt mixture treatment increased the antioxidant activity and phenolics concentration of unstressed mushroom fruiting bodies, and invigorated them in stressed bodies.

Mineral content

The impact of salinity on mushroom is tabulated (Table 6). Estimation of minerals content showed a variation in their concentration among macronutrient level (K, P, S and Mg) and micronutrients level (Cu, Mn, Na Fe, Cl, Ca and Zn) in descending order. Salinity application retarded the uptake of all investigated minerals except both of Na and Cl, which were increased, and this effect was concentration dependent. On the other hand, ascorbate mixture enhanced all mineral uptakes in unstressed fungi and improved their uptake in stressed fungi.

Discussion

Inhibition of mushroom growth due to salinity can be explained by the limitation of water and nutrient uptake for cell growth beside the toxicity due to the accumulation of Na (Kaymakanova, 2012). Mitigating this negative effect of salinity due to ascorbate mixture application can be attributed to the osmotic activity of accumulated

K for adjustment of cell osmosis (Amtmann & Rubio, 2012). Moreover, accumulation of the soluble carbohydrates is a known physiological mechanism to conquer the retardation occurred in cell-free water potential (Hamed & AbdElgawad, 2017), especially those molecules which are classified as osmoprotectants such as glucose, galactose, trehalose and mannitol (Zhou et al., 2016). Exogenous ascorbate application enhanced the yield of glucose, xylulose and gluconic acid (Smirnoff, 2018). The presence of both Ca and K ions also accelerates the sugar related enzymatic reactions like carbohydrate synthesizing enzymes, while, under stress conditions, the antioxidant activity of ascorbate alleviated the stress effect which means more energy was directed to the anabolic activities (Hamed & AbdElgawad, 2017). On the other hand, salinity induced inhibition occurred in the polysaccharides and total carbohydrates may be due to an inactivation of their specific synthesizing enzymes and/or to a general anabolic inhibition (Al-Tamie, 2014). Thus, the medical and nutritional benefits were reduced due to the reduction in glucan and total carbohydrates in response to stress.

TABLE 3. Effect of ascorbate mixture on total and individual amino acids (µg/ g F Wt) and protein content (mg/ g F Wt) of *Pleurotus ostreatus* grown under salinity stress

Amino acids	Spraying solution					
	Water	50mM NaCl	100mM NaCl	Ascorbate mixture	50mM NaCl + ascorbate mixture	100mM NaCl + ascorbate mixture
Asp	112.60±0.32^{a}	132.40±0.35^{b}	152.49±0.09^{c}	117.62±0.49^{d}	127.51±0.46^{b}	149.43±0.16^{b}
Glu	118.80±0.33^{a}	140.49±0.50^{ac}	163.49±0.80^{c}	120.65±0.51^{d}	136.28±0.36^{a}	152.76±0.17^{a}
Ser	145.44±0.41^{a}	177.38±0.39^{b}	192.65±0.11^{d}	161.35±0.19^{c}	167.89±0.18^{b}	184.53±0.21^{b}
Gly	138.44±0.39^{a}	168.48±0.42^{b}	199.92±0.10^{c}	150.13±0.60^{d}	157.45±0.55^{ab}	174.68±0.19^{ab}
Ala	405.52±1.14^{a}	587.40±0.38^{bc}	629.70±0.32^{cd}	447.78±1.76^{d}	493.78±1.64^{ab}	531.85±0.30^{ab}
Pro	84.11±0.23^{a}	242.23±0.52^{bc}	261.56±0.13^{bc}	92.98±0.36^{c}	204.80±0.68^{a}	222.83±0.23^{ab}
Cys	346.70±0.96^{a}	395.70±1.06^{b}	418.46±0.26^{bc}	380.55±0.41^{c}	374.57±0.82^{b}	401.71±0.47^{b}
Val	119.48±0.33^{a}	155.46±0.36^{bc}	179.31±0.09^{cd}	131.96±0.51^{d}	143.40±0.48^{ab}	175.64±0.16^{b}
Met	12.32±0.03^{a}	14.45±0.03^{ab}	16.69±0.008^{b}	13.71±0.05^{c}	13.42±0.06^{ab}	15.18±0.01^{ab}
Phe	26.30±0.27^{a}	36.35±0.08^{bc}	38.76±0.02^{c}	28.52±0.11^{c}	35.78±0.10^{ab}	36.39±0.32^{bc}
Ilu	30.07±0.08^{a}	39.58±0.09^{bc}	43.38±0.02^{cd}	33.38±0.13^{d}	37.59±0.12^{ab}	40.83±0.04^{bc}
Leu	17.67±0.99^{a}	25.56±0.05^{bc}	27.64±0.01^{cd}	19.36±0.07^{d}	21.60±0.34^{ab}	23.56±0.02^{ab}
Lys	41.66±0.22^{a}	61.47±0.12^{bc}	65.66±0.03^{cd}	45.54±0.24^{d}	53.40±0.21^{ab}	59.50±0.05^{ab}
Thr	76.56±0.21^{a}	122.30±0.23^{bc}	132.49±0.05^{cd}	84.83±0.07^{d}	116.74±0.31^{ab}	128.31±0.10^{bc}
Trp	28.04±0.07^{a}	40.36±0.08^{bc}	43.44±0.02^{cd}	30.64±0.21^{d}	34.35±0.23^{ab}	37.32±0.29^{ab}
Asn	98.25±0.27^{a}	136.47±0.30^{bc}	146.16±0.07^{cd}	108.51±0.42^{d}	122.38±0.39^{ab}	129.80±0.13^{b}
Gln	201.58±0.56^{a}	247.73±0.62^{bc}	264.28±0.15^{cd}	222.56±0.87^{d}	235.87±0.81^{ab}	245.67±0.27^{bc}
Arg	109.34±0.30^{a}	119.76±0.33^{ab}	128.20±0.08^{bc}	121.98±0.47^{c}	113.78±0.44^{bc}	123.69±0.14^{ab}
His	66.40±0.18^{a}	85.45±0.20^{bc}	99.91±0.05^{cd}	73.17±0.28^{d}	80.62±0.26^{ab}	87.73±0.09^{b}
Tyr	134.45±0.37^{a}	193.19±0.41^{bc}	208.76±0.10^{cd}	148.66±0.58^{d}	163.29±0.54^{ab}	177.50±0.18^{ab}
TAA	2338.8±6.51^{a}	3448.5±7.41^{bc}	3720.7±1.85^{cd}	2556.9±10.04^{d}	2913.2±9.67^{ab}	3155.3±3.31^{ab}
EAA	352.09±1.42^{a}	495.52±1.09^{bc}	547.34±0.27^{cd}	387.95±0.65^{d}	456.28±0.94^{ab}	516.72±0.40^{b}
FAA	231.47±0.23^{a}	272.82±0.71^{a}	315.97±0.17^{b}	238.27±1.00^{c}	263.39±0.93^{a}	302.18±0.32^{a}
SAA	773.50±0.56^{a}	1172.38±2.65^{bc}	1283.84±0.66^{cd}	852.24±3.36^{d}	1024.99±3.46^{ab}	1113.89±1.18^{ab}
HAA	1042.16±2.93^{a}	1493.36±3.47^{bc}	1615.50±0.86^{cd}	1148.24±4.52^{d}	1324.93±4.54^{ab}	1447.99±1.55^{b}
Protein	149.93±0.32^{cd}	116.75±0.42^{bc}	88.81±0.45^{ab}	171.66±0.26^{a}	137.68±0.14^{d}	111.67±0.43^{cd}

- Data are mean of three replicates.
- Means followed by different letter in the same column are significantly different at 0.05% level of significance according to Duncan's method.

TABLE 4. Effect of ascorbate mixture on total fatty acids content, lipids (mg/ g F Wt) and individual acids (µg/ g F Wt) of *Pleurotus ostreatus* grown under salinity stress

Spraying solution	Water	50mM NaCl	100mM NaCl	Ascorbate mixture	50mM NaCl + ascorbate mixture	100mM NaCl + ascorbate mixture
Lauric acid. C12	0.131±0.005^{c}	0.117±0.004^{bc}	0.091±0.003^{a}	0.180±0.007^{d}	0.121±0.003^{c}	0.104±0.004^{ab}
Trydelic acid. C13	0.157±0.002^{e}	0.138±0.001^{c}	0.110±0.002^{a}	0.216±0.003^{f}	0.147±0.002^{d}	0.124±0.002^{b}
Myrestic acid. C14	0.171±0.003^{e}	0.148±0.003^{c}	0.116±0.002^{a}	0.237±0.005^{f}	0.159±0.003^{d}	0.132±0.003^{b}
Palmitic acid. C16	2.347±0.035^{e}	2.089±0.031^{c}	1.549±0.023^{a}	3.216±0.048^{f}	2.218±0.033^{d}	1.819±0.027^{b}
Palmic acid. C16-1	0.266±0.008^{d}	0.234±0.006^{c}	0.178±0.005^{a}	0.370±0.01^{e}	0.250±0.007^{cd}	0.206±0.005^{b}
Margaric acid. C17	0.609±0.01^{e}	0.524±0.009^{c}	0.390±0.007^{a}	0.834±0.014^{f}	0.566±0.01^{d}	0.457±0.008^{b}
Stearic acid. C18	1.142±0.02^{e}	1.016±0.017^{c}	0.742±0.013^{a}	1.599±0.027^{f}	1.079±0.018^{d}	0.879±0.015^{b}
Oleic acid. C18-1	1.298±0.026^{e}	1.143±0.023^{c}	0.818±0.017^{a}	1.779±0.035^{f}	1.220±0.024^{d}	0.980±0.02^{b}
Linoleic acid. C18-2	3.387±0.06^{e}	2.913±0.05^{c}	2.168±0.04^{a}	4.572±0.08^{f}	3.150±0.05^{d}	2.540±0.04^{b}
SFA:TFA	0. 216±0.001^{a}	0.225±0.001^{c}	0.233±0.001^{e}	0.218±0.001^{ab}	0.220±0.001^{b}	0.229±0.001^{d}
MSFA:TFA	0.058±0.00	0.060±0.00	0.06±0.00	0.058±0.00	0.059±0.00	0.06±0.00
PSFA:TFA	0.152±0.001^{b}	0.154±0.001^{c}	0.159±0.0003^{e}	0.150±0.001^{a}	0.153±0.001^{bc}	0.156±0.0003^{d}
Total fatty acids	22.302±0.44^{e}	18.957±0.38^{c}	13.60±0.27^{a}	30.554±0.61^{f}	20.629±0.41^{d}	16.281±0.32^{b}
Total lipids	34.308±0.68^{e}	29.383±0.42^{c}	19.555±0.39^{a}	48.255±0.86^{f}	31.845±0.55^{d}	24.469±0.40^{b}

- Data are mean of three replicates.
- Means followed by different letter in the same column are significantly different at 0.05% level of significance according to Duncan's method.

TABLE 5. Effect of ascorbate mixture on antioxidant activity [DPPH radical scavenging (as % of catechin inhibition), hydroxyl radical scavenging (as inhibition % of deoxyribose oxidation) and superoxide anion radical scavenging (as inhibition % of nitroblue tetrazolium)] and total phenols (mg/ 100 g F Wt) of *Pleurotus ostreatus* grown under salinity stress

Spraying solution	DPPH radical scavenging	Hydroxyl radical scavenging	Superoxide anion radical scavenging	Total phenols
Water	14.83±0.29^{a}	7.68±0.29^{a}	10.27±0.06^{a}	3.46±0.14^{a}
50mM NaCl	27.13±0.30^{b}	14.66±0.28^{b}	17.90±0.31^{b}	8.63±0.28^{c}
100mM NaCl	61.44±0.48^{e}	33.11±0.29^{e}	37.32±0.09^{e}	13.50±0.19^{e}
Ascorbate mixture	34.34±0.29^{c}	19.01±0.06^{c}	21.37±0.08^{c}	6.12±0.05^{b}
50mM NaCl + ascorbate mixture	51.70±0.19^{d}	26.27±0.01^{d}	30.62±0.29^{d}	10.70±0.22^{d}
100mM NaCl + ascorbate mixture	79.21±0.13^{f}	38.68±0.29^{f}	41.34±0.09^{f}	16.52±0.16^{f}

- Data are mean of three replicates.
- Means followed by different letter in the same column are significantly different at 0.05% level of significance according to Duncan's method.

TABLE 6. Effect of ascorbate mixture on mineral constituent (as mg/100g D Wt.) of *Pleurotus ostreatus* grown under salinity stress

Spraying solution	K	Na	Ca	Mg	P	S	Cl	Cu	Fe	Mn	Zn
Water	339.3±0.85e	7.9±0.01a	3.8±0.07e	6.2±0.02e	204.6±0.84e	136.3±0.56e	6.2±0.05a	22.7±0.09c	6.5±0.02e	20.0±0.08e	0.5±0.001c
50mM NaCl	278.8±0.68c	15.1±0.02e	2.9±0.09e	5.1±0.02e	168.3±0.50e	112.2±0.33e	11.8±0.07d	18.7±0.05c	4.9±0.03c	16.5±0.04c	0.4±0.003e
100mM NaCl	226.7±3.98a	24.3±0.02f	1.9±0.05a	4.2±0.07a	136.7±2.50a	91.1±1.66a	20.5±0.04f	15.2±0.27a	3.3±0.03a	13.4±0.24a	0.3±0.003a
Ascorbate mixture	546.8±0.68f	8.5±0.02b	5.6±0.21f	7.0±0.01f	230.7±0.26f	153.7±0.17f	6.9±0.02b	25.6±0.02f	9.6±0.04f	22.5±0.02f	0.8±0.004f
50mM NaCl + ascorbate mixture	323.7±0.17d	10.3±0.007c	3.4±0.09d	5.9±0.003d	195.5±0.11d	130.3±0.07d	9.5±0.14c	21.7±0.01d	5.7±0.01d	19.1±0.01d	0.5±0.001d
100mM NaCl + ascorbate mixture	268.5±1.85b	13.3±0.01d	2.4±0.07b	4.8±0.007b	160.8±0.24b	107.2±0.16b	12.5±0.05e	17.9±0.02b	4.0±0.03b	15.7±0.02b	0.3±0.002b

- Data are mean of three replicates.
- Means followed by different letter in the same column are significantly different at 0.05% level of significance according to Duncan's method.

Generally, the increment of essential amino acids EAA may increase the nutritive and medicinal values of this treated fungus (Sun et al., 2017). Increase of flavor amino acids (FAA) and sweet amino acids (SAA) under salinity stress may acquire mushroom more sweetly taste. Generally, hydrophobic amino acids (HAA) have imperative antioxidant impacts (Zhuang et al., 2009), thus their accumulation is a mechanism by which mushroom overcomes stress impact. Contrary, the increment of amino acids was not accompanied by more protein biosynthesis. The decrease of protein concentration due to salinity application may be attributed to an inactivation in its synthesizing enzymes (Kersten et al., 2000), which reduce finally the fungal growth (Ayodele & Ojoghoro, 2007). The enhancing impact of ascorbate salt mixture on amino acid metabolism, especially tyrosine, phenyl alanine and tryptophan (Smirnoff, 2018) can explain the improvement of growth and nutritive value of treated mushroom. Similarly, the variation in the values of fatty acids composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), between the untreated and ascorbate treated mushrooms indicated that the variation in their nutritive values was congruent with their individual response, which means that the more nutritive values the more medicinal application (Barros et al., 2007). The activator impact of ascorbate mixture on both stressed and nonstressed samples may be attributed to its antioxidant activity and/or to its direct contribution to the lipid metabolism (Pehlivan, 2017). The coincident inhibition of fatty acids and lipid metabolism with carbohydrate and protein biosynthesis may be through the general retardation that occurred in the overall catabolism inhibition, specifically in acetyl coenzyme A link (Sidorov & Tsydendambaev, 2014). The positive impact of ascorbate mixture may be due to the activation in the uptake process from the growth media to supply different activated metabolic reactions with their requirements. Also, the activation in the biosynthetic enzymes may need more concentrations of minerals necessary for their reactions (Hamed & AbdElgawad, 2017).

Increment of free radical-scavenging potency can be ascribed as a mechanism to overcome the negative effects of salinity stress. Such increment is closely correlated to phenolic constituents of mushroom samples that attack ROS by their free protons which react with H_2O_2, O_2^-, OH and

OH* (Hassan et al., 2017). The positive response of mushroom to ascorbate treatment may be attributed to that ascorbate acts as total phenols and as an antioxidant, besides it acts as a signaling molecule involved in the regulation of oxidative stress (Hamed & AbdElgawad, 2017; Hassan et al., 2017). Simultaneously, the antioxidant activity of ascorbate was significantly succeeded to ameliorate the bad impact of salinity stress. Such amelioration effect may be attributed to the fact that ascorbate protects cells by reducing ROS damage to essential proteins and/or nucleic acids as well as it enhances the biosynthesis of carbohydrates, which can participate in cell osmotic potential regulation (Hameed et al., 2016).

Conclusion

Generally, the salinity stress significantly inhibited the growth and activity of mushroom and reduced its nutritive value. The results in this study indicated that ascorbate salt mixture can apply as an efficient approach to improve the growth of mushroom under normal conditions as well as to mitigate salinity induced growth reduction and oxidative damage.

Finally, it can be recommended that ascorbate mixture (1:2, K-ascorbate: Ca-ascorbate, w/w) can be used to improve the growth and the nutritive value of mushroom under normal condition and to alleviate the negative effect of osmotic stress under salt stress conditions.

Ethical approval: Not applicable.

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تأثير بعض أملاح حمض الأسكوربيك على عيش الغراب المحارى النامي تحت الإجهاد الملحى

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تم رى فطرة عيش الغراب المحارى بمخلوط من مركبات الأسكوربات (بنسبة 1 أسكوربات البوتاسيوم : 2 أسكوربات الكالسيوم، وزن: وزن) والذى يحتوى على 50 مللى مول أو 100 مللى مول من كلوريد الصوديوم، وذلك لدراسة تأثير هذه المعاملات على نمو ونشاط فطرة عيش الغراب المحارى. وقد أظهرت النتائج أنَّ المعاملة قد أثرت سلباً على الوزن الطازج والجاف ونسبة المحتوى المائى والرماد، وكذلك كمية الجلوكان والمحتوى الكلى للكربوهيدرات والبروتين والدهون. وفى المقابل أدت الملوحة إلى زيادة كل من الجلوكوز، الجالاكتوز، التريهالوز والمانيتول، وكذلك الأحماض الأمينية والدهنية. بينما أظهرت العناصرالمعدنية المقدرة (K، Ca، Mg،P، S، Cu، Fe، Mn، Zn) نقصا بإستثناء الصوديوم والكلور الزائدين نتيجة وفرتهما فى بيئة النمو.

ومن الجدير بالذكر أنَّ تغير بعض خصائص الاجسام الثمرية نتيجة الملوحة ربما يؤدى إلى تحسن طعمها نظراً لزيادة الأحماض الأمينية المسببة للمذاق الحلو وكذلك المسئولة عن الطعم المميز لها. كما تجدر الإشارة إلى أنَّ زيادة هذه الأحماض الأمينية المسؤلة عن المذاق وكذلك بعض السكريات لم يصاحبها زيادة فى البروتينات والكربوهيدرات الكلية مما يعنى نقصان القيمة الغذائية بالرغم من تحسن المذاق. كما أدت الملوحة إلى إرتفاع النظـام الـدفاعى المضـاد للأكسدة لمواجهة هذا الإجهاد الملحى.

وفى المقابل أدت المعاملة بمخلوط الأسكوربات إلى تحسن نمو الاجسام الثمرية لفطرة عيش الغراب فى نموها بعيداً عن الملوحة، وكذلك قللت المعالجة بمخلوط الأسكوربات الأثر السلبى الناجم عن الملوحة بفروق إحصائية معنوية. ومما سبق يمكن التوصية باستخدام مخلوط الأسكوربات كعامل مخفف للإجهاد الملحى فى مدى التركيزات الملحية المستخدمة.