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MORPHO-PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF H_2S – POLLUTED LETTUCE (*Lactuca Sativa* L.) PLANTS TO ASCORBIC ACID

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ABSTRACT: Pot experiments were conducted in a greenhouse with the aim to study the role of antioxidant ascorbic acid under two levels (0 and 100 ppm) in minimizing and/or overcoming the phytotoxic effects of H_2S (0, 50 and 100 ppm) on growth characters, some physiological and biochemical aspects; and yield quantity and quality parameters of lettuce plants. Vegetative growth parameters of H_2S-polluted lettuce plants were significantly reduced with increasing the H_2S level. The phytotoxic effect of H_2S at the high level was more pronounced and greater in head length (-31%), stem length (-34%), leaf area (-42%), fresh whole plant (-35%) and dry whole plants (-34%) matters of lettuce plant than other growth characters. Treating the H_2S- polluted lettuce plants with ascorbic acid not only counteracted the harmful effect on the above-mentioned parameters, but also induced a greatly increase in them, head length (40%), stem length (104%), leaf area (82%), fresh whole plant (148%) and dry whole plant (96%) matters compared with the untreated plants. Leaf water deficit (LWD), osmotic pressure and membrane integrity (MI) were increased, whereas total water content (TWC), relative water content (RWC), transpiration rate, and the administration of H_2S reduced the succulence of the leaves. Using ascorbic acid led to regulate and improve the plant water status by increasing RWC (66%) and osmotic pressure (16%), and decreasing LWD (40%), and MI (28%). All H_2S levels had a deleterious effect on leaf photosynthetic pigments; chlorophyll a, b and total chlorophylls, carotenoids, total photosynthesis as well as chlorophyll stability index (CSI). Application of ascorbic acid overcame the inhibitory effect of H_2S and enhanced the biosynthesis and concentration of photosynthetic pigments; chl. a (170%), chl. b (303%), total chlorophyll (186%) as well as carotenoids (64%). Exposure of lettuce plants to H_2S pollutant significantly reduced the head yield, more at 100 ppm (-50%) than that at 50 ppm (-33%). The values of chemical components, i.e., vitamin C, total protein, total soluble sugars (SS), total amino acids and N, P, K minerals contents in head lettuce leaves significantly decreased with increasing the H_2S level. While the Sulphur (S) contents, soluble phenols, NO_3-N and NO_2-N in lettuce leaves were increased as H_2S rates increased. Adding ascorbic acid to the H_2S-polluted lettuce plants not only led to overcome the deleterious effect of intolerable H_2S levels (50 and 100 ppm) on most above-mentioned characters, but also increased the yield (150 and 169%) and improved the qualitative characters of head lettuce leaves.

Key words: H_2S pollutant, Ascorbic Acid, lettuce plant, vegetative growth, plant water relations, photosynthetic pigments, yield.

INTRODUCTION

The concentration of gaseous and particle pollutants increased further as a result of ongoing increases in the human population, road traffic, automobile traffic, and industries (Joshi *et al.*, 2009). One of the major air contaminants in the world is hydrogen sulfide, or H_2S. Conventionally thought to be a poisonous gas, hydrogen sulfide is identified as a gas transmitter. Generally, studies on lettuce, rocket and/or radish at low, moderate, and high H_2S levels (0.25, 0.3, 15, 25, 50, and/or 100 ppm H_2S or 1 mM H_2S) have revealed detrimental impacts on growth, yield, and physiological parameters (Liu and Lal, 2015). Additionally, it was found that plants exposed to H_2S had decreased levels of photosynthetic pigments (carotenoids, chlorophyll a and b), photosynthetic efficiency, stomatal conductance, water relations, most N fractions, accumulation of sugars, and activity of

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peroxidase and carbohydrase (Seyyednejad *et al*., 2011). H_2S inhibits cytochrome C oxidase, which in turn stops the transfer of electrons within the cell's mitochondria. This prohibits both cellular respiration and the synthesis of ATP (Jiang *et al.*, 2016). High levels of H_2S can also cause oxidative cellular stress, which can be harmful to plants (Jiang et al., 2016). Furthermore, it was found that elevated levels of H_2S impede the growth of primary roots by generating reactive oxygen species (ROS) and disrupt the auxin distribution during seedling development (Zhang *et al.*, 2017).

Sulphur can hinder the uptake of phosphorus (Van Der Heide *et al.*, 2012), nitrogen (Koch *et al.*, 1990), iron (Lamers *et al.*, 2012), magnesium, and calcium (Lamers *et al.*, 2013), it can also enhance the uptake of sulphur and other sulphur-compounds in plants (Lamers *et al.*, 2013). Nevertheless, certain researchers have demonstrated that extremely low concentrations of H_2S help plants by increasing their resistance to heat, salinity, and copper stress (Dooley *et al.*, 2013; Li *et al.*, 2014). Furthermore, it has been demonstrated that very low concentrations of H_2S benefit a wide range of plant species, including soybeans and peas (Singh *et al.*, 2015; Zou *et al.*, 2019). H_2S has been demonstrated to produce oxidative stress by overproducing reactive oxygen species (ROS), which deteriorates macromolecules and reduces physiological and biochemical activities. Consequently, crop plants have reduced growth and productivity (Zhang *et al.*, 2017). Enzymes, antioxidants, and non-enzyme substances can all scavenge reactive oxygen species. Apart from its antioxidant properties, ascorbic acid plays a crucial function in the growth and development of plants, making it the most significant non-enzymatic antioxidant (Pavet *et al.*, 2005). Numerous biological processes, including cell division, differentiation, and senescence, are regulated by ascorbic acid (Venkatesh and Park, 2014). Ascorbic acid was found to protect lipids and proteins and plays a crucial role in protection against various abiotic stresses such as, salinity (Venkatesh *et al.*, 2012), drought (Hussein and Khursheed, 2014), low/high temperatures (Larkindale *et al.*, 2005) high light intensity (Talla *et al.*, 2011), heavy metals (Álvarez-Robles *et al.*, 2022), ozone (Feng *et al.*, 2010), and various abiotic stresses (Hemavathi *et al.*, 2011). Plant growth, transpiration, photosynthesis, oxidative defense capacity, photosynthetic pigments, and element nutrition uptake are all stimulated by ascorbic acid (Naz *et al.*, 2016; Al-Taweel *et al.*, 2022). However, the studies concerning the ability of ascorbic acid to minimize or alleviate the negative and harmful effects of H_2S on plants are scarce. Therefore, the aim of this study was to better understand how ascorbic acid helps lettuce plants tolerate H_2S toxicity by enhancing the physiology, growth, and yield quantity and quality of H_2S-polluted lettuce plants.

MATERIAL AND METHODS

Pot experiments were conducted at the Experimental Farm, Faculty Agriculture, Shibin El-Kom, Menoufia University, Egypt. Three bottom drainage holes were covered with sponge to impede drainage in 30-cm-diameter, 30-cm-deep polyethylene pots. Eight kg of clay loam soil (ECe = 2.6 dS.m-1, pH = 7.8, soluble salts = 0.17%) from the Experimental Farm of the Faculty of Agriculture, Shebin El-Kom was placed into each pot. The recommended fertilizers were applied to all pots: potassium sulphate (48% K_2O) at a rate of 0.81 g K_2O/pot; N in the form of ammonium nitrate (33% N) at a rate of 1.36 g N/pot; and calcium superphosphate (15.5% P_2O_5) at a rate of 1.6 g P_2O_5/pot prior to planting. These additions were made twice during the growth period. Pots were divided into two sets: 1st seeds soaked in tap water and 2nd seeds soaked in 100 ppm ascorbic acid, then each set divided into three groups: 1st irrigated with tap water, 2nd irrigated with 50 ppm H_2S and 3rd irrigated with 100 ppm H_2S. The plant was used in this study: lettuce (*Lactuca sativa* L. *cv* Balady). The seeds were soaked in ascorbic acid for 24 hours (0 and 100 ppm) then germinated in peatmoss media in a greenhouse. On November 20 and 22, 2021 and 2022, respectively the uniform seedlings of plants were transplanted in the aforementioned plastic pots under the same conditions. Each treatment was employed five times, and the pots were set up in a completely randomized block design. Pots were irrigated with aqueous Na_2S solutions containing 0, 50 and 100 ppm H_2S three times (after 25, 50 and 75 days from transplanting) during the growing seasons, then tap water was used for normal irrigation whenever to keep the moisture in soil at about 65% of the total water holding capacity of the soil during the experimental period. The aqueous Na_2S solutions containing 0, 50 and 100 ppm H_2S was prepared according to the methods of (Chen *et al.*, 2017). Following 115 days of transplanting, a thorough random sample of plants from each treatment was selected, and the following measurements were done:

Vegetative growth characteristics

Head length (cm), root length and diameter (cm), stem length and diameter (cm), leaves number per plant, leaf length and max. width (cm), fresh and dry weights of root, stem, and leaves (dried in an electric oven at 70˚C for 72 h) (g/plant), total leaf area (cm^2/plant) using the disc method of Bremner and Taha (1966), the shoot/root ratio. Relative growth rate (RGR) [RGR= (LN w_2 - LN w_1) / (t_2-t_1)], Net assimilation rate (NAR) [NAR = [(w_2 - $w_{1)}$ ($\log_e$ A_2- $\log_e$ $A_{1)}$] / [(A_2-$A_{1)}$ (t_2-t_1)] Where: w_1 and w_2 = Total dry weight of plant at t_1 and t_2, A_1 and A_2 = Leaf area of plant (cm^2) at t_1 and t_2, respectively Log_e = The normal logarithm (2.7185)], dry weight of whole plant (g). Relative growth rate (RGR, $mg.g^{-1}.week^{-1}$) and net assimilation rate (NAR, $g.cm^{-2}.week^{-1}$) during the period of 80-115 days were estimated according to Simane *et al.* (1993) and leaf area index was calculated.

Leaf water relations

The Kalapos (1994) formula was used to calculate the leaf water deficit (LWD, %), relative water content (RWC, %), and total water content (TWC, %). Using an Abbe Refractometer, the total soluble solids of the cell sap were measured for the squeezed sap of the middle fresh leaves of the plants under examination. Special tables were used to determine the osmotic pressure (OP) values (bar), using the methodology outlined by Gosev (1960). We calculated the transpiration rate (TR) by applying Kreeb's (1990) weight technique. integrity of the membrane (permeability, MI): Using the Leopold *et al.* (1981) approach, the absorption of solute leakage across tissue cell membranes was measured at an ultraviolet wavelength of 273 nm. In accordance with Putz (1996), the leaf succulence (LSc) was computed as follows: Fresh weight (fwt) / Dry weight (dwt) equals leaf succulence (LSc).

Photosynthetic pigments

Pigments were extracted from middle fresh leaves using acetone 80% and estimated using spectrophotometer as described by Von Wettstein (1957), then calculated as mg/g dry weight. Chlorophyll stability index (CSI, %) was estimated according to (Kaloyereas, 1958) as follows:

$$\text{CSI (\%)} = \frac{\text{Total chlorophyll content (treated)}}{\text{Total chlorophyll content (control)}} \times 100$$

Yield measurements

The measurements of yield of lettuce and its attributes were recorded as follows:

Head yield ($g.plant^{-1}$, $kg.m^{-2}$ and $ton.fed.^{-1}$).

The percentage of total soluble solids (T.S.S.) using a hand Abbe refractometer were estimated in fresh lettuce leaves according to the methods of A.O.A.C. (1995).

Vitamin C. (mg/100 g fwt.) was determined in the fresh leaves using the methods of A.O.A.C. (1995).

Chemical Analysis

For the ensuing chemical analysis, lettuce leaves were air dried, pulverized into a fine powder, and stored in tiny plastic bags.

Total N in leaves was estimated then total protein (%) was calculated according to the method of Sadasivam and Manickam (1992).

P, K and S were determined in lettuce leaves using the method described by Sadasivam and Manickam (1992).

Total soluble sugar, total amino acids and total phenols (mg/g Dwt.) was assessed in leaves using the techniques outlined by Sadasivam and Manickam (1992).

NO_3-N- and NO_2 -N in leaves were determined as follow:

For 16 hours, at room temperature and constant shaking at 300 rpm, 0.2 g of the powdered air-dried lettuce leaves from each treatment was homogenized in 20.0 ml of phosphate buffer solution (0.1M, pH 7.0). Following a 20-minute centrifugation at 5,000 rpm, the sample was filtered using Whatman filter paper and refrigerated at 4 °C. It was then utilized to determine the following chemical components:

- Nitrate nitrogen (ppm) was determined spectrophotometrically at 410 nm according to the procedure of Cataldo *et al.* (1975).
- Nitrite nitrogen (ppm) was determined spectrophotometrically at 520 nm according to the procedure adopted by Flamerz and Bashir (1981).

Statistical analysis

The data were analyzed using the version 9.2 of the SAS software (SAS, 2014) conducting the analysis of variance tests. Data of each season was analyzed separately. The Duncan's New Multiple Range Test was used to compare individual treatment mean differences at a 5% significance level.

RESULTS AND DISCUSSION

Growth characteristics

Tables 1 and 2 show the vegetative growth measurements of lettuce plants treated with ascorbic acid, different levels of H_2S and their interactions. The findings demonstrate that as the H_2S level rose, growth parameters dramatically ($P < 0.05$) dropped. At 50 ppm H_2S, the % of decrease in head length, root length and diameter, stem length and diameter, leaves number, leaf length and width, leaf area (cm^2/plant), LAI, RGR, NAR, fresh and dry weights of whole plant reached about 16, 13, 15, 24, 4, 27, 13, 8, 13, 8, 4, 5, 24, 12, 33 and 27%, respectively, whereas the shoot/root ratio increased by 18%, compared to the control plants. Meanwhile, at 100 ppm H_2S, the % of reduction was about 31, 18, 27, 32, 9, 37, 16, 19, 43, 32, 40, 30, 33 and 3%, respectively, compared to the untreated plants. It can be noticed that the deleterious effect of H_2S at 100 ppm on the growth of lettuce plants was more harmful than that at 50 ppm. Similar results were obtained in the 2nd season with similar reduction values. These findings align with the findings published by Liu and Lal (2015), who found negative effects on growth in lettuce at low, moderate and high H_2S levels (0.25, 0.3, 15, 25, 50 and/or 100 ppm H_2S or 1 mM H_2S). Furthermore, it was discovered that elevated levels of H_2S impede the growth of primary roots by generating reactive oxygen species and disrupt the auxin distribution during seedling development (Zhang *et al.*, 2017). The deleterious effect of H_2S on growth may be attributed to its inhibitory effect on auxins in plant tissues (Zhang *et al.*, 2017) and that inhibited cell division and cell elongation (Liu and Lal, 2015) and/or act as inhibitors of electron transport chain, inhibitors of enzymes, and other metabolism processes (Jiang *et al.*, 2016) which leads to a reduction in plant growth. Our data in Table 3; Liu and Lal (2015) suggest that the inhibition in fresh and dry matter may be caused by the toxic effect of H_2S on cell mass formation and an increase in water loss through transpiration. Alternatively, our results in Table 4 Seyyednejad *et al.* (2011) and/or (Zhang *et al.*, 2017) suggest that H_2S is a metabolic inhibitor and has an inhibitory effect on photosynthetic activity and rate. When compared to untreated plants, ascorbic acid administration boosted the development of both polluted and non-polluted plants with all H_2S levels (Tables 3 and 4). When compared to normal control plants, the percentage of increase in head length, root length, stem length, leaf area (cm^2/plant), RGR, NAR, fresh and dry weights of the whole plant at the high H_2S level was around 30, 20, 122, 115, 118, 493, 135 and 105%, respectively. Similar trends were observed in the 2nd season. This suggests that applying ascorbic acid to the H_2S-contaminated plants was a helpful technique for mitigating the negative effects of the H_2S and promoting plant growth. Accordingly, Venkatesh and Park (2014) found that plants treated with ascorbic acid or having a greater ascorbate content are better able to scavenge the excess ROS produced under stress circumstances, thereby increasing their tolerance to abiotic challenges. In addition to its role in enhancing the photosynthetic system, which raises the net assimilation rate (Yabuta *et al.*, 2007), ascorbic acid also promotes a variety of cellular activities, including differentiation, senescence, cell division, elongation, and expansion of the cell wall (Venkatesh and Park, 2014). This may have a positive effect on the growth of polluted plants.

Table (1): Effect of H_2S and Ascorbic acid (AsA) treatments on some vegetative growth characters of lettuce plants 115 days from transplanting during the growing seasons 2020/2021 and 2021/2022.

Characters / Treatments		Head length (cm)	Root		Stem		Leaves No per plant	Leaf		Leaf area (cm^2/plant)	LAI	RGR (mg/g/week)	NAR (g/m^2/week)
			length (cm)	Diameter (cm)	Length (cm)	diameter (cm)		Length (cm)	diameter (cm)				
AsA (ppm)	H_2S (ppm)	**Season 2020/2021**											
00	00	32.63^{ab}	13.40^{ab}	1.77^{a}	19.83^{b}	1.97^{a}	41.00^{c}	23.12^{ab}	8.24^{ab}	1109.60^{c}	2.65^{ab}	1.74^{ab}	0.25^{b}
	50	27.35^{bc}	11.70^{ab}	1.50^{a}	15.10^{c}	1.90^{a}	30.00^{d}	20.13^{b}	7.60^{ab}	1060.30^{d}	2.53^{ab}	1.32^{b}	0.2^{b}
	100	22.60^{c}	11.00b	1.30^{a}	13.40^{c}	1.80^{a}	26.00^{d}	19.42^{b}	6.64^{b}	636.04^{e}	1.52^{b}	1.19^{b}	0.15^{b}
100	00	33.85^{a}	14.35^{ab}	1.85^{a}	25.45^{a}	2.05^{a}	47.50^{b}	26.47^{a}	9.98^{ab}	1404.40^{ab}	3.35^{a}	2.59^{a}	0.86^{a}
	50	34.50^{a}	17.25^{a}	1.80^{a}	27.95^{a}	1.75^{a}	53.00^{a}	24.28^{ab}	9.10^{ab}	1427.80^{a}	3.41^{a}	2.58^{a}	0.93^{a}
	100	29.30^{ab}	13.25^{ab}	1.55^{a}	29.75^{a}	1.70^{a}	47.50^{b}	22.55^{ab}	9.05^{ab}	1367.10^{b}	3.27^{a}	2.59^{a}	0.89^{a}
Season 2021/2022													
00	00	32.63^{a}	13.73^{a}	1.77^{a}	19.83^{b}	1.97^{a}	46.00^{b}	21.12^{abc}	8.50^{ab}	1234.30^{b}	2.95^{c}	1.30^{b}	0.50^{c}
	50	25.05^{b}	12.75^{a}	1.35^{ab}	14.50^{c}	1.80^{ab}	37.50^{c}	19.04^{bc}	7.67^{ab}	921.79^{d}	2.20^{e}	1.27^{b}	0.29^{d}
	100	22.60^{b}	11.75^{a}	1.10^{b}	13.00^{c}	1.45^{b}	26.50^{d}	18.10^{c}	6.96^{b}	733.88^{e}	1.75^{f}	1.08^{b}	0.22^{d}
100	00	30.85^{a}	14.35^{a}	1.75^{a}	23.45^{a}	2.05^{a}	49.50^{b}	24.47^{a}	9.91^{a}	1387.10^{a}	3.62^{b}	2.97^{a}	1.22^{b}
	50	32.00^{a}	14.30^{a}	1.85^{a}	25.25^{a}	1.85^{ab}	59.50^{a}	23.21^{abc}	8.33^{ab}	1326.45^{a}	2.62^{d}	2.98^{a}	1.11^{b}
	100	34.00^{a}	12.75^{a}	1.65^{ab}	24.25^{a}	1.75^{ab}	59.00^{a}	24.19^{ab}	8.47^{ab}	1092.72^{c}	4.54^{a}	2.98a	1.55^{a}

Table (2): Effect of H_2S and Ascorbic acid (AsA) treatments on the fresh and dry weights as well as the shoot/ratio of lettuce plants 115 days from transplanting during the growing seasons 2020/2021 and 2021/2022.

Characters / Treatments		Fresh weight (g/plant)				Dry weight (g/plant)				Shoot/Root ratio
		Root	Stem	Leaves	Whole	Root	Stem	Leaves	Whole	
AsA (ppm)	**H_2S (ppm)**	**Season 2020/2021**								
00	00	24.50^{d}	42.70^{a}	35.10^{d}	102.30^{c}	3.24^{c}	5.51^{b}	8.01^{b}	16.76^{b}	2.47^{b}
	50	21.00^{e}	18.96^{d}	28.60^{e}	68.56^{d}	2.38^{e}	2.93^{c}	6.93^{b}	12.24^{c}	2.91^{ab}
	100	18.55^{f}	16.60^{e}	21.30^{f}	71.30^{d}	2.86^{d}	1.56^{d}	6.86^{b}	11.28^{c}	2.40^{b}
100	00	30.75^{b}	27.50^{c}	81.90^{c}	140.15^{b}	3.06^{cd}	2.48^{cd}	11.45^{a}	16.99^{b}	3.74^{a}
	50	26.90^{c}	30.90^{b}	93.20^{b}	141.80^{b}	4.66^{a}	3.29^{c}	10.61^{a}	18.56^{b}	2.28^{b}
	100	36.70^{a}	41.10^{a}	103.80^{a}	167.30^{a}	3.86^{b}	7.61^{a}	11.61^{a}	23.08^{a}	3.01^{ab}
Season 2021/2022										
00	00	18.55^{c}	26.60^{b}	41.30^{c}	86.45^{c}	2.63^{d}	2.97^{ab}	8.91^{c}	14.51^{c}	3.39^{a}
	50	16.40^{cd}	20.00^{c}	34.50^{d}	80.90^{c}	2.00^{e}	2.11^{bc}	6.49^{d}	10.60^{d}	3.25^{a}
	100	13.80^{d}	12.55^{d}	23.05^{e}	52.95^{d}	1.89^{e}	1.91^{c}	5.70^{d}	9.50^{d}	3.02a
100	00	29.70^{a}	27.50^{b}	91.90^{a}	149.20^{a}	5.29^{c}	3.03^{a}	11.78^{a}	20.10^{a}	2.23^{b}
	50	24.80^{b}	21.50^{c}	97.50^{a}	153.00^{a}	6.10^{a}	2.66^{abc}	11.27^{ab}	20.03^{a}	1.85^{b}
	100	18.90^{c}	31.60^{a}	75.00^{b}	138.50^{b}	5.70^{b}	2.85^{ab}	9.28^{bc}	17.83^{b}	1.63^{b}

Table (3): Effect of H_2S and Ascorbic acid (AsA) treatments on water relations in leaves of lettuce plants 115 days from transplanting during the growing seasons 2020/2021 and 2021/2022.

Characters / Treatments		T. Water content (%)	Rel. water content (%)	Leaf water def. (%)	Osmotic Pressure C.S.(bar)	Transpiration rate $mg/cm^2.h$	Leaf Succulence	M.I. %
AsA (ppm)	**H_2S (ppm)**	**Season 2020/2021**						
00	00	87.52^{ab}	74.78^{ab}	25.22^{d}	3.58^{b}	2.87^{b}	4.38^{bc}	16.56^{b}
	50	80.83^{bc}	63.61^{c}	36.39^{b}	4.81^{ab}	2.33^{bc}	4.13^{bc}	26.28^{a}
	100	75.83^{c}	52.12^{d}	47.88^{a}	4.98^{a}	1.79^{c}	3.10^{c}	25.56^{a}
100	00	92.04^{a}	70.17^{bc}	29.83^{c}	4.74^{ab}	4.28^{a}	7.15^{a}	15.65^{b}
	50	89.69^{ab}	77.26^{a}	22.74^{d}	5.77^{a}	3.33^{ab}	5.78^{ab}	19.39^{b}
	100	90.61^{a}	68.14^{bc}	31.86^{c}	5.27^{a}	3.13^{b}	4.94^{abc}	20.00^{b}
Season 2021/2022								
00	00	88.61^{ab}	74.71^{ab}	25.29^{e}	3.63^{d}	3.22^{bc}	4.64^{b}	17.66^{b}
	50	80.47^{bc}	62.15^{d}	37.85^{b}	4.33^{bc}	2.38^{cd}	4.61^{b}	28.38^{a}
	100	78.98^{c}	34.07^{e}	65.93^{a}	4.18^{bc}	1.79^{d}	3.44^{b}	29.67^{a}
100	00	92.06^{a}	70.18^{bc}	29.82^{d}	4.62^{c}	4.57^{a}	7.45^{a}	19.65^{b}
	50	87.50^{a}	76.97^{a}	43.03^{f}	5.23^{a}	3.59^{b}	6.69^{ab}	20.40^{b}
	100	87.31^{a}	69.81^{cd}	30.19^{c}	5.01^{b}	3.36^{b}	6.37^{ab}	20.34^{b}

Table (4): Effect of H_2S and Ascorbic acid (AsA) treatments on the concentrations and the ratios of the photosynthetic pigments in the leaves of lettuce plants 115 days from transplanting during the growing seasons 2020/2021 and 2021/2022.

Characters / Treatments		Chlorophyll a	Chlorophyll b	Total Chlorophyll a+b	Carotenoids	total Photosynthetic pigments	Chlorophyll stability index(CSI%)	Chlorophyll a/b	T.Chl/Car.
		mg/g DW							
AsA (ppm)	H_2S (ppm)	Season 2020/2021							
00	00	1.20^{a}	0.29^{ab}	1.49^{a}	0.57^{a}	2.06^{b}	100.00^{b}	4.19^{b}	2.60^{b}
	50	0.39^{cd}	0.06^{b}	0.45^{c}	0.29^{b}	0.74^{e}	30.51^{d}	6.09^{a}	1.59^{c}
	100	0.34^{d}	0.05^{b}	0.39^{c}	0.28^{b}	0.66^{e}	25.87^{d}	6.70^{a}	1.38^{c}
100	00	1.27^{b}	0.44^{a}	1.72^{a}	0.59^{a}	2.30^{a}	115.32^{a}	2.87^{c}	2.92^{b}
	50	1.13^{a}	0.38^{a}	1.51^{a}	0.38^{b}	1.89^{c}	101.41^{b}	2.95^{c}	3.99^{a}
	100	0.78^{b}	0.29^{ab}	1.07^{b}	0.37^{b}	1.43^{d}	71.71^{c}	2.73^{c}	2.92^{b}
Season 2021/2022									
00	00	1.20^{b}	0.26^{b}	1.46^{c}	0.57^{a}	2.03^{c}	100.00^{c}	4.67^{a}	2.56^{c}
	50	0.46^{c}	0.14^{c}	0.60^{d}	0.27^{b}	0.87^{d}	41.32^{d}	3.33^{b}	2.22^{d}
	100	0.34^{d}	0.10^{c}	0.44^{e}	0.23^{b}	0.67^{e}	30.13^{e}	3.39^{b}	1.91^{e}
100	00	1.29^{b}	0.45^{a}	1.74^{a}	0.59^{a}	2.33^{a}	119.01^{a}	2.86^{c}	2.92^{b}
	50	1.45^{a}	0.31^{b}	1.76^{a}	0.56^{a}	2.32^{a}	120.73^{a}	4.69^{c}	3.12^{a}
	100	1.30^{b}	0.30^{b}	1.60^{b}	0.53^{a}	2.14^{b}	109.95^{a}	4.27^{c}	3.01^{ab}

Water relations

The data presented in Table 3 demonstrate how the H_2S levels, particularly the high level, negatively impacted the total water content (TWC), relative water content (RWC), transpiration rate (TR), and leaf succulence (LSc), osmotic pressure (OP), leaf water deficit (LWD), and membrane integrity (MI) of lettuce plants. In the first season, the TWC, RWC, TR, and LSc were significantly reduced and reached approximately 14, 30, 38, and 29% at the high H_2S level of 100 ppm; in the second season, they reached approximately 11, 55, 45, and 26%. Furthermore, compared to the control, there was a notable increase in OP, LWD, and MI, which reached roughly 90, 39, and 54% (in the first season), 161, 15 and 68% (in the second season), respectively. It is clear that H_2S had a greater detrimental impact on RWC, LWD, TR, and MI than on the other water related factors. Liu and Lal (2015) reported similar findings, observing that plants exposed to H_2S saw an increase in water loss through transpiration. Tiwari *et al.* (2006) found that H_2S may negatively impact plant water relations because it can cause stomatal damage, leaf injury, and disruption of membrane permeability, all of which can affect a plant's capacity to maintain its water status.

On the other hand, ascorbic acid treatment caused a noteworthy rise or fall in the water relations values when compared to the control. Ascorbic acid application resulted in a significant increase in TWC, RWC, OP, TR, and LSc relative to the control by approximately 20, 31, 6, 75, and 59% (in the 1st season), 11, 105, 20, 88, and 85% (in the 2nd season), respectively under the level of 100 ppm H_2S. However, LWD and MI decreased by about 34 and 22% (in the 1st season), 54 and 32% (in the 2nd season), respectively. Ascorbic acid clearly reduced the negative effects of H_2S while also controlling and enhancing the water relations of the H_2S-polluted lettuce plants. The results obtained are consistent with the research conducted by Asada (2006) and Asada (2006) and Neubauer and Yamamoto (1992). The amelioration of the water relations of ascorbic acid-treated lettuce plants under H2S stress may be explained by the following theory: A stressful environment increased the concentration of abscisic acid (ABA), which seals the stomata and increases the production of hydrogen peroxide. Applying ascorbic acid stopped hydrogen peroxide-induced stomatal closure because ascorbate detoxifies hydrogen peroxide (Zhang *et al.*, 2001). Fotopoulos *et al.* (2008) found that transgenic tobacco leaves with elevated concentrations of ABA and hydrogen peroxide, which lowered stomatal conductance and water loss rates, expressed a cucumber ascorbic oxidase located in the cell wall.

Photosynthetic pigments

Table 4 shows that when H_2S levels increased relative to the control, the concentration of photosynthetic pigments in lettuce leaves reduced considerably. The percentage decrease in total photosynthetic pigments, carotenoids, chlorophyll a, chlorophyll b, and total chlorophyll (a+b) reached at 50 ppm H_2S as 68, 78, 70, 50, and 50%; at 100 ppm H_2S as 72, 83, 74, 51, and 68%, respectively. Similar trend was observed in the 2nd season. In this concern, several researches have recorded reduction in chlorophyll content under air pollution (Tripathi and Gautam, 2007; Joshi *et al.,* 2009). Furthermore, Joshi and Swami (2007) found that air pollution may lower the concentration of photosynthetic pigments including carotenoids and chlorophyll when absorbed by plants. One of the most prevalent effects of air pollution is the progressive loss of chlorophyll and the resulting yellowing of leaves, which may be linked to a corresponding reduction in the ability to perform photosynthesis (Joshi and Swami, 2007). Through the stomata, air pollutants enter the tissues, partially denaturing the chloroplast and reducing the pigment content of the cells in contaminated leaves. Furthermore, De Kok *et al.* (1983) proposed that photosynthetic electron transport is inhibited by high concentrations of accumulated sulfide.

However, ascorbic acid treatment of the lettuce plants resulted in a significant increase in photosynthetic pigments under both H_2S levels in addition to counteracting H_2S toxicity. Comparing the corresponding controls at the first season, the concentrations of chlorophyll a, chlorophyll b, total chlorophyll (a+b), carotenoids, and total photosynthetic pigments increased by 189, 497, 233, 33, and 155% at 50 ppm H_2S, respectively: 133, 472, 177, 31 and 116% at 100 ppm H_2S, respectively. Ascorbic acid treatment leads to an increase in photosynthetic pigments, which can be attributed to its function in photosynthesis as a substitute electron donor for PSII in abiotic stress conditions. It also protects the photosynthetic apparatus in chloroplasts by reducing ROS activity (Venkatesh and Park, 2014), improving absorption of essential elements, particularly iron, magnesium, potassium, nitrogen, and other cations (Al-Taweel *et al.*, 2022), which are essential for the activation of enzymes and the formation of both chloroplasts and chlorophyll. Furthermore, ascorbic acid is essential for scavenging harmful ROS produced as byproducts of photosynthesis and is a fundamental element of excess photonic energy dissipation mechanisms like the xanthophyll cycle (Yabuta *et al.*, 2007) and the water-water cycle (Neubauer and Yamamoto, 1992; Asada, 2006).

Yield quantity

The yield quantity and quality of lettuce plants exposed to different levels of H_2S and ascorbic acid treatments are shown in Table 5. Table 5 illustrates how the head output of lettuce plants dropped dramatically when H_2S levels increased in terms of head yield per plant (g), head yield per m^2 (kg), and head yield/feddan (tons). At 50 ppm H_2S and 100 ppm H_2S levels, the yield loss percentage varied from 33 to 48% and 41 to 51% respectively. Similar findings were observed in the 2nd season. These findings are consistent with those of Kord and Abbass (1993), who found that tomato, radish, and rocket plants fumigated with 50–100 ppm H_2S showed yield-inhibiting effects. In comparison to control plants, Bennett *et al.* (1980) observed a reduction in a range of yield metrics in field-grown snap beans exposed to different doses (300 to 700 ppm) of H_2S for 4 hours per day for 40 days. Taylor and Selvidge (1984) exposed bush beans to concentrations of H_2S ranging from 6.1 to 81.8 ppm and found that yield of various plants was impaired at all concentrations and the degree of impairment increased with increasing H_2S concentration.

Table (5): Effect of H_2S and Ascorbic acid (AsA) treatments on yield attributes of lettuce plants during the two growing seasons 2020/2021 and 2021/2022.

AsA (ppm)	H_2S (ppm)	Head yield/ Plant (gm)	Head yield/ m^2 (kg)	Head yield/ Feddan (ton)	Vit.C (mg ascorbic acid/100g FWt)	T.S.S (%)	Total soluble sugars (mg/g dwt)	Total protein (%)	Total amino acids (mg/g dwt)	Soluble phenols (mg/g dwt)	NO_3 - N content (ppm)	NO_2 - N content (ppm)	Mineral content mg/g dwt N	P	K	S
Season 2020/2021																
00	00	77.80^{c}	1.86^{c}	7.80^{b}	15.60^{b}	4.75^{a}	35.31^{a}	16.88^{ab}	13.91^{b}	2.70^{bc}	114.2^{ab}	1.36^{ab}	27.0^{ab}	5.25^{c}	15.60^{b}	5.60^{c}
	50	50.10^{d}	1.24^{d}	4.03^{c}	11.97^{c}	5.36^{a}	33.90^{ab}	15.25^{bc}	12.83^{b}	2.85^{ab}	118.7^{ab}	1.38^{ab}	24.4^{ab}	4.34^{d}	12.10^{c}	6.60^{bc}
	100	37.90^{e}	1.08^{e}	3.81^{c}	10.08^{c}	5.41^{a}	31.35^{b}	13.94^{c}	11.33^{c}	3.01^{a}	130.8^{a}	1.89^{a}	22.3^{b}	4.10^{d}	10.34^{d}	7.00^{bc}
100	00	109.40^{a}	2.61^{a}	10.97^{a}	25.98^{a}	5.83^{a}	36.08^{a}	17.69^{a}	15.75^{a}	2.65^{bcd}	110.4^{ab}	1.20^{b}	28.3^{a}	6.12^{b}	17.80^{a}	6.80^{bc}
	50	114.10^{a}	2.06^{b}	7.45^{b}	22.68^{a}	5.95^{a}	34.89^{ab}	17.00^{a}	15.99^{a}	2.51^{cd}	109.5^{ab}	1.10^{b}	27.2^{ab}	7.15^{a}	16.34^{b}	8.60^{b}
	100	100.90^{b}	1.76^{c}	6.54^{bc}	16.38^{b}	6.01^{a}	34.95^{ab}	16.56^{ab}	13.43^{b}	2.45^{d}	100.2^{b}	1.00^{b}	26.5^{ab}	5.34^{c}	16.23^{b}	10.70^{a}
Season 2021/2022																
00	00	67.90^{c}	1.62^{cd}	6.81^{c}	17.22^{c}	4.78^{b}	33.52^{a}	17.06^{a}	14.86^{ab}	2.73^{ab}	115.5^{ab}	1.62^{abc}	27.3^{ab}	5.45^{ab}	15.90^{ab}	5.20^{e}
	50	47.40^{d}	1.17^{de}	4.76^{d}	15.39^{d}	5.76^{a}	31.20^{a}	15.75^{ab}	13.97^{ab}	3.01^{a}	117.2^{ab}	1.78^{ab}	25.2^{b}	4.50^{b}	13.34^{bc}	6.40^{d}
	100	35.60^{d}	0.85^{e}	3.57^{e}	12.96^{e}	5.71^{a}	30.08^{a}	12.63^{b}	12.67^{b}	2.67^{ab}	121.3^{a}	1.98^{a}	20.2^{c}	4.20^{b}	11.23^{c}	7.40^{c}
100	00	119.40^{a}	2.85^{a}	11.98^{a}	30.16^{a}	5.96^{a}	36.72^{a}	18.38^{a}	15.96^{a}	2.71^{ab}	109.8^{bc}	1.45^{bcd}	29.4^{a}	6.67^{a}	18.01^{a}	6.60^{d}
	50	110.00^{ab}	2.54^{ab}	9.94^{b}	29.16^{a}	5.83^{a}	32.08^{a}	17.19^{a}	16.23^{a}	2.63^{ab}	102.2^{cd}	1.32^{cd}	27.5^{ab}	6.97^{a}	17.23^{a}	9.80^{b}
	100	96.60^{b}	1.95^{bc}	7.69^{c}	21.06^{b}	5.85^{a}	32.00^{a}	16.31^{a}	15.34^{a}	2.34^{b}	100.1^{d}	1.21^{d}	26.1^{b}	6.10^{ab}	17.10^{a}	11.10^{a}

Table 5 shows that treating lettuce plants with ascorbic acid recorded highly significant increases in head yield per plant, per m^2 and per feddan (ton), at all H_2S levels which reached about 128, 66 and 85% at 50 ppm H_2S; 166, 63 and 72% at 100 ppm H_2S, respectively compared to the corresponding controls. A similar trend was observed in the 2nd season. These results are consistent with those of Amin *et al.* (2008) in wheat plants, who reported that foliar treatment of ascorbic acid up to 400 ppm resulted in a progressive increase in spike length, grain and straw production. Additionally, Abdel-Hameed *et al.* (2004) found that applying ascorbic acid topically to wheat plants in doses between 50 and 200 ppm significantly increased yield and its constituent parts.

Yield quality

Table 5 and Fig. 1 demonstrate how the chemical contents of lettuce leaves, such as vitamin C, total protein, total amino acids, and N, P, and K mineral contents, dramatically dropped when the H_2S level rose in comparison to the control. The percentages of vitamin C, total protein, total soluble sugars (SS), total amino acids, N, P, and K content decreased by approximately 11, 8, 4, 8, 8, 18, and 16% at 50 ppm H_2S level, and by approximately 15, 26, 11, 19, 17, 20, and 23% at 100 ppm, respectively, in comparison to the untreated control plants. With an increase in H_2S rates, lettuce leaves' levels of total soluble solids (TSS), soluble phenols, NO_3-N, NO_2-N, and Sulphur (S) increased. The percentage increases in the previously indicated chemical levels were approximately 14, 12, 15, 39, and 25% at 100 ppm H_2S, respectively. These findings are consistent with those of Kord and Abbass (1993), who reported that plants exposed to H_2S showed a decrease in the majority of N fractions, sugar buildup, and the activities of peroxidase and carbohydrase. The drop in soluble sugar concentration in polluted stations can be ascribed to a decline in CO_2 fixation due to chlorophyll deterioration and an increase in respiration. It has been noted that in hardening conditions, pollutants like SO_2, NO_2, and H_2S can lead to a greater depletion of soluble sugars in the leaves of plants cultivated in polluted areas. Tripathi and Gautam (2007) noted that the reactivity of sulfite with the aldehydes and ketones of carbohydrates can also lead to reductions in the amount of carbs.

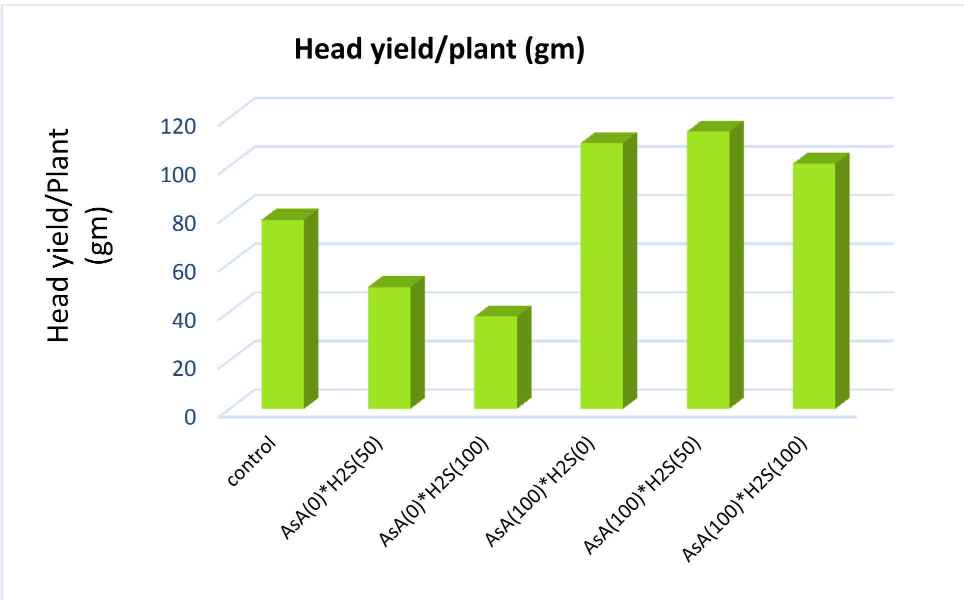

Fig. (1). Interaction effect of H_2S and Ascorbic acid (AsA) on head yield per plant (gm) of lettuce plants (average the two growing seasons 2020/2021 and 2021/2022).

However, applying ascorbic acid resulted in a noticeable improvement in the chemical content values of the lettuce plants contaminated with all H_2S levels. In comparison to the owing control, at 50 ppm H_2S, the contents of vitamin C, TSS, SS, total protein, total amino acids, N, P, K, and S increased by approximately 90, 11, 3, 12, 25, 12, 65, 35, and 30%, respectively. Ascorbic acid treatment at 100 ppm increased the features' percentages by approximately 63, 11, 11, 19, 19, 19, 30, 57, and 53%, respectively, in comparison to the corresponding control. Furthermore, application of ascorbic acid led to a marked decrease in soluble phenols, NO_3-N and NO_2-N by about 12, 8 and 20% (at 50 ppm H_2S), and 19, 24 and 47% (at 100 ppm H_2S), respectively. Similar findings were observed in the 2nd season (Table 5). These results are consistent with those of Elkelish *et al.* (2020), who reported that exogenous ascorbic acid administration can enhance the endogenous ascorbic acid concentration under various stress conditions, hence increasing plant tolerance to a variety of stresses. Al-Taweel *et al.* (2022) discovered that applying 2.0 mM ascorbic acid for integrative (seed soaking + foliar spraying) significantly enhanced the contents of nutrients, soluble sugars, free proline, and ascorbic acid, as well as growth, yields, and essential oil fractions in parsley plants. It was also more successful in giving parsley plants a higher degree of resistance to unfavorable circumstances. As a result, ascorbic acid might aid in promoting the absorption of nutrients. Through regulating essential functions like enzyme activity, hormone, protein, and chlorophyll biosynthesis, as well as osmosis regulation, the increased uptake of nutrients (*e.g.,* N, P, K, Fe, Mn, and Zn) in ascorbic acid-treated plants may have had a direct effect on metabolism (Mazid *et al.*, 2011; Farooq *et al.*, 2013). Both the amount and quality of lettuce produced demonstrate these positive benefits.

CONCLUSION

Our results suggest that H_2S is a phytotoxic pollutant that causes oxidative stress and has detrimental impacts on photosynthetic pigments, plant water relations, growth, and the quantity and quality of lettuce plants' produce. In addition to counteracting the inhibitory effects of H_2S, treating the stressed lettuce plants with ascorbic acid enhanced their growth, improved their physiological and biochemical behavior, boosted their uptake of nutrients, produced a greater yield, and enhanced their quality.

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الإستجابات المورفولوجية والفسيولوجية والبيوكيميائية لنباتات الخس الملوثة بكبريتيد الهيدروجين لحمض الاسكوربيك (*Lactuca sativa* L.)

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الملخص العربى

أجريت تجارب أصص تحت ظروف الصوبة بهدف دراسة دور حمض الأسكوربيك المضاد للأكسدة تحت مستويين (٠ و ١٠٠ جزء في المليون) في تقليل و/أو التغلب على تأثيرات السمية النباتية لكبريتيد الهيدروجين بتركيزات (٠ و ٥٠ و ١٠٠ جزء في المليون) على بعض صفات النمو، الصفات الفسيولوجية البيوكيمائية وكمية وصفات الجودة لمحصول نباتات الخس. انخفضت مؤشرات النمو الخضري لنباتات الخس الملوثة بكبريتيد الهيدروجين بشكل ملحوظ مع زيادة مستوى كبريتيد الهيدروجين. كان التأثير السمي النباتي لكبريتيد الهيدروجين عند المستوى العالي أكثر وضوحًا على الصفات الآتية: طول الرأس (-٣١ ٪)، وطول الساق (-٣٤ ٪)، ومساحة الورقة (-٤٢ ٪)، والوزن الطازج للنبات (٣٥ ٪)، والوزن الجاف للنبات (-٣٤% لنباتات الخس بالنسبة لصفات النمو الأخرى. إن معاملة نباتات الخس الملوثة بكبريتيد الهيدروجين بحمض الأسكوربيك لم تبطل التأثير الضار على العوامل المذكورة أعلاه فحسب، بل أدت أيضًا إلى زيادة كبيرة فيها، طول الرأس (٤٠ ٪)، طول الساق (١٠٤ ٪)، مساحة الورقة (٨٢ ٪). ، الوزن الطازج للنبات (١٤٨ ٪)، والوزن الجاف للنبات (٩٦ ٪) مقارنة بالنباتات غير المعاملة. ازداد النقص المائي للأوراق (LWD)، والضغط الأسموزي ونفاذية الأغشية (MI) ؛ في حين انخفض محتوى الماء الكلي(TWC)، ومحتوى الماء النسبي (RWC)، ومعدل النتح، و غضاضة الأوراق كنتيجة للمعاملة بكبريتيد الهيدروجين. أدى استخدام حامض الأسكوربيك إلى تنظيم وتحسين الحالة المائية للنبات عن طريق زيادة محتوى الماء النسبي (RWC) (٦٦%) والضغط الأسموزي (١٦٪)، وتقليل نقص الماء الورقي (LWD) (٤٠%) و نفاذية الأغشية (MI) (٢٨%). جميع مستويات كبريتيد الهيدروجين لها تأثير ضار على صبغات التمثيل الضوئي للأوراق (الكلوروفيل a ، b والكلوروفيلات الكلية، الكارتنويدات، صبغات التمثيل الضوئي الكلية وكذلك مؤشر ثبات الكلوروفيل (CSI). وجد أن حمض الاسكوربيك يثبط التأثير السام لكبريتيد الهيدروجين ويعزز التخليق الحيوي وتركيز صبغات التمثيل الضوئي مثل: كلوروفيل a (١٧٠٪)، كلوروفيل b (٣٠٣٪)، الكلوروفيل الكلي (١٨٦٪) وكذلك الكارتنويدات (٦٤٪). أدى تعرض نباتات الخس لملوث كبريتيد الهيدروجين إلى انخفاض كبير في محصول الخس، أكثر عند مستوى ١٠٠ جزء في المليون (-٥٠%) من ذلك عند ٥٠ جزء في المليون (-٣٣%). إنخفضت معنوياً المكونات الكيميائية لفيتامين C والبروتين الكلي والسكريات الكلية الذائبة (SS) والأحماض الأمينية الكلية ومحتوى العناصر الغذائية N، P، K في أوراق الخس مع زيادة مستوى كبريتيد الهيدروجين. بينما ازداد محتوي الكبريت (S) والفينولات القابلة للذوبان وNO_3-N وNO_2-N في أوراق الخس مع زيادة معدلات كبريتيد الهيدروجين. إن إضافة حامض الأسكوربيك إلى نباتات الخس الملوثة بكبريتيد الهيدروجين لم يؤد فقط إلى التغلب على التأثيرات الضارة لمستويات كبريتيد الهيدروجين (٥٠ و١٠٠ جزء في المليون) على معظم الصفات المذكورة أعلاه، بل أدى أيضًا إلى زيادة المحصول بنسبة تتراوح من (١٥٠ إلى ١٦٩%) وتحسين صفات جودة محصول أوراق الخس.

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