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Spray Golden Pothos and Croton Plants with Glutathione and Bilirubin to Purify the Indoor Air from Pollutants



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ABSTRACT

Indoor air pollution is considered as one of the greatest risks to human health by many international health organizations. Therefore, there is a global trend to increase awareness among people of the importance of indoor plants as well as its important role in the purification of indoor air pollutants. So, this investigation was performed in Plexiglas chamber for evaluating the effects of foliar spraying of golden pothos and croton plants with non-enzymatic antioxidants: glutathione(GSH)and bilirubin(BI)at 300 mg L⁻¹ and 600mg L⁻¹ to increase their resistance and abilities to absorb formaldehyde and ammonia gases. Results showed that pothos and croton plants exposed to ammonia gas were extremely injured with a high number of dead leaves and decreased in fresh and dry weights as well as the total chlorophyll and carotenoids compared with the plants exposed to formaldehyde gas. GSH increased the total chlorophyll and carotenoids of pothos and croton under the formaldehyde gas. Treated pothos and croton plants with glutathione or bilirubin before exposure to the gases decreased the generation of reactive oxygen species(ROS),increase the guaiacol peroxidase and reduced polyphenol oxidase activities. Chloroplasts composition and stomatal index were injured with exposing croton and pothos plants to formaldehyde and ammonia gases with partially opening of stomata and moderately decrease the number of stomata. Pothos plants were greatly resistant and more growth efficiency when treated with BI at 600mgL⁻¹ before exposed to formaldehyde gas.In conclusion, spraying pothos and croton plants with GSH at 600mgL⁻¹ increased their resistance to formaldehyde and ammonia gases.

Keywords: air pollution, formaldehyde, ammonia, golden pothos, croton.

INTRODUCTION

There is a great realization among the government's agencies, international health organizations, ecologists and the research centers about climate change and the continuous increase of gaseous emissions and their relation with the increase of air pollution. According to the latest air quality database, 97% from cities in low and middle-income countries with more than 100,000 inhabitants do not meet World Health Organization (WHO) air quality guidelines, however, in high-income countries that percentage decreases to 49% (WHO 2018). In fact, indoor air quality recognized as important risk factor for human health in all countries. Now, most environmental scientists and government agencies agree that indoor air pollution is a realistic threat to human health. There are many sources of indoor air pollution in homes including: combustion sources, building materials and furnishings, products for household cleaning and maintenance, personal care, central heating and cooling systems and humidification devices. Mostly, indoor air pollutants including volatile organic compounds (VOCs) (Wolkoff *et al.*, 2006), particulate matter ≤ 2.5 μm in diameter (PM_{2.5}) (Habre *et al.*, 2014), bioaerosols (Van Kampen *et al.*, 2016). Formaldehyde sources are in newly built housing and from clothing or furniture stores poorly ventilated, cosmetics, detergents and wallpaper. Formaldehyde is harmful and classified as a mutagen and carcinogen. In the 1980's, an NASA's research revealed that low level of formaldehyde in air could be removed by plant leaves alone, while higher concentrations of the toxic chemical can be filtered by activated carbon firstly and the plant roots and associated microorganisms degrade and

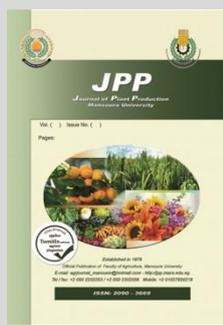
assimilate remained chemicals (Wolverton,1988). Plants are absorbing formaldehyde through stomata where rapidly metabolized in the leaves by conjugating the formaldehyde with nucleophiles, such as glutathione, to form S-hydroxymethyl glutathione, which is then converted to S-formylglutathione (Haslam *et al.*, 2002). Ammonia (NH₃) is a colorless gas which is occurring in both natural and manufactured. Ammonia has an important role in the formation of particulate matter (PM_{2.5}) and climate change, consequently, affects the air quality and implications on human health and life expectancy. In the atmosphere ammonia can bind to other gases, such as nitrogen dioxide (NO_x) and sulphur dioxide (SO₂) to form ammonium containing fine particulate matter (Guthrie *et al.*, 2018).

Recently, the ornamental plants are considered as a green liver of the earth because it is the global repository for environmental chemicals (Kvesitadze *et al.*, 2009). The major health benefits of interior plants include absorption of air pollutants, filtration of dust, releasing oxygen and add humidity and increase positive feelings and reduce feelings of stress. So, indoor plants play an important role in counteracting building syndrome diseases. Golden Pothos (*Epipremnum aureum* L.) family *Araceae* and croton (*Codiaeum variegatum* L.) family *Euphorbiaceae* are the most popular indoor plants and does not need constant attention. They act as air Bio-filtration and absorbing formaldehyde, xylene and benzene. Glutathione (GSH) and bilirubin (BI) are prominent endogenous antioxidant cytoprotectants. Glutathione and bilirubin are coming under second-line defense as non-enzymatic antioxidants because they are able to scavenge directly or indirectly reactive oxygen species (ROS). GSH is contributed to the

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detoxification of different toxic compounds, such as herbicides and air pollutants (Cummins *et al.* 2011). Bilirubin enhanced the antioxidant enzymes activities (Wu *et al.*, 2011) and inhibit protein oxidation (Wang and Liao, 2016).

Acting these facts, chamber experiments were carried out to investigate the effects of spraying pothos and croton plants with glutathione and bilirubin antioxidants on increasing the absorption and resistance to air pollutants with formaldehyde and ammonia gases.

MATERIALS AND METHODS

1. This investigation was carried out at Mansoura Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center, Egypt in Plexiglas chambers during 2017 season to evaluate the effects of treated pothos and croton plants with glutathione and bilirubin antioxidants on increasing their ability to absorb and resistant to the air polluted with formaldehyde and ammonia gases.

2. Plant materials:

Two species of indoor ornamental plants were used in this investigation: golden pothos (*Epipremnum aureum* L., family *Araceae*) and croton (*Codiaeum variegatum* L., family *Euphorbiaceae*). The plants transplanted in 20cm diameter pots filled with peatmoss were obtained from the local nurseries. The average number of leaves was 30±5 leaf/plant for pothos and 13±3 for croton. Plants were acclimatized to the interior environment used for the experiment by placing them in the above trial room for 30 days before beginning of the trials. During this period plants were thoroughly watered every two days and fertilized as recommended doses.

3. Antioxidants:

Plants sprayed with glutathione reduced (C10H17N3O6S) and bilirubin (C33H36N4O6) at 300mgL⁻¹ and 600mgL⁻¹ in twice time before exposed to gas pollutants; the first time before 6 days and the second before one day of the beginning of gas pollutant treatments. Distilled water was used for preparing the antioxidant solutions supplemented with 0.02% Tween 20, (polyoxy ethylene sorbitan monolaurate), as a surfactant. Bilirubin solution was prepared by solute 0.3 or 0.6g in 1ml methyl alcohol before adding the distilled water.

4. **Air pollution gases:** Plants were exposed to the gas pollutants (formaldehyde or ammonia gases) for 4 hrs daily for 10 days.

Formaldehyde gas treatment: Plants were exposed to the gas by injecting 5ml of formaldehyde (HCHO) solution (34-38%) on the glass-petri dish into the chamber.

Ammonia gas treatment: Plants were treated with ammonia (NH₃) gas by injecting 1ml of ammonia solution (25%) on the betri-glass dish into the chamber. Air circulation within the chamber was continued through half hour at the beginning of each trial period.

5. Test chamber layout:

Treatment was conducted in Plexiglas chambers which were made perfectly airtight. The construction of the chambers was as the following dimensions: 60*60*70cm. The chamber's frame was made of aluminum metal and the sides from clear glass 3mm in thickness. The front side of the chamber was sliding door, sealed by adhesive foam-rubber insulation tape, for input the plants. One small fan (4" with 3 feathers) fixed inside the chamber to distribute the trial gases

around the plants. The air pump was used for injecting fresh air inside the chamber through the plastic tube. Chambers were situated within a trial room used to simulate an indoor environment. The air temperature and relative humidity within the room were 25±5°C and 60-70%, respectively. Two white fluorescent lamps with light intensity at 1150±50 lux were used as the light source for 12 hrs daily.

The treatments were arranged as follow:

- 1- Control (non-polluted): Plants not exposed to gases and not sprayed with antioxidants.
- 2- Plants exposed to formaldehyde gas only (control-polluted).
- 3- Plants sprayed with glutathione at 300mgL⁻¹ + exposed to formaldehyde gas.
- 4- Plants sprayed with glutathione at 600mgL⁻¹ + exposed to formaldehyde gas.
- 5- Plants sprayed with bilirubin at 300mgL⁻¹ + exposed to formaldehyde gas.
- 6- Plants sprayed with bilirubin at 600 mgL⁻¹ + exposed to formaldehyde gas.
- 7- Plants exposed to ammonia gas only (control-polluted).
- 8- Plants sprayed with glutathione at 300mgL⁻¹ + exposed to ammonia gas.
- 9- Plants sprayed with glutathione at 600mgL⁻¹ + exposed to ammonia gas.
- 10- Plants sprayed with bilirubin at 300mgL⁻¹ + exposed to ammonia gas.
- 11- Plants sprayed with bilirubin at 600mgL⁻¹ + exposed to ammonia gas.

During the experiment of croton plants, the treatments of 2, 3 and 10 numbers were died or not enough number of leaves for data analysis.

6. Measurements:

At the end of the experiment the above ground biomasses were decapitated at the surface of the medium for estimating the morphological and physiological parameters.

Morphological parameters:

leaf area/plant: It was calculated using ImageJ software version 1.50i according to Ferreira and Rasband (2012). Average of one leaf area was calculated by sum of the 5 leaves area and divided on 5. The total leaf area/plant was calculated by using the following equation:

$$\text{Total leaf area/plant} = \frac{\text{One leaf area} \times \text{F.W./plant}}{\text{F.W./leaf}}$$

Mean area injury/leaf: All leaves with any spot injury were collected and measured by using the ImageJ software according to Ferreira and Rasband (2012).

Percentage (%) of area injury/leaf: Calculated as the following formula:

$$\text{Percentage (\% of area injury/leaf} = \frac{\text{mean area injury for one leaf}}{\text{mean area of one leaf}} \times 100$$

Fresh and dry weights: Shoot fresh weight was estimated by weight the above ground biomass just after decapitated them. The dry weight was determined after dried shoots for 48hrs at 70°C in ventilated oven.

Photosynthesis pigments: Total Chlorophyll and carotenoids were determined according to Lichtenthaler and Wellburn (1983). The samples were collected from the blade of the 4th upper leaf of croton plants and from the middle of the stem of pothos plants.

Activities of antioxidant enzymes and histochemical analysis of reactive oxygen species (ROS): Randomly samples were collected from each treatment at the end of the

second experiment and were immediately transferred in a cool dry container to the EPCRS (Excellence Center, Plant Pathology and Biotechnology Lab., Fac. Agric., Kafrelsheikh University) for measuring the enzymes activities and analysis of reactive oxygen species (ROS).

Biochemical Assays of Antioxidant Enzymes:

- -The total soluble enzyme activities were measured spectrophotometrically according to Hafez *et al.*, (2014). All measurements were carried out at 25°C, using the model UV-160A spectrophotometer (Shimadzu, Japan).
- -Polyphenol oxidase activity was measured according to Malik and Singh (1980).
- -Ascorbate peroxidase activity was determined spectrophotometrically according to Asada (1984).
- -Catalase activity was determined spectrophotometrically according to Aebi (1984).
- -Activity of guaiacol peroxidase was directly determined of the crude enzyme extract according to a typical procedure proposed by Hammerschmidt *et al.*, (1982). Changes in absorbance at 470nm were recorded every 30sec intervals for 3min. Enzyme activity was expressed as increase in absorbance $\text{min}^{-1}\text{g}^{-1}$ fresh weight.
- -Detection of O_2^- and H_2O_2 were visualized as a purple coloration of nitro blue tetrazolium (NBT) and a reddish-brown coloration of 3,3-diaminobenzidine (DAB), respectively as described with Hückelhoven *et al.* (1999).

Anatomical studies: Anatomical characteristics were taken in the treatments that showed a good and poor response to the gas pollutants. Leaves anatomy were investigated with scanning and transmission electron microscopes according to Karnovsky (1965). Samples (5mm²) were taken from the middle blade of the middle mature leaf for the pothos plants and from the fourth lower leaf for croton plants. Samples were dehydrated with passing by ascending concentrations of ethanol (30-100%). Specimen preparation for scanning and transmission electron microscopy were observed at E. M. Unit, Mansoura University, Egypt.

Statistical analysis: A completely randomized design was used with for pothos and croton plants experiments. Three plants of each species were used as replicates for each treatment. The experiment was repeated one time. For

pothos plants, data were analyzed by two-way ANOVA, while for croton plants data were analyzed by one-way and using the analysis of variance technique by means of CoStat Computer Software (Cohort, Berkeley, CA,USA). Mean values were compared by using Duncan’s multiple range test method (Duncan, 1955) at least significance difference ($p \leq 0.05$).

RESULTS AND DISCUSSION

Results

1. Effects of treated pothos and croton plants with glutathione (GSH) and bilirubin (BI) before exposure to formaldehyde or ammonia gases on morphological parameters: leaf area injury, percentage of area injuries and fresh and dry weights:

The data recorded in the Table (1) showed that the pothos plants were a variable response to the gas pollutants and to spraying with the antioxidants treatments. The plants were injured significantly when exposed to the ammonia pollutant during the two experiments (5.89 and 5.95 cm² area injury/leaf), in percentage, 12.83 and 13.79, respectively (Table, 1 & Fig. 1).

Also, the number of dead leaves was recorded in the same trend (12.67 and 12 leaves/plants, respectively). Pothos plants spraying with BI at 300mgL⁻¹ concentration significantly were decreased in the leaf injury and the number of dead leaves comparing with the other antioxidants treatments. Concerning the interactions between the antioxidants of GSH or BI and formaldehyde or ammonia, the results indicated that, spraying plants with BI at 300mgL⁻¹ before exposing to the ammonia were the lowest in the leaf injury. The highest values of the leaf injury were recorded in plants exposed to ammonia gas alone (10.30 and 9.37 cm² area injury/leaf), in percentage, 22.21 and 22.18, respectively during the two experiments. Also, spraying pothos plants with GSH at 300mgL⁻¹ concentration before exposure to ammonia takes out the same trend in leaf injury. The highest number of dead leaves was incident when pothos plants were treated with BI at 600mg⁻¹L before exposing to ammonia. The leaf area per plant of pothos was decreased among all treated plants with ammonia (Table 1). The exception of this, the plants treated with GSH or BI at 600mg L⁻¹ before exposing to the formaldehyde showed the lowest decreased in leaf area compared with the control plants (non-polluted).

Table 1. Effects of treated pothos plants with glutathione (GSH) and bilirubin (BI) on morphological parameters under formaldehyde or ammonia gases during the two experiments.

Parameters	Leaf area (cm ²)/plant		Mean area injury(cm ²)/leaf		Area injury% /leaf		No.of dead leaves/plant	
	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment
Treatments	Air gas pollutants (A)							
Formaldehyde (Fmd)	1143.76 a	1146.22 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 a	0.0 a
Ammonia (Amm)	678.55 b	693.03 b	5.89 a	5.95 a	12.83 a	13.79 a	12.67 b	12.0 b
Antioxidants (B)								
Control (non-polluted) ^y	1408.07 a	1471.00 a	0.0 c	0.0 b	0.0 d	0.0 e	0.0 e	0.0 e
Control -polluted	796.54 e	858.75 b	5.15 a	4.94 a	11.10 a	11.09 a	8.0 c	8.50 b
Glutathione (GSH) 300 mg L ⁻¹	852.73 b	842.44 c	4.26 ab	4.23 a	8.99 b	11.14 a	7.5 c	6.50 c
Glutathione (GSH) 600 mg L ⁻¹	834.93 c	746.92 d	3.29 b	3.44 a	8.24 bc	8.50 b	9.0 b	7.67 bc
Bilirubin (BI) 300 mg L ⁻¹	821.89 d	840.64 c	1.40 c	1.51 b	3.14 d	2.99 c	3.0 d	3.17 d
Bilirubin (BI) 600 mg L ⁻¹	752.79 f	758.02 d	3.59 ab	3.74 a	7.01 c	7.65 b	10.5 a	10.17 a
Pollution gases combined with antioxidants (A*B)								
Control (non-polluted)	1408.07 a	1471.00 a	0.0 d	0.0 d	0.0 e	0.0 d	0.0 e	0.0 e
Fmd (control -polluted)	1007.12 d	1124.60 bc	0.0 d	0.0 d	0.0 e	0.0 d	0.0 e	0.0 e
Fmd + GSH 300mg L ⁻¹	1106.77 c	1112.43 c	0.0 d	0.0 d	0.0 e	0.0 d	0.0 e	0.0 e
Fmd + GSH 600 mg L ⁻¹	1313.50 b	1133.74 b	0.0 d	0.0 d	0.0 e	0.0 d	0.0 e	0.0 e
Fmd +BI 300 mg L ⁻¹	922.83 e	940.22 e	0.0 d	0.0 d	0.0 e	0.0 d	0.0 e	0.0 e
Fmd + BI 600 mg L ⁻¹	1104.28 c	1095.33 d	0.0 d	0.0 d	0.0 e	0.0 d	0.0 e	0.0 e
Amm (control-polluted)	585.96 g	592.89 g	10.30 a	9.87 a	22.21 a	22.18 a	16.0 c	17.0 b
Amm + GSH 300 mg L ⁻¹	598.69 g	572.44 h	8.51 ab	8.46 ab	17.97 b	22.28 a	15.0 c	13.0 c
Amm + GSH 600 mg L ⁻¹	356.37 i	360.10 j	6.57 b	6.88 b	16.49 b	17.01 b	18.0 b	15.33 b
Amm + BI 300 mg L ⁻¹	720.95 f	741.07 f	2.80 c	3.03 c	6.28 d	5.99 c	6.0 d	6.33 d
Amm + BI 600 mg L ⁻¹	401.29 h	420.71 i	7.17 b	7.47 b	14.02 c	15.30 b	21.0 a	20.33 a

Mean values followed by the same letter in each column are not different significantly at $P \leq 0.05$ based on Duncan’s multiple range test, y : Control (non-polluted); plants not exposed to gas pollutants.



Fig. 1. leaf injury symptoms of pothos plants treated with glutathione (GSH) and bilirubin (BI) antioxidants before exposed to ammonia (Amm) gas.

Data recorded in Table (2) shows that spraying croton plants with BI at both concentrations before exposing to the formaldehyde were, significantly decreased the leaf abscission comparing with the both of control plants and with the other treatments. The highest values of leaf abscission were incidence when exposed croton plants to formaldehyde or ammonia gases and sprayed with GSH at 600mg L⁻¹. Additionally, as shown in Table (2) croton plants exposed to ammonia were clearly decreased in the leaf area.

The results presented in Table (3) indicate that pothos plants fresh weight significantly decreased when exposed to ammonia gases comparing with plants exposed to formaldehyde.

The plants treated with antioxidants increased significantly in fresh and dry weights with BI treatment at

300mgL⁻¹ in comparison to the other treatments during the first experiment. In focus with the interaction between gases and antioxidants, sprayed plants with BI at 300mgL⁻¹ before exposure to formaldehyde increased significantly in fresh and dry weights in comparison to the other treatments followed by the treatment of exposing to formaldehyde only. Furthermore, the pothos plants sprayed with BI at the same concentration before exposure to the ammonia gas were the best in fresh and dry weights when compared with the other antioxidants supplemented with ammonia gas treatments. On the otherwise, the lowest values of fresh and dry weights were recorded with plants treated with GSH at 600mgL⁻¹ before exposing to ammonia gas.

Table 2. Effects of spraying with glutathione (GSH) and bilirubin (BI) on morphological parameters of croton plants under formaldehyde (Fmd) or ammonia (Amm) gases during the two experiments.

Parameters	Leaf area/plant (cm ²)		No. abscission leaves/plant		Fresh weight (g/plant)		Dry weight (g/plant)	
	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment
Control (non-polluted) ^y	283.82 c	266.68 d	0 d	0 c	8.32 ab	7.94 a	1.212 ab	1.109 ab
Fmd + GSH 600 mgL ⁻¹	315.34 b	345.40 b	4 a	5 a	8.61 ab	9.59 a	0.895 bc	0.907 bc
Fmd + BI 300 mgL ⁻¹	401.57 a	375.09 a	1 cd	0 c	6.89 ab	7.11 a	0.429 d	0.508 d
Fmd+ BI 600 mgL ⁻¹	331.00 b	365.51 a	2 bc	3 b	8.78 ab	7.94 a	1.235 ab	1.318 a
Ammonia (Amm)	316.26 b	336.77 b	3 ab	3 b	9.96 a	9.46 a	1.184 ab	1.098 ab
Amm+ GSH 300 mgL ⁻¹	279.93 c	310.84 c	3 ab	4 ab	8.82 ab	9.90 a	1.305 a	1.451 a
Amm+ GSH 600 mgL ⁻¹	183.58 e	201.66 f	4 a	3 b	5.33 b	6.05 a	0.539 cd	0.619 cd
Amm+ BI 600 mgL ⁻¹	208.93 d	230.12 e	4 a	5 a	7.58 ab	8.35 a	0.760 cd	0.752 bcd

Mean values followed by the same letter in each column are not different significantly at P≤0.05 based on Duncan's multiple range test. Y: Control (non-polluted); plants not exposed to gas pollutants.

Table 3. Effects of treated pothos plants with glutathione (GSH) and bilirubin (BI) on fresh and dry weights and total chlorophyll and carotenoids concentrations under formaldehyde or ammonia gases during the two experiments.

Parameters	Fresh weight (g/plant)		Dry weight (g/plant)		Total chlorophyll mg/g F.W.		Total carotenoids mg/g F.W.	
	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment
Air gas pollutants (A)								
Formaldehyde (Fmd)	38.20 a	37.52 a	2.33 a	2.17 a	1.99 a	2.00 a	0.117 a	0.115 a
Ammonia (Amm)	20.78 b	21.01 b	1.57 b	1.55 b	1.46 b	1.51 b	0.082 b	0.087 b
Antioxidants (B)								
Control(non-polluted) ^y	35.74 b	37.72 a	2.26 b	2.31 b	1.96 b	2.04 a	0.062 cd	0.078 bc
Control -polluted	28.47 c	26.74 b	1.60 d	1.49 cd	1.03 e	1.00 c	0.042 d	0.056 c
Glutathione (GSH) 300 mg L ⁻¹	28.15 c	27.48 b	1.90 c	1.76 c	1.76 cd	1.81 b	0.158 a	0.150 a
Glutathione e(GSH) 600 mg L ⁻¹	23.32 d	25.34 b	1.67 d	1.55 cd	1.83 c	1.83 b	0.108 bc	0.108 ab
Bilirubin (BI) 300 mg L ⁻¹	38.79 a	38.73 a	2.64 a	2.71 a	2.13 a	2.10 a	0.151 ab	0.137 a
Bilirubin (BI) 600 mg L ⁻¹	22.49 d	22.57 c	1.62 d	1.37 d	1.65 d	1.76 b	0.079 cd	0.079 bc
Pollution gases combined with antioxidants (A*B)								
Control (non-polluted)	35.74 c	37.72 bc	2.26 bc	2.31 b	1.96 c	2.04 c	0.062 cd	0.078 cde
Fmd (control-polluted)	40.88 b	35.17 cd	2.45 b	2.11 b	1.34 e	1.30 e	0.019 d	0.017 e
Fmd + GSH 300 mg L ⁻¹	37.10 c	38.43 bc	2.30 b	2.15 b	2.67 a	2.73 a	0.211 a	0.184 a
Fmd + GSH 600 mg L ⁻¹	36.28 c	40.62 b	2.24 bc	2.11 b	2.40 b	2.35 b	0.146 ab	0.136 abc
Fmd + BI 300 mg L ⁻¹	47.35 a	45.00 a	2.80 a	2.91 a	1.81 c	1.79 d	0.162 ab	0.162 ab
Fmd + BI 600 mg L ⁻¹	31.82 d	28.15 e	1.92 c	1.44 c	1.79 c	1.76 d	0.106 bc	0.113 bcd
Amm (control-polluted)	16.05 ef	18.31 f	0.76 f	0.87 d	0.71 f	0.70 g	0.065 cd	0.095 bcd
Amm + GSH 300 mg L ⁻¹	19.19 e	16.53 f	1.50 d	1.36 cd	0.84 f	0.88 f	0.105 bc	0.116 abcd
Amm + GSH 600 mg L ⁻¹	10.35 g	10.06 g	1.10 e	0.98 cd	1.25 e	1.30 e	0.070 cd	0.080 cde
Amm + BI 300 mg L ⁻¹	30.22 d	32.46 d	2.47 b	2.50 ab	2.46 b	2.40 b	0.141 b	0.111 bcd
Amm + BI 600 mg L ⁻¹	13.15 fg	16.98 f	1.31 de	1.29 cd	1.52 d	1.76 d	0.051 cd	0.045 de

Mean values followed by the same letter in each column are not different significantly at P≤0.05 based on Duncan's multiple range test. y: Control (non-polluted); plants not exposed to gas pollutants.

For croton plants, the differences between treatments were non-significant in fresh weights (Table 2). However, the highest values of fresh weight were recorded with the plants exposed to ammonia gas only during the first experiment, while during the second experiment, recorded with the plants treated with GSH at 300mgL⁻¹ before exposure to ammonia gas (Table 2). Concerning the effects of antioxidant treatments on dry weights of croton plants, mostly of treated plants showed significant differences. But in general, the highest values of dry weights were recorded with the plants sprayed with GSH at 300mgL⁻¹ before exposure to ammonia.

2. Effects of treated pothos and croton plants with glutathione (GSH) and bilirubin (BI) before exposure to formaldehyde or ammonia gases on chlorophyll and carotenoids concentrations:

As the above results presented in Table (3), the pothos plants take the same trend in total chlorophyll and carotenoids when exposed to the formaldehyde and ammonia. The chlorophyll and carotenoids concentrations were, significantly decreased with exposing to ammonia comparing with the formaldehyde. Treated pothos plants with BI at 300mg L⁻¹, significantly gave the highest value of

total chlorophyll content during the two experiments. While, GSH at 300mg L⁻¹ led to increase the carotenoids concentration during the two experiments. Concerning the effects of interactions between the gas pollutants and antioxidants, the data presented in Table (3) shows that spraying plants with GSH at 300mg L⁻¹ before exposing to formaldehyde increased significantly chlorophyll concentration followed by treatment of BI at 300mg L⁻¹ with exposing to ammonia during the two experiments. Moreover, the highest values of carotenoids concentration were recorded with plants treated with the same treatments before exposed to formaldehyde or ammonia comparing with the other treatments during the two experiments.

Croton plants treated with GSH at 600mg L⁻¹ showed a significant increase in total chlorophyll and carotenoids under formaldehyde comparing with the other treatments during the two experiments (Table 4). Moreover, croton plants exposed to ammonia with or without antioxidants decreased in total chlorophyll and carotenoids concentrations, but plants treated with GSH 300mg L⁻¹ increased in chlorophyll and carotenoids during exposing to ammonia.

Table 4. Effects of treated croton plants with glutathione (GSH) and bilirubin (BI) on total chlorophyll and carotenoids concentrations under formaldehyde (Fmd) or ammonia (Amm) gases during the two experiments.

Parameters Treatments	Total chlorophyll mg/g F.W.		Total carotenoids mg/g F.W.	
	First experiment	Second experiment	First experiment	Second experiment
Control (non-polluted)	2.46 d	2.60 b	0.131 bc	0.140 bc
Fmd + GSH 600 mgL ⁻¹	3.18 a	3.35 a	0.281 a	0.328 a
Fmd + BI 300 mgL ⁻¹	2.71 c	2.29 c	0.180 b	0.173 b
Fmd+ BI600 mgL ⁻¹	2.30 e	1.67f	0.048 d	0.042 d
Ammonia (Amm)	1.62 g	1.58 g	0.105 cd	0.101 cd
Amm+ GSH 300 mgL ⁻¹	2.75 b	1.98 e	0.193 b	0.103 cd
Amm+ GSH 600 mgL ⁻¹	1.48 h	1.69 f	0.079 cd	0.064 d
Amm+ BI 600 mgL ⁻¹	1.99 f	2.12 d	0.091 cd	0.092 cd

Mean values followed by the same letter in each column are not different significantly at P≤0.05 based on Duncan's multiple range test. y : Control(non-polluted): plants not exposed to gas pollutants.

3. Effects of treated pothos and croton plants with glutathione (GSH) and bilirubin (BI) before exposure to formaldehyde or ammonia gases on histochemical analysis of reactive oxygen species (ROS):

The reactive oxygen species were imagined, using naked eyes, as purple discoloration for superoxide (O₂^{•-}) and as brown discoloration for hydrogen peroxide (H₂O₂) of

pothos and croton plants (Fig. 2 & 3). The brown discoloration in the pothos plants was clearly decreased when treated plants with GSH or BI before exposing them to the formaldehyde comparing with the other treatments. The brown and purple discolorations were highly increased with exposing plants to ammonia (Fig. 2).

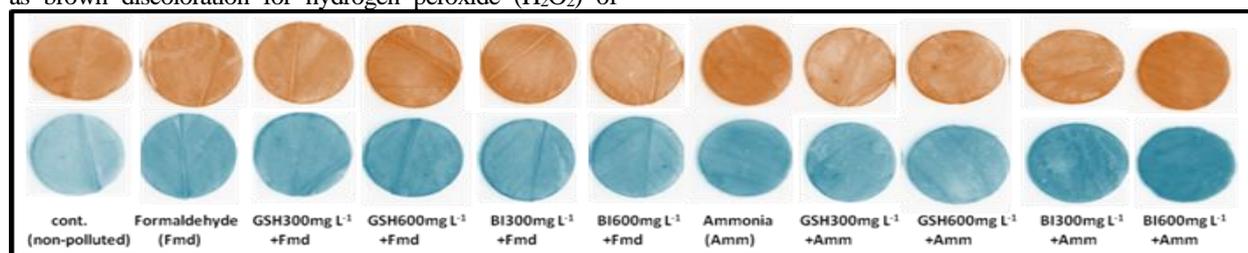


Fig. 2. Accumulation of reactive oxygen species (ROS), brown discoloration of hydrogen peroxide (H₂O₂) (upper row) and purple discoloration of superoxide(O₂^{•-}) (lower row) in pothos leaves treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases.

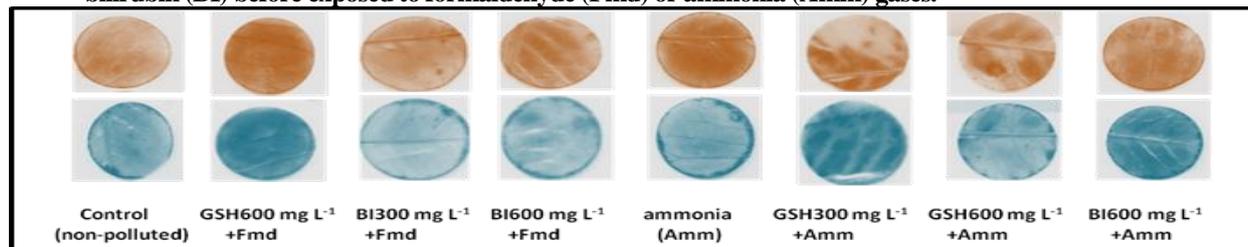


Fig. 3. Accumulation of reactive oxygen species (ROS), brown discoloration of hydrogen peroxide (H₂O₂) (upper row) and purple discoloration of superoxide (O₂^{•-}) (lower row) in croton leaves treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases.

For croton plants, spraying with BI at both concentrations was positively effects on decreasing the accumulation of the ROS of either H₂O₂ or O₂⁻ with exposing plants to formaldehyde. These are clearly show in the less of brown and purple discolorations as presented in Fig.(3). Also, the brown and purple discolorations were decreased with spraying plants with GSH at 600mg L⁻¹ before exposing them to ammonia. On the otherwise, these discolorations were highly increased when treated plants with GSH at 600mg L⁻¹ before exposing to the formaldehyde.

4. Effects of treated pothos and croton plants with glutathione (GSH) and bilirubin (BI) before exposure to formaldehyde or ammonia gases on antioxidant enzymes activities:

The activities of antioxidant enzymes of catalase (CAT), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and guaiacol peroxidase (GPOX) in pothos plants were impacted with gas pollution treatments (Fig. 4). The CAT activity was significantly increased when exposed pothos plants to the formaldehyde comparing with ammonia, while the GPOX activity was increased with ammonia. APX and PPO activities were non-significant between the two gases. Among all the antioxidants treatments (GSH and BI), the activity of CAT was increased significantly with GSH at 300mg L⁻¹ and with BI at 600mg L⁻¹ comparing with the control plants (non-polluted) and other treatments. On the other hand, all antioxidant treatments led to increase in the APX activity comparing with the control plants. The PPO activity was decreased among all antioxidant treatments comparing with the non-polluted plants. GPOX activity was highly increased with GSH at 600mg L⁻¹ comparing with the

non-polluted plants and with the other treatments. In focus on the effects of the interactions between exogenously application of antioxidants (GSH and BI) before exposing pothos plants to formaldehyde or ammonia on the activity of antioxidant enzymes, the data presented in Fig. (4) clearly indicate to significantly increased in CAT activity when spraying plants with GSH at 300mgL⁻¹ or with BI at 600mgL⁻¹ before exposing them to the formaldehyde comparing with the plants exposed to formaldehyde only or with the other treatments. It is worth mentioning that, the APX activity was increased in all polluted plants comparing with the non-polluted plants while the PPO activity was decreased in all treated plants except of the treatment of formaldehyde or ammonia only. Spraying plants with GSH at both concentrations, significantly increased the GPOX activity in plants exposed to ammonia followed by BI at 600mg L⁻¹ with plants exposed to either ammonia or formaldehyde gases.

In croton plants the activity of catalase (CAT) was decreased in all treated plants (Fig. 5) comparing with the control plants (non-polluted). On the otherwise, the ascorbate peroxidase (APX), significantly increased in all treated croton plants except for the treatment of BI at 600mg L⁻¹ under ammonia gas comparing with the control plants. Also, the GPOX activity showed the same trend which was increased in all treatments but the highest activity was recorded with croton plants treated with GSH or BI at 600mg L⁻¹ concentration before exposing them to ammonia gas. There were no differences in PPO activity among all treatments.

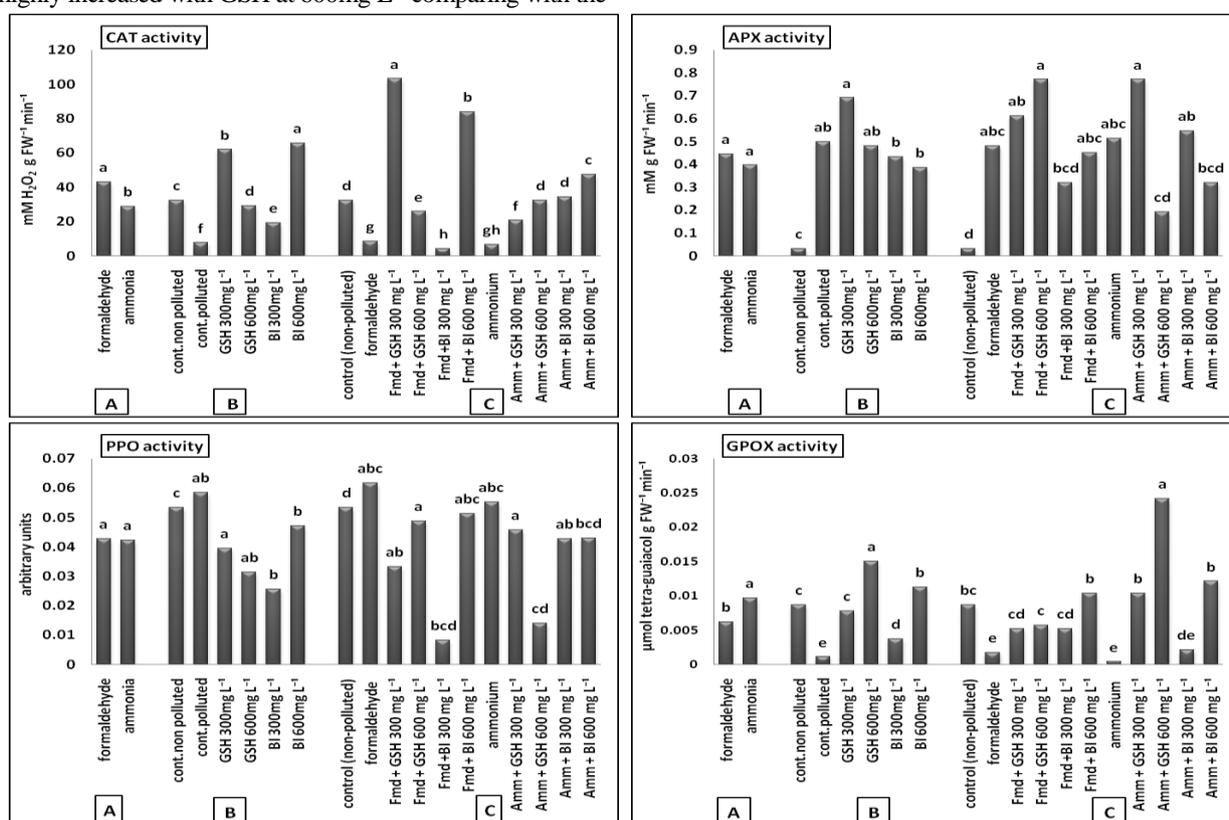


Fig. 4. Activities of antioxidant enzymes of catalase (CAT), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and guaiacol peroxidase (GPOX) in pothos plants treated with glutathione (GSH) and bilirubin (BI) under formaldehyde (Fmd) or ammonia (Amm) gases. A: gas pollutants, B: glutathione and bilirubin treatments, C: interaction between gas pollutants and glutathione or bilirubin. Treatments within each group (A, B or C) set marked with same letter are not different significantly at P≤0.05 based on Duncan's multiple range test, Control(non-polluted): plants not exposed to gas pollutants.

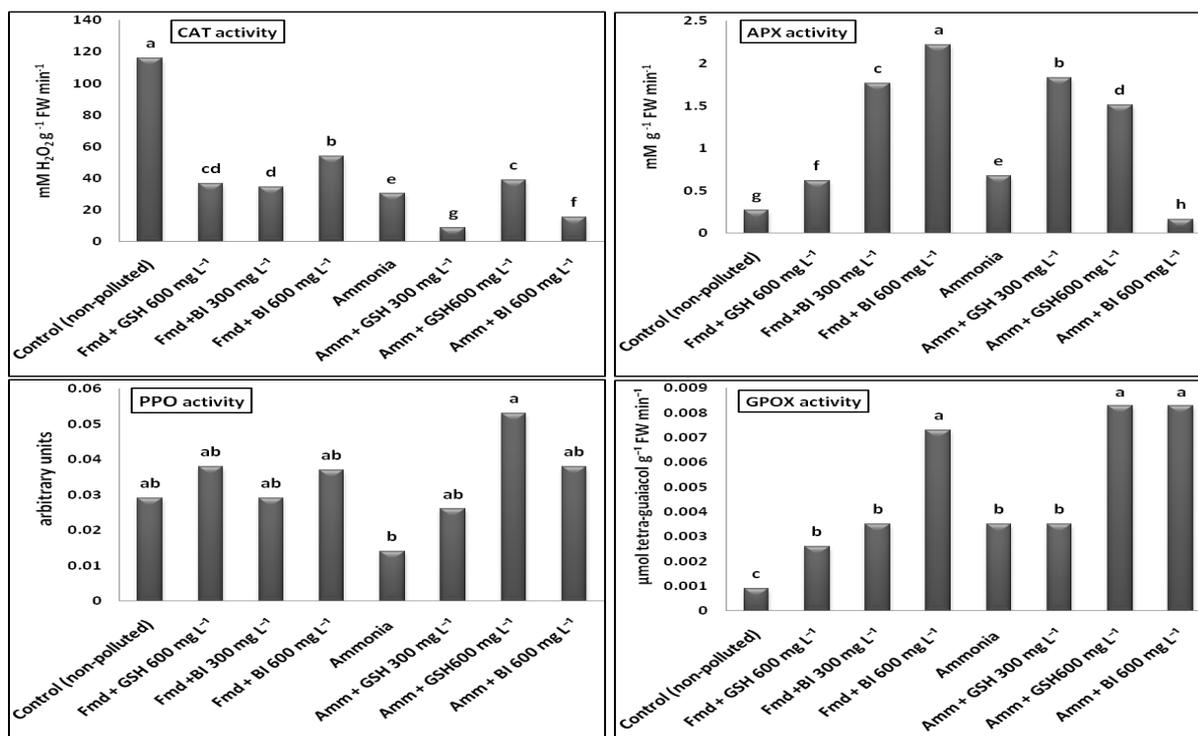


Fig. 5. Activities of antioxidant enzymes of catalase (CAT), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and guaiacol peroxidase (GPOX) in croton plants treated with glutathione (GSH) and bilirubin (BI) under formaldehyde (Fmd) or ammonia (Amm) gases.

Treatments within each group (A, B or C) set marked with same letters are not different significantly at $P \leq 0.05$ based on Duncan's multiple range test. Control (non-polluted): plants not exposed to gas pollutants.

5. Effects of treated pothos and croton plants with glutathione (GSH) and bilirubin (BI) before exposure to formaldehyde or ammonia gases on anatomical characters:

Scanning electron microscopy:

Plants exposed to formaldehyde or ammonia gases showed irregular and hetero-dimensional epidermal cells on both of the abaxial and adaxial surfaces (Figs. 6 & 7) comparing with the non-polluted plants. However, the adaxial surface of pothos plants treated with GSH at 600mg L⁻¹ before exposing to formaldehyde (Fig. 6, B) showing highly similar regular epidermal cells as shown in control (non-polluted) (Fig. 6, A) plants. As shown in Table (5) and Fig. (8) there were clearly decreased in stomata numbers and its dimensions and the stomata pore size dimensions in pothos plants exposed to formaldehyde or ammonia gases comparing with the control plants. In spite of that its clearly notice that treated pothos plants with GSH at 600mg L⁻¹ before exposure to formaldehyde or ammonia gases led to decrease the stomata pore size width (Table 5). Plants treated with BI at 600mg L⁻¹ before exposure to formaldehyde were the highest in both stomatal's length and width as well as stomata pore size width in spite of it were the lowest in stomata numbers comparing with the other treatments (Fig.

8, C). The guard cell width was decreased in all treated plants comparing with the non-polluted plants.

Concerning the effects of exposing croton plants to formaldehyde or ammonia gases on the adaxial surface there were partially irregular and hetero-dimensional epidermal cells as well as there were no stomata appeared on the surface (Fig., 9, B and C) comparing with the non-polluted plants (Fig., 9, A). On the contrary, there were a high appearance of stomata at the abaxial surface of treated croton plants with GSH or BI (Fig.,10, B and C). The number of stomata was the highest on the leaf abaxial surface of croton plants treated with GSH at 600mgL⁻¹ before exposure to ammonia gas (Table 6). However, these stomata were the lowest in dimensions (length and width) and were the highest in pore width. On the other hand, the plants treated with GSH at 600mgL⁻¹ before exposing to formaldehyde gas showed an increasing in stomata length and width. However, this trial led to increase the stomata pore width and the guard cell thickness (Table 6 and Fig., 11, B). Generally, the stomata were appeared, mostly, in partial close in all treated croton plants comparing with the control plants (Figs., 10 and 11).

Table 5. Effects of treated pothos plants with glutathione (GSH) and bilirubin (BI) on number of stomata, stomata dimensions, stomata pore size dimensions and guard cell width(µm) under formaldehyde (Fmd) or ammonia (Amm) gases.

Parameters Treatments	number of stomata	stomata dimensions		stomata pore size dimensions		guard cell width
		length	width	length	width	
control (non-polluted)	28	28.24	16.59	21.74	3.41	7.47
Fmd+GSH 600 mg L ⁻¹	17	25.68	10.99	18.18	1.84	4.42
Fmd+ BI 600 mg L ⁻¹	2	27.39	14.78	18.58	2.633	5.30
Amm+ GSH 600 mg L ⁻¹	3	26.04	11.70	16.51	1.29	5.50
Amm+ BI 600 mg L ⁻¹	23	25.43	9.95	11.52	2.47	4.03

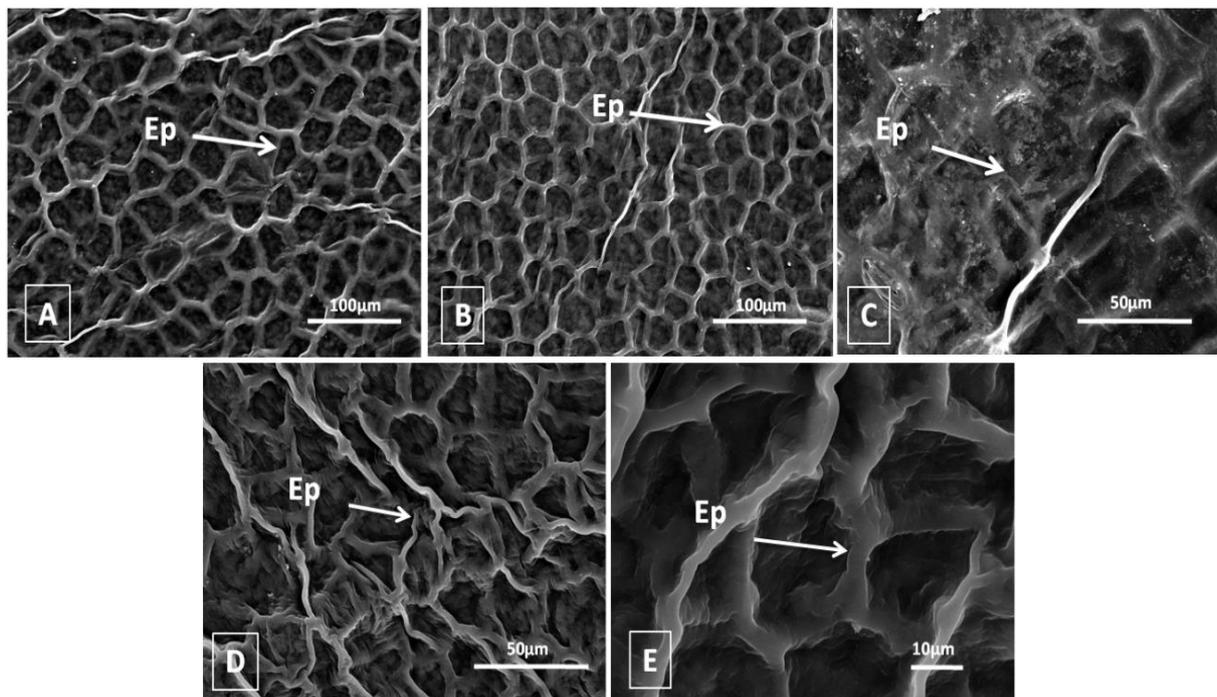


Fig. 6. Scanning electron microscopy of adaxial leaf epidermis of pothos plants treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. Arrows indicate to the regular, irregular and hetero-dimensional epidermal cells of the adaxial surface. A: control non-polluted (plants not exposed to (Fmd) or (Amm) gases). B: plants sprayed with GSH at 600mg L^{-1} before exposed to Fmd. C: plants sprayed with BI at 600mg L^{-1} before exposed to Fmd. D: plants sprayed with GSH at 600mg L^{-1} before exposed to Amm . E: plants sprayed with BI at 600mg L^{-1} before exposed to Amm. (Ep: epidermal cells) . (Magnification of adaxial epiderms: 200 X for A and B, 500X for C and D, 1000X for E).

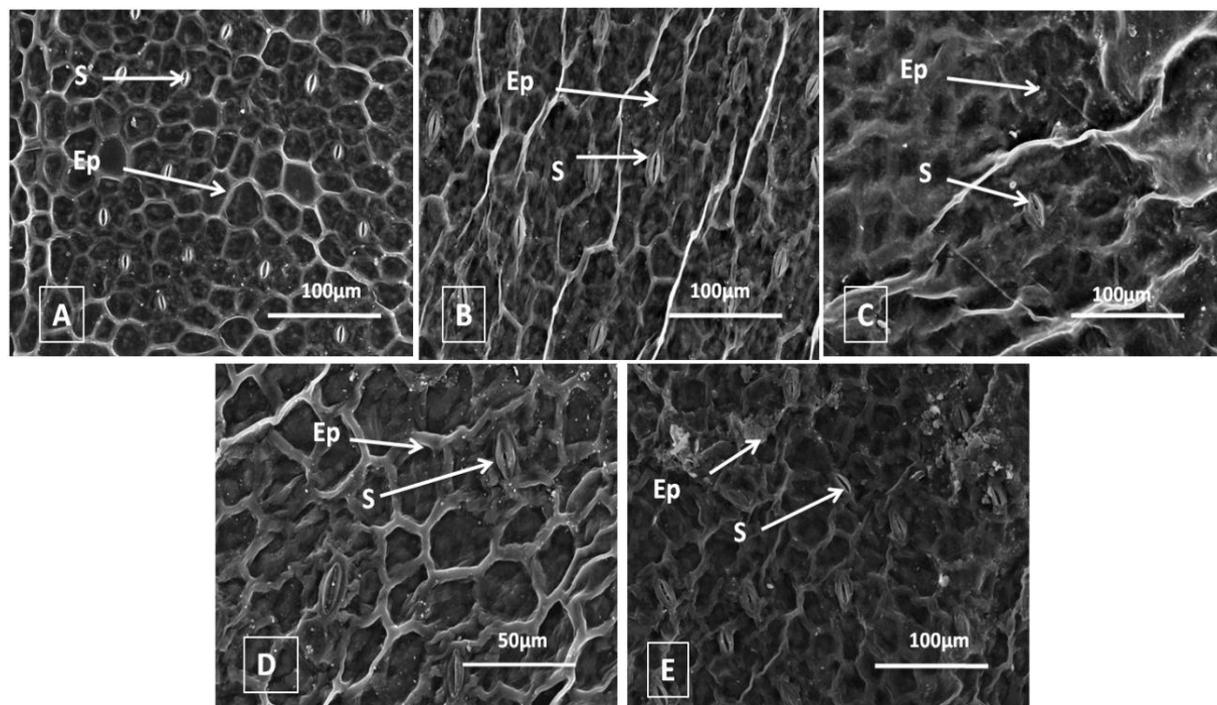


Fig. 7. Scanning electron microscopy of abaxial leaf epidermis of pothos plants treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. Arrows indicate to the regular, irregular and hetero-dimensional epidermal cells and the clogged and unclogged stomata of the abaxial surface. A: control non-polluted (plants not exposed to (Fmd) or (Amm) gases). B: plants sprayed with GSH at 600mg L^{-1} before exposed to Fmd. C: plants sprayed with BI at 600mg L^{-1} before exposed to Fmd. D: plants sprayed with GSH at 600mg L^{-1} before exposed to Amm . E: plants sprayed with BI at 600mg L^{-1} before exposed to Amm. (Ep: epidermal cells, S: stomata). (magnification of abaxial epiderms: 130 X for A, 250X for B and E, 200X for C, 500X for D).

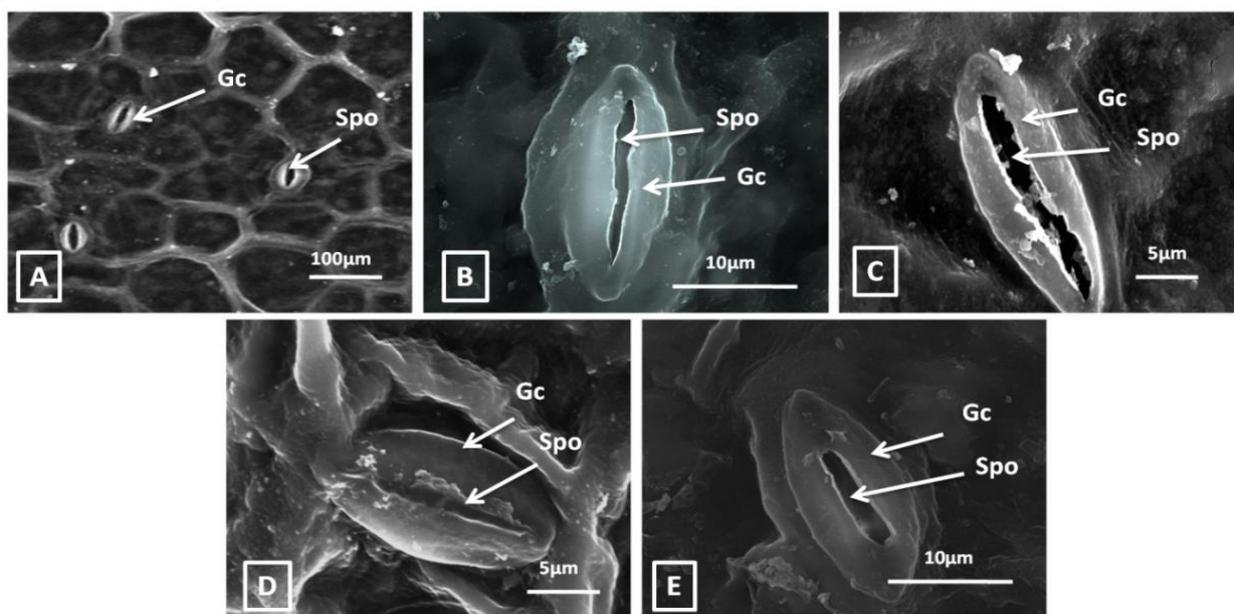


Fig. 8. Scanning electron microscopy of the stomata of pothos plants treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. Arrows indicate to the guard cell, stomata pore, and the clogged and unclogged stomata of the abaxial surface. A: control non-polluted (plants not exposed to (Fmd) or (Amm) gases). B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with BI at 600mg L⁻¹ before exposed to Fmd. D: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm . E: plants sprayed with BI at 600mg L⁻¹ before exposed to Amm. (S: stomata, Gc: guard cell; Spo: stomata pore). (magnification for stomata: 1000X for A, 2500X for B and E, 2700X for C and 3000X for D).

Table 6. Effects of treated croton plants with glutathione (GSH) on number of stomata, stomata dimensions, stomata pore dimensions and guard cell thickness (µm) of the abaxial surface under formaldehyde (Fmd) or ammonia (Amm) gas.

Parameters Treatments	stomata numbers	stomata dimensions		stomata pore size dimensions		guard cell width
		length	Width	length	width	
control (non-polluted)	37	20.00	13.44	5.56	3.44	6.25
Fmd+GSH 600 mgL ⁻¹	45	18.26	12.34	7.21	4.22	4.09
Amm+ GSH 600 mgL ⁻¹	83	17.35	10.28	9.17	2.64	3.56

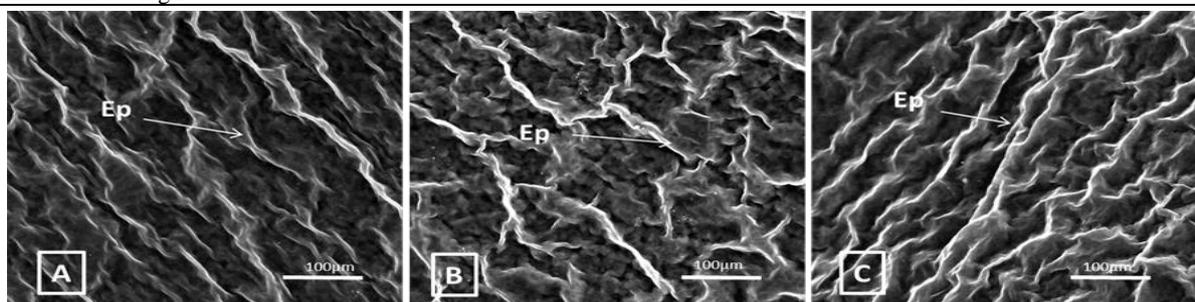


Fig. 9. Scanning electron microscopy of the adaxial epidermis cells of croton plants treated with glutathione (GSH) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. Arrows indicate to the regular, irregular and hetero-dimensional epidermal cells. A: control non-polluted (plants not exposed to (Fmd) or (Amm) gases). B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm . (Ep: epidermal cells). (Magnification: 150x for A and B, 110x for C).

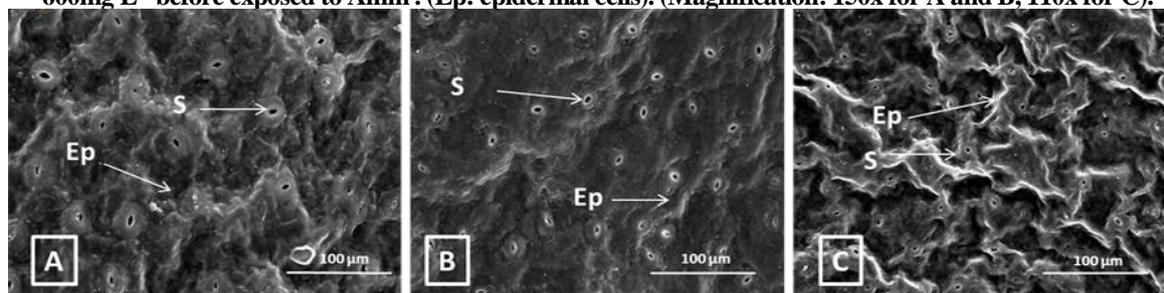


Fig. 10. Scanning electron microscopy of the abaxial epidermis cells of croton plants treated with glutathione (GSH) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. Arrows indicate to the regular, irregular and hetero-dimensional epidermal cells and the clogged and unclogged stomata . A: control non-polluted (plants not exposed to (Fmd) or (Amm) gases). B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm . (S: stomata, Ep: epidermal cells). (Magnification: 250x for A, 200x for B, 150x for C).

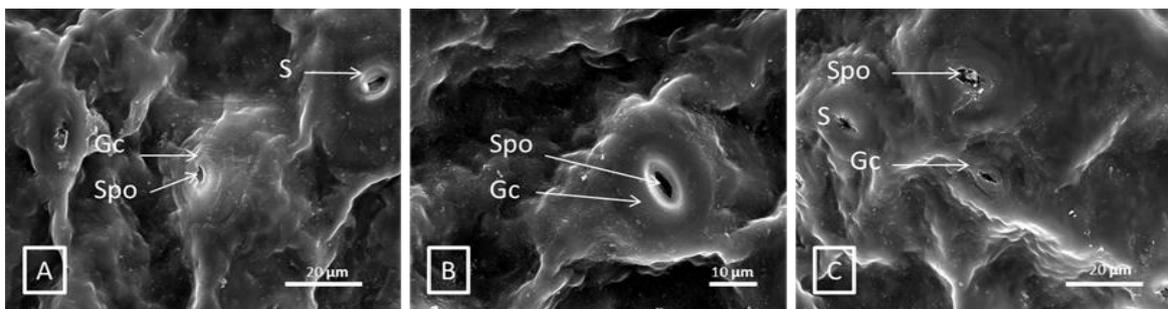


Fig. 11. Scanning electron microscopy of the stomata of croton plants treated with glutathione (GSH) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. Arrows indicate to the guard cell, stomata pore, and the clogged and unclogged stomata of the abaxial surface. **A:** control non-polluted (plants not exposed to (Fmd) or (Amm) gases). **B:** plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. **C:** plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm . (S: stomata, Gc: guard cell, Spo: stomata pore). (Magnification: 800x for A, 1000x for B, 700x for C).

Transmission electron microscopy:

Air pollution stress clearly caused changes in the structure of mesophyll cells and chloroplasts as well as the thylakoids structure comparing to the non-polluted plants (control). The mesophyll cells of pothos plants exposed to ammonia gas appeared in wavy shape membranes with dark

deposits areas (Fig. 12,E). Additionally, the chloroplasts were separated from the plasma membrane in pothos plants exposed to ammonia gas (Fig. 12,D & E). In contrast, the plants treated with the GSH at 600mg L⁻¹ and exposed to the both gases that were contacted with the membrane (Fig. 12, B & D).

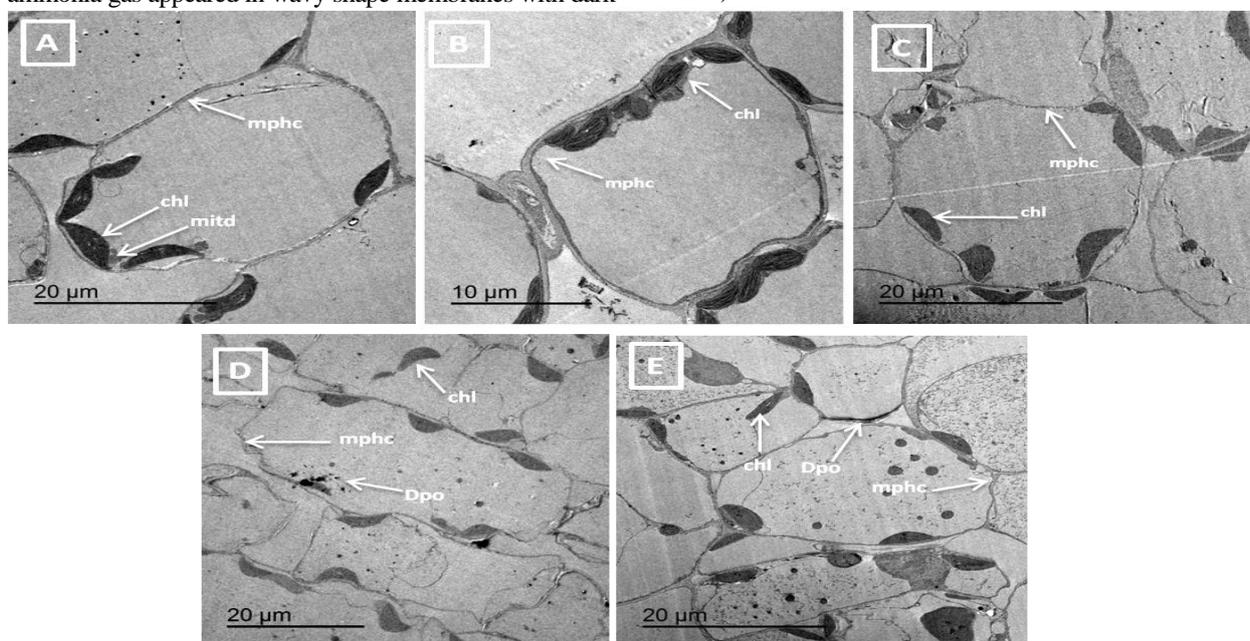


Fig. 12. Transmission electron microscopy of the mesophyll cells of pothos plants treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. **A:** control non-polluted (plants not exposed to (Fmd) or (Amm) gases). **B:** plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. **C:** plants sprayed with BI at 600mg L⁻¹ before exposed to Fmd. **D:** plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm . **E:** plants sprayed with BI at 600mg L⁻¹ before exposed to Amm. (mphc: mesophyll cell membrane, mitd: mitochondria, chl: chloroplast, Dpo: dark deposits). (Magnification: 400x for A, C and E, 600x for B, 300x for D).

Additionally, there were clearly showed numerous osmiophilic plastoglobuli within chloroplasts with BI treatments under ammonia and formaldehyde gases (Fig. 13, C and E). Moreover, the internal lamellae of stromal thylakoids were damaged with disintegration of the stacked granal thylakoid (Fig. 14). In general, the pothos plants which treated with GSH at 600mg L⁻¹ before exposing to formaldehyde gas had oval and normal chloroplasts and contained numerous well compartmentalized grana stacks as similar to the control plants (non-polluted) (Figs. 13,B & 14, B).

Concerning the effects of treated croton plants with GSH with exposure to formaldehyde or ammonia gases on the mesophyll cells and chloroplasts structures, it is clearly noticed from Fig. (15, B & C) that the mesophyll cells were larger in size with naturally walls as well as the chloroplasts

were fusiform and contacted with the plasma membrane under formaldehyde comparing with plants under ammonia gas. Moreover, there were noticed a little of plastoglobuli under formaldehyde gas and a dark deposits areas within the mesophyll cells under ammonia gas. Additionally, there were a numerous of brown big starch grains and a large numbers of mitochondria under both of gases (Fig 16, B & C). Also, the internal lamellae of stroma thylakoids were slightly damaged and the stacked grana thylakoids were clear and partly folded regularly of treated plants with GSH under Fmd (Fig. 17, B). In contrast, the chloroplasts of croton plants exposed to ammonia gas, became swelling with degradation of the outer membrane and the grana thylakoids were apparently rupture and disorganized with fewer thylakoids stocks (Fig., 17, C).

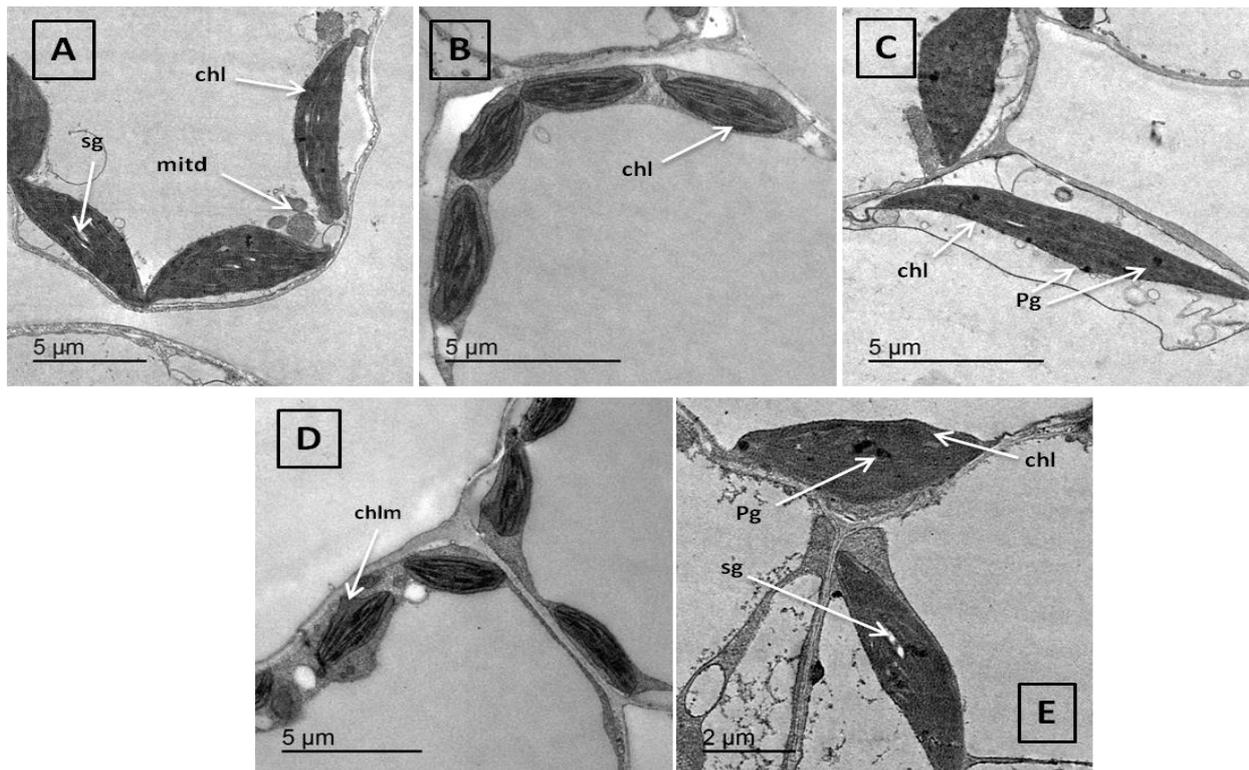


Fig. 13. Transmision electron microscopy of the chloroplasts of pothos plants treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehide (Fmd) or ammonia (Amm) gases. A: control non-polluted (plants not exposed to (Fmd) or (Amm) gases).. B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with BI at 600mg L⁻¹ before exposed to Fmd. D: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm . E: plants sprayed with BI at 600mg L⁻¹ before exposed to Amm. (chl: chloroplast, Pg: plastoglobuli, chlm: chloroplast membrane, mitd: mitochonderia, sg: starch grain). (Magnification: 800x for A, C and E, 1500x for B, 1200x for D).

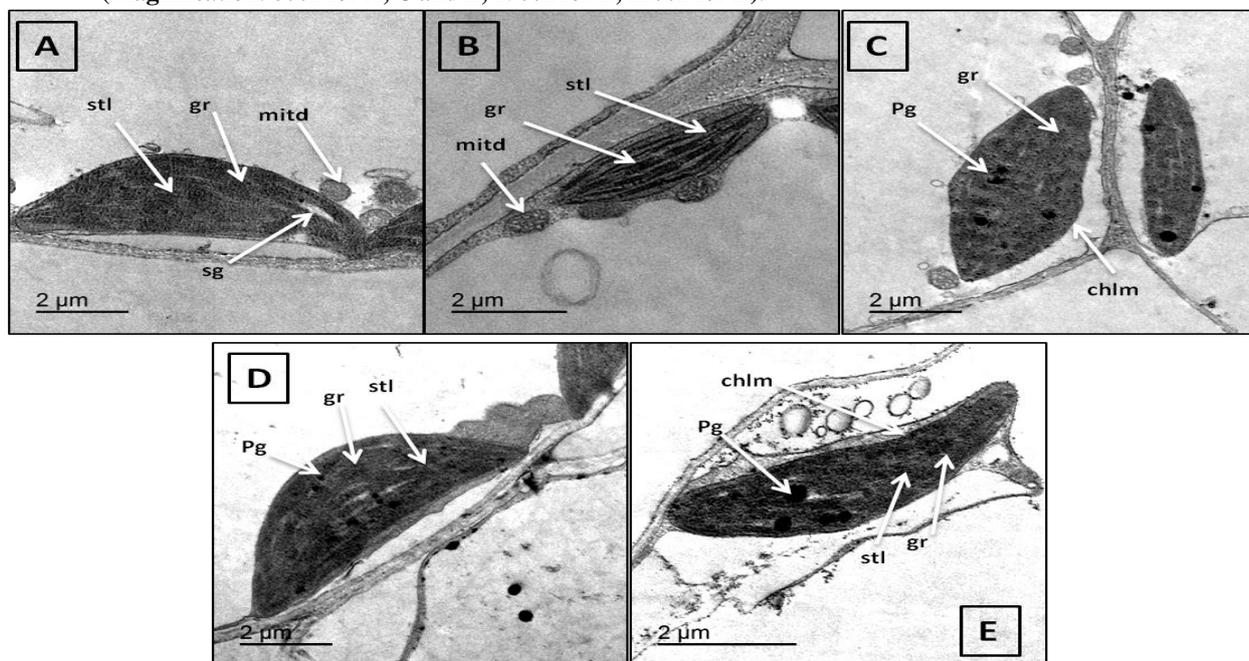


Fig. 14. Transmision electron microscopy of the thylakoids structure of pothos plants treated with glutathione (GSH) and bilirubin (BI) before exposed to formaldehide (Fmd) or ammonia (Amm) gases. A: control non-polluted (plants not exposed to (Fmd) or (Amm) gases).. B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with BI at 600mg L⁻¹ before exposed to Fmd. D: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm . E: plants sprayed with BI at 600mg L⁻¹ before exposed to Amm. (Pg: plastoglobuli, chlm: chloroplast membrane, mitd: mitochonderia, sg: starch grain, stl: stroma lamellae, gr: granum). (Magnification: 2000x for A, C and D, 2500x for B, 3000x for E).

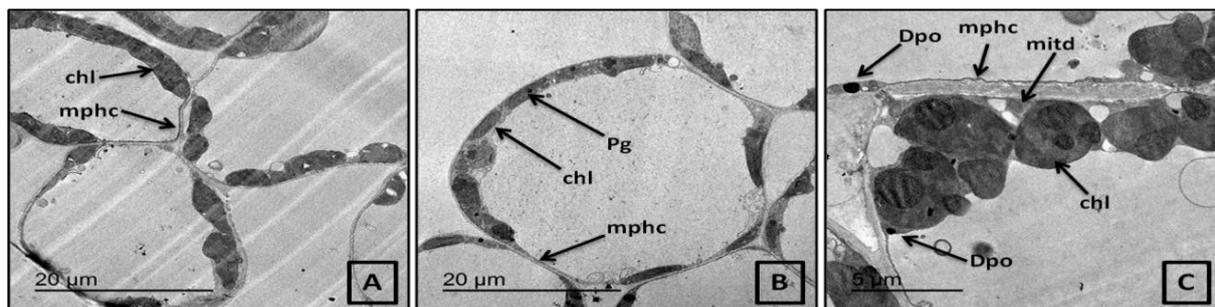


Fig. 15. Transmission electron microscopy of the mesophyll cells of croton plants treated with glutathione (GSH) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. A: control non-polluted (plants not exposed to (Fmd) or (Amm)). B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm. (mphc: mesophyll cell wall, chl: chloroplast, Pg: plastoglobuli, mitd: mitochondria, Dpo: dark deposits areas). (Magnification: 400x for A, and B, 1000x for C).

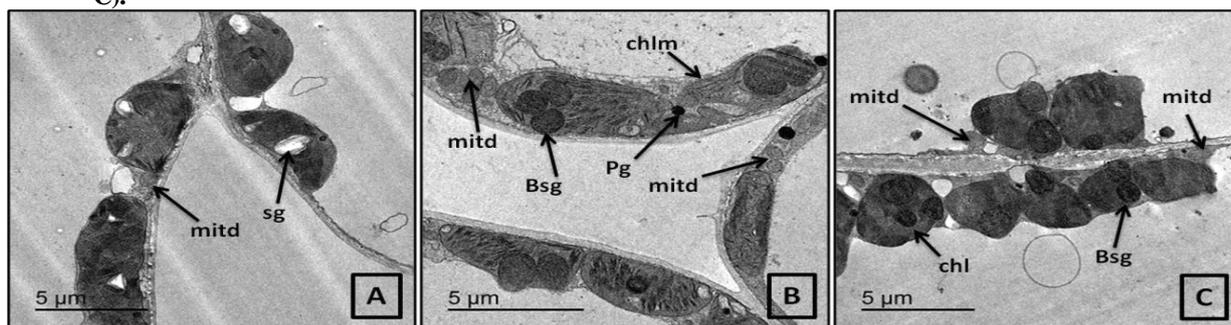


Fig. 16. Transmission electron microscopy of the chloroplast of croton plants treated with glutathione (GSH) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. A: control non-polluted (plants not exposed to (Fmd) or (Amm)). B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm. (chl: chloroplast, sg: starch grain, Bsg: brown starch grain, Pg: plastoglobuli, mitd: mitochondria, chlm: chloroplast membrane). (Magnification: 1000x for A, and C, 1200x for B).

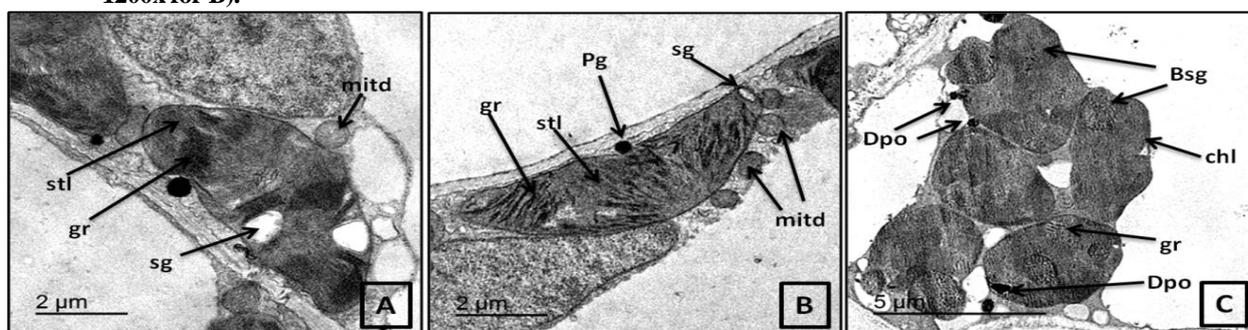


Fig. 17. Transmission electron microscopy of the thylakoids of croton plants treated with glutathione (GSH) before exposed to formaldehyde (Fmd) or ammonia (Amm) gases. A: control non-polluted (plants not exposed to (Fmd) or (Amm)). B: plants sprayed with GSH at 600mg L⁻¹ before exposed to Fmd. C: plants sprayed with GSH at 600mg L⁻¹ before exposed to Amm. (Pg: plastoglobuli, chlm: chloroplast membrane, mitd: mitochondria, sg: starch grain, stl: stroma lamellae, gr: granum, Bsg: brown starch grain, Dpo: dark deposits areas). (Magnification: 2000x for A, and B, 1500x for C).

Discussion

The presented results showed that pothos and croton plants exposed to ammonia gas were extremely injured and highly number of dead leaves comparing with the formaldehyde gas pollutant. Fresh and dry weights were decreased with exposing plants to ammonia gas as well as total chlorophyll and carotenoids. These results mainly attributed to the leaf injury caused by pollutants which lead to decreasing in photosynthetic activity, stomata damage, and premature senescence, disarrange membrane permeability and reduce growth and yield in some plant species (Tiwari *et al.* 2006). Also, exposing plants to air

pollutants may be induced some physiological changes in most plants before exhibiting visible damage to leaves (Liu & Ding, 2008). Joshi and Swami, (2009) reported that when plants are absorbed air pollutants by their leaves, it may be resulted in reducing the concentration of photosynthetic pigments which directly affected to the plant quality and productivity. Plants can directly absorb formaldehyde at low level (Wolverton, 1988) and transform into organic acids, sugars or CO₂ and H₂O (Wei *et al.*, 2017). In this investigation, spraying pothos and croton plants with non-enzymatic antioxidants (GSH and BI) led to increase their ability to absorb the air gas pollutants. The good tolerance of pothos and croton plants to formaldehyde gas related to the

ability of GSH to detoxify formaldehyde to formate and further to carbon dioxide (Tada and Kidu, 2011). Data recorded in present investigation clearly show that treated pothos and croton plants with GSH or BI before exposing to air pollutants decreased the ROS generation and increased the total chlorophyll and carotenoids contents which reflected on increasing their abilities to resistance to the formaldehyde gas. This attributed to the ability of GSH on scavenging the excessive ROS production during exposing plants to air gas pollutants (Gupta and Sharma, 2006). It can directly function as a free radical scavenger by reacting with $^1\text{O}_2$, O_2^- and HO^\cdot . In this concern, Hasanuzzaman *et al.* (2013) reported that increasing endogenous GSH level to adequate concentration helps plants to tolerate oxidative air pollution stress.

Concerning the effects of BI (BI) on plant resistance to air gas pollutants, the pothos plants were greatly resistance with more growth efficiency only at the high concentration of BI ($600\text{mg}^{-1}\text{L}$) when exposed to gas formaldehyde. These results may be attributed to the role of BI in detoxify and scavenging the ROS produced during stress conditions (Daridon and Veyrier, 2013). BI play a role in regulating against various abiotic stresses in plants (Wang and Liao, 2016). Moreover, carotenoids were considered as natural antioxidants in plants and it have an important role in air pollution resistance. The function of carotenoids in chloroplasts is as pigments to capture the light but it may contribute to protect the plant cells and tissues against the deleterious effects of free radical oxidative (Flechin *et al.*, 2003). When exposed plants to ammonia gas led to decrease the total chlorophyll and carotenoids contents. Tripathi and Gautam, (2007) reported that carotenoids content reduced under air pollution.

Furthermore, exogenously with GSH or BI led to increase the GOPX, CAT and APX synthesis before exposed pothos plants to formaldehyde or ammonia gases compared with control polluted plants. These reactions may be attributed to the function of GSH and BI of scavenging the excessive of ROS and/or increasing the antioxidant enzymes activities or synthesis (Daridon and Veyrier, 2013). When activities of antioxidant enzymes increase, the tolerance of plants would be enhanced against oxidative stress (Korniyev *et al.*, 2003). Gupta and Sharma (2006) reported that catalase (CAT) mainly acts by catalyzing the decomposition of hydrogen peroxide (H_2O_2) to H_2O and O_2 . Catalase and ascorbate peroxidase (APX) can completely reduce (H_2O_2) to H_2O (Edreva, 2005). Bamniya *et al.* (2012) reported that polyphenol oxidase enzyme activity was a minimum in low pollution areas and increased under pollution stress. Sofó *et al.* (2005) suggested that the decreasing in PPO activity following abiotic stress was associated with improved antioxidant capacity. This is in agreement with the present investigation on pothos and croton plants as the PPO activity was reduced when treated plants with non-enzymatic enzymes of GSH and BI in comparing with the polluted control plants. In the present investigation, the bad effects of BI were associated with the increasing in ROS combined with the decreasing in contents of antioxidant enzymes: CAT, PPO and GPX which led to disrupt the equilibrium between ROS generation and detoxification Moller *et al.*, (2007).

The results presented here clearly demonstrated that chloroplasts composition and stomatal density were injured with exposing croton and pothos plants to formaldehyde and

ammonia. Pollution stress changed the leaf anatomy of *Plantago lanceolata* plants (Irina, 2009). Verma *et al.* (2006) reported that stomatal density were significantly decreased of *Ipomea pestigridis* grown under various degrees of coal-smoke pollutants. The decrease of the stomatal size may be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis (Verma *et al.*, 2006). In plants under air pollution stress, decreasing of stomata density can decrease the entry of pollutants, whereas its increase, almost always followed by a reduction of stomata size, represents a way to maximize its closure efficiency, an important feature for plants under air contaminants (Larcher, 2000). Spraying pothos and croton plants with GSH and BI resulted in increasing the plant resistance to formaldehyde or ammonia gases which clearly appearance in partially opening of stomata and moderately decrease in number of stomata. Additionally, stomatal pore dimensions in the presently investigated pothos plants were also reduced when treated plants with GSH or BI before exposing to ammonia or formaldehyde. Ahmad *et al.*, (2005) revealed that stomatal length and width and its number showed a significant reduction of *Trigonella foenum graecum* under metal stress. Pourkhabbaz *et al.*, (2010) noticed that the guard cells appeared more shrunken on the polluted leaves. The reduction of the stomatal pore dimensions under pollution stress as observed during the present investigation, it has been regarded as a protective measure against the pollutants (Gupta and Ghouse, 1987) as well as the decrease in stomatal size opening or the stomatal closure may be consider as an avoidance mechanism the action of the pollutants (Ahmad *et al.*, 2005). It was noticed in this investigation that spraying pothos and croton plants with GSH before exposing to ammonia or formaldehyde gases maintenance the mesophyll cells and chloroplasts structures including: thylakoid membranes, granum and stroma lamellae. Photosynthesis complement is generally induced in thylakoid membranes of the chloroplasts. So, the thylakoids structural is a major factor that affects functionality and efficiency of the photosynthetic apparatus (Ioannidis *et al.* 2009). The chloroplast and mitochondria are the major sources of ROS production in plants (Rathore and Chaudhary, 2019; Das and Roychoudhury, 2014). ROS induced oxidative damage to the main components of the cells (Hasanuzzaman *et al.* 2017). GSH in chloroplast plays an important role in the organelle's protection from possible oxidative damages caused by ROS (Pietrini *et al.* 2003). In fact, during stress conditions that induce high ROS production in chloroplasts (Pospisil, 2012), GSH accumulates not only in the stroma but also in the thylakoid lumen (Zechmann, 2014). Therefore, it should be protected the chloroplasts and mitochondria through exogenously of GSH and BI which scavenging the excessive production of ROS and consequently increasing the plant resistance to air pollution. It is evident from the above results that the indoor gas pollutants (formaldehyde and ammonia gases) cause physiological and anatomical changes of pothos and croton plants. The study elucidates that spraying pothos and croton plants with GSH at 600mgL^{-1} before exposure to formaldehyde or ammonia gas increased the plant tolerance to air pollution stress. Additionally, the pothos and croton plants can extremely resistance to the formaldehyde gas comparing with the ammonia gas pollutant.

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رش نباتات البوتس والكروتين بالجلوتاثيون والبليروبين لتنقية الهواء الداخلي من الملوثات. نعيمه إسماعيل السيد إسماعيل قسم بحوث نباتات الزينة وتنسيق الحدائق- معهد بحوث البساتين- مركز البحوث الزراعية- مصر

اعتبرت منظمات الصحة العالمية أن تلوث الهواء الداخلي أحد أهم المخاطر على حياة الإنسان. من أجل ذلك فإن هناك اتجاه عالمي لزيادة المساحات الخضراء على كوكب الأرض وكذلك زيادة الوعي عند الناس لأهمية نباتات التنسيق الداخلي لما لها من دور كبير في تنقية الهواء الداخلي من ملوثات الهواء. ولذلك أجرى هذا البحث في شمبر زجاجي لدراسة تأثير رش نباتات البوتس والكروتين بمضادات الأكسدة الغير انزيمية: الجلوتاثيون (GSH) والبليروبين (BI) بتركيزات 300 ملجرام/لتر و 600 ملجرام/لتر على زيادة مقاومة وقدرة تلك النباتات على إمتصاص غازات الفورمالدهيد والأمونيا الملوثة للهواء. أوضحت النتائج أن نباتات البوتس والكروتين التي عرضت لغاز الأمونيا قد تضررت بشدة وكانت الأعلى في عدد الأوراق الميتة مع النقص الشديد في الأوزان الطازجة والجافة بالإضافة إلى المحتوى الكلي من الكلوروفيل والكاروتين مقارنة بالنباتات التي عرضت لغاز الفورمالدهيد. معاملة نباتات البوتس والكروتين بالجلوتاثيون أو البليروبين قبل تعرضها لملوثات الهواء أدت إلى نقص أنواع الأكسجين الحرة (ROS) مع زيادة نشاط إنزيم البيروكسيديز ونقص نشاط إنزيم البولي فينول. وقد لوحظ أن عدد الثغور وحجم فتحة الثغر وتركيب البلاستيدات الخضراء قد تضررت نتيجة تعرض نباتات البوتس والكروتين لغازات الفورمالدهيد والأمونيا. وقد وجد أن نباتات البوتس كانت أكثر مقاومة مع زيادة كفاءة النمو مع التركيز العالي من المعاملة بالبليروبين (600 ملجرام/لتر) قبل التعرض لغاز الفورمالدهيد. بوجه عام أدى رش نباتات البوتس والكروتين بالجلوتاثيون بتركيز (600 ملجرام/لتر) قبل التعرض لملوثات الهواء الغازية إلى زيادة مقاومة النباتات لإجهاد التلوث الهوائي. بالإضافة إلى ذلك تستطيع نباتات البوتس والكروتين أن تقاوم إلى حد كبير غاز الفورمالدهيد مقارنة بغاز الأمونيا.