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Purification of Indoor Air from Pollutants by Areca Palm (*Chrysalidocarpus lutescens* L.) Treated with some Non-Enzymatic Antioxidants

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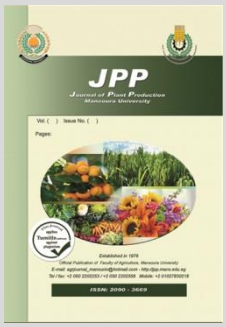

ABSTRACT

Indoor plants play an important role in purification the indoor air pollutants. Therefore, the main objective of this investigation was to increase the resistance of areca palm plants to the excessive concentrations of air gaseous pollutants. The present study was carried out in plastic chambers (80cm length×80cm width×100cm height) for exposing plants to formaldehyde or ammonia gasses after spraying with non-enzymatic antioxidants (glutathione and bilirubin at 300mg L^{-1} and 600mg L^{-1}). Plants exposed to ammonia were highest in the number of dead leaves and leave injury percentage comparing with the plants exposed to formaldehyde. Plants treated with glutathione at 600 mg L^{-1} or bilirubin at 300mg L^{-1} before exposure to formaldehyde gas were not injured leaves, as well as, increased in dry weight and chlorophyll concentration compared with the plants exposed to formaldehyde gas without spraying with antioxidants. Spraying plants with bilirubin at 300mg L^{-1} before exposure to formaldehyde were increased in total carotenoids as well as increased guaiacol peroxidase (GOPX) and catalase (CAT) activities under formaldehyde whereas it increased the activeties of ascorbate peroxidase (APX) and polyphenol oxidase (PPO) under ammonia. Also, plants treated with bilirubin or glutathione increased in stomata density and pore length under formaldehyde gas. In conclusion, spraying areca plants with bilirubin at 300 mg L^{-1} enhanced the plant resistance to the formaldehyde and ammonia air pollutants followed by glutathione treatments. Areca palm plants were higher sensitivity to ammonia gas than with formaldehyde gas.

Keywords: indoor air pollution, formaldehyde, ammonia, areca palm.

INTRODUCTION

Some indoor plants can be safely exploited as a good Bio-purification system for reducing indoor air pollution (Jim and Chen, 2008). Moreover, it more effective on absorbing pollutants, inexpensive and require no electricity to operate it comparing with the air purifiers and filters which consume electricity and frequently need maintenance. Also, ornamental plants have psychological effects on human as it can be reduced psychological stress and increased tolerance to the illness pain (Bringslimark *et al*., 2009).

Areca Palm (*Chrysalidocarpus Lutescens* L.) is a member of the *Arecaceae* family and it is widely used as indoor plant. This plant with its feathery fronds is best known as a humidifier. Although the plant grows slowly and needs to care yearly, it can be kept in any corner in the workplace, hospitals, schools, or anywhere in houses (especially next to newly varnished furniture or carpeted areas). The areca palm helps remove deadly toxins like formaldehyde and xylene.

Volatile organic compounds (VOCs) considered as one of the pollutants that is widely found indoors (Wolkoff *et al*., 2006) ,a problem that is aggravated by the decreased air exchange in newer, more tightly constructed buildings. VOCs are emitted by various types of products that can be found indoors, such as paints and lacquers, cleaning agents, building materials and new furnishings, office equipment like printers and copiers, carbonless copy paper and correction fluids, permanent markers, and photographic solutions (Jones, 1999; Zabiegała, 2006). The most common VOCs sources are formaldehyde, benzene, trichloroethylene and methane. Formaldehyde is a popular raw material used in the manufacture of urea-type or phenol-type synthetic resins, both of which are used as sticky agents in manufacturing plywood, flooring, particleboard and fiberboard. Urea-formaldehyde resin also is used in textile synthesis for clothing, as a preservative in paper and as a foam-type heat insulator. Furthermore, formaldehyde is often added to products, such as wall-paper, adhesive agents, cosmetics and detergents, as a preservative or fungicide. As the ambient temperature is increased, formaldehyde volatilization and decomposition from these products is increased and liberate formaldehyde, which is released into room air. Also, formaldehyde concentration up to the high level with Cigarette smoke and combustion exhaust gas and reach several times greater than those present in outdoor environment (Sakamoto *et al*., 1999). Formaldehyde has been considered as a probable human carcinogen. Ammonia (NH_3) is a common substance that occurs naturally and manufactured. Pure ammonia is a colorless, pungent-smelling and caustic gas. It is easily soluble in water and reacts with acids to form ammonium salts. It is used for bleaching or cleaning and in the production of fertilizers, pharmaceuticals and plastics. Ammonia is released with the decomposition of organic matter, combustion and manmade sources. Gaseous ammonia in

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the atmosphere leads to the secondary inorganic aerosol formation of ammonium compounds, including ammonium sulfate, ammonium bisulfate and ammonium nitrate (Henze *et al*., 2009) which are the main compositions of the fine particulate matter ($PM_{2.5}\mu m$) which affects of the air quality and human health (World Health Organisation, 2013).

Glutathione and bilirubin are considered as a non-enzymatic antioxidants and come under the second line defense, because they able to scavenge directly or indirectly reactive oxygen species (ROS). GSH is contributed in the detoxification of different toxic compounds, such as herbicides and air pollutants (Cummins *et al*., 2011). Bilirubin antioxidant activity is predominantly due to their ability to scavenge free radical species such as superoxide anion and peroxide radicals (Farrera *et al*., 1994)

The aim of our study was to evaluate the ability of areca palm plants to purify indoor air pollutants with formaldehyde and ammonia gases under controlled conditions that also allow determination of physiological effects of these gaseous pollutants on plants.

MATERIALS AND METHODS

The pot experiment was conducted at Mansoura Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center, Egypt in clear, plastic chambers during 2017 season to evaluate the areca palm plant's ability to purify air from formaldehyde and ammonia gases after spraying with glutathione or bilirubin antioxidants.

1. Plant materials:

Areca plants (*Chrysalidocarpus Lutescens* L.) family *Arecacea* were used as indoor plant in this investigation. Plants were obtained from the commercial nurseries in a retail-ready stage and transplanted individually in 20cm pots in diameter. The average number of leaves was 4±1 leaf/plant. Plants were acclimatized to the same interior environment condition used for 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.

2. Antioxidants:

Plants sprayed with glutathione reduced ($C_{10}H_{17}N_3O_6S$) and bilirubin ($C_{33}H_{36}N_4O_6$) at 300 mgL^{-1} and 600 mgL^{-1} concentrations in twice time before exposed to air gas pollutants: the first time before 6 days and the second time before one day before the beginning of gas pollutants treatments. The antioxidant solutions were prepared by using distilled water. The bilirubin solution was prepared by solute 0.3 or 0.6g in 1ml methyl alcohol before adding the distilled water. Tween 20, (polyoxyethylenesorbitan monolaurate), at 0.02% was added to the solutions as a surfactant.

3. Air pollutant gases:

Plants were exposed to the gases pollutantes (formaldehyde (HCHO) or ammonia (NH_3)) for 4 hrs daily for 10 days. Plants were exposed to the gas pollutants by injecting 7ml of formaldehyde solution (34-38%) or 1ml of ammonia solution (25%) on glass-petri dish into the chambers. Air circulation within the chambers was continued through half hour at the beginning of each trial period.

4. Test chamber layout:

Clear, plastic chambers were used to investigate the effects of exposed areca plants to gas pollutants of formaldehyde and ammonia. Chambers were made perfectly airtight. The construction of the chambers was as the following dimensions: 80*80*100cm. The chamber's frame was made of wood and covered with 100 micron clear plastic. The chamber's front cover was removable for plant loading and fitted with transparent adhesive tape and bolts and wing-nuts to ensured complete sealing of the front cover and to provide an airtight seal of chambers for testing. 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. The white fluorescent lamps Light levels at 1150±50 lux were used as the light source for 12 hrs daily.

The treatments were arranged as follow:

1- Control non-polluted: the plants were not exposed to gases and not sprayed with antioxidants.
2- Plants exposed to formaldehyde gas.
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.
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.

At the end of the experiment, the treatments numbers of 9 and 11 were died or not enough number of leaves for data analysis.

5. Measurements: The biomasses above ground were decapitated at the surface of the pot medium at the end of the experiments for estimating the morphological and physiological parameters.

Morphological parameters:

Mean of one leaf area: it was calculated according to Ferreira and Rasband (2012).

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

Percentage (%) of area injury/leaf: it was calculated as the following formula:

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

Fresh and dry weights: it were estimated by weight the above ground biomass just after decapitated them for fresh weight and then dried at 70ºC for estimate the dry weight.

Pigments concentration : Total chlorophyll and carotenoids concentrations were determined according to

Lichtenthaler and Wellburn (1983).These samples were collected from the middle blade leaflet of the middle mature leave.

Activities of antioxidant enzymes and 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., Kafr-elsheikh 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 in the supernatant (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). Activity of ascorbate peroxidase was determined spectrophotometrically according to Asada (1984). Activity of catalase 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 470 nm were recorded every 30 sec intervals for 3min. Enzyme activity was expressed as increase in absorbance $min^{-1}g^{-1}$fresh weight.

Analysis of reactive oxygen species (ROS): Detection of $O_2^{\bullet-}$ 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 electron microscopy and with transverse sections by light microscopy. Samples ($5mm^2$) were taken from the middle of first upper mature leave at the middle of the leaflet blade and immediately fixed in solution of glutaraldehyde + 2 % paraformaldehyde in 0.1 M sodiumphosphate buffer pH 7.4 for 24 hrs at 4°C as described with Karnovsky (1965).

Specimen preparation for scanning electron microscopy: Specimens were prepared and observed at EM Unit, Mansoura University, Egypt using a Jeol JSM-6510 L.V SEM.

Specimens preparation for transverse sections: Samples were prepared and post-contrast of sections according to Reynolds (1963). Ultrathin sections were observed using a JEOL JEM -2100 at EM Unit, Mansoura University, Egypt.

6. Statistical analysis:

A completely randomized design with one-way ANOVA was used with this experiment. Two individual pot plants were used for each treatment as replicates. The experiment was repeated one time. The data were analyzed by using the analysis of variance technique by means of CoStat Computer Software (Cohort, Berkeley, CA,USA). The treatments mean values were compared by Duncan's multiple range test method at least significance difference $p \leq 0.05$ as published by Duncan, (1955).

RESULTS AND DISCUSSION

Results

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

The leaf area injury data as well as the fresh and dry weights parameters of areca palm plants were presented in Table (1). Effectiveness of areca plants on resistance to the air gas pollutants and keeping their quality were varied along with antioxidant treatments. The areca plants showed no injuries on their leaves when exposed to formaldehyde gas after spraying with either GSH or BI at $600mgL^{-1}$ and $300mgL^{-1}$ concentrations, respectively. The highest injury incidence was on the plant leaves exposed to ammonia gas (Fig. 1), especially, with the plants treated with BI at $300mgL^{-1}$ before exposed to the gas (73.11 and $68.44cm^2$ per leaf), in percentage, 32.02 and 27.82 at the end of the first and the second experiments respectively. Plants exposed to gases extremely decreased in fresh weight comparing with the non-polluted plants. However, the areca plants fresh weight was relatively increased when treated with antioxidants before exposed to formaldehyde gas compared with the control polluted plants (exposed to formaldehyde only). Except, for the plants sprayed with GSH at $300mgL^{-1}$ decreased significantly in fresh biomass in comparing with the other treatments.

The highest increase in fresh weight was obtained when spraying areca plants with GSH at $300mgL^{-1}$ concentration before exposure to the ammonia gas pollutant (29.05 and 27.19 gm/plant respectively of the two experiments). Meanwhile, concerning the dry biomass, it was non-significantly different among the treatments and the control plants (Table 1).

Table 1. Effects of exposure areca plants to formaldehyde or ammonia gases after treated with glutathione (GSH) and bilirubin (BI) on morphological parameters during first and second experiments.

Parameters	Mean area/one leaf (cm^2)		Mean area Injury/ Leaf (cm^2)		Area injury % /leaf		Fresh weight (g/plant)		Dry weight (g/plant)	
Treatments	Frs. exp.	Sec. exp.	Frs. exp.	Sec. exp.	Frs. exp.	Sec. exp.	Frs. exp.	Sec. exp.	Frs. exp.	Sec. exp.
Control (non-polluted)[y]	244 g	275 f	0.0 g	0.0 d	0.0 g	0.0 c	28.51 a	25.31 a	5.55 ab	5.19 ab
Formaldehyde (Fmd)	328 d	319 e	2.98 d	3.07 c	0.77 d	0.83 c	17.15 c	19.25 b	4.11 ab	4.26 ab
Ammonia (Amm)	293 e	328 d	9.68 b	10.59 b	3.31 b	3.23 b	19.23bc	21.16 b	4.27 ab	4.42 ab
Fmd + GSH 300 mg L^{-1}	388 b	368 b	1.46 e	1.29 cd	0.45 e	0.41 c	16.76 c	17.82 bc	4.40 ab	3.88 ab
Fmd + GSH 600 mg L^{-1}	436 a	451 a	0.0 g	0.0 d	0.0 g	0.0 c	18.64 bc	20.21 b	3.68 ab	3.46 ab
Fmd +BI 300 mg L^{-1}	247 g	263 g	0.0 g	0.0 d	0.0 g	0.0 c	21.61 b	20.49 b	4.60 ab	4.83 ab
Fmd + BI 600 mg L^{-1}	342 c	364 c	8.69 c	8.38 b	2.54 c	2.30 b	19.82 bc	17.76 bc	4.31 ab	4.22 ab
Amm + GSH 300 mg L^{-1}	281 f	210 i	0.92 f	0.84 cd	0.33 f	0.40 c	29.05 a	27.19 a	6.51 a	6.73 a
Amm + BI 300 mg L^{-1}	231 h	246 h	73.11 a	68.44 a	32.02 a	27.82 a	12.53 d	15.34 c	2.38 b	2.50 b

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

Fig. 1. Leaflet injury symptoms of areca plants exposed to formaldehyde or ammonia gases after spraying with glutathione (GSH) and bilirubin (BI).

In general, the highest values were recorded with the plants sprayed with GSH at 300mgL^{-1} before exposed to the ammonia gas. In addition, the lowest values were recorded when plants were exposed to ammonia gas before spraying with BI at 300mgL^{-1} concentration.

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

The total chlorophyll concentration in areca leaves exposed to the formaldehyde gas was not significant when were compared to the non-polluted plants (Table 2).

Table 2. Effects of exposure areca plants to formaldehyde or ammonia gases after treated with glutathione (GSH) and bilirubin (BI) on total chlorophyll and carotenoids concentration (mg/g F. W.) during first and second experiments.

Parameters	Total chlorophyll concentration		Total carotenoids concentration	
Treatments	Frs. exp.	Sec. exp.	Frs. exp.	Sec. exp.
Control (non-polluted)^{Y}	2.87 ab	2.65 abc	0.296 b	0.287 ab
Formaldehyde (Fmd)	2.96 a	2.80 ab	0.239 bc	0.247 bcd
Ammonia (Amm)	2.46 c	2.92 a	0.204 c	0.182 d
Fmd + GSH 300 mg L^{-1}	2.85 ab	2.92 a	0.287 b	0.263 bc
Fmd + GSH 600 mg L^{-1}	3.08 a	2.92 a	0.398 a	0.338 a
Fmd +BI 300 mg L^{-1}	3.21 a	3.03 a	0.291 b	0.255 bcd
Fmd + BI 600 mg L^{-1}	2.57 bc	2.50 bc	0.280 b	0.254 bcd
Amm + GSH 300 mg L^{-1}	2.37 cd	2.08 d	0.251 bc	0.277 ab
Amm + BI 300 mg L^{-1}	2.06 d	2.35 cd	0.237 bc	0.188 cd

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

However, the highest increase in chlorophyll concentration was recorded in the plants treated with BI at 300mgL^{-1} before exposed to formaldehyde gas followed by the treatment of GSH at 600mgL^{-1}. In addition, the total carotenoids was significantly increased to the highest value in the areca leaves exposed to formaldehyde gas after treated with GSH at 600mgL^{-1} compared with the non-polluted plants, follow it the plants treated with BI at 300mgL^{-1} (Table 2). In general, areca plants exposed to ammonia gas decreased total chlorophyll and carotenoids concentrations when compared with the control plants and the other treatments.

3. Effects of exposure areca plants to formaldehyde or ammonia gases after treated with glutathion (GSH) and bilirubin (BI) on antioxidant enzymes activities:

Data recorded in the Fig.(2) and Table (3) show the activities of antioxidant enzymes of catalase (CAT), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and guaiacol peroxidase (GPOX) in the areca plants treated with antioxidants before exposed to the air gas pollutants. Spraying areca plants with BI at 300mgL^{-1} concentration before exposed to the formaldehyde gas increased the CAT and GPOX enzymes activities up to the significant values compared with the control plants and the other treatments. Also, spraying areca plants with glutathione at 300mgL^{-1} significantly increased the CAT activity before exposed plants to both gases. APX and PPO activities were non-significant from all treated plants comparing with the non-polluted plants except the plants sprayed with BI 300mgL^{-1} before exposed to ammonia gas which increased the activities of these enzymes.

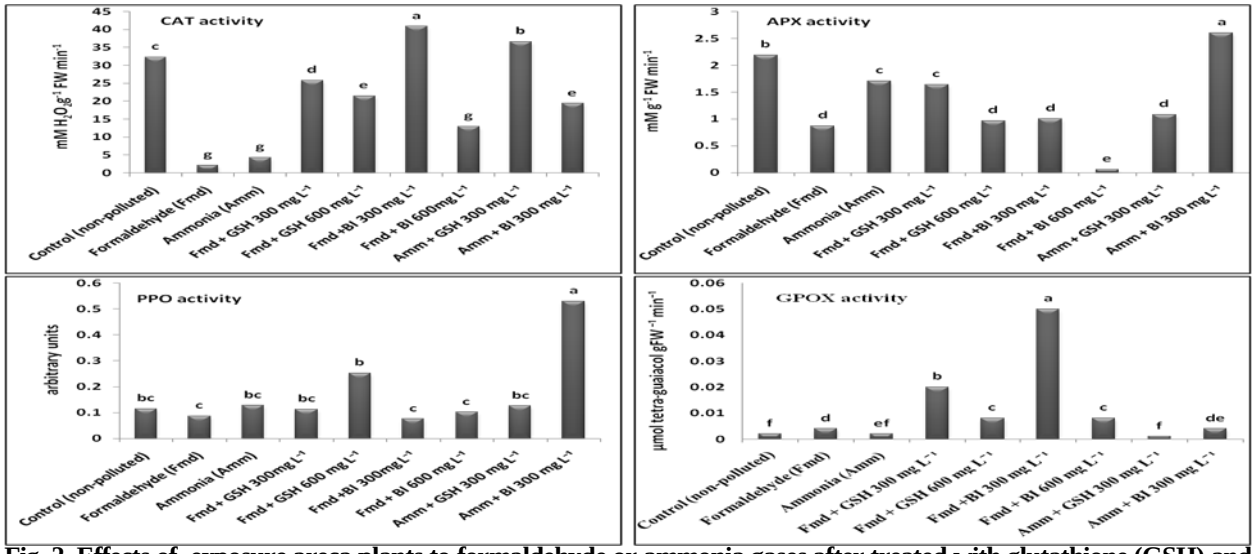

Fig. 2. Effects of exposure areca plants to formaldehyde or ammonia gases after treated with glutathione (GSH) and bilirubin (BI) on activities of antioxidant enzymes of catalase (CAT), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and guaiacol peroxidase (GPOX).

Treatments set marked with same letter within each parameter are not different significantly at P≤0.05 based on Duncan's multiple range test, Control(non-polluted): plants not exposed to gas pollutants.

4. Effects of exposure areca plants to formaldehyde or ammonia gases after treated with glutathion (GSH) and bilirubin (BI) on the histochemical analysis of reactive oxygen species(ROS):

The brown discoloration was used for estimating the hydrogen peroxide (H2O2) and the purple discoloration for estimating the superoxide ($O2^{\cdot-}$) as examples of reactive oxygen species (ROS) of the areca leaves exposed to the air gases (Fig.2). As shown in Fig.(3), the brown and purple discolorations were increased in plants treated with BI at 600mgL^{-1} before exposed to formaldehyde gas and with BI at 300mgL^{-1} before exposed to ammonia gas compared with the other treatments. The highly increasing in discoloration of purple and brown colors was recorded in areca leaves exposed to ammonia gas-only comparing with the plants treated with GSH and BI.

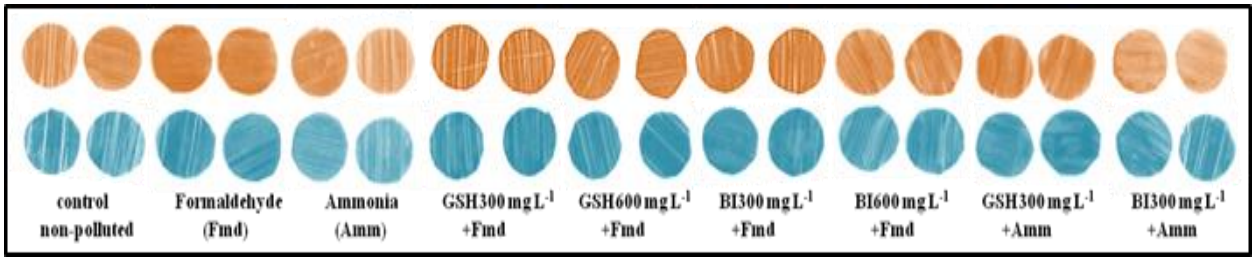

Fig. 3. Effects of exposure areca plants to formaldehyde or ammonia gases after treated with glutathione (GSH) and bilirubin (BI) on accumulation of reactive oxygen species (ROS), brown discoloration of hydrogen peroxide (H_2O_2) (upper row) and purple discoloration of superoxide($O_2^{\cdot-}$) (lower row).

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

Scanning electron microscopy:

At adaxial leaf surface of areca plants exposed to formaldehyde showed no appearance of stomata and the epidermal cells have un-strait wall and irregular shape (Fig. 4, A, B & C). On the other hand, the stomata clearly appear on the abaxial leaf surface and arranged and organized in longitudinal rows of plants treated with BI or GSH before exposure to the formaldehyde (Fig. 5, B & C).

Table 3. Effects of exposure areca plants to formaldehyde gas after treated with glutathione (GSH) and bilirubin (BI) on number of stomata, stomata dimensions, stomata pore dimensions and guard cell thickness at abaxial leaf surface.

Parameters	Stomata number	Stomata dimensions (µm)		Stomata pore size dimensions (µm)		Guard cells width
Treatments	(mm2)	length	width	length	width	(µm)
Control-polluted with formaldehyde (Fmd)	–	–	–	14.96	6.24	–
Fmd + GSH 300mgL^{-1}	8	20.52	10.08	9.29	3.70	3.89
Fmd+ BI 300mgL^{-1}	12	22.61	9.45	13.45	2.88	3.67

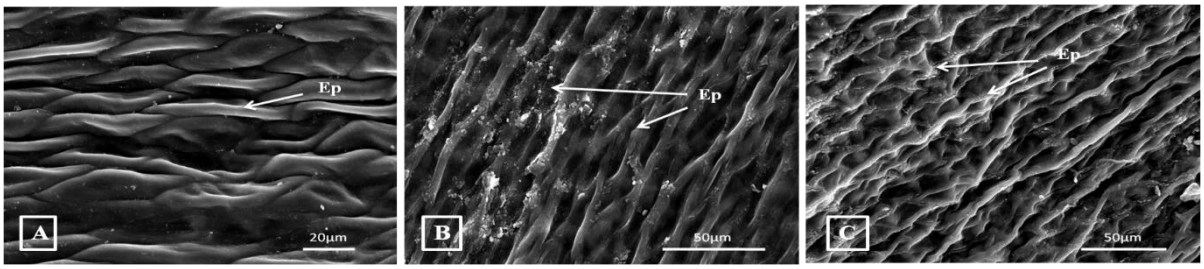

Fig. 4. Scanning electron microscopy of adaxial leaf surface focusing the epidermis shape of areca plants treated with glutathione (GSH) or bilirubin (BI) before exposed to formaldehyde gas. Arrows indicate to the regular, irregular and hetero-dimensional epidermal cells. A: control-polluted (plants exposed to formaldehyde gas), B: plants sprayed with GSH at 300mg L^{-1} before exposed to formaldehyde, C: plants sprayed with BI at 300mg L^{-1} before exposed to formaldehyde. (Ep: epidermal cells). (Magnification: A= 750x , B,= 500x, C= 400x).

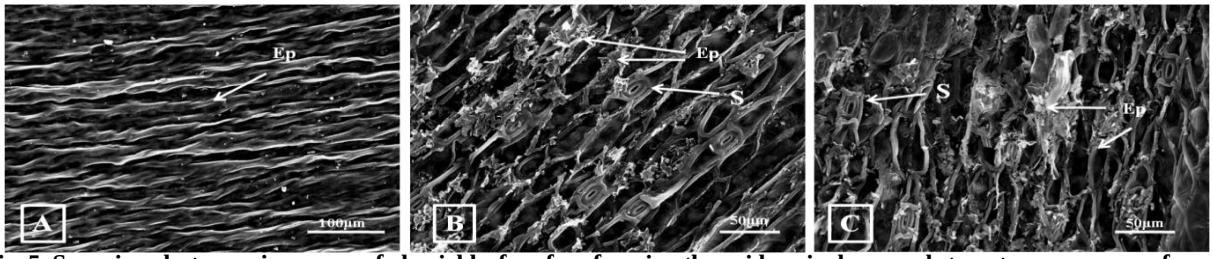

Fig. 5. Scanning electron microscopy of abaxial leaf surface focusing the epidermis shape and stomata appearance of areca plants treated with glutathion (GSH) or bilirubin (BI) before exposed to formaldehyde gas. Arrows indicate to the regular, irregular and hetero-dimensional epidermal cells and clogged and unclogged stomata. A: control-polluted (plants exposed to formaldehyde gas), B: plants sprayed with GSH at 300mgL^{-1} before exposed to formaldehyde, C: plants sprayed with BI at 300mg L^{-1} before exposed to formaldehyde. (Ep: epidermal cells). (Magnification: A = 250x , B and C = 400x).

Otherwise, the stomata were sunken and not appeared of plants exposed to formaldehyde only (Fig. 6, A). The stomata number and its length as well as the stomata pore length were the highest of the plants treated with BI at 300mgL^{-1} before exposure to formaldehyde gas compared with the control polluted plants (Table 3, Fig. 6, C). Guard cell width showed partially increase by treated plants with GSH at 300mgL^{-1} (Fig. 6, B).

Transversal sections:

The transversal sections data presented in Table (4) and shown in Fig.(7) revealed that the plants treated with GSH or BI at 300 mgL^{-1} before exposed to formaldehyde were decreased in the blade thickness by 16.15 and 9.55% compared with the plants which were exposed to the formaldehyde gas only. In the contrary, the upper cuticle thickness was increased by 6.02% in plants treated with GSH and highly increased by 52.57% with BI treatment comparing with the control-polluted plants. These results were accompanied by the occurrence of decreasing in mesophyll thickness. Otherwise, there were increases in the upper and lower hypodermis by 16.52 and 9.48% respectively with BI treatment compared with treatment of exposure to formaldehyde only. Additionally, large black deposits and some of the lower epidermis cells detached from the mesophyll were also noticed on the transverse leaflet sections of polluted plants with the formaldehyde gas (Fig.7, A) while plants treated with the BI at 300mgL^{-1} has a semi-normal and healthy upper and lower epidermal cells (Fig. 7, C). Furthermore, it is noticed that there were both of crushed and normal upper epidermal cells when treated plants with GSH at 300 mgL^{-1} before exposed to formaldehyde gas (Fig. 7, B).

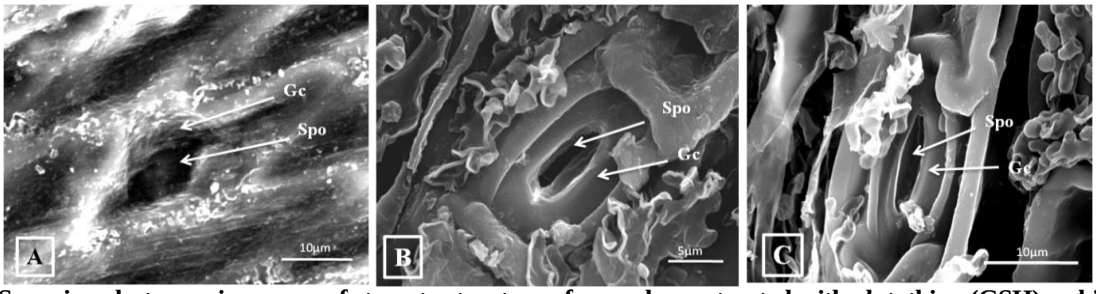

Fig. 6. Scanning electron microscopy of stomata structure of areca leaves treated with glutathion (GSH) or bilirubin (BI) before exposed to formaldehyde gas. Arrows indicate to the guard cells and stomata pore. A: control-polluted (plants exposed to formaldehyde gas), B: plants sprayed with GSH at 300mg L^{-1} before exposed to formaldehyde, C: plants sprayed with BI at 300mg L^{-1} before exposed to formaldehyde. (Gc: guard cell; Spo: stomata pore). (Magnification: A = 2000x , B = 3000x and C = 2500x).

Table 4. Effects of exposure areca plants to formaldehyde gas after treated with glutathione (GSH) and bilirubin (BI) on the thicknesses of leaflet transversal sections structures (µm).

Treatments Parameters	Formaldehyde (Fmd) (control-polluted)	Fmd+GSH 300 mgL^{-1}	± % to control- polluted	Fmd+BI 300 mgL^{-1}	± % to control-polluted
Blade thickness	56.33	47.23	- 16.15	50.95	- 9.55
Cuticle thickness	0.565	0.599	+ 6.02	0.862	+ 52.57
Upper epidermis thickness	3.90	2.58	- 33.85	2.10	- 46.15
Lower epidermis thickness	3.11	3.56	+ 14.47	3.11	0.0
Mesophyll thickness	35.70	29.29	- 17.96	31.28	- 12.38
Upper hypodermis thickness	5.69	4.57	- 19.68	6.63	+ 16.52
Lower hypodermis thickness	5.17	4.79	- 7.35	5.66	+ 9.48

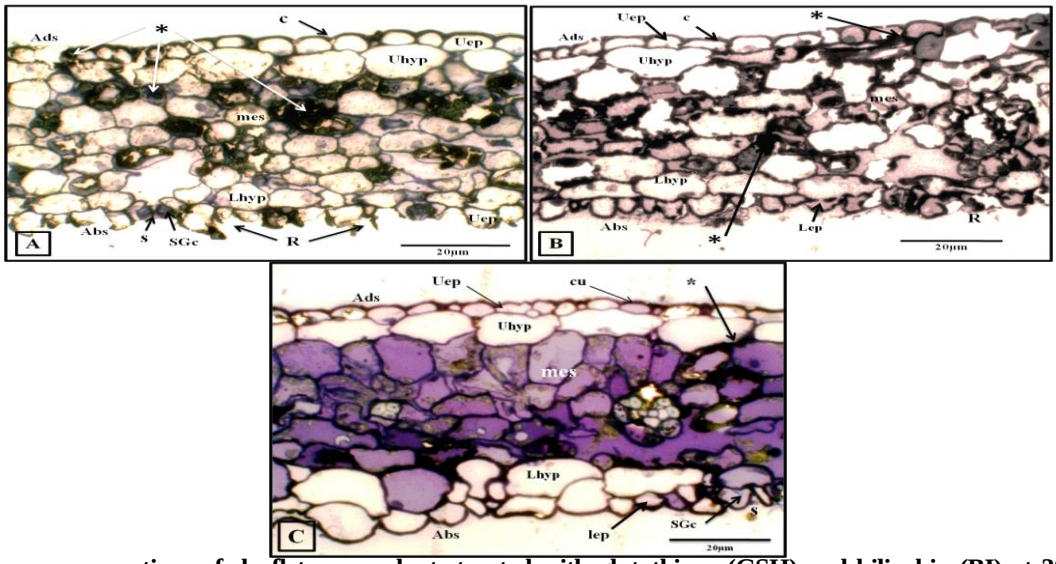

Fig. 7. Transverse sections of leaflet areca plants treated with glutathione (GSH) and bilirubin (BI) at 300 mgL^{-1} before exposed to formaldehyde gas. A: control-polluted (plants exposed to formaldehyde gas only); looked the crushed lower epidermal cells (R) with large dark deposits in the mesophyll (*). B: plants sprayed with GSH at 300mg L^{-1}. C: plants sprayed with BI at 300mg L^{-1}. (Ads: adaxial surface, Abs: abaxial surface, Uep: upper epidermal cells, Lep: lower epidermal cells, Uhyp: upper hypodermis cells, Lhyp: lower hypodermis cells, mes: mesophyll, S: stomata, SGc: stomata guard cell. Scale bar = 20 µm, Magnification= 400x.

Discussion

The main objective of this investigation was to study the effects of foliar spraying areca palm plants with non-enzymatic antioxidants (GSH and BI) on increasing their resistance to air pollution during exposing to formaldehyde and ammonia gases. Exposing plants to ammonia was the highest in the number of dead leaves and percentage of leave injury comparing with the plants exposed to formaldehyde. Otherwise, the leaves were not died or injured when treated with GSH at 600 mg L^{-1} or BI at 300mg L^{-1} before exposure to formaldehyde gas, as well as, increased in the dry weight and total chlorophyll concentration compared with the plants exposed to formaldehyde without non-enzymatic antioxidants (control-polluted plants). These results may be related to the impact of GSH and BI on increasing the plant's ability to absorb formaldehyde gas and transform it into organic acids, sugars or CO_2 and H_2O (Wei *et. al,* 2017). Additionally, increasing chlorophyll pigments was reflected in increasing the photosynthesis level and consequently increasing the physiological processes in plants resulting in increasing the dry weights. Previous researchers stated that photosynthetic pigments at higher levels enhance the plants resistance to pollution stress (Yılmaz, 2018; Tripathi and Gautam, 2007). Glutathione (GSH) able to detoxify formaldehyde to formate carbon dioxide (Tada and Kidu, 2011). Furthermore, the endogenous GSH level to adequate concentration helps plants to tolerate oxidative air pollution stress (Hasanuzzaman *et al*., 2013). The experimental data indicates that total carotenoids concentration was increased when treated plants with BI at 300mg L^{-1} before exposure to formaldehyde gas compared with the other treatments. Also, carotenoids concentration was highly increased when exposed plants to ammonia gas. These results attributed to the glutathione and BI effect on increasing plant capability against air pollution stress through its role in decreasing the ROS generation under air pollution stress (Gupta and Sharma, 2006). Areca plants that exposed to ammonia gas were extremely decreased in the fresh and dry weights as well as in the total chlorophyll and carotenoids concentrations. These results may be a result of the excessive generation of ROS under ammonia gas. Akpoghelie *et al*., (2017) reported that absorption air pollutants may cause a reduction in the concentration of photosynthetic pigments that directly affect plant productivity. Areca plants treated with BI at 300mg L^{-1} before exposure to formaldehyde or ammonia gases showed an increase in activities of GOPX and CAT enzymes under formaldehyde gas, whereas increased the activities of APX and PPO under ammonia gas. Farhan *et al.* (2001) have shown that BI possesses both antioxidant and prooxidant properties. BI may function as a strong antioxidant and inhibiting protein oxidation (Wang and Liao, 2016). Daridon and Veyrier, (2013) specified that the adaptive responses of plants in reaction to biotic or abiotic stress bring about the production of reactive ROS including the involvement of small antioxidant molecules (GSH, carotenoids, BI, ect.,) and the involvement of antioxidant enzymes.

The internal structure of the leaf blades is an important indicator for determines the response and resistance of the plants to air pollution. Treated areca plants with BI or glutathione before exposure to formaldehyde gas showed a clear appearance of stomata with increasing their density and the pore length. Pääkköen *et al*.(1997) recorded an increase of stomata density in plants exposed to air pollutants, while Matyssek *et al*. (1993) observed an initial decrease in stomata density in plants. The decrease in stomata densities and their pore sizes considered an adaptation for controlling absorption of pollutants (Verma et al., 2006), but will limit photosynthesis (Pourkhabbaz *et al*., 2010). Therefore, as shown in this investigation, the decreasing in chlorophyll concentrations with exposing areca plants to ammonia gas pollutants was due to the reduction of the stomatal index (Manjunath and Reddy, 2019) and the blockage of stomata opening in response to air pollutants (Leghari and Zaidi, 2013). The upper cuticle and the mesophyll thicknesses are the main properties that distinguish the tolerant and resistant plant species from the sensitive ones to air pollution (Ferdinand *et al*., 2000). The areca plants treated with GSH or BI at 300 mg L^{-1} before exposure to formaldehyde were decreased the blade thickness and mesophyll thicknesses compared with the plants exposed to formaldehyde gas only. Otherwise, the upper cuticle thickness was increased with BI treatment. The cuticle responds to some abiotic stresses by changes in cuticle thickness and deposition (Dominguez *at al.*, 2011). Pourkhabbaz *et al*., (2010) reported that the cuticle layer was much thinner in leaves from trees at the urban (polluted area) than on those of leaves from trees grown at the rural site. Leaf anatomy of *Lotus corniculatus* , *Trifolium montanum* and *T. pretense* showed reduction in epidermis, palisade and spongy parenchyma in highly polluted sites and sometimes, the sub-stomatal chamber from *Lotus corniculatus* leaves was filled with dark deposits (Irina 2009,a). Iqbal (1985) has shown a significant reduction in palisade and spongy parenchyma in leaves of white clover of a polluted area. The transverse sections in our investigation showed that the epidermal cells were destroyed and some of the lower epidermis cells detached from the mesophyll as well as large black deposits were also observed in leaves which exposed to formaldehyde gas. Gao *et al*., (2016) suggested that ROS degrades the pectin of the middle lamella inducing separation of the mesophyll cells. They added that the hydrogen bounds between cellulose molecules and other cell wall components may also be affected resulted in softening the cell which becomes deformed. Furthermore, plants exposed to ammonia gas showed high spaces of necrotic areas with highly dead cells. In an experiments related to the influence of different pollutants on plants grown in a polluted area, Irina (2009,b) observed the presence of the phenolic compounds (dark deposits from the epidermis, assimilatory and vascular tissues) in *Plantago lanceolata* leaves from pollutes sites. Also, Tosserams *et al*., (2001) concluded that increased accumulation of carbon in nutrient-stressed plants may lead to enhanced accumulation of phenolic compounds and leaf thickening. The most common visible symptoms of ammonia gas pollutants in conifers are black discoloration, usually sharply bordered tip burn and abscission of needles (Gheorghe and Ion, 2011).

In conclusion, the results obtained in this investigation indicated that spraying areca plants with bilirubin at 300mg L^{-1} enhanced the plant resistance to the formaldehyde and ammonia air pollutants. Also the

glutathione has fewer effects on areca plant resistance to air gases pollutants. Additionally, the areca plants were higher sensitivity to ammonia gas pollutants.

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تنقية الهواء الداخلى من الملوثات باستخدام نخيل الأريكا المعامل ببعض مضادات الأكسدة الغير إنزيمية

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لنباتات التنسيق الداخلى دور مهم فى تنقية الهواء الداخلى من الغازات الملوثة. لذلك كان الهدف الرئيسى لهذه الدراسة هو زيادة مقاومة نباتات الأريكا لتلوث الهواء. أجرى هذا البحث فى شمبر بلاستيكى (٨٠ سم طول, ٨٠ سم عرض, ١٠٠ سم إرتفاع) و عرضت نباتات الأريكا لغازات الفورمادهيد أو الأمونيا بعد رشها بمضادات الأكسدة الغير إنزيمية (الجلوتاثيون والبليروبين بتركيز ٣٠٠ملجرام/لتر و٦٠٠ملجرام/لتر). ولقد وجد أن نباتات الأريكا التى عرضت لغاز الأمونيا (NH_3) كانت الأعلى فى عدد الأوراق الميتة ونسبة المساحات الورقية المصابة مقارنة بالنباتات التى عرضت لغاز الفورمالدهيد. نباتات الأريكا المعاملة بالجلوتاثيون تركيز ٦٠٠ملجرام/لتر أو البليروبين تركيز ٣٠٠ملجرام/لتر قبل تعرضها لغاز الفورمالدهيد لم يحدث بها موت أو إصابات للأوراق بالإضافة إلى زيادة كلا من الوزن الجاف والكلوروفيل مقارنة بالنباتات التى عوملت بغاز الفورمالدهيد بدون الرش بمضادات الأكسدة (الكنترول). أدى رش النباتات بالبليروبين تركيز ٣٠٠ملجرام/لتر قبل تعرضها لغاز الفورمالدهيد إلى زيادة محتوى النبات من الكاروتين مقارنة بباقى المعاملات وإلى زيادة واضحة فى نشاط إنزيمات الجويكول بيروكسيديز (GOPX) والكتاليز (CAT) بينما أدت إلى زيادة نشاط إنزيمات الأسكوربيك بيروكسيديز (APX) والبولى فينول أوكسيديز (PPO) عند التعرض لغاز الأمونيا. معاملة النباتات بالجلوتاثيون أو البليروبين أدت إلى زيادة أعداد الثغور و زيادة طول فتحة الثغر عند التعرض لغاز الفورمالدهيد. نستنتج من هذه الدراسة أن رش نباتات الأريكا بالبليروبين تركيز ٣٠٠ملجرام/لتر يؤدى إلى زيادة مقاومة النباتات لتلوث الهواء بغازات الفورمالدهيد والأمونيا يليها المعاملة بالجلوتاثيون. وقد سجلت نباتات الأريكا حساسية أعلى لغاز الأمونيا من غاز الفورمالدهيد.