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Physiological and Biochemical Responses of Maize *(Zea mays)* to Phenanthrene Toxicity

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Abstract

Polycyclic aromatic hydrocarbons (PAHs), a class of organic pollutants, have been observed to exert deleterious effects on plant growth and various physiological processes. In this study, the effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on growth parameters, photosynthetic pigments, some antioxidant enzymes, and some biochemical compounds of the maize plant were investigated. The experiments were conducted as pot cultures of plants under controlled conditions using a completely randomized design (CRD) with three replications for each treatment. Increasing phenanthrene concentration reduced all studied growth parameters and significantly increased photosynthetic pigment contents. Phenanthrene led to the increase in catalase, peroxidase, and superoxide dismutase activities in the roots but in shoots, only the peroxidase activity was increased. Moreover, higher phenanthrene concentrations were associated with elevated levels of malondialdehyde and hydrogen peroxide, coupled with a marked reduction in soluble sugar content in both shoot and root tissues ($p< 0.05$). Also, increase in phenanthrene concentration in the shoots decreased the total phenol and flavonoid contents compared to anthocyanin. Phenanthrene treatment led to a significant reduction in the concentrations of lauric acid, meric acid, palmitic acid, and oleic acid in maize shoots. In conclusion, it seems that high concentrations of phenanthrene induce oxidative stress in the maize, and plants improve their enzymatic antioxidant system to moderate the stress condition. In addition, damage of cell membranes by phenanthrene leads to weakening of plants root system as well as disordering in water and nutrient uptake and finally reduction in the plant growth.

Keywords: Antioxidant system, Oxidative stress, Maize plant, Phenanthrene, Physiological responses, Polycyclic aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of organic compounds composed exclusively of carbon and hydrogen (Ansari *et al.*, 2023). These compounds form a

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major group of environmental pollutants, mainly produced by the incomplete fuel combustion and various anthropogenic sources (Kumar *et al.*, 2021). Some compounds of PAHs are carcinogenic and mutagenic and therefore may possibly be important in the context of human health concerns. Due to their high persistence, long-term toxicity and accumulation in the environment, PAHs have attracted more attention in recent years (Gitipour *et al.*, 2018; Thacharodi *et al.*, 2023). Polycyclic aromatic hydrocarbons consist of two groups including low molecular weight (LMW) and high molecular weight (HMW) compounds containing 2-3 and 4-7 rings, respectively. Phenanthrene (PHE), a representative of the LMW PAHs, is made of three benzene rings and thus it is determined as one of the central pollutants listed in the Environmental Protection Agency (Houshani and Salehi-Lisar, 2020; Tarigholizadeh *et al.*, 2022).

Plants can uptake PAHs both through their roots and leaves, subsequently transfer these compounds into food chains (Jia *et al.*, 2021). A number of factors such as concentration and physicochemical properties of the compound, soil type, temperature, plant species, stage of ontogenesis, and lipid content of plants can influence the rate of PAHs uptake by plants (Patowary *et al.*, 2017; Wu and Zhu, 2019). Indeed, PAHs can exert their influence throughout the entire lifecycle of a plant, from germination to maturity (Kummerova *et al.*, 2012). Accordingly, some known effects of PAHs on plants resulted in biochemical and physiological changes such as alterations in enzyme activities, reduction in photosynthesis and respiration rates, generation of reactive oxygen species, and damage to cellular membranes via lipid oxidation (Houshani *et al.*, 2019; Sushkova *et al.*, 2020). While substantial research has addressed the well-known adverse effects of PAHs on plants, there remains a critical need to delve deeper into the physiological aspects of these effects and the complicated mechanisms controlling plant responses to PAH contamination and toxicity. This study aims to contribute to the existing body of knowledge by providing valuable insights into these crucial areas. Maize (*Zea mays* L.) is a very important annual crop with thermophilic and photophilic characteristics which can adapt to various types of soils. Due to the relative resistance of maize to salt, drought stress, and its short growth cycle, it is usually grown as a remediation crop in various adverse conditions. Due to the importance of corn as food resource for animal, bird and human feeding; the entrance of pollutants into this species can lead to some concerns on food safety (Houshani et al., 2019). Therefore, we examined the effects of different concentrations of phenanthrene (PHE) as one of the abundant PAHs in the environment (Tarigholizadeh *et al.*, 2022) on the

growth of maize in this study. Moreover, the evaluation of the biochemical, lipid content and, physiological responses of plants to PHE toxicity was another aim of this study.

2. Materials and methods

2.1. Experimental design

The experiments were conducted as pot cultures of plants under controlled conditions using a completely randomized design (CRD) with three replications for each treatment.

2.2. The treatment

In order to provide different concentrations of PHE (25, 50, 75 and, 100 ppm), the suitable amount of the compound was dissolved in ethanol for each treatment and then the solutions were sprayed on sterile perlite in pots. Treated perlite was used for plant cultivation after evaporation of ethanol for 72 h.

2.3. Plant culture

The seeds of maize (*Zea mays* L.) were obtained from the East Azerbaijan Research and Education Centre for Agriculture and Natural Resources (Tabriz, Iran) and stored at 4 $^{\circ}$C until cultivation. Appropriate numbers of seeds were chosen with respect to their vigor and uniformity, sterilized using 1% (v/v) sodium-hypochlorite solution for 5 minutes, and washed sufficiently using sterile distilled water. Then, sterilized seeds were planted in uncontaminated (control) and PHE-contained perlite. After 3 days, all germinated seeds were transferred to growth chambers under controlled conditions (25-30°C, 16/8 h light/dark photoperiod and relative humidity of 60%) for 3 weeks. The water content of the pots was adjusted to 100% field capacity every two days by sterile distilled water. After 4 and 10 days, the water of pots was replaced with 50% and 100% Hoagland solution respectively.

2.4. Harvesting of plants and assays

The cultivation period of plants lasted for 21 days, when the PAHs toxicity symptoms were observed in plants. Biochemical and physiological assays were carried out using fresh samples prior to plant harvesting. After the evaluation of shoot height and root length, the harvested plants were divided into the roots and shoots. The samples were washed appropriately with water, instantly dried on the towel paper and after determining the fresh weight transferred to 70 $^{\circ}$C chamber. The dry weight of samples was measured after 72 h.

2.5. Measurement of photosynthetic pigments content

Photosynthetic pigments content (chlorophyll a, b, total chlorophyll, and total carotenoids) was measured according to of Hartmut (1987). Briefly, 0.1 g of fresh leaf samples was homogenized with 5 ml of >99.5% acetone using a mortar and pestle on ice bath. Homogenates were filtered using a number 42 Whatman filter paper. The determination of pigment contents was carried out using the absorbance of extracts at 645, 663, and 470 nm by spectrophotometer (Analytic Jena, Specol 1500, and Germany).

2.6. Measurement of total protein content and antioxidant enzyme assays

Approximately 0.1 g of samples were homogenized in ice-cold phosphate-buffered solution (50 mM, pH = 7) using mortar and pestle. The homogenates were centrifuged at 10,000 g for 10 min at 4 $^{\circ}$C. The supernatants were used immediately for determination of the total soluble protein content (Bradford, 1976), as well as the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

SOD activity was evaluated by determination of nitro-blue-tetrazolium (NBT) photoreduction inhibition by extracts (Winterbourn *et al.*, 1976). The reaction mixture contained 2.7 ml sodium phosphate solution (1 M, pH=7.8), 100 µl NBT (1.5 mM), 200 µl NaCN (0.3 mM), EDTA (1 M), 50 µl of riboflavin (0.12 mM) and 50 µl of enzyme extract. The mixture was illuminated at light intensity of 40 W for 12 minutes and the absorbance of solution was recorded at 560 nm. The amount of the enzyme causing 50% protection of NBT photoreduction was considered as one unit of SOD and the activity is expressed as U mg^{-1} protein.

The activity of POD was determined by Obinger and colleagues *(*1997) method. The reaction mixture (1 ml) containing 300 µl of guaiacol (4 mM), 350 µl of phosphate buffer (10 mM, pH= 7), 300 µl of H_2O_2 (50 mM) and 50 µl of enzyme extract. The reaction was initiated by adding H_2O_2 to reaction mixture and POD specific activity (expressing as U mg^{-1} protein) was calculated using the extinction coefficient of 26.6 $mM^{-1}cm^{-1}$ for guaiacol (Obinger *et al.*, 1997).

CAT activity was assayed according to the methods of Chance and Maehly (1955). The reaction mixture contained 2.5 ml potassium phosphate buffer (50 mM, pH= 7), 1 ml H_2O_2 (10 mM) and 500 µl of enzyme extract. CAT activity was expressed as U mg^{-1} protein.

2.7. Measurement of malondialdehyde content (MDA)

Approximately, 0.1 g of samples were homogenized with 0.1% (W/V) trichloroacetic acid (TCA) and centrifuged for 5 minutes at 10000 g. Subsequently, 0.5 ml of supernatants was mixed with 2 ml of 20% TCA containing 0.5% of 2-thiobarbituric acid and was heated in hot

water at 95 °C for 30 minutes. Mixture was immediately transferred to ice bath and then was centrifuged at 10000 g for 15 min. Finally, the absorbance of supernatant was recorded at 532 nm and MDA concentration was calculated according to a standard curve prepared using 3,1,1,3-tetraethoxy propane (0-100 nmol) and expressed as µmol g^{-1} FW (Boominathan and Doran 2002).

2.8. Estimation of hydrogen peroxide (H_2O_2) content

0.5 ml of supernatant prepared from section 2-7 was immediately mixed with 0.5 ml of phosphate buffered solution (10 mM, pH = 7) and 1 ml of KI solution (1 M) and the mixture was kept at 25ºC for 15 minutes. Finally, the absorbance was recorded at 390 nm and the concentration of H_2O_2 was calculated according to a standard curve prepared using H_2O_2 (0-120 µmol). The data was expressed as µmol g^{-1} FW (Harinasut *et al.*, 2003).

2.9. Measurement of total flavonoid, phenol, and anthocyanin contents

For the measurement of total flavonoid and phenol contents, 0.1 g of sample was homogenized in 80% methanol using a mortar and pestle. The homogenate was centrifuged at 10000 g for 5 min and then 500 µl of supernatant was mixed with 1.5 ml of 80% methanol, 100 µl of 10% aluminum chloride solution, 100 µ of 1 M potassium acetate, and 2.8 ml of distilled water. After 40 minutes, absorbance of the mixture was measured at 415 nm compared to the control. The total flavonoid content of the extract was described as mg quercetin equivalents (QE) g^{-1} FW (Chang *et al.*, 2002).

The total phenol content was determined by the Folin–Ciocalteu method (Meda *et al.*, 2005). A volume of 2.8 ml of distilled water, 100 µl of Folin–Ciocalteu reagents and 2 ml of sodium carbonate 2% were added to 100 µl of supernatant and incubated for 30 minutes. The absorbance of sample was measured at 720 nm compared to the control. The data was expressed as mg gallic acid equivalents (GAE) g^{-1} FW.

To measure the total anthocyanin content, 0.02 g of dried plant sample was pulverized with 4 ml of hydrochloric acid containing 1% methanol in a porcelain mortar. The solution was kept in a refrigerator for 24 hours and then was centrifuged for 10 minutes at 13000 g. The supernatant was separated and the absorbance of the extract was measured at 530 and 657 nm compared to the control (hydrochloric acid containing 1% methanol). Anthocyanin content of each extract was calculated using the following equation (Mita *et al.*, 1997).

$$A = A_{530} - (0.25 \times A_{657})$$

A is the absorbance of the solution (subscripts indicate the wavelengths at which the absorbance is measured).

2.10. Measurement of soluble sugars content

The soluble sugar content was determined by phenol-sulfuric acid technique (Kochert 1978). 5 ml of ethanol (70%) was added to 50 mg of dry sample and incubated in refrigerator for one week. The sample was centrifuged at 10000 g for 15 minutes at room temperature. Then, 0.5 ml of the plant extract was added to 1.5 ml distilled water and then 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid were added to the extract. The mixture was vortexes and incubated for 30 minutes at room temperature. The absorption of solution was recorded at 485 nm. The data was expressed as mg g^{-1} DW.

2.11. Measurement of fatty acid

2.11.1. Preparation of phenacyl esters of fatty acids

The fresh plant tissues were homogenized using chloroform-methanol solvent (2: 1) and filtered by a filter paper. The filtrates was poured into the decanter, 20 ml of distilled water was added and shaken slightly. The lower phase was saponified with 50 ml potassium hydroxide in 70% ethanol and boiled in a water-bath for 1 h. After cooling, 50 ml of distilled water was added and the solution was poured into a decanter. Then, 30 ml of ether was added, shaken well and the ether phase was collected (for 3 times). Then, the solution was acidified using 30 ml hydrochloric acid (4N) and 30 ml of diethyl ether was added and shaken to form two phases. The upper phase, which is ether containing fatty acids, was separated and the content of decanter was washed by 30 ml of distilled water. The solution was dehydrated by a rotary evaporator at 80 °C using absolute ethanol. Finally, 10 ml of diethyl ether was added and stored in the refrigerator (Hamilton *et al.*, 1992).

The ether of the samples was evaporated, and one ml of methanol was added to each sample. Then, 100 µl phenol phthalene was added and the solutions were alkaline by adding NaOH (0.2 mM). The samples were evaporated and 200 µl of 18-crown-6 (200 µl 2mg ml^{-1} in acetonitrile) and 200 µl of 4-bromophenacyl bromide (200 µl 2mg ml^{-1} in acetone) were added. The samples were heated hot water-bath at 80 °C for 15 min. 600 µl acetonitrile was added to all samples and kept in -20 °C until analyses (Bodoprost and Rosemeyer, 2007; Rioux *et al.*, 1999; Hanist *et al.*, 1988).

2.11.2. Fatty acid phenacyl ester separation on HPLC

To investigate changes in fatty acids in plants treated with PHE, 6 available fatty acid standards including lauric acid (C12: 0), meristic acid (C14: 0), palmitic acid (C16: 0), oleic acid (C18: 1), linoleic acid (C18: 2), and arachidonic acid (C20: 4) were considered. Fatty acids were prepared as a phenyl derivative and then injected into the high-performance liquid chromatograph (HPLC) and standard chromatograms were obtained. Fatty acid phenacyl esters were analyzed using a HPLC (KNAUER, Germany) equipped with a UV detector and a Φ 4.6 × 150 mm reverse phase C18 column. The UV-detector wavelength was set at 242 nm and the column temperature was maintained at 40 °C. The mobile phase with flow-rate 0.8 mL min^{-1} and with a gradient of methanol/acetonitrile/water started at 80:10:10 (v/v/v) which increased linearly to 86:10:4 (v/v/v) in 30 min, then increased linearly to 90:10:0 (v/v/v) in 10 min holding at 90:10:0 (v/v/v) for 5 min and returning to the initial conditions (80:10:10) in 5 min. the whole running time for each injection was set to 50 min (Bodoprost and Rosemeyer, 2007; Rioux *et al.*, 1999; Hanist *et al.*, 1988).

2.12. Statistical analysis

All measurements were conducted with three replications and data were reported as mean ± standard deviation (SD). The data were analyzed using GLM procedure by SPSS software (Ver.16) and Tukey's multiple range tests was used for mean comparisons at 1 % probability level. Microsoft excel 2013 software was used for the preparation of figures.

3. Results

3.1. Growth parameters

The findings obtained from this study indicate a significant decline in all assessed growth parameters with an increase in PHE concentration compared to the control (p<0.05) (Table 1). Accordingly, treatment of plants with 100 ppm of PHE led to a 68.89% and 56.07% reduction in the shoot and root length. The fresh weights of shoot and root were also decreased by 80% and 72.28%, respectively. Also, by the exposure to 100 ppm of PHE, dry weights of shoots and roots were decreased by 85.78% and 74.83%. However, no significant differences in root dry and fresh weights were seen among 50, 75 and 100 ppm of PHE treatments.

Table 1. The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on the growth parameters of maize.

Phenanthrene (ppm)	Shoot Length (cm)	Root Length (cm)	Shoot FW (mg)	Root FW (mg)	Shoot DW (mg)	Root DW (mg)
0	0.25 ^{a} ± 39.1	1.59 ^{a} ±28	64.75 ^{a} ±995	35.45 ^{a} ±888	16.99 ^{a} ± 197	24.03 ^{a}± 155
25	1.03 ^{b} ± 30.6	0.03 ^{b} ± 19.6	38.64 ^{b} ±648	17.25 ^{b}±601	5.46 ^{b} ± 93	12.16 ^{ab} ±80
50	0.06 ^{c} ± 20.3	0.51 ^{b} ± 18.3	58.52 ^{c} ±388	13.91 ^{c}± 260	9.07 ^{c}± 62.6	1.36 ^{b} ± 52.3
75	0.21 ^{d} ± 13.3	0.51 ^{c} ± 10.6	45.3 ^{d}± 220	16.13 ^{c} ±235	2.25 ^{d} ± 43.6	4.73 ^{b} ±47.3
100	0.26 ^{d} ± 13.3	0.26 ^{c} ± 12.3	18.81 ^{d} ±199	19.33 ^{c} ± 201	1.94 ^{e} ±28	2.28 ^{b} ± 39

The data represent the mean of three replications ± SD and similar upper-case letters indicates no significant difference at $p < 0.05$. DW: Dry Weight, FW: Fresh Weight.

3.2. Photosynthetic pigments content

Chlorophyll a content exhibited significant increases in plants treated by 50, 75, and 100 ppm of PHE in comparison with the control plants, with the most significant elevation (+128.85%) observed at 100 ppm PHE treatment ($p < 0.05$). In contrast, all applied levels of PHE significantly decreased chlorophyll b content. Such content in the plants treating with 25, 50, 75, and 100 ppm of PHE were decreased to 27.04, 72.26, 75.71, and 76.36 %, respectively. Moreover, the highest value of carotenoids (+126.98 %) was recorded in plants exposed to 100 ppm of PHE (Fig. 1).

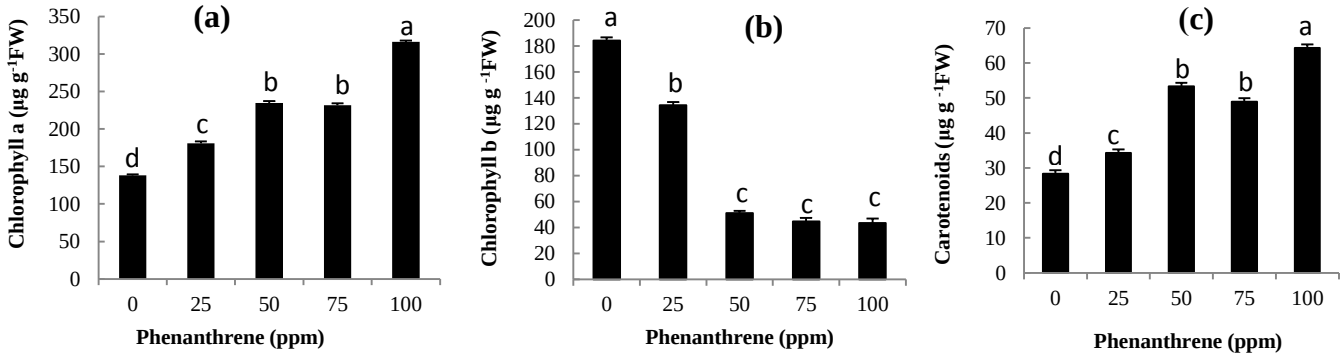

Fig 1. The effects of different concentrations of phenanthrene on photosynthetic pigments content of maize plants. (a) Chlorophyll a, (b) Chlorophyll b, and (c) Carotenoids. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences (p<0.05).

3.3. The content of malondialdehyde (MDA)

Malondialdehyde was measured as an indicator to assess the severity of oxidative stress induced by PHE. A statistically significant increase in MDA content was found after exposure to PHE (p<0.05). The results also indicated that MDA content in the shoots of treated plants exceeded that in the roots. Compared to the control, MDA contents of the shoots were increased by 86.1, 104.4, 115.15, and 128.81% when plants treated with 25, 50, 75, and 100 ppm PHE, respectively. A similar trend was also seen in the MDA content in the roots, with the highest

increase occurring at 50 ppm PHE exposure (Fig. 2). Differences in MDA contents were not statistically significant among treatments with higher concentrations of PHE.

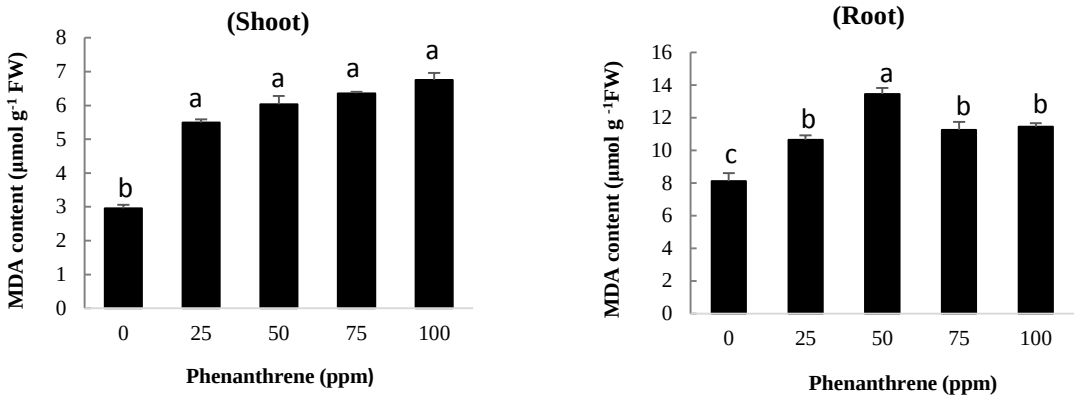

Fig 2. The effects of different concentrations of phenanthrene on malondialdehyde (MDA) contents of maize plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences (p<0.05).

3.4. Hydrogen peroxide (H_2O_2) content

Phenanthrene had a significant effect on the content of H_2O_2 in treated plants. The enhanced content of H_2O_2 in plants had been observed through the increasing concentrations of PHE (p<0.05). The H_2O_2 contents in the shoots of plants treated with 25, 50 75, and 100 ppm of PHE were increased by 160.67, 171, 171.58, and 207.21%, respectively. The values for the roots of the same plants recorded increases of126.73, 130.01, 240.38, and 260.17%, respectively (Fig. 3).

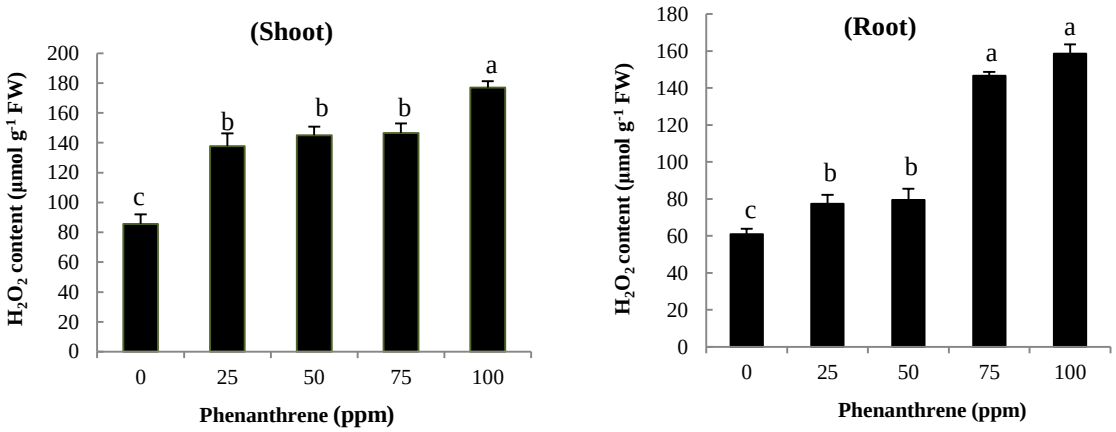

Fig 3. The effects of different concentrations of phenanthrene on hydrogen peroxide (H_2O_2) contents of maize plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences (p<0.05).

3.5. Soluble sugars content

The results revealed that PHE had significant effect on the content of soluble sugars ($p<0.05$). The content of soluble sugars of both shoots and roots were decreased at various levels of phenanthrene in comparison with the control, and the greatest reduction in soluble sugars was observed in the shoots and roots of plants subjected to 50 (38.77%) and 100ppm (46.07%), respectively($p<0.05$) (Fig. 4).

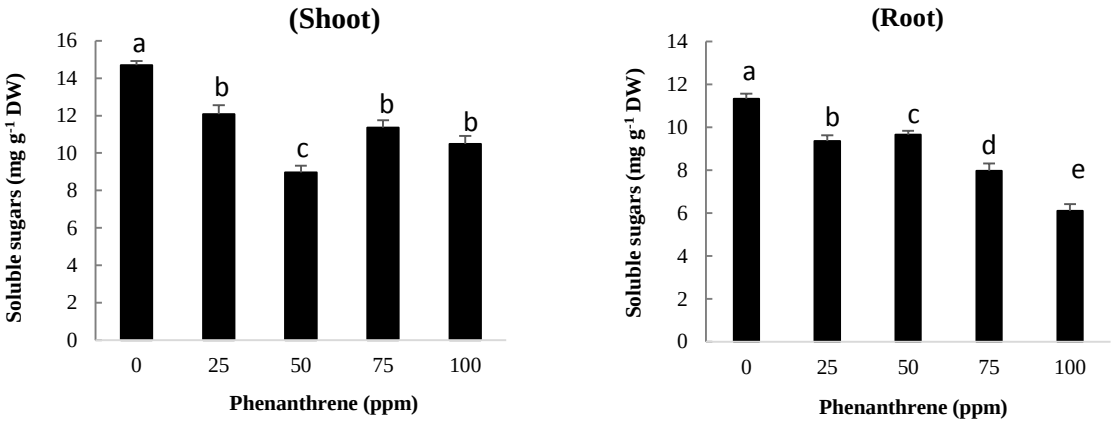

Fig 4. The effects of different concentrations of phenanthrene on soluble sugars contents of maize plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences ($p<0.05$).

3.6. Activity of antioxidant enzymes

The changes in the activity of CAT, POD, and SOD within the shoot and root of maize plants after treatment with PHE were shown in Table 2. The CAT activity was declined in the shoots of the plants exposed to 50, 75, and 100 ppm of PHE. Conversely, in the roots, CAT activity responded differently, with significant increases of 91.55%, 86.88%, and 48.44% observed at 50, 75, and 100 ppm of phenanthrene, respectively, compared to the control ($p<0.05$).

POD activity in the shoots of treated plants with various concentrations of PHE was significantly higher in comparison with the control. The highest POD activities were observed in plants treated by 50 (+112%) and 75 ppm (+128%) of PHE, respectively ($p<0.05$). In roots only significant increase in POD activity was seen at 75 ppm (+57.26%) of phenanthrene (Table 2).

PHE treatments affected SOD activity in the shoots. SOD activity was increased at a low concentration (25 ppm) of PHE and then declined at higher levels (75 and 100 ppm). Up to 28% increase in SOD activity was detected in plants treated with 25 ppm of PHE, but in plants treated by 75 and 100 ppm of PHE, SOD activity was decreased by 47.16 and 54.91%, respectively,

compared with the control. Interestingly, SOD activity in the root was significantly higher in plants treated with different PHE concentrations and the highest activity was found in plants treated with 75 ppm of phenanthrene ($p<0.05$) (Table 2).

Table 2. The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on antioxidant enzymes activity (U mg^{-1} protein) in the shoot and root of maize plants.

Phenanthrene (ppm)	Shoot			Root		
	CAT	POD	SOD	CAT	POD	SOD
0	0.002 ^{a}± 0.183	0.052 ^{d} ± 2.5	0.112 ^{b} ± 15.9	0.032 ^{d} ± 0.452	7.69^{b} ±80.5	1. 6 ^{c}± 47.8
25	0.051 ^{a}± 0.183	0.031 ^{c} ±3.5	2.35 ^{a} ± 20.5	0.029^{c}± 0.576	5.98 ^{bc} ± 65.7	3.25 ^{c}± 47.8
50	0.021^{c} ± 0.108	0.022 ^{a}± 5.3	1.95 ^{b} ± 16.1	0.033^{a}± 0.862	2.45 ^{b} ± 79.6	2.33 ^{b}±81.3
75	0.019 ^{bc} ± 0.121	0.041 ^{a} ±5.7	1.22 ^{c} ± 8. 43	0.127 ^{a} ± 0.841	6.26 ^{a} ± 126	6.9 ^{b} ±90.7
100	0.014 ^{b} ± 0.137	0.045 ^{ab}± 4.4	0.521 ^{c}± 8.61	0.012 ^{b} ± 0.668	7.33 ^{c} ± 46.6	3.1 ^{a} ±117

The data represent the mean of three replications ±SD and similar upper case letters indicates no significant difference at $p < 0.05$. CAT: catalase, POD: peroxidase and SOD: superoxide dismutase.

3.7. Total flavonoids, phenols and anthocyanin contents

By increasing the PHE concentration, total flavonoids and phenol contents in shoots were significantly reduced compared to the control plants ($p<0.05$). In roots, flavonoids content was increased after exposure to 25 ppm of PHE, but no statistically significant difference was evident between the plants treated with higher levels of PHE and the control plants. Except for the level of 50 ppm, there was no significant increase detected in root phenol content at the other concentrations. It was also seen that anthocyanin content was increased in the shoots compared to the control, with the highest value (121.31%) recorded at the concentration of 100 ppm, but anthocyanin in the roots was decreased through the increasing levels of PHE up to 75 ppm (Table 3).

Table 3. The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on total flavonoid (mg EQ g^{-1} FW), total phenol (mg EGA g^{-1} FW) and, total anthocyanin contents (mg g^{-1} FW) in the shoot and root of maize plant.

Phenanthrene (ppm)	Shoot			Root		
	Total Flavonoid	Total Phenol	Total Anthocyanin	Total Flavonoid	Total Phenol	Total Anthocyanin
0	0.002 ^{a} ±0.292	0.106^{a} ± 4.30	0.002 ^{c} ± 0.61	0.001 ^{b} ± 0.027	0.184^{b} ± 2.86	0. 001 ^{b} ± 1.01
25	0.009 ^{b} ± 0.207	0.076^{b} ± 3.72	0.012 ^{a} ± 1.25	0.009^{a} ± 0.040	0.051^{b} ± 2.71	0.049 ^{bc} ± 0.86
50	0.004^{b} ± 0.189	0.159^{b} ± 3.47	0.019 ^{a} ± 1.33	0.005^{b} ± 0.030	0.092 ^{a} ± 3.18	0.031 ^{c} ± 0.61
75	0.003 ^{c} ± 0.149	0.184^{c} ± 2.99	0.012 ^{a} ± 1. 25	0.001 ^{b} ± 0.028	0.106^{c} ± 1.98	0.089 ^{bc} ± 0.79
100	0.005 ^{d} ± 0.128	0.046^{d} ± 2.69	0.048 ^{a} ± 1.35	0.002 ^{b} ± 0.028	0.205^{c} ± 2.81	0.096 ^{a} ± 1.3

The data represent the mean of three replications ±SD and similar upper case letters indicates no significant difference at $p < 0.05$.

3.8. Fatty acid concentration

Samples of both shoots and roots from control maize plants and those exposed to PHE at 50 ppm were injected to HPLC in three replications. The results of fatty acid analysis showed that oleic acid is the most abundant fatty acid in both shoots and roots of maize. Alongside oleic

acid, other fatty acids were also detected in maize tissues including lauric acid, meristic acid, and palmitic acid and due to the lack of identification of arachidonic acid and linoleic acid fatty acids; it was not possible to investigate the changes in the concentration of these fatty acids. PHE treatment significantly decreased the concentrations of lauric acid, meric acid, palmitic acid, and oleic acid in shoots of maize plants compared to the control plants ($p \leq 0.05$). In contrast, the roots of PHE-treated plants exhibited a significant increase in the concentrations of lauric acid, meric acid, and palmitic acid by 79.79, 36.36, and 23.58%, respectively. However, oleic acid concentration in the roots decreased in response to phenanthrene treatment ($p \leq 0.05$) (Table 4).

Table 4. The effect of concentration of phenanthrene (50 ppm) on fatty acid concentration (µg g^{-1}FW) in the shoot and root of maize plant.

Fatty acid (µg g^{-1}FW)	Phenanthrene (ppm)	Shoot	Root
Lauric acid	0	$0.485^{a} \pm 0.012$	$0.268^{b} \pm 0.013$
	50	$0.098^{b} \pm 0.028$	$3.665^{a} \pm 0.554$
Myristic acid	0	$0.716^{a} \pm 0.025$	$0.885^{b} \pm 0.007$
	50	$0.047^{b} \pm 0.003$	$1.306^{a} \pm 0.125$
Palmitic acid	0	$0.543^{a} \pm 0.002$	$0.106^{b} \pm 0.004$
	50	$0.007^{b} \pm 0.009$	$0.131^{a} \pm 0.005$
Oleic acid	0	$196.83^{a} \pm 7.57$	$174.80^{a} \pm 5.07$
	50	$62.151^{b} \pm 6.21$	$158.85^{b} \pm 9.45$

The data represent the mean of three replications ±SD and similar upper-case letters indicates no significant difference at $p < 0.05$.

3.9. Analysis correlation

The correlation analysis conducted to assess the relationship between MDA and H_2O_2 contents, as well as between CAT and SOD activities in both shoots and roots (at significance levels of 1% and 5%), revealed a negative correlation coefficient between enzyme activities and MDA and H_2O_2 contents. These findings demonstrated that CAT and SOD involving in plants resistance to oxidative stress are induced by PHE toxicity. Moreover, no correlation was seen between POD activity and MDA and H_2O_2 contents of both shoots and roots of maize (Table 5).

Table 5. Statistical analysis for correlation between the activity of antioxidant enzyme and MDA and H_2O_2 contents in the shoot and root of maize plant.

	CAT Shoot	CAT Root	POD Shoot	POD Root	SOD Shoot	SOD Root	MDA Shoot	MDA Root	H_2O_2 Shoot	H_2O_2 Root
H_2O_2 Root	-0.222^{ns}	-0.308^{ns}	-0.024^{ns}	-0.267^{ns}	-0.736**	-0.341^{ns}	0.599*	0.547*	0.747**	1
H_2O_2 Shoot	-0.541*	0.002^{ns}	-0.185^{ns}	-0.181^{ns}	-0.830**	-0.435^{ns}	0.904**	0.822**	1	
MDA Root	-0.690**	0.314^{ns}	0.009^{ns}	0.189^{ns}	-0.893**	-0.434^{ns}	0.892**	1		
MDA Shoot	-0.539*	0.110^{ns}	-0.180^{ns}	-0.011^{ns}	-0.816**	-0.391^{ns}	1			
SOD Root	0.140^{ns}	0.300^{ns}	0.647**	-0.078^{ns}	0.555*	1				
SOD Shoot	0.624*	-0.075^{ns}	-0.306^{ns}	-0.166^{ns}	1					
POD Root	-0.274^{ns}	0.697**	0.246^{ns}	1						
POD Shoot	-0.267^{ns}	0.438^{ns}	1							
CAT Root	-0.446^{ns}	1								
CAT Shoot	1									

Notes: **Correlation is significant at 0.01 levels, *Correlation is significant at 0.05 levels, ^{ns} correlation is not significant.

4. Discussion

PAHs constitute a substantial class of pollutants recognized for their detrimental effects on both animals and plants. These compounds can adversely affect plants growth, disrupt photosynthesis, and inhibit enzyme activities (Ahammed *et al.*, 2012; Sushkova *et al.*, 2021). In the current study, PHE treatments negatively affected the growth parameters, especially at higher concentrations. The reduction of growth and biomass production in the presence of PAHs was consistent with earlier studies involving different plant species such as *Arabidopsis thaliana* (Alkio *et al.*, 2005; Liu *et al.*, 2009), rice (Li and Yi, 2012), and tomato (Ahammed *et al.*, 2012). PHE and possibly other compounds of this group can disrupt root development in the early stages of plant growth. Since roots play a crucial role in mineral nutrition, this disruption can lead to growth impairment (Dupuy *et al.*, 2016).

Photosynthetic pigments content was changed in a different way by phenanthrene concentrations. Accordingly, chlorophyll a and carotenoids contents were increased and chlorophyll b content was decreased. Elevated levels of reactive oxygen species (ROS) can contribute to chlorophyll decline, thereby impairing photosynthesis. This is associated with the significant reduction of the light-harvesting complex protein within photosystem II under stressful conditions. Part of this complex protein, chlorophyll b, is embedded in the chloroplast membrane, and increased ROS levels within the chloroplast, resulting from oxidative stress, accelerate chloroplast membrane degradation. Thus, stress-induced degradation of complex protein results in a reduction of chlorophyll b (Sharma *et al.*, 2020; Moustakas *et al.*, 2022). On the other hand, pigments accumulation could be due to negative effect of PHE on growth. As plant growth decreases, the concentration of the pigmentation tends to increase.

Our results have shown that H_2O_2 and MDA contents in both shoots and roots enhanced through increasing PHE levels. Therefore, MDA accumulation resulting from oxidative stress and ROS accumulation was a reliable marker for determining of the negative effect of PHE on the growth of maize plants. Generally, plants equipped with enzymatic and non-enzymatic mechanisms for scavenging ROS accumulation under oxidative stress (Tarigholizadeh *et al.*, 2021). Several enzymes like SOD, CAT, and POD as enzymatic mechanisms play important roles during seed germination. SOD, as the first line of defense against ROS, acts on superoxide radicals producing under stress conditions, catalyzing their conversion into H_2O_2 and O_2 (Hasanuzzaman *et al.*, 2021). CAT is the primary H_2O_2 scavenging enzyme in plant cells (Li and Yi, 2012). In this study, CAT, POD, and SOD activities were increased in the roots, while CAT and SOD activities were decreased in the shoots by different levels of PHE. Also, the correlation analysis (Table 4) demonstrated a negative correlation between H_2O_2 and MDA contents, as well as between CAT and SOD activities, in both the shoot and root of maize, emphasizing the role of these enzymes in ROS detoxification to mitigate stress conditions and enhance plant resistance to oxidative stress. Moreover, the decrease in SOD and CAT activities induced by PHE occurred exclusively in the shoots, while phenol and flavonoid levels contributed to this decrease in shoots and roots, consequently reducing the H_2O_2 scavenging ability and leading to lipid peroxidation. Therefore, it can be said that in the maize plant, the antioxidant system of maize in the shoots was not strong enough to eliminate all produced ROS at high concentrations, resulting in oxidative damage, diminished photosynthesis, reduced soluble sugar content, and compromised growth parameters. Similar results have been reported for sunflower, alfalfa, and wheat plants (Salehi-Lisar and Deljoo, 2015).

PHE significantly influenced soluble sugar content ($p<0.05$), with reduced levels detected in both shoots and roots. This reduction can be linked to the decreased demand for photosynthetic materials due to reduced root growth (Table 1). In addition, lower carbohydrate content in roots can be attributed to higher consumption of energy for resistance of PHE toxicity. Carbohydrates in plants, in addition to energy production, regulate various gene expressions (Rolland *et al.*, 2006) and may have antioxidant activity (Lang-Mladek *et al.*, 2010).

Regarding fatty acids, the decrease in oleic acid concentration in the roots of maize plants treated with PHE, compared to the control plants, may be caused by the oxidative stress induced by PHE. Therefore, the increases of saturated fatty acids are likely to moderate stress conditions of root. Moreover, their concentrations treated with pollutants decreased in the shoots and this can be attributed to lipid peroxidation in the shoots confirming by MDA results. On the other

hand, induced stress in the shoots of plants treated with contaminants decreased photosynthesis and soluble sugars, which may be due to carbon dioxide fixation decrease in the Calvin cycle. The triosphosphates of this cycle by changing into acetyl-CoA provide the primary substrate required for biosynthesis of fatty acids (Du *et al.*, 2020). Therefore, a decrease in photosynthesis and thiophosphates may also be another reason for the reduction in fatty acids in the shoots.

5. Conclusions

Based on the obtained results, high concentrations of phenanthrene (75 and 100 ppm) have inhibitory effects on plants. Reduced growth, lowere chlorophyll b content, and elevated contents of MDA and H_2O_2 in root are some negative effects of phenanthrene. Further, the increase in the activity of antioxidant enzymes due to phenanthrene treatment indicated the oxidative stress caused by this compound. However, the decrease in the activity of CAT and the non-destruction of the produced hydrogen peroxide due to stress indicated a decrease in the resistance of the maize plant to this compound. On the other hand, the decrease in the amount of MDA in the shoot and then the increase in the roots also demonstrated higher accumulation of phenanthrene in the roots leading to weakening of plants root system as well as disordering in water and nutrient uptake and finally reduction in the plant growth.

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پاسخ های فیزیولوژیکی و بیوشیمیایی ذرت (*Zea mays*) به سمیت فنانترن

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هیدروکربن‌های آروماتیک چند حلقه‌ای (PAHs)، دسته‌ای از آلاینده‌های آلی، مشاهده شده‌اند که اثرات مضری بر رشد گیاه و فرآیندهای فیزیولوژیکی مختلف دارند. در این تحقیق اثر غلظت های مختلف فنانترن (0، 25، 50، 75 و 100 پی پی ام) بر پارامترهای رشد، رنگدانه های فتوسنتزی، برخی آنزیم های آنتی اکسیدانی و برخی ترکیبات بیوشیمیایی گیاه ذرت مورد بررسی قرار گرفت. آزمایش ها به صورت کشت گلداني گیاهان در شرایط کنترل شده در قالب طرح کاملاً تصادفي (CRD) با سه تکرار براي هر تیمار انجام شد. افزایش غلظت فنانترن تمام پارامترهای رشد مورد مطالعه را کاهش داد و محتوای رنگدانه فتوسنتزی را به طور قابل توجهی افزایش داد. فنانترن منجر به افزایش فعالیت کاتالاز، پراکسیداز و سوپراکسید دیسموتاز در ریشه شد اما در اندام هوایی تنها فعالیت پراکسیداز افزایش یافت. علاوه بر این، غلظت‌های بالاتر فنانترن با افزایش سطح مالون دی‌آلدئید و پراکسید هیدروژن همراه با کاهش محسوس در محتوای قند محلول در هر دو بافت اندام هوایی و ریشه همراه بود ($p< 0/05$). همچنین افزایش غلظت فنانترن در اندام هوایی باعث کاهش محتوای کل فنل و فلاونوئید نسبت به آنتوسیانین شد. تیمار فنانترن منجر به کاهش قابل توجه غلظت اسید لوریک، مریک اسید، اسید پالمیتیک و اسید اولئیک در اندام هوایی ذرت شد. در نتیجه به نظر می رسد که غلظت بالای فنانترن باعث ایجاد استرس اکسیداتیو در ذرت می شود و گیاهان سیستم آنتی اکسیدانی آنزیمی خود را برای تعدیل شرایط تنش بهبود می بخشند. علاوه بر این، آسیب غشای سلولی توسط فنانترن منجر به تضعیف سیستم ریشه گیاهان و اختلال در جذب آب و عناصر غذایی و در نهایت کاهش رشد گیاه می شود.