

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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33 major group of environmental pollutants, mainly produced by the incomplete fuel combustion
34 and various anthropogenic sources (Kumar *et al.*, 2021). Some compounds of PAHs are
35 carcinogenic and mutagenic and therefore may possibly be important in the context of human
36 health concerns. Due to their high persistence, long-term toxicity and accumulation in the
37 environment, PAHs have attracted more attention in recent years (Gitipour *et al.*, 2018;
38 Thacharodi *et al.*, 2023). Polycyclic aromatic hydrocarbons consist of two groups including low
39 molecular weight (LMW) and high molecular weight (HMW) compounds containing 2-3 and
40 4-7 rings, respectively. Phenanthrene (PHE), a representative of the LMW PAHs, is made of
41 three benzene rings and thus it is determined as one of the central pollutants listed in the
42 Environmental Protection Agency (Houshani and Salehi-Lisar, 2020; Tarigholizadeh *et al.*,
43 2022).

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

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

67

68 2. Materials and methods

69 2.1. Experimental design

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

72

73 2.2. The treatment

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

78

79 2.3. Plant culture

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

90

91 2.4. Harvesting of plants and assays

92 The cultivation period of plants lasted for 21 days, when the PAHs toxicity symptoms were
93 observed in plants. Biochemical and physiological assays were carried out using fresh samples
94 prior to plant harvesting. After the evaluation of shoot height and root length, the harvested
95 plants were divided into the roots and shoots. The samples were washed appropriately with
96 water, instantly dried on the towel paper and after determining the fresh weight transferred to
97 70 °C chamber. The dry weight of samples was measured after 72 h.

98

99

100 **2.5. Measurement of photosynthetic pigments content**

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

107

108 **2.6. Measurement of total protein content and antioxidant enzyme assays**

109 Approximately 0.1 g of samples were homogenized in ice-cold phosphate-buffered solution
110 (50 mM, pH = 7) using mortar and pestle. The homogenates were centrifuged at 10,000 g for
111 10 min at 4 °C. The supernatants were used immediately for determination of the total soluble
112 protein content (Bradford, 1976), as well as the activities of superoxide dismutase (SOD),
113 peroxidase (POD), and catalase (CAT).

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

121 The activity of POD was determined by Obinger and colleagues (1997) method. The reaction
122 mixture (1 ml) containing 300 µl of guaiacol (4 mM), 350 µl of phosphate buffer (10 mM, pH=
123 7), 300 µl of H₂O₂ (50 mM) and 50 µl of enzyme extract. The reaction was initiated by adding
124 H₂O₂ to reaction mixture and POD specific activity (expressing as U mg⁻¹ protein) was
125 calculated using the extinction coefficient of 26.6 mM⁻¹cm⁻¹ for guaiacol (Obinger *et al.*, 1997).

126 CAT activity was assayed according to the methods of Chance and Maehly (1955). The
127 reaction mixture contained 2.5 ml potassium phosphate buffer (50 mM, pH= 7), 1 ml H₂O₂ (10
128 mM) and 500 µl of enzyme extract. CAT activity was expressed as U mg⁻¹ protein.

129

130 **2.7. Measurement of malondialdehyde content (MDA)**

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

134 water at 95 °C for 30 minutes. Mixture was immediately transferred to ice bath and then was
135 centrifuged at 10000 g for 15 min. Finally, the absorbance of supernatant was recorded at 532
136 nm and MDA concentration was calculated according to a standard curve prepared using
137 3,1,1,3-tetraethoxy propane (0-100 nmol) and expressed as $\mu\text{mol g}^{-1}$ FW (Boominathan and
138 Doran 2002).

139 **2.8. Estimation of hydrogen peroxide (H₂O₂) content**

140 0.5 ml of supernatant prepared from section 2-7 was immediately mixed with 0.5 ml of
141 phosphate buffered solution (10 mM, pH = 7) and 1 ml of KI solution (1 M) and the mixture
142 was kept at 25°C for 15 minutes. Finally, the absorbance was recorded at 390 nm and the
143 concentration of H₂O₂ was calculated according to a standard curve prepared using H₂O₂ (0-
144 120 μmol). The data was expressed as $\mu\text{mol g}^{-1}$ FW (Harinasut *et al.*, 2003).

145

146 **2.9. Measurement of total flavonoid, phenol, and anthocyanin contents**

147 For the measurement of total flavonoid and phenol contents, 0.1 g of sample was homogenized
148 in 80% methanol using a mortar and pestle. The homogenate was centrifuged at 10000 g for 5
149 min and then 500 μl of supernatant was mixed with 1.5 ml of 80% methanol, 100 μl of 10%
150 aluminum chloride solution, 100 μl of 1 M potassium acetate, and 2.8 ml of distilled water. After
151 40 minutes, absorbance of the mixture was measured at 415 nm compared to the control. The
152 total flavonoid content of the extract was described as mg quercetin equivalents (QE) g^{-1} FW
153 (Chang *et al.*, 2002).

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

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

$$165 \quad A = A_{530} - (0.25 \times A_{657})$$

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

168

169 **2.10. Measurement of soluble sugars content**

170 The soluble sugar content was determined by phenol-sulfuric acid technique (Kochert 1978).
171 5 ml of ethanol (70%) was added to 50 mg of dry sample and incubated in refrigerator for one
172 week. The sample was centrifuged at 10000 g for 15 minutes at room temperature. Then, 0.5
173 ml of the plant extract was added to 1.5 ml distilled water and then 1 ml of 5% phenol and 5 ml
174 of concentrated sulfuric acid were added to the extract. The mixture was vortexed and incubated
175 for 30 minutes at room temperature. The absorption of solution was recorded at 485 nm. The
176 data was expressed as mg g⁻¹ DW.

177

178 **2.11. Measurement of fatty acid**

179 **2.11.1. Preparation of phenacyl esters of fatty acids**

180 The fresh plant tissues were homogenized using chloroform-methanol solvent (2: 1) and
181 filtered by a filter paper. The filtrates was poured into the decanter, 20 ml of distilled water was
182 added and shaken slightly. The lower phase was saponified with 50 ml potassium hydroxide in
183 70% ethanol and boiled in a water-bath for 1 h. After cooling, 50 ml of distilled water was
184 added and the solution was poured into a decanter. Then, 30 ml of ether was added, shaken well
185 and the ether phase was collected (for 3 times). Then, the solution was acidified using 30 ml
186 hydrochloric acid (4N) and 30 ml of diethyl ether was added and shaken to form two phases.
187 The upper phase, which is ether containing fatty acids, was separated and the content of
188 decanter was washed by 30 ml of distilled water. The solution was dehydrated by a rotary
189 evaporator at 80 °C using absolute ethanol. Finally, 10 ml of diethyl ether was added and stored
190 in the refrigerator (Hamilton *et al.*, 1992).

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

198

199

200 **2.11.2. Fatty acid phenacyl ester separation on HPLC**

201 To investigate changes in fatty acids in plants treated with PHE, 6 available fatty acid
202 standards including lauric acid (C12: 0), meristic acid (C14: 0), palmitic acid (C16: 0), oleic
203 acid (C18: 1), linoleic acid (C18: 2), and arachidonic acid (C20: 4) were considered. Fatty acids
204 were prepared as a phenyl derivative and then injected into the high-performance liquid
205 chromatograph (HPLC) and standard chromatograms were obtained. Fatty acid phenacyl esters
206 were analyzed using a HPLC (KNAUER, Germany) equipped with a UV detector and a Φ 4.6
207 \times 150 mm reverse phase C18 column. The UV-detector wavelength was set at 242 nm and the
208 column temperature was maintained at 40 °C. The mobile phase with flow-rate 0.8 mL min⁻¹
209 and with a gradient of methanol/acetonitrile/water started at 80:10:10 (v/v/v) which increased
210 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
211 at 90:10:0 (v/v/v) for 5 min and returning to the initial conditions (80:10:10) in 5 min. the whole
212 running time for each injection was set to 50 min (Bodoprost and Rosemeyer, 2007; Rioux *et*
213 *al.*, 1999; Hanist *et al.*, 1988).

214
215 **2.12. Statistical analysis**

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

220
221 **3. Results**

222 **3.1. Growth parameters**

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

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234

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

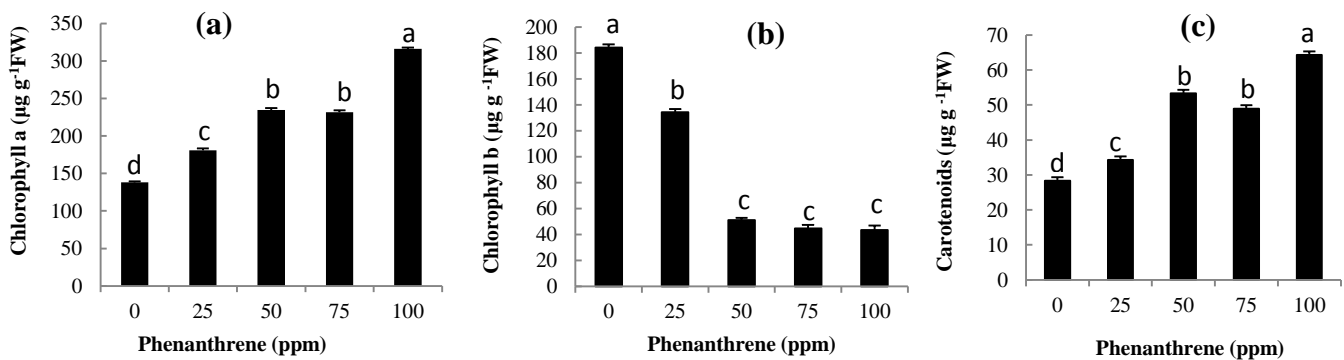
Phenanthrene (ppm)	Shoot Length (cm)	Root Length (cm)	Shoot FW (mg)	Root FW (mg)	Shoot DW (mg)	Root DW (mg)
0	0.25 ^a ± 39.1	1.59 ^a ± 28	64.75 ^a ± 995	35.45 ^a ± 888	16.99 ^a ± 197	24.03 ^a ± 155
25	1.03 ^b ± 30.6	0.03 ^b ± 19.6	38.64 ^b ± 648	17.25 ^b ± 601	5.46 ^b ± 93	12.16 ^{ab} ± 80
50	0.06 ^c ± 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

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

240 **3.2. Photosynthetic pigments content**

241 Chlorophyll a content exhibited significant increases in plants treated by 50, 75, and 100 ppm
242 of PHE in comparison with the control plants, with the most significant elevation (+128.85%)
243 observed at 100 ppm PHE treatment ($p < 0.05$). In contrast, all applied levels of PHE
244 significantly decreased chlorophyll b content. Such content in the plants treating with 25, 50,
245 75, and 100 ppm of PHE were decreased to 27.04, 72.26, 75.71, and 76.36 %, respectively.
246 Moreover, the highest value of carotenoids (+126.98 %) was recorded in plants exposed to 100
247 ppm of PHE (Fig. 1).

248

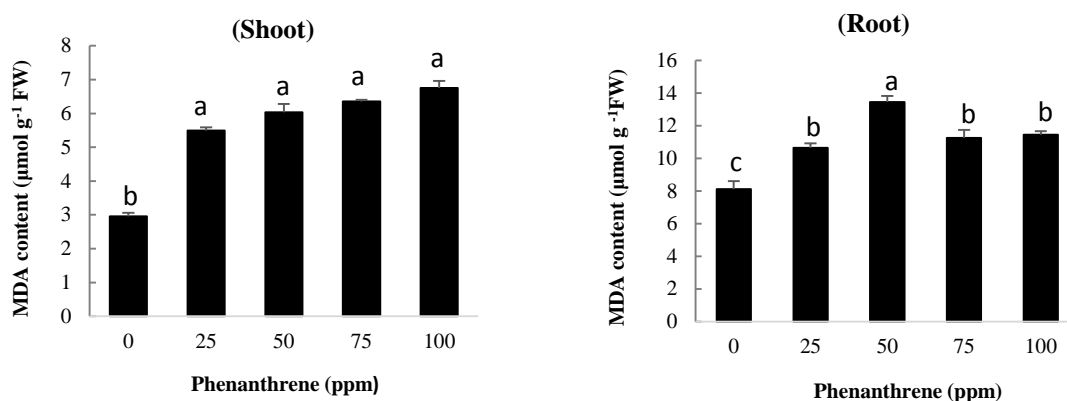


249 **Fig 1.** The effects of different concentrations of phenanthrene on photosynthetic pigments content of maize plants.
250 (a) Chlorophyll a, (b) Chlorophyll b, and (c) Carotenoids. The data represent the mean of three replications and
251 error bars indicate SD. The same letters above the bars indicate no significant differences ($p < 0.05$).
252

254 **3.3. The content of malondialdehyde (MDA)**

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

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

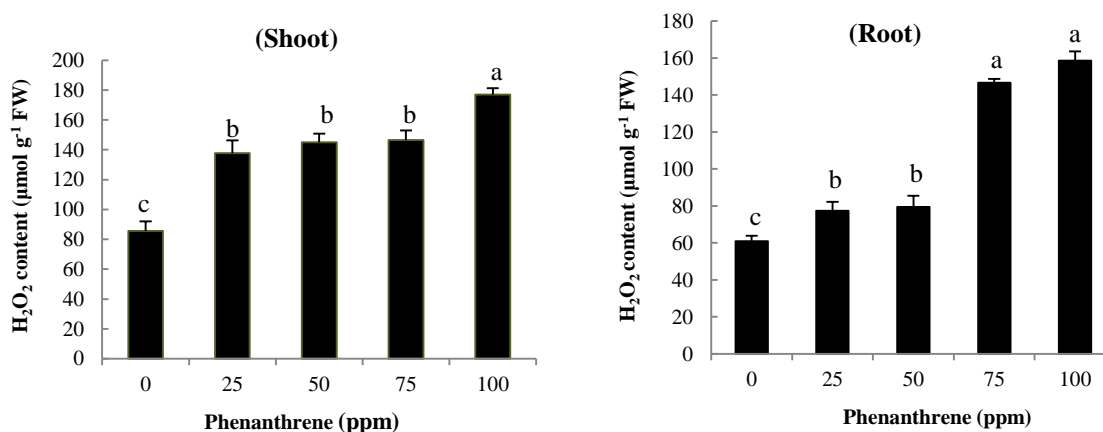


263

264 **Fig 2.** The effects of different concentrations of phenanthrene on malondialdehyde (MDA) contents of maize
 265 plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the
 266 bars indicate no significant differences ($p < 0.05$).
 267

268 3.4. Hydrogen peroxide (H₂O₂) content

269 Phenanthrene had a significant effect on the content of H₂O₂ in treated plants. The enhanced
 270 content of H₂O₂ in plants had been observed through the increasing concentrations of PHE
 271 ($p < 0.05$). The H₂O₂ contents in the shoots of plants treated with 25, 50 75, and 100 ppm of PHE
 272 were increased by 160.67, 171, 171.58, and 207.21%, respectively. The values for the roots of
 273 the same plants recorded increases of 126.73, 130.01, 240.38, and 260.17%, respectively (Fig.
 274 3).



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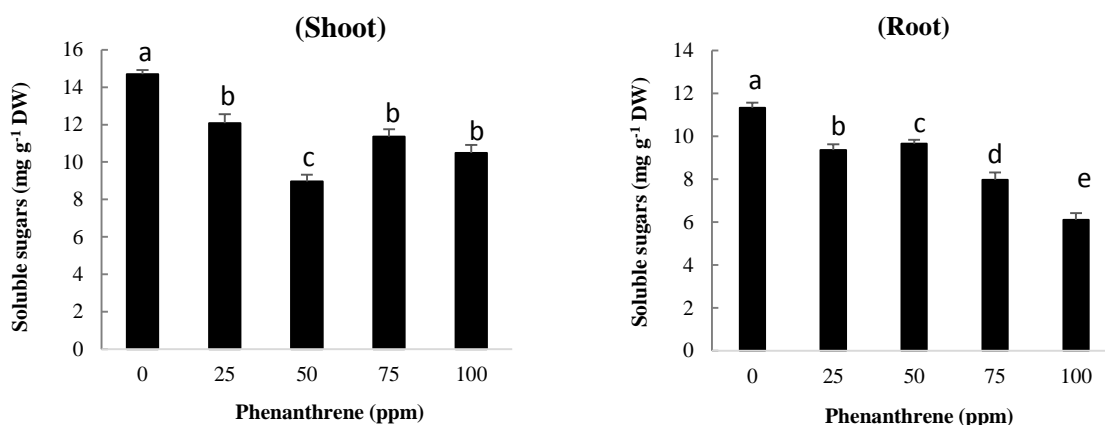
276 **Fig 3.** The effects of different concentrations of phenanthrene on hydrogen peroxide (H₂O₂) contents of maize
 277 plants. The data represent the mean of three replications and error bars indicate SD. The same letters above the
 278 bars indicate no significant differences ($p < 0.05$).
 279

280

281

282 3.5. Soluble sugars content

283 The results revealed that PHE had significant effect on the content of soluble sugars ($p < 0.05$).
 284 The content of soluble sugars of both shoots and roots were decreased at various levels of
 285 phenanthrene in comparison with the control, and the greatest reduction in soluble sugars was
 286 observed in the shoots and roots of plants subjected to 50 (38.77%) and 100ppm (46.07%),
 287 respectively ($p < 0.05$) (Fig. 4).
 288



289

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

293

294 3.6. Activity of antioxidant enzymes

295 The changes in the activity of CAT, POD, and SOD within the shoot and root of maize plants
 296 after treatment with PHE were shown in Table 2. The CAT activity was declined in the shoots
 297 of the plants exposed to 50, 75, and 100 ppm of PHE. Conversely, in the roots, CAT activity
 298 responded differently, with significant increases of 91.55%, 86.88%, and 48.44% observed at
 299 50, 75, and 100 ppm of phenanthrene, respectively, compared to the control ($p < 0.05$).

300 POD activity in the shoots of treated plants with various concentrations of PHE was
 301 significantly higher in comparison with the control. The highest POD activities were observed
 302 in plants treated by 50 (+112%) and 75 ppm (+128%) of PHE, respectively ($p < 0.05$). In roots
 303 only significant increase in POD activity was seen at 75 ppm (+57.26%) of phenanthrene (Table
 304 2).

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

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

312
 313 **Table 2.** The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on antioxidant
 314 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

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

318 3.7. Total flavonoids, phenols and anthocyanin contents

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

328
 329 **Table 3.** The effect of different concentrations of phenanthrene (0, 25, 50, 75, and 100 ppm) on total flavonoid
 330 (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
 331 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

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

335 3.8. Fatty acid concentration

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

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

348
 349 **Table 4.** The effect of concentration of phenanthrene (50 ppm) on fatty acid concentration ($\mu\text{g g}^{-1}\text{FW}$) in the
 350 shoot and root of maize plant.

Fatty acid ($\mu\text{g g}^{-1}\text{FW}$)	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

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

353

354 3.9. Analysis correlation

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

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372 **Table 5.** Statistical analysis for correlation between the activity of antioxidant enzyme and MDA and H₂O₂
 373 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 ₂ O ₂ Shoot	H ₂ O ₂ Root
H ₂ O 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 ₂ O 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									

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

376

377 4. Discussion

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

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

399 Our results have shown that H₂O₂ and MDA contents in both shoots and roots enhanced
400 through increasing PHE levels. Therefore, MDA accumulation resulting from oxidative stress
401 and ROS accumulation was a reliable marker for determining of the negative effect of PHE on
402 the growth of maize plants. Generally, plants equipped with enzymatic and non-enzymatic
403 mechanisms for scavenging ROS accumulation under oxidative stress (Tarigholizadeh *et al.*,
404 2021). Several enzymes like SOD, CAT, and POD as enzymatic mechanisms play important
405 roles during seed germination. SOD, as the first line of defense against ROS, acts on superoxide
406 radicals producing under stress conditions, catalyzing their conversion into H₂O₂ and O₂
407 (Hasanuzzaman *et al.*, 2021). CAT is the primary H₂O₂ scavenging enzyme in plant cells (Li
408 and Yi, 2012). In this study, CAT, POD, and SOD activities were increased in the roots, while
409 CAT and SOD activities were decreased in the shoots by different levels of PHE. Also, the
410 correlation analysis (Table 4) demonstrated a negative correlation between H₂O₂ and MDA
411 contents, as well as between CAT and SOD activities, in both the shoot and root of maize,
412 emphasizing the role of these enzymes in ROS detoxification to mitigate stress conditions and
413 enhance plant resistance to oxidative stress. Moreover, the decrease in SOD and CAT activities
414 induced by PHE occurred exclusively in the shoots, while phenol and flavonoid levels
415 contributed to this decrease in shoots and roots, consequently reducing the H₂O₂ scavenging
416 ability and leading to lipid peroxidation. Therefore, it can be said that in the maize plant, the
417 antioxidant system of maize in the shoots was not strong enough to eliminate all produced ROS
418 at high concentrations, resulting in oxidative damage, diminished photosynthesis, reduced
419 soluble sugar content, and compromised growth parameters. Similar results have been reported
420 for sunflower, alfalfa, and wheat plants (Salehi-Lisar and Deljoo, 2015).

421 PHE significantly influenced soluble sugar content ($p < 0.05$), with reduced levels detected in
422 both shoots and roots. This reduction can be linked to the decreased demand for photosynthetic
423 materials due to reduced root growth (Table 1). In addition, lower carbohydrate content in roots
424 can be attributed to higher consumption of energy for resistance of PHE toxicity. Carbohydrates
425 in plants, in addition to energy production, regulate various gene expressions (Rolland *et al.*,
426 2006) and may have antioxidant activity (Lang-Mladek *et al.*, 2010).

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

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

437

438 5. Conclusions

439 Based on the obtained results, high concentrations of phenanthrene (75 and 100 ppm) have
440 inhibitory effects on plants. Reduced growth, lower chlorophyll b content, and elevated
441 contents of MDA and H₂O₂ in root are some negative effects of phenanthrene. Further, the
442 increase in the activity of antioxidant enzymes due to phenanthrene treatment indicated the
443 oxidative stress caused by this compound. However, the decrease in the activity of CAT and
444 the non-destruction of the produced hydrogen peroxide due to stress indicated a decrease in the
445 resistance of the maize plant to this compound. On the other hand, the decrease in the amount
446 of MDA in the shoot and then the increase in the roots also demonstrated higher accumulation
447 of phenanthrene in the roots leading to weakening of plants root system as well as disordering
448 in water and nutrient uptake and finally reduction in the plant growth.

449

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453

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- 565 پاسخ های فیزیولوژیکی و بیوشیمیایی ذرت (*Zea mays*) به سمیت فنانترن
- 566 مهدیه هوشانی، سید یحیی صالحی لیسار، علی موقفی، و روح الله متفکر زاد
- 567 هیدروکربن های آروماتیک چند حلقه ای (PAHs)، دسته ای از آلاینده های آلی، مشاهده شده اند که اثرات مضر بر رشد
- 568 گیاه و فرآیندهای فیزیولوژیکی مختلف دارند. در این تحقیق اثر غلظت های مختلف فنانترن (0، 25، 50، 75 و 100 پی
- 569 پی ام) بر پارامترهای رشد، رنگدانه های فتوسنتزی، برخی آنزیم های آنتی اکسیدانی و برخی ترکیبات بیوشیمیایی گیاه
- 570 ذرت مورد بررسی قرار گرفت. آزمایش ها به صورت کشت گلدانی گیاهان در شرایط کنترل شده در قالب طرح کاملاً
- 571 تصادفی (CRD) با سه تکرار برای هر تیمار انجام شد. افزایش غلظت فنانترن تمام پارامترهای رشد مورد مطالعه را
- 572 کاهش داد و محتوای رنگدانه فتوسنتزی را به طور قابل توجهی افزایش داد. فنانترن منجر به افزایش فعالیت کاتالاز،
- 573 پراکسیداز و سوپراکسید دیسموتاز در ریشه شد اما در اندام هوایی تنها فعالیت پراکسیداز افزایش یافت. علاوه بر این،
- 574 غلظت های بالاتر فنانترن با افزایش سطح مالون دی آلدئید و پراکسید هیدروژن همراه با کاهش محسوس در محتوای قند
- 575 محلول در هر دو بافت اندام هوایی و ریشه همراه بود ($p < 0/05$). همچنین افزایش غلظت فنانترن در اندام هوایی باعث
- 576 کاهش محتوای کل فنل و فلاونوئید نسبت به آنتوسیانین شد. تیمار فنانترن منجر به کاهش قابل توجه غلظت اسید لوریک،
- 577 مریک اسید، اسید پالمیتیک و اسید اولئیک در اندام هوایی ذرت شد. در نتیجه به نظر می رسد که غلظت بالای فنانترن
- 578 باعث ایجاد استرس اکسیداتیو در ذرت می شود و گیاهان سیستم آنتی اکسیدانی آنزیمی خود را برای تعدیل شرایط تنش
- 579 بهبود می بخشند. علاوه بر این، آسیب غشای سلولی توسط فنانترن منجر به تضعیف سیستم ریشه گیاهان و اختلال در
- 580 جذب آب و عناصر غذایی و در نهایت کاهش رشد گیاه می شود.
- 581