Morphophysiological and Redox Modulation of *Abelmoschus esculentus* Under Graded Crude Oil Soil Contamination

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ABSTRACT   Original Research Article

This study evaluated the morphophysiological and biochemical responses of Abelmoschus esculentus to graded crude oil contamination under a controlled pot experiment. Five treatments were established: 0% (control), 0.1%, 0.2%, 0.3% and 0.4% v/w contamination, arranged in a completely randomised design with five replicates. Growth dynamics over seven weeks showed progressive increases in plant height and stem girth across treatments; however, higher contamination levels (0.3–0.4% v/w) significantly reduced final height (≈32 cm) relative to the control (≈36 cm). Stem girth followed a similar trend, with reduced thickening at higher contamination by week 7. Relative water content declined dose-dependently from 92% in the control to 51% at 0.4% v/w, indicating substantial impairment of plant water status. Biochemical analyses revealed tissue-specific modulation of oxidative stress markers and antioxidants. Malondialdehyde (MDA) concentrations ranged from 0.0034–0.0051 $\mu mol\ g^{-1}$ FW (leaf), 0.0098–0.0121 $\mu mol\ g^{-1}$ FW (stem) and 0.0020–0.0089 $\mu mol\ g^{-1}$ FW (root), reflecting variable lipid peroxidation across treatments. Conversely, Vitamin C increased progressively with contamination, reaching 94 $\mu mol\ g^{-1}$ FW (leaf), 271 $\mu mol\ g^{-1}$ FW (stem) and 336 $\mu mol\ g^{-1}$ FW (root). Reduced glutathione (GSH) also exhibited substantial induction, particularly in roots at 0.2% v/w (20.4 $\mu mol\ g^{-1}$ FW) and stems at 0.4% v/w (15.3 $\mu mol\ g^{-1}$ FW). Overall, crude oil contamination induced dose-dependent growth inhibition and water deficit, accompanied by activation of antioxidant defence systems. The findings demonstrate that A. esculentus exhibits measurable redox plasticity and moderate tolerance to petroleum stress, although higher contamination levels compromise morphophysiological performance.

Keywords: Crude Oil Contamination, *Abelmoschus esculentus*, Oxidative Stress Biomarkers, Antioxidant Defence System, Relative Water Content, Phytoremediation Potential.

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Introduction

Crude oil contamination of agricultural soils remains a persistent environmental challenge in petroleum-producing regions, particularly in sub-Saharan Africa, where exploration, transportation and artisanal refining frequently result in hydrocarbon release into terrestrial ecosystems. Petroleum hydrocarbons alter soil structure, reduce aeration, disrupt nutrient cycling and impair microbial activity, collectively compromising soil fertility and crop productivity (Falih et al., 2024; Orire, 2024). In addition to physicochemical alterations, crude oil contamination exerts phytotoxic effects through the induction of oxidative stress, osmotic imbalance and impaired water relations, ultimately constraining plant growth and yield (Agbaji et al., 2024; Orocio-Carrillo et al., 2022).

One of the primary mechanisms underlying hydrocarbon-induced phytotoxicity is the excessive generation of reactive oxygen species (ROS), including superoxide radicals,

hydrogen peroxide and hydroxyl radicals. These reactive intermediates attack membrane lipids, proteins and nucleic acids, resulting in cellular dysfunction (Alam et al., 2025; Vicidomini et al., 2024). Lipid peroxidation, commonly assessed via malondialdehyde (MDA) accumulation, is widely recognised as a reliable indicator of oxidative membrane damage under environmental stress (Rizzo, 2024; Valgimigli, 2023). Elevated MDA levels in plants exposed to petroleum hydrocarbons have been associated with disrupted membrane integrity and reduced physiological performance (Correa et al., 2022; Haider et al., 2021).

To counteract oxidative injury, plants activate a coordinated antioxidant defence system comprising enzymatic and non-enzymatic components. Among the latter, ascorbic acid (Vitamin C) and reduced glutathione (GSH) play central roles in maintaining cellular redox homeostasis. Ascorbate participates in direct ROS scavenging and regeneration of other antioxidants, while GSH functions in detoxification reactions, xenobiotic conjugation and operation of the ascorbate–glutathione cycle (Foyer & Kunert, 2024; Yemelyanov et al., 2024). Enhanced accumulation of these metabolites under pollutant stress reflects adaptive metabolic reprogramming aimed at mitigating oxidative damage. However, the extent to which antioxidant induction offsets growth inhibition under graded petroleum contamination remains incompletely characterised.

Beyond biochemical responses, crude oil contamination also affects morphophysiological traits such as plant height, stem girth and relative water content (RWC). Reduced RWC under hydrocarbon stress has been linked to impaired root water uptake, altered osmotic potential and membrane destabilisation (Hoang et al., 2021; MORELOS-MORENO et al., 2021). Since water status directly influences turgor-driven growth, changes in RWC may provide mechanistic insight into observed reductions in biomass accumulation.

Abelmoschus esculentus (okra) is an economically important vegetable crop widely cultivated in tropical regions. Its rapid growth cycle and sensitivity to soil conditions make it a suitable model for evaluating plant responses to petroleum-contaminated soils. Although previous studies have examined the general effects of hydrocarbons on crop performance, integrated assessments linking growth dynamics, water status and redox biomarkers under graded contamination levels are limited.

Therefore, this study investigated the morphophysiological and biochemical responses of A. esculentus to increasing concentrations of crude oil in soil. By integrating growth parameters with indices of lipid peroxidation and antioxidant status, the study aimed to elucidate the adaptive capacity and tolerance thresholds of okra under petroleum-induced stress.

Materials and Methods

Experimental Design and Soil Contamination

The study was conducted as a controlled pot experiment to evaluate the effects of graded crude oil contamination on the morphophysiological and biochemical responses of *Abelmoschus esculentus*. Topsoil (0–20 cm depth) was collected from an uncontaminated site, air-dried, homogenised, and sieved through a 2 mm mesh to remove debris. Physicochemical properties of the soil were determined prior to contamination using standard analytical procedures (Bremner, 1996; Sparks et al., 1996) and well documented (Adeyemi and Akpobaro, 2026).

Bonny Light crude oil was obtained from a certified petroleum facility and thoroughly mixed with 10 kg of soil per pot to achieve volumetric contamination levels of 0% (control), 0.1%, 0.2%, 0.3%, and 0.4% v/w. The contaminated soils were equilibrated for seven days to allow hydrocarbon–soil interaction before planting, following established petroleum contamination protocols (Adam & Duncan, 2002). The experimental layout followed a completely randomised design with five treatments (A–E), each in five replicates.

Planting and Growth Monitoring

Certified seeds of *A. esculentus* were surface-sterilised in 1% sodium hypochlorite for 2 min and rinsed thoroughly with distilled water prior to sowing. Three seeds were planted per pot and later thinned to one uniform seedling per pot after emergence. Pots were maintained under natural photoperiod conditions and irrigated with distilled water to field capacity as required. Five replicate pots per treatment was used.

Plant height (cm) was measured weekly from the soil surface to the apical meristem using a calibrated ruler, while stem girth (cm) was measured at 2 cm above the soil surface using a digital Vernier calliper. Measurements were recorded for seven consecutive weeks.

Determination of Relative Water Content (RWC)

Leaf relative water content was determined according to the method described by Barrs and Weatherley (1962). Fresh weight (FW) of leaf discs was recorded immediately after excision. Samples were floated on distilled water for 4 h to obtain turgid weight (TW), then oven-dried at 70°C to constant weight to determine dry weight (DW). RWC was calculated as:

$$RWC\,(\%) = \frac{FW - DW}{TW - DW} \times 100$$

Leaf Chlorophyll Determination

Leaf chlorophyll content was determined non-destructively using a portable SPAD chlorophyll meter (SPAD-502 Plus, Konica Minolta, Japan). The instrument operates at dual wavelengths of 650 nm (red light, chlorophyll absorption peak) and 940 nm (near-infrared reference), enabling estimation of relative chlorophyll concentration based on differential light transmittance.

Biochemical Analyses

Fresh leaf, stem, and root tissues were harvested at week 7, rinsed with distilled water, blotted dry, and homogenised in ice-cold phosphate buffer (0.1 M, pH 7.0). Homogenates were centrifuged at $10,000 \times g$ for 15 min at 4°C, and supernatants were used for biochemical assays.

Malondialdehyde (MDA) was quantified as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method described by Heath and Packer (1968). Absorbance was measured spectrophotometrically at 532 nm and corrected for non-specific turbidity at 600 nm. Results were expressed as $\mu mol\ g^{-1}$ fresh weight (FW).

Reduced glutathione (GSH) was determined using the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) method of Ellman (1959), as modified for plant tissues. Absorbance was read at 412 nm, and concentrations were calculated using the molar extinction coefficient of reduced glutathione.

Vitamin C (ascorbic acid) was quantified using the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method as described by AOAC (2016). Results were expressed as $\mu mol\ g^{-1}$ FW.

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM) for five independent replicates. Statistical analyses were performed using one-way analysis of variance (ANOVA) to assess treatment effects, followed by Tukey's honestly significant difference (HSD) test for post hoc comparisons at $p < 0.05$.

Results

Plant height increased progressively across the seven-week growth period in all treatments (Figure 1). However, contamination level significantly influenced growth magnitude. The control (A) consistently exhibited the greatest height throughout the study, culminating in approximately 36 cm at week 7. Treatments B (0.1% v/w) and C (0.2% v/w) showed moderate reductions relative to the control but maintained comparable growth trajectories. In contrast, higher contamination levels (D: 0.3% v/w and E: 0.4% v/w) resulted in comparatively reduced final plant heights, particularly evident from week 5 onwards. Although early growth differences (weeks 1–3) were minimal, divergence became pronounced during the exponential growth phase (weeks 5–7), indicating that crude oil stress exerted cumulative inhibitory effects over time.
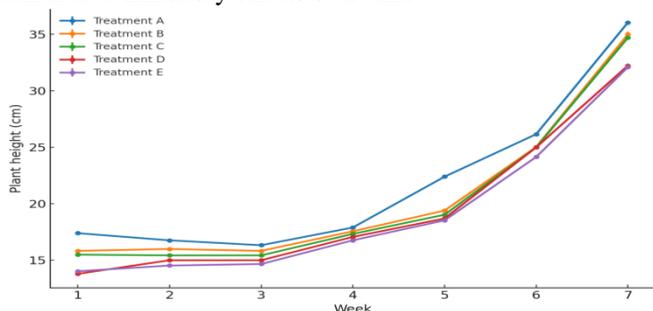

Figure 1: Okra Plant Height over Seven Weeks under Graded Bonny Light Crude Oil Treatments (Mean ± SEM)

Stem girth followed a similar temporal pattern (Figure 2). Initial reductions were observed at week 2 across all treatments, likely reflecting transplant establishment dynamics. Thereafter, girth increased steadily until week 6. The control maintained relatively stable and progressive thickening, reaching approximately 3.4 cm at week 7. Moderate contamination levels (B and C) exhibited comparable or slightly enhanced girth relative to the control at mid-growth stages (weeks 5–6), suggesting possible compensatory thickening under mild stress. However, the highest contamination levels (D and E) showed reduced girth at week 7, indicating compromised structural development under prolonged hydrocarbon exposure.
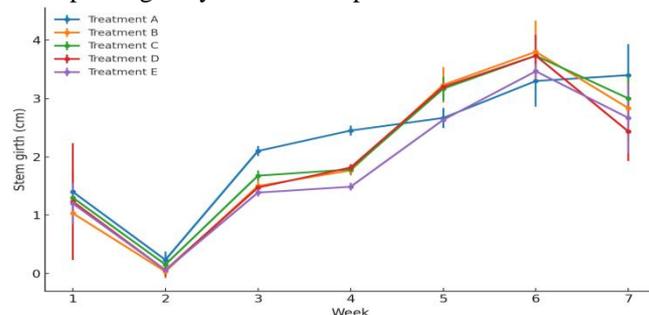

Figure 2: Okra Plant stem girth over Seven Weeks under Graded Bonny Light Crude Oil Treatments (Mean ± SEM)

Relative water content (RWC) declined significantly and progressively with increasing contamination (Figure 3). The control recorded the highest RWC (~92%), while treatments B, C, D, and E demonstrated stepwise reductions to approximately 80%, 67%, 56%, and 51%, respectively. The graded decrease, accompanied by distinct statistical groupings, indicates a strong dose-dependent impairment of plant water status under crude oil contamination.
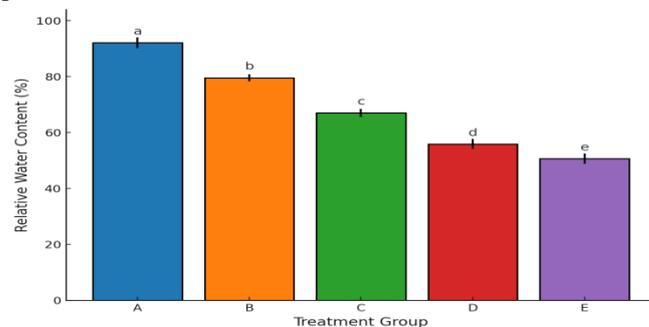

Figure 3: Relative water content (%) in okro plants exposed to varying concentrations of Bonny Light crude oil (0–4% v/w). Plotted values are means ± SEM. Bars bearing different alphabetical notations are significantly different (p≤0.05).
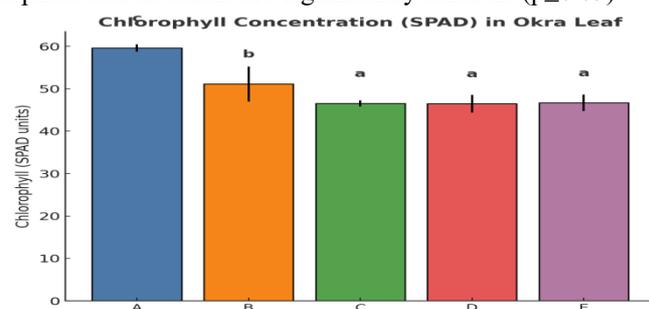

Figure 4: Concentration of chlorophyll in leaf of okra plants exposed to varying concentrations of Bonny Light crude oil

(0–4% v/w). Plotted values are means ± SEM. Bars bearing different alphabetical notations are significantly different (p≤0.05).

Graded crude oil contamination significantly altered lipid peroxidation and non-enzymatic antioxidant responses in *Abelmoschus esculentus* (Tables 1–3).

Table 1 presents the concentration of malondialdehyde (MDA), an established index of lipid peroxidation and oxidative membrane damage, in the leaf, stem and root tissues of **Abelmoschus esculentus** subjected to graded crude oil contamination (0–0.4% v/w). The data demonstrate clear tissue-specific and concentration-dependent responses, with statistically significant differences among treatments within each organ (p < 0.05). In the **leaf**, MDA levels ranged from 0.0034 to 0.0051 μmol g^{-1} FW. The control (0%) recorded 0.0043 ± 0.0001 μmol g^{-1} FW ([b]). A significant increase was observed at 0.1% (0.0051 ± 0.0001 μmol g^{-1} FW; [c]) and 0.3% (0.0050 ± 0.0001 μmol g^{-1} FW; [c]), indicating enhanced lipid peroxidation under mild to moderate contamination. Interestingly, MDA declined significantly at 0.2% and 0.4% (both 0.0034 ± 0.0001 μmol g^{-1} FW; [a]), suggesting either activation of antioxidant defence mechanisms or reduced metabolic activity at higher stress levels. The biphasic pattern implies an initial oxidative surge followed by adaptive or suppressive physiological adjustment. In the **stem**, MDA concentrations were consistently higher than in leaves, ranging from 0.0098 to 0.0121 μmol g^{-1} FW. The highest value occurred at 0.2% contamination (0.0121 ± 0.0001 μmol g^{-1} FW; [c]), representing a significant elevation relative to control (0.0103 ± 0.0001 μmol g^{-1} FW; [a]). Moderate elevation was also observed at 0.3% (0.0115 ± 0.0001 μmol g^{-1} FW; [b]). However, at 0.4%, MDA declined to 0.0098 ± 0.0001 μmol g^{-1} FW ([a]), statistically comparable to control. This pattern suggests that the stem exhibits pronounced oxidative sensitivity at intermediate contamination levels, possibly due to its role in translocation of hydrocarbons and associated reactive oxygen species (ROS). In the **root**, MDA exhibited marked variability, ranging from 0.0020 to 0.0089 μmol g^{-1} FW. The control recorded 0.0089 ± 0.0001 μmol g^{-1} FW ([c]), the highest baseline among treatments. Exposure to 0.1% and 0.3% resulted in moderate but significant reductions (0.0074 ± 0.0001 μmol g^{-1} FW; [b]). A pronounced decline was observed at 0.2% (0.0020 ± 0.0001 μmol g^{-1} FW; [a]), indicating minimal lipid peroxidation at this concentration. At 0.4%, MDA increased slightly to 0.0039 ± 0.0001 μmol g^{-1} FW ([d]), though still significantly lower than control. The reduction in root MDA under contamination may reflect altered membrane lipid composition, restricted oxygen diffusion in oil-impacted rhizosphere, or induction of root-specific antioxidant systems.

Table 1: MDA Concentration (μmol g^{-1} FW) in Leaf, Stem and Root of *A. esculentus* under Graded Crude Oil Contamination

Treatment (v/w)	Leaf (μmol g^{-1} FW)	Stem (μmol g^{-1} FW)	Root (μmol g^{-1} FW)
A (0%)	0.0043 ± 0.0001[b]	0.0103 ± 0.0001[a]	0.0089 ± 0.0001[c]
B (0.1%)	0.0051 ± 0.0001[c]	0.0103 ± 0.0001[a]	0.0074 ± 0.0001[b]
C (0.2%)	0.0034 ± 0.0001[a]	0.0121 ± 0.0001[c]	0.0020 ± 0.0001[a]
D (0.3%)	0.0050 ± 0.0001[c]	0.0115 ± 0.0001[b]	0.0074 ± 0.0001[b]
E (0.4%)	0.0034 ± 0.0001[a]	0.0098 ± 0.0001[a]	0.0039 ± 0.0001[d]

Values are expressed as mean ± SEM (n = 5). Different superscript letters within each tissue indicate significant differences among treatments (p < 0.05; Tukey's HSD).

Vitamin C concentrations increased progressively with contamination intensity across all tissues (Table 2). In leaves, ascorbate content rose significantly from the control to the highest contamination level, with the greatest accumulation observed at 0.4% v/w. Stem tissues showed a pronounced elevation at 0.3% v/w, representing the peak response, while root tissues exhibited a strong dose-dependent increase, reaching maximum values at 0.4% v/w.

Table 2: Vitamin C Concentration (μmol g^{-1} FW) in Leaf, Stem and Root of *A. esculentus* under Graded Crude Oil Contamination

Treatment (v/w)	Leaf (μmol g^{-1} FW)	Stem (μmol g^{-1} FW)	Root (μmol g^{-1} FW)
A (0%)	42.0 ± 3.0[a]	118.0 ± 8.0[a]	122.0 ± 9.0[a]
B (0.1%)	58.0 ± 4.0[b]	167.0 ± 10.0[b]	168.0 ± 11.0[b]
C (0.2%)	77.0 ± 5.0[c]	167.0 ± 11.0[b]	174.0 ± 12.0[b]
D (0.3%)	75.0 ± 4.0[c]	271.0 ± 13.0[c]	286.0 ± 15.0[c]
E (0.4%)	94.0 ± 6.0[d]	240.0 ± 12.0[d]	336.0 ± 16.0[d]

Values are expressed as mean ± SEM (n = 5). Different superscript letters within each tissue indicate significant differences among treatments (p < 0.05; Tukey's HSD).

Reduced glutathione (GSH) also demonstrated a general dose-dependent elevation (Table 3). In leaves, GSH increased progressively across treatments, with the highest value recorded at 0.4% v/w. Stem tissues followed a similar pattern, with incremental increases

culminating at 0.4% v/w. In roots, however, GSH peaked at 0.2% v/w, followed by a decline at 0.3% v/w and a secondary increase at 0.4% v/w, indicating a biphasic response.

Collectively, contamination significantly modulated oxidative stress markers and antioxidant metabolites in a concentration- and tissue-dependent manner.

Table 3: Reduced Glutathione (GSH) Concentration (μmol g^{-1} FW) in Leaf, Stem and Root of *A. esculentus* under Graded Crude Oil Contamination

Treatment (v/w)	Leaf (μmol g^{-1} FW)	Stem (μmol g^{-1} FW)	Root (μmol g^{-1} FW)
A (0%)	0.95 ± 0.07^a	2.60 ± 0.20^a	2.50 ± 0.18^a
B (0.1%)	1.65 ± 0.15^b	4.70 ± 0.50^b	4.80 ± 0.45^b
C (0.2%)	2.90 ± 0.30^c	7.90 ± 0.80^c	20.40 ± 2.10^c
D (0.3%)	3.40 ± 0.35^c	11.10 ± 1.10^d	11.10 ± 1.20^d
E (0.4%)	6.20 ± 0.60^d	15.30 ± 1.50^e	15.40 ± 1.60^e

Values are expressed as mean ± SEM (n = 5). Different superscript letters within each tissue indicate significant differences among treatments (p < 0.05; Tukey's HSD).

Discussion

The growth responses illustrated in Figures 1–3 demonstrate that Abelmoschus esculentus retained the capacity for vegetative development under graded crude oil contamination, although performance declined with increasing hydrocarbon concentration. The progressive increase in plant height across treatments confirms sustained meristematic activity; however, the consistent reduction in final height at 0.3–0.4% v/w indicates that crude oil exerts inhibitory effects on cell elongation and biomass accumulation. Such growth suppression under petroleum stress has been attributed to reduced nutrient bioavailability, impaired root respiration and hydrocarbon-induced soil hydrophobicity, which collectively limit resource acquisition (Kamranifar et al., 2025; Odukoya et al., 2019). The greater divergence observed during later growth stages suggests cumulative soil-mediated constraints, consistent with reports that chronic hydrocarbon exposure progressively disrupts plant development (Bahar et al., 2024; Kamranifar et al., 2025).

Stem girth patterns (Figure 2) further reveal structural adjustments under contamination. The comparable or slightly enhanced girth at moderate contamination during mid-growth stages may reflect stress-induced assimilate reallocation towards supportive tissues, enhancing mechanical stability and vascular conductivity. Similar compensatory responses have been observed in plants exposed to sub-lethal abiotic stress (Du et al., 2024; Zhang et al., 2025). However, the decline in girth at higher contamination levels by week 7 indicates that prolonged hydrocarbon exposure suppresses cambial activity and secondary growth, likely due to impaired carbon assimilation and hormonal imbalance.

The marked, dose-dependent decline in relative water content (Figure 3) demonstrates significant disruption of plant water relations. Reduced RWC is indicative of impaired root hydraulic conductivity, osmotic imbalance and altered membrane permeability under hydrocarbon stress (Sherri et al., 2023; Григориади et al., 2023). Because turgor pressure directly drives cell expansion, compromised hydration status plausibly explains the concomitant reductions in height and girth at higher contamination levels. The sensitivity of RWC underscores its utility as an early physiological indicator of petroleum-induced stress.

The significant decline in leaf chlorophyll concentration with increasing crude oil contamination (Figure 4) indicates impaired photosynthetic capacity under hydrocarbon stress. The reduction from the control to contaminated treatments suggests disruption of chlorophyll biosynthesis and enhanced pigment degradation, likely mediated through oxidative stress and membrane lipid peroxidation (Ali et al., 2024; Dąbrowski et al., 2024). Petroleum hydrocarbons can alter soil nutrient availability, particularly nitrogen and magnesium, which are essential for chlorophyll formation (Yong et al., 2024). The plateau observed at higher contamination levels may reflect physiological threshold effects, where chloroplast damage and metabolic inhibition reach a saturation point (Ghassemi-Golezani et al., 2025).

Biochemical responses (Tables 1–3) provide mechanistic insight into these morphophysiological alterations. Elevated MDA concentrations observed in selected tissues under crude oil exposure substantiate the occurrence of enhanced lipid peroxidation and increased generation of reactive oxygen species (ROS), consistent with established evidence that petroleum hydrocarbons disrupt membrane integrity through oxidative pathways (Janbazi et al., 2024; Oleforuh-Okoleh et al., 2024). In the present dataset, this effect was particularly evident in the stem, where MDA peaked significantly at 0.2% contamination, indicating heightened oxidative vulnerability at intermediate exposure levels. This pattern suggests that the stem, functioning as the principal conduit for assimilate and solute translocation, may experience intensified oxidative burden due to systemic distribution of hydrocarbon-derived toxicants and associated ROS. In the leaf, moderate elevations at 0.1% and 0.3% further support the induction of oxidative stress under low-to-moderate contamination.

However, the absence of a progressive increase with concentration and the significant reduction in MDA at 0.2% and 0.4% indicate a non-linear response. Such biphasic dynamics may reflect activation of enzymatic and non-enzymatic antioxidant defence systems, including superoxide dismutase, catalase and peroxidases, which can suppress lipid peroxidation when effectively induced. Similarly, the marked decline in root MDA at 0.2% and 0.4% compared with the control suggests either enhanced antioxidative regulation or altered rhizospheric conditions that limit oxidative propagation. Given that roots are the primary interface with contaminated soil, their response may involve adaptive modulation of membrane composition or metabolic downregulation under sustained stress. Collectively, the tissue-specific peaks—most pronounced in stems at intermediate contamination—and the reduction at higher doses in certain organs align with the concept of differential oxidative susceptibility and compensatory antioxidant activation under graded crude oil stress (Janbazi et al., 2024; Oleforuh-Okoleh et al., 2024).

The progressive elevation of Vitamin C and GSH across treatments reflects inducible redox buffering. Ascorbate functions in direct ROS scavenging and regeneration of antioxidant systems, while GSH is central to the ascorbate–glutathione cycle and xenobiotic detoxification (Chen et al., 2023; Foyer & Kunert, 2024). The biphasic GSH response in roots indicates transient oxidative overload followed by compensatory synthesis, highlighting dynamic redox regulation.

Collectively, the inverse association between MDA and antioxidant metabolites demonstrates coordinated oxidative stress modulation. Moderate contamination appears to impose maximal oxidative challenge, whereas higher levels stimulate stronger antioxidant induction, partially stabilising membrane integrity. These findings indicate substantial redox plasticity in A. esculentus, supporting its moderate tolerance to petroleum-contaminated soils.

In conclusion, this study demonstrated that graded crude oil contamination significantly alters the morphophysiological and redox status of *Abelmoschus esculentus*. While the crop maintained measurable growth across contamination levels, increasing hydrocarbon intensity progressively reduced plant height, stem girth, and relative water content, indicating impaired hydration and structural development. Biochemically, crude oil exposure induced lipid peroxidation alongside marked upregulation of non-enzymatic antioxidants, particularly Vitamin C and reduced glutathione, reflecting activation of adaptive redox buffering mechanisms. The coordinated modulation of oxidative stress markers suggests that *A. esculentus* possesses moderate tolerance mediated through inducible antioxidant defence systems. However, the energetic cost of sustained detoxification responses appears to constrain biomass accumulation under higher contamination levels.

Overall, the findings highlight a threshold-dependent balance between stress injury and metabolic adaptation, underscoring both the resilience and physiological limits of okra cultivated in petroleum-impacted soils.

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