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Phytodegradation of *Spent Engine Oil* in the soil by lady finger *(Abelmoschus esculentus (L.) Moench)* through *exogenous* application of *melatonin*

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Abstract

The *spent engine oil* (SEO) is a major source of pollution and impacts the soil quality and plant growth. *Phytoremediation* is an environmentally friendly technique for soil remediation. The aim of this study was to assess how the *phytoremediation* ability and stress resistance of okra (*Abelmoschus esculentus (L.)* could be improved with the use of *exogenous melatonin* (MT). P. *Moench*) cultivated in a soil contaminated with SEO. Four concentrations of SEO (0, 100, 200 and 300 µM) and three MT levels (0, 100 and 200 µM) were investigated in a 4×3 factorial pot experiment. SEO stress had significant effects on plant growth, plant physiological activity and biochemical activity. MT application dose-dependently however mitigated these negative effects. Seedling exposure to MT resulted in up to 11.51%, 15.85% and 32.71% increase in shoot length, root length and number of leaves, respectively, as compared to the stressed control. Physiological parameters also enhanced, including *photosynthetic rate* (13.12%), *chlorophyll content* (26.32%), *stomatal conductance* (26.41%) and *transpiration rate* (31.62%). The maximum improvement was with the total *amino acid* content (107.78) in severe stress. Moreover, it is important to point out that the removal efficiency of *anthracene* has risen from XX% (SEO-only treatment) to XX% (SEO + 200 µM MT) showing an improvement of the *phytodegradation* efficiency. Two-way ANOVA showed that there were significant ($p < 0.05$) differences among the various SEO, MT and SEO x MT for all parameters. These results indicate that the use of *exogenous melatonin* in the field of phytoremediation with A. *esculentus* in soils contaminated with SEO could be a good option for sustainable remediation.

Introduction

Spent engine oil (SEO) is primarily composed of 80-95% petroleum-based hydrocarbons and 5-20% additives formulated to enhance lubricant performance [3, 20]. During engine operation, the oil undergoes thermo-oxidative degradation, accumulating toxic byproducts including *polycyclic aromatic hydrocarbons* (PAHs) and heavy metals [18]. In the engine, the oil is subjected to oxidative processes, high temperature and pressure, and over time becomes degraded in quality converting it into spoiled or used engine oil (SEO), and thereby couldn't lubricate the engine parts properly [7]. Globally, an estimated 24-28 million tons of used engine oil are generated annually, with significant proportions released into the environment through improper disposal, particularly in developing nations [21]. The toxic components of *Spent Engine Oil* include partially decomposed hydrocarbons, *polycyclic aromatic hydrocarbons* (PAHS), and high levels of certain heavy metals such as *chromium* (Cr), *zinc* (Zn), *lead* (Pb), and *barium* (Ba). which are due to fuel combustion, oil degradation, and engine wear. The concentration of the PAHs will further increase because of high temperatures and incomplete combustion, which also makes PAHs persistent, toxic and *carcinogenic* Heavy metals made PAHs more toxic and slowed the rate at which the oil breaks down in the environment [23]. *Polycyclic aromatic hydrocarbons* (PAHs) are a family of planar organic hydrocarbons that consist of two or more fused carbon and hydrogen rings, are non-polar, non-ionic and *lipophilic (hydrophobic)* [6]. Because of these, they are known for their extremely high adherence to soil and organic matter both, which brings them a high staying power in the environment. Over 130 PAHs have been identified, more than 16 of which are considered to be priority pollutants and are deemed to be more toxic, such as benzo[a]pyrene and chrysene [8]. Diluted and used engine oil in soil affects the beneficial soil organisms and nutrient cycling, affects soil structure and lowers soil fertility. It is also known to have a negative impact on plant

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growth, by disrupting germination, biomass production and root development [1]. PAHs can be absorbed by food animals and plants and are harmful to animals and people. Human exposure can be through contaminated food, inhalation and skin contact, and long-term exposure has been associated with cancer and immune, nervous or endocrine system damage, to the lung, skin, liver and bladder [17]. The ability of the plants to uptake pollutants from soil and water, such as *phytoextraction, phytostabilization*, *Phytodegradation* and *plant–microbe interaction in the rhizosphere*), all of which are environmentally friendly and less costly than conventional cleanup methods contribute to it being a better and more sustainable options [16].

Plants also have natural growth and stress hormones and *melatonin*'s antioxidant properties enhance plant stress tolerance. *Melatonin* has been found to improve the plant's ability to withstand stress both by decreasing the level of oxidative damage and increasing the activity of *antioxidant enzymes* and by protecting the components of the cell and regulating the stomatal function [2,4]. It has been demonstrated to stimulate plant growth in contaminated soil and to enrich the soil's *rhizosphere microbial* communities, which in turn would enhance pollutant degradation. Its application in *phytoremediation* with edible plants, such as *Abelmoschus esculentus Moench* (L.) *Moench* for hydrocarbon removal remains limited [19].

The separate effects of SEO toxicity and *melatonin* mediated stress tolerance have been reported individually, however their integration in the context of a phytoremediation application is not well explored. Specifically, there is still little known about: (1) the potential of A. *esculentus* to remove hydrocarbons from SEO contaminated soils; (2) the dose dependent effect of the *exogenous* melatonin on the efficiency of phytoremediation; and (3) the correlation between *melatonin*-induced physiological improvement and pollutant degradation. Although, *melatonin* was investigated in the alleviation of abiotic stress in different crops [9, 12], but its application in the hydrocarbon-contaminated soil of edible plants is still scarce.

SEO contamination inhibits plant growth and photosynthesis; *melatonin* enhances antioxidant defense under various stresses; certain plants can participate in hydrocarbon remediation. Whether *exogenous* melatonin can enhance both the hydrocarbon remediation capacity and physiological tolerance of A. *esculentus* under SEO contamination, and the dose-dependent nature of such responses. We hypothesized that *exogenous* melatonin would dose-dependently mitigate SEO-induced physiological stress and enhance the removal of hydrocarbon contaminants from soil. This study aimed to evaluate the effects of *melatonin* on the growth, physiology, biochemistry, and anthracene degradation potential of A. *esculentus* grown in SEO-contaminated soil.

Methodology

❖ Experimental Site and Conditions

The experiment was carried out in Environmental Sciences Department, Bahauddin Zakariya University, Multan, Pakistan. The study was conducted in the greenhouse at mild temperature, low humidity, less cloudiness and low rainfall (Rabi season from December to March).

❖ Experimental Design

A pot experiment was conducted using a *Completely Randomized Design* (CRD) with 4 × 3 factorial arrangements, comprising four *Spent Engine Oil* (SEO) concentrations and three *melatonin* (MT) levels, replicated three times (total 36 pots). The treatment factors were:

Factor A - SEO concentration (UEO):

UEO0: 0 μM (control, no SEO)

UEO1: 100 μM

UEO2: 200 μM

UEO3: 300 μM

Factor B - *Melatonin* concentration (MT):

MT0: 0 μM (control, no *melatonin*)

MT1: 100 μM

MT2: 200 μM

This resulted in 12 treatment combinations:

UEO0×MT0, UEO0×MT1, UEO0×MT2

UEO1×MT0, UEO1×MT1, UEO1×MT2

UEO2×MT0, UEO2×MT1, UEO2×MT2

UEO3×MT0, UEO3×MT1, UEO3×MT2

Plastic pots measuring 12 inches in height and 5.5 inches in diameter were used. To prevent experimental bias, all pots were identical in size and arranged randomly within the greenhouse.

❖ Soil Collection and Characterization

A soil sample was collected from the experimental site at 6” depth. To remove the plant residues and debris, the homogenization and sieving (2 mm mesh) process was used. The basic *physicochemical* characteristics of the soil used were measured before the experiment:

Soil pH: Measuring through the pH Reader in the 1:2.5 soil: water mixture.

Electrical conductivity (EC): Obtained by conductivity meter.

Organic matter: Measured using Walkley-Black oxidation method

Total nitrogen: by Kjeldahl method

Available phosphorus: Based on Olsen method

Available *potassium*: *Potassium* determined by flame photometry

Initial concentrations of *hydrocarbons*: Extracted and analyzed by HPLC

❖ ***Spent Engine Oil* Collection and Characterization**

The used engine oil was collected from local auto workshop of Multan, Pakistan, with recorded history of usage (about 5000km engine run). The oil was analyzed for the presence of PAH, including the concentration of anthracene, with HPLC (Agilent 1260 Infinity) as described in section 2.10. To achieve concentration of the target (0, 100, 200, 300 µM) the oil was well mixed in soil.

❖ **Soil Contamination and Stabilization**

The quantity of soil in each pot was 2kg. There was a need to determine the application rate (RE) of *Spent Engine Oil* (SEO) on the basis of the desired concentration. The polluted soil was left partially stabilized for 4 weeks (at field capacity (FC) soil moisture) to achieve uniformity and ensure interaction with the soil matrix.

❖ **Plant Material and Sowing**

The certified okra seeds (*Abelmoschus esculentus (L.)* The seeds of these cultivars (Brassica napus, L. 'Sabz Pari' and B. napus, napifer, L.) were secured from Sun Crops Production, Multan Cantt. Seeds were surface-sterilized with 70% ethanol for 2 min and then with 1% *sodium hypochlorite* for 5 min and washed three times with sterile distilled water. In each pot, 10 seeds were planted at the same depth. The pots were kept under isolation and uniform germination was produced. Irrigation water was distilled water to keep soil moisture at optimum. Thinning was done to three uniform seedlings per pot after germination to improve uniformity and minimize intra-specific competition.

❖ ***Melatonin* Application**

Melatonin (MT; *Sigma-Aldrich*, purity ≥98%) was dissolved in 0.1% ethanol solution and foliar-sprayed. In order to use *melatonin* solution in MT1 (100 µM) and MT2 (200 µM) treatments, the spray of *melatonin* solution was applied on the leaves at 2-day interval from 14 days after sowing until harvest as 50 mL per pot. Plants such as control (MT0) were also sprayed with the same amount of 0.1% ethanol solution.

❖ **Growth Parameters**

Shoot length (cm): Measured from the soil surface to the shoot tip using a ruler.

Root length (cm): Plants were carefully uprooted, and roots were washed with distilled water. Root length was measured using a ruler.

Number of leaves: Counted manually by considering only fully expanded leaves.

Root fresh weight (g): Measured after washing and drying with an electronic balance.

Root dry weight (g): Determined after oven drying the samples at 70°C for 72 hours.

❖ **Physiological Parameters**

Photosynthetic rate: Measured using an Infra-Red Gas Analyzer (IRGA, LCi-SD) following Munger et al. [27].

Total chlorophyll content (mg/g FW): Estimated using 80% acetone extraction and *spectrophotometric measurement* at 663 and 645 nm according to Maclachlan and Zalik [26].

Stomatal conductance (cm/s): Measured following Hussain et al. (2021).

Transpiration rate (mmol H_2O m^{-2} s^{-1}): Measured according to Hussain et al. (2021).

CO_2 exchange rate (ppm): Measured following Hussain et al. (2021).

❖ **Biochemical Parameters**

Total amino acids content (mg/g FW): Estimated by the method of Hamilton and Van (1969), with absorbance measured using a UV-visible spectrophotometer at 570 nm.

❖ ***Anthracene Analysis* and *Phytoremediation Efficiency***

Plant and soil samples were collected after 30 days to evaluate phytoremediation efficiency. Anthracene concentration was analyzed using an Agilent 1260 Infinity HPLC system equipped with a *Zorbax Eclipse Plus C18 column* (250 mm × 4.6 mm, 5 µm particle size).

HPLC conditions:

Mobile phase: Acetonitrile: water (80:20 v/v)

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Column temperature: 30°C

Detection wavelength: 254 nm

Calibration and quality control: Calibration curve was prepared using anthracene standards (0.01-10 mg/L) with $R^2 > 0.999$

Limit of detection (LOD): 0.01 ng/mL

Limit of quantification (LOQ): 0.03 ng/mL

Recovery experiments: Soil samples spiked with known anthracene concentrations achieved recoveries of 85-95%

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Quality control samples were analyzed with each batch (n = 3)

Data were processed using OpenLab CDS software (version A.01.01).

Phytoremediation efficiency (anthracene removal) was calculated using the formula:

Anthracene removal (%) = [(Concentration in control − Concentration in treatment) / Concentration in control] × 100

❖ **Statistical Analysis**

The analyses of the results were conducted using R software version 4.3.1 (R Core Team, 2023). Two-way analysis of variance (ANOVA) was used to analyze data and test the effect of SEO concentration, *melatonin* concentration and the interaction of these two factors. Before ANOVA, it was observed that the normality and homogeneity of variance assumptions were met (Shapiro-Wilk test and Levene's test). When the overall ANOVA results were significant, Tukey's *Honestly Significant Difference* (HSD) post-hoc test was used to compare treatments two at a time ($p < 0.05$). To estimate the sizes of the treatment effects, effect sizes (partial eta-squared, $\eta^2 p$) were also calculated. Correlation analysis (Pearson) was performed to examine relationships between physiological and biochemical parameters. The treatment group clustering patterns were examined using *Principal Component Analysis* (PCA). The results are the average of three replicates, with *standard error* (SE) and significance ($p < 0.05$) indicated.

Results

Melatonin significantly enhanced the growth characteristics of *Abelmoschus esculentus* both under normal and *Spent Engine Oil* polluted conditions. The longer shoots were produced, the higher the concentration of *melatonin*. *Melatonin* at 100µM and 200µM consistently increased the shoot length when compared to control under low, moderate, high and severe stress conditions, concentration-dependently increasing the enhancement and reducing the effect of stress. There were also increased root lengths with the *melatonin* treatment under all stress levels. The root length was affected by the oil stress; however, this effect was alleviated by *melatonin* in the two highest concentrations (100 and 200 µM), which indicates that the application of *melatonin* assisted plants with hydrocarbon stress. The *melatonin*-induced increase in number of leaves under all conditions was the highest under severe stress condition. Better leaf development was also observed due to *melatonin* which can form bonds with *Spent Engine Oil* and minimized its negative effects. *Melatonin* treatment under both stressed and non-stressed conditions significantly decreased the toxicity effect while increasing both the fresh weight and dry weight of the root, which led to increased biomass of root.

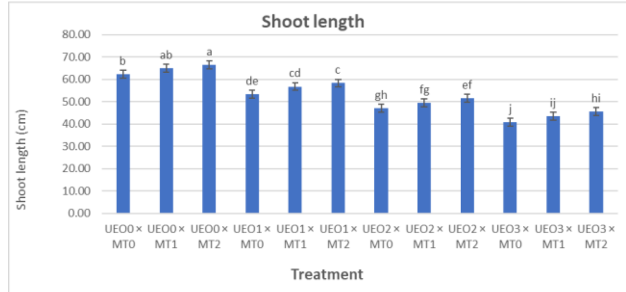

Figure 1.0: Effects of SEO and MT on shoot length of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

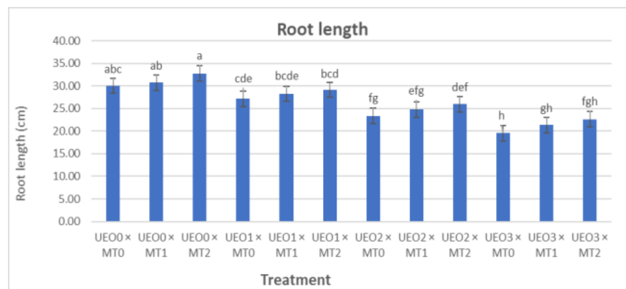

Figure 2.0: Effects of SEO and MT on root length of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

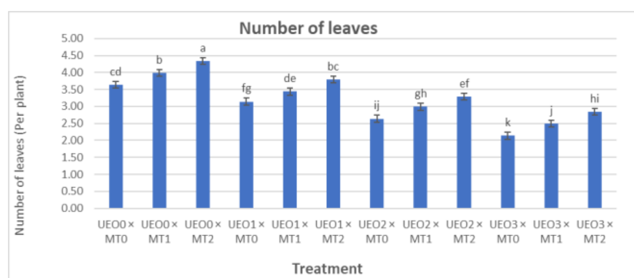

Figure 3.0: Effects of SEO and MT on number of leaves per plant of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

The positive effects on physiological parameters were also observed. In all conditions of stress, the photosynthetic rate increased with *melatonin*, thus signifying the protection of the photosynthetic system. Under oil stress, total *chlorophyll* content decreased while it was restored to normal levels after *melatonin* treatment, showing potent protection against the degradation of chlorophyll. Oil stress reduced stomatal conductance, transpiration rate and CO_2 exchange rate, while the application of *melatonin* significantly decreased these parameters due to the better gas exchange and water regulation especially at 200 µM. Stress led to a reduction in the total amino acids, whereas *melatonin* significantly increased the total amino acids with high metabolic activity and tolerance to stress.

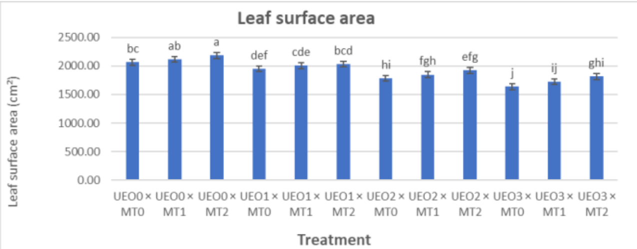

Figure 4.0: Effects of SEO and MT on leaf surface area (cm²) of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 μM); MT0-2: MT concentrations (0, 100, 200 μM).

In general, *melatonin* alleviated the toxic effects of used engine oil and enhanced growth, physiology and stress responses of the okra under applied levels of spent oil, which was concentration-dependent. The results on growth, physiological and biochemical parameters of *Abelmoschus esculentus* under non-stressed and *Spent Engine Oil* (SEO) stress conditions revealed significant improvement with application of *melatonin* (100 and 200 μM) which was dose-dependent in all the cases. Under all the conditions, the length of shoots was found to be longer when treated with *melatonin*. Without stressing the situation, there were increases of 4.51% and 6.91%, respectively. Shoot length showed moderate changes, ranging up to 9.54%, becoming more effective under higher stress conditions, with the highest amount found under severe stress (11.51%). Roots length also has improved regularly. Increase under non-stress were 2.33% and 8.98%.

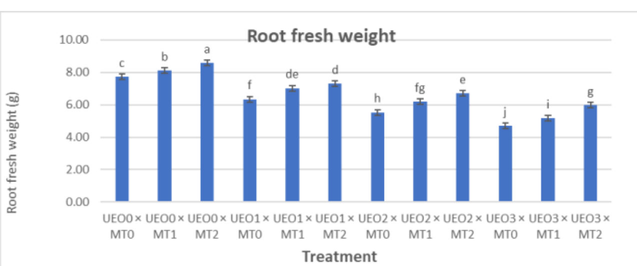

Figure 5.0: Effect of different UE and MT treatment combinations on root fresh weight (g). Bars represent means ± error bars. Means followed by different letters are significantly different ($p < 0.05$).

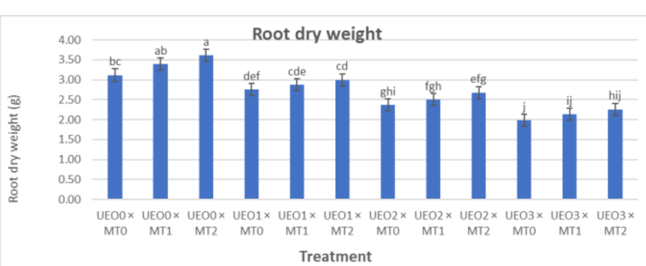

Figure 6.0: Root dry weight across treatment groups. Treatments are divided into four UE levels (UE0-UE3), each with three MT levels (MT0-MT2). Similar to fresh weight, root dry weight generally increased with higher MT levels within most UE groups. The highest biomass was achieved in the UE0 × MT2 group, whereas the lowest was observed in the UE3 × MT0 group. Statistical significance is indicated by the letters above the error bars.

They found that under stress they had improvements of 4.05% – 15.85% (with the highest improvement at the most severe stress indicating clear stress-alleviating influence of *melatonin*). *Melatonin* application significantly increased amount of leaves/plant. The improvements were from 9.62% to 32.71% under non-stress and severe stress conditions respectively. Leaf surface area was also enhanced by treatment with *melatonin*, and stress induced by SEO caused a decrease, but still *melatonin* resulted in the restoration of leaf area with an improvement of up to 10.74% under high stress levels. The growth of root biomass was strongly improved. The fresh weight of roots rose from 4.91% when plants do not face stress to 26.69% when they face severe stress.

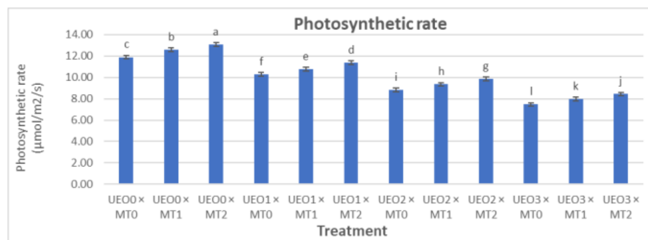

Figure 7.0: Effects of SEO and MT on photosynthetic rate (μmol m^{-2} s^{-1}) of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at $p < 0.05$ (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 μM); MT0-2: MT concentrations (0, 100, 200 μM).

Meanwhile, in the same direction, the root dry weight also showed a gradual increase from 8.97% to 15.70% reflecting the increase of the overall plant biomass under the stress. *Physiological parameters* were significantly affected. *Photosynthetic* rate enhanced by up to 13.12 per cent, the ability of *chlorophyll* content to up 26.32 per cent, *stomatal conductance* up 26.41 per cent, transpiration rate up 31.62 per cent and CO_2 exchange rate up 9.23 per cent under severe stress conditions, thus revealing enhancement in photosynthetic efficiency and gas exchange.

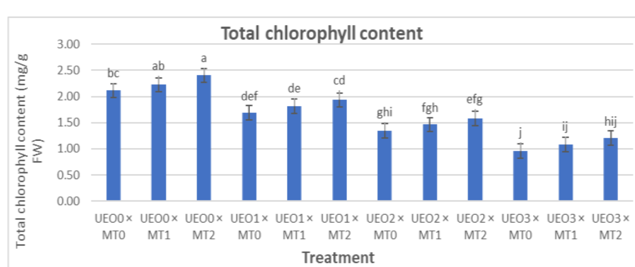

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Figure 8.0: Effects of SEO and MT on total *chlorophyll* content (mg/g FW) of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at p < 0.05 (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

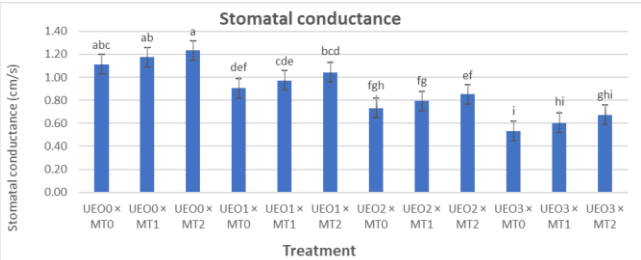

Figure 9.0: Effects of SEO and MT on stomatal conductance (cm/s) of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at p < 0.05 (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

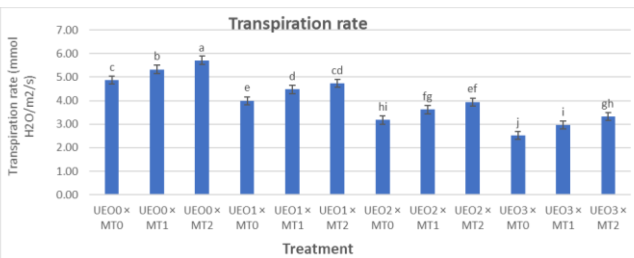

Figure 10.0: Effects of SEO and MT on transpiration rate (mmol H_2O m^{-2} s^{-1}) of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at p < 0.05 (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

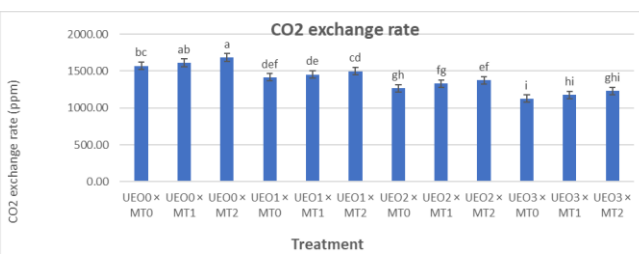

Figure 11.0: Effects of SEO and MT on CO_2 exchange rate (ppm) of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at p < 0.05 (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

The biochemical analysis revealed that the content of total amino acids had the highest response levels with values ranging from 33.33% for control to 107.78% for severe stress, reflecting high metabolic activation and stress recovery. In general, all the growth, physiology and biochemistry parameters improved in all cases with the constant supplementation of *melatonin* in both normal and contaminated environments in Abelmoschus *esculentus*.

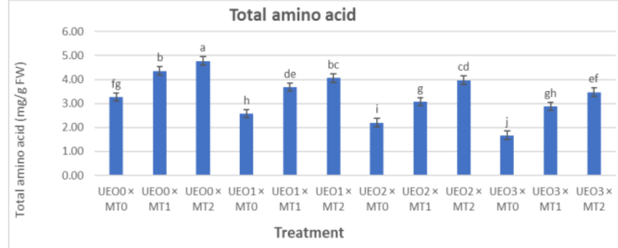

Figure 12.0: Effects of SEO and MT on total amino acid content (mg/g FW) of A. *esculentus*. Data are mean ± SE (n = 3). Different letters indicate significant differences at p < 0.05 (Tukey's HSD). UEO0-3: SEO concentrations (0, 100, 200, 300 µM); MT0-2: MT concentrations (0, 100, 200 µM).

The most significant improvements were under the extremes of stress conditions, especially at the amino acid, leaf number and root biomass levels, whereas the lowest responses were found under the non-stress conditions. The findings above is clear evidence of the efficacy of application of *Melatonin* in reduction of the harmful effects of contaminated mixture of *Spent Engine Oil* and improvement of plant growth and stress tolerance.

Discussion

The present study has shown that the application of *melatonin* was effective in decreasing the negative effects of used engine oil stress in the plant *Abelmoschus esculentus*. Stress conditions caused an increase in shoot length, root length, leaf area and biomass, which greatly improved plant growth. A clear titration effect was seen with the increasing *melatonin*, that is, the higher the dose of *melatonin*, the greater the good outcome. In comparison to the previous studies, the growth responses were relatively moderate in this experiment. However, with respect to the improvement in growth earlier studies reported more significant increase when subjected to nutrient deficiency and salinity stress, as in the study conducted by [10, 13, 22, 24]. The difference can be attributed to the complex and highly toxic used engine oil giving rise to both chemical and oxidative stress to plants. However, *melatonin* remained protective and sustained growth in contaminated growth environment.

The observed improvements in growth and physiological parameters under *melatonin* treatment are consistent with but generally more moderate than those reported in other stress contexts. [11] found maize growing 18-25% better under salinity stress, with [25] seeing maize growth responding more strongly to nutrient deficiency stress. This lesser improvement in our study could be attributed to the fact that the type of SEO contamination we are dealing with is highly potent and complicated as it compounds both chemical toxicity and oxidative stress. These research findings also showed *melatonin*'s ability to preserve *chlorophyll* pigments and cut down chlorophyll breakdown while under stress. The improvement in transpiration rate and CO_2 exchange, which was reported by both [5, 14] on gas exchange traits was equally observed. This indicates improved stomatal regulation in plant under stress conditions due to *melatonin*. The answer, however, was slightly less than reported value by [15], which might be attributed to the higher destructive impact of oil pollution on the plant tissues in this study. In all biochemical characterization, Amino acid content was the most responsive to *melatonin*.

While our results demonstrate the potential of *melatonin*-assisted phytoremediation, several limitations must be acknowledged. The greenhouse pot experiment does not fully replicate field conditions, and the 30-day experimental period may not capture long-term remediation dynamics. Additionally, contaminant accumulation in edible tissues was not measured, which is a critical concern given that okra is an edible crop. Future research should address these limitations through field-scale trials, long-term studies, and food safety assessments before recommending this approach for agricultural applications. In overall conclusion, the study authorizes that the negative effects of used engine oil stress are mitigated in *Abelmoschus esculentus* and the improvements are slightly lesser than other abiotic stresses stress, *melatonin* definitely boosted plant growth, physiology and biochemical activity in *Abelmoschus esculentus*. This highlights its potential role in improving plant tolerance in polluted soil environments.

Conclusion

The present study demonstrated that *Spent Engine Oil* (SEO) contamination significantly inhibits growth, photosynthesis, and biochemical processes in *Abelmoschus esculentus*. However, *exogenous melatonin* application effectively mitigated these stress effects in a dose-dependent manner. *Melatonin* at 200 μM most effectively improved shoot length (11.51%), root length (15.85%), leaf number (32.71%), root fresh weight (26.69%), root dry weight (15.70%), and *photosynthetic* parameters including *photosynthetic* rate (13.12%), chlorophyll content (26.32%), *stomatal conductance* (26.41%), transpiration rate (31.62%), and CO_2 exchange rate (9.23%) under severe stress conditions. The most pronounced response was observed in total *amino acid* content, which increased by up to 107.78% under *melatonin* treatment, indicating enhanced metabolic recovery and stress tolerance. Two-way ANOVA confirmed significant ($p < 0.05$) main effects of SEO concentration and *melatonin* concentration, as well as significant interactive effects for all measured parameters. The significant SEO × MT interaction indicates that *melatonin*'s beneficial effects were amplified under higher stress conditions, suggesting that *melatonin* is particularly effective when plants face severe contamination stress. Additionally, *melatonin* treatment significantly enhanced anthracene removal from contaminated soil, demonstrating improved *Phytodegradation* capacity. These findings indicate that *melatonin* could serve as a valuable tool for improving the *phytoremediation* potential of A. *esculentus* in SEO-contaminated soils. The application of *melatonin* represents a promising, sustainable, and cost-effective strategy for enhancing plant tolerance and remediation efficiency in hydrocarbon-contaminated environments. However, further field-scale studies are necessary to validate these results under real-world conditions, and food safety assessments are essential before recommending this approach for agricultural applications. This research contributes to the development of sustainable remediation strategies for hydrocarbon-contaminated soils and provides a foundation for future investigations into *melatonin*-assisted *phytoremediation*.

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Declarations:

Authors' Contribution:

- All Authors Conceptualization, data collection, interpretation, drafting of the manuscript and intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed
- **Data Availability**: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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