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Corymbia citriodora Induces Oxidative Stress in *Senna occidentalis*: A Weed Management Strategy

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ABSTRACT

Background and Objective: The essential oils (EOs) are known for their bioactive properties and have been widely studied for their effects on plant physiology, especially in oxidative stress responses. This study investigated the impact of the essential oil of *Corymbia citriodora* from the family Myrtaceae, on the stress physiology of the common wasteland weed *Senna occidentalis*. **Materials and Methods:** The essential oil of *C. citriodora* was extracted through hydro-distillation and seeds of *S. occidentalis* were treated with its various concentrations (0.1, 0.2 and 0.5 mg/mL). Oxidative stress-related parameters were studied on the seventh day after seed germination. **Results:** The dose-dependent increase in malondialdehyde (MDA), H_2O_2 and proline content was observed in treated plants. However, plants treated with 0.1 mg/mL of essential oil exhibited hormesis, suggesting a potential adaptive response to mild oxidative signaling. The results suggested that the essential oil of *C. citriodora* can be a pro-oxidant at high and a biostimulant at lower concentrations. **Conclusion:** Understanding how these intricate interactions work gives us a better idea of using essential oils to make plant-based bioherbicides, that are considered eco-friendly alternatives to chemical herbicides.

KEYWORDS

Corymbia citriodora, *Senna occidentalis*, oxidative stress, weed management, proline, hydrogen peroxide, lipid peroxidation

INTRODUCTION

Corymbia citriodora, commonly known as lemon-scented gum belongs to the family Myrtaceae, endemic to Australia[1,2]. It is known for its wood, essential oils and decorative applications. The tree's vertical growth, smooth bark and lemon-scented leaves set it apart. The *C. citriodora* thrives in diverse climates, especially in areas with limited water resources. Traditional medicine and pharmaceutical research have studied its essential oil (EO) for potential use in addressing microbial infections, respiratory ailments and disorders related to oxidative stress. The major components of essential oil include citronellal, dl-isopulegol, citronellol, β-pinene, γ-terpinene, methyleugenol, eucalyptol, (+)-cis-p-menthane and citronellic acid[3]. Citronellal and limonene are known for their antibacterial, anti-inflammatory, antimicrobial and insectrepellent properties[4-8].

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During biotic or abiotic stress, living cells generate reactive oxygen species (ROS) in many locations and at an elevated rate[9]. The oxidative stress appears when there is an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses. It is one of the critical responses of plants, especially when they are exposed to bioactive chemicals that stress them out. This imbalance frequently leads to oxidative damage to cellular constituents, such as lipids, proteins and nucleic acids, hence hindering plant development and activity[10]. Monitoring the levels of malondialdehyde (MDA), proline and hydrogen peroxide serves as a significant indication of oxidative stress and cellular damage in treated plants[11,12].

Recently, EOs gained attention as oxidative stress modulators in plants because of their high antioxidant capacity and wide range of bioactive constituents[13]. Depending on their composition and concentration, EOs can either increase oxidative stress, acting as pro-oxidants or mitigate it by alleviating antioxidant defenses[14]. Among the aromatic species of interest, *C. citriodora* EO (CCEO) is notable for its significant therapeutic potential, including antimicrobial, antioxidant and anti-inflammatory properties. This study aimed to investigate the impact of CCEO on oxidative stress-related parameters in *Senna occidentalis* (commonly known as Coffee Senna, Septicweed), a problematic wasteland weed. Lipid peroxidation, H_2O_2 and proline content measurements will be used as markers of oxidative damage. Examining, these parameters provides insights into the oxidative modifications induced by CCEO treatments and elucidates the potential of using CCEO as bioherbicides, thereby advancing their application in biotechnological and agricultural contexts.

MATERIALS AND METHODS

Study area: The effect of *Corymbia citriodora* (Hook.) K.D. Hill and L.A.S. Johnson essential oil was studied on seeds of *Senna occidentalis* (L.) link in June, 2022. The experimental procedures were performed at the Department of Botany, DAV College, Jalandhar, Punjab, India (31.3499°N, 75.5583°E).

Essential oil extraction and treatment: Freshly chopped leaves of *C. citriodora* were used for extraction of EO through hydrodistillation using the Clevenger apparatus. Extracted CCEO was kept at 4°C till further use. The chemical composition of CCEO was confirmed by Head Space Gas Chromatography-Mass Spectrometry (HS GC-MS) from CSIR-IHBT, Palampur, Himachal Pradesh, India (32.1052°N, 76.5564°E) to ensure consistency.

The CCEO solutions were prepared at varying concentrations (e.g., 0.1, 0.2 and 0.5 mg/mL w/v) by dissolving the oils in distilled water with Tween 80 as an emulsifier. Pre-imbibed seeds of *S. occidentalis* were treated with 4 mL of different concentrations (0.1, 0.2 and 0.5 mg/mL) of CCEO emulsion in 9 cm Petri dishes. A parallel control was maintained with distilled water containing Tween 80. All Petri dishes were sealed immediately after treatment with brown adhesive tape to prevent volatilization of EO. Petri dishes were kept under controlled conditions with a photoperiod of 16 hrs light/8 hrs dark, a temperature of 25±2°C and relative humidity of 60%. After the seventh day of the seed germination, data of germination percentage was recorded and different assays viz., lipid peroxidation, H_2O_2 content and proline content were performed.

Bioassays measurements

Lipid peroxidation determination: The lipid peroxidation was assessed by measuring malondialdehyde (MDA) content using the thiobarbituric acid (TBA) assay procedure given by Heath and Packer[15] for the determination of lipid peroxidation. The study involved macerating 100 mg of plant tissue with 0.1% TCA, centrifuging at 10,000 g and adding 0.5% TBA in 20% TCA. The mixture was then heated to 95°C for 30 min. The mixture was immediately placed in an ice bath and then centrifuged again at 10,000 g. The 3 replicates were maintained for each concentration including control. The absorbance was measured using a dual-wavelength of 532 and 600 nm and malondialdehyde content was expressed as nmol/g fresh weight.

Determination of Hydrogen Peroxide (H_2O_2) content: The H_2O_2 content was determined by Velikova *et al*.[16] method. The 100 mg of tissues from the roots and leaves of *S. occidentalis* were homogenized with 5 mL of 0.1% TCA over an ice bath. The homogenate was centrifugation at 12,000 g for 15 min. To 0.5 mL of the supernatant, 0.5 mL of phosphate buffer and 1 mL of potassium iodide (KI) were added. The absorbance was measured at 390 nm. Three replicates were maintained for each concentration. The H_2O_2 concentration was expressed in nmol/g fresh weight.

Proline content: The proline content was determined by using the procedure of Bates *et al*.[17]. The 100 mg of dried roots or leaf tissues were digested in 3% sulfosalicylic acid for 30 min at 100°C. Both tissues were centrifuged at 2000 g for 5 min at 25°C. To 0.2 mL of the supernatant, added 0.4 mL of distilled water, 2 mL of the reagent mixture (which included 0.5 g of ninhydrin, 20 mL of distilled water and 30 mL of glacial acetic acid) and incubated for 1 hr over a boiling water bath. The mixture was cooled and extracted using 6 mL of toluene. The absorbance of the toluene phase was measured at 520 nm and proline concentration was calculated using the proline standard and reported as mg/g dry weight.

Statistical analysis: Data were analyzed using one-way ANOVA followed by Duncan's *post hoc* test to determine significant differences between treated and control plants. Each treatment was performed in triplicate and values were expressed as Means±Standard Deviation. Statistical significance was set at $p \leq 0.05$.

RESULTS

Under laboratory growth studies, corresponding to complete germination in control (100±0%), seed germination was 96.67±5.77, 80.00±10 and 40±0% at 0.1, 0.2 and 0.5 mg/mL CCEO concentrations. The seedlings at 0.5 mg/mL concentration were less than 1 cm in length hence, the stress-related parameters viz. lipid peroxidation (LPO), hydrogen peroxide and proline were analyzed for 0, 0.1 and 0.2 mg/mL CCEO concentrations shown in Table 1.

Stress related parameters

Lipid peroxidation (measured by the malondialdehyde concentration): In the current study, malondialdehyde (MDA) formed during lipid peroxidation increased in treated tissues (leaves and roots) in response to CCEO treatment. The MDA content produced in leaves of *S. occidentalis*, at concentrations of 0, 0.1 and 0.2 mg/mL was 9.23±0.68, 10.43±0.39 and 11.97±0.15 nmol/g fresh weight, respectively and an increase of 1.13 and 1.30 times was detected compared to control. The MDA content produced in control roots of *S. occidentalis* was 5.21±0.65, 13.85±0.26 and 16.75±0.78 nmol/g fresh weight, respectively.

Hydrogen Peroxide (H_2O_2) content: Treatment with CCEO increased the amount of H_2O_2 in the leaves of *S. occidentalis*. The increase in treated samples depended on the dose. The amount of H_2O_2 in control leaves of *S. occidentalis* was 125.24±1.35 nmol/g fresh weight. In *S. occidentalis*, values of H_2O_2 obtained at concentrations 0.1 and 0.2 mg/L (CCEO) were 127.86±0.36 and 132.14±1.64 nmol/g fresh weight and an increase of 1.02 and 1.06 times was observed, respectively, with respect to control. The amount of H_2O_2 produced in the roots of *S. occidentalis* at 0, 0.1 and 0.2 mg/L was 115.36±0.51, 121.43±0.36 and 124.64±0.90 nmol/g fresh weight, respectively. An increase of 1.05 and 1.08 times was observed at 0.1 and 0.2 mg/mL compared to the control group.

Proline content: In *S. occidentalis*, an increase in proline content was observed in roots treated with CCEO in comparison to the control. The proline content in leaves of *S. occidentalis* was measured to be 192.29±1.67, 203.31±2.48 and 215.99±1.72 mg/g dry weight at 0, 0.1 and 0.2 mg/mL concentration, respectively.

Table 1: Effect of CCEO on lipid peroxidation, hydrogen peroxide and proline content in *S. occidentalis* roots and leaves

	Lipid peroxidation (nmoles/g f.wt.)		Hydrogen peroxide (nmoles/g f.wt.)		Proline content (mg/g d.wt.)	
Concentration (mg/mL)	Roots	Leaves	Roots	Leaves	Roots	Leaves
0.0	5.21s±0.65^{c}	9.23±0.68^{c}	115.36±0.51^{c}	125.24±1.35^{c}	29.20±0.63^{c}	192.29±1.67^{c}
0.1	13.85±0.26^{b}	10.43±0.39^{b}	121.43±0.36^{b}	127.86±0.36^{b}	32.92±1.86^{b}	203.31±2.48^{b}
0.2	16.75±0.78^{a}	11.97±0.15^{a}	124.64±0.90^{a}	132.14±1.64^{a}	35.12±0.82^{a}	215.99±1.72^{a}
0.5	0	0	0	0	0	0

Data was expressed as Means±Standard Deviation and analyzed by One-way Analysis of Variance (ANOVA) and the difference among the means was determined by Duncan's *post hoc* test ($p \leq 0.05$) in IBM SPSS 26.0 for windows statistical software package software

DISCUSSION

Using synthetic herbicides for weed management has led to weed resistance, environmental pollution, adverse impacts on beneficial organisms and ecological imbalance[18]. Recently natural plant products have been explored to offer bioherbicides as alternatives to chemical herbicides. The EOs from many genera have been shown to have varying phytotoxicity towards agricultural and wasteland weeds[19,20] generally reflected in the development of reactive oxygen species (ROS) and stress-related metabolites in target weeds[18]. Intending to use CCEO as a bioherbicide for managing *S. occidentalis*, the present study was planned. The CCEO induced oxidative stress in the leaf and root tissues of *S. occidentalis* as evidenced by the increased content of hydrogen peroxide and malondialdehyde, a by-product of lipid peroxidation. The reactive oxygen species (ROS) are reactive compounds that in excess lead to the degradation of macromolecules such as carbohydrates and proteins[21]. Treatment with CCEO increased the MDA content in test weed suggesting that essential oils, particularly at higher concentrations, induce oxidative stress by generating ROS that target membrane lipids. A hormetic effect at lower concentrations of CCEO suggested a potential adaptive response of weed potentially triggering beneficial oxidative signals and defense responses in *S. occidentalis*.

Headspace GC-MS analysis revealed that the essential oil was monoterpenoid type with citronellal and citronellol as the major monoterpenoids[22]. Many researchers in the past have discussed the link between EO, monoterpenes, ROS generation and MDA as an indicator of lipid peroxidation and oxidative stress[23-26]. In response to stress, plants have developed defense mechanisms, such as metabolic acclimation via proline build-up. Proline is a solute that can scavenge free radicals, chelate metals, activate reactive oxygen species detoxification pathways, balance cell redox, provide energy and function as a signaling molecule[27,28]. In the present study, proline content increased significantly in treated root and leaf tissues of *S. occidentalis*. Its comparison with increased lipid peroxidation and hydrogen peroxide content establishes the phytotoxic potential of CCEO.

CONCLUSION

The study demonstrates that the essential oil of *Corymbia citriodora* has a dose-dependent effect on the oxidative stress physiology of *Senna occidentalis*, with higher concentrations inducing pro-oxidant activity and lower concentrations promoting a hormetic response. These findings suggest that at low concentrations, the essential oil acts as a biostimulant, potentially aiding plant stress resilience, while at higher concentrations, it triggers oxidative damage. The results support the potential of *C. citriodora* essential oil as an eco-friendly alternative for developing plant-based bioherbicides, offering a sustainable approach to managing weeds.

SIGNIFICANCE STATEMENT

Weeds are unwanted plants in the wasteland and agricultural areas that cause significant damage to the environment, native life forms, human health and the health of other organisms. Since last century, synthetic herbicides have remained a prevalent method to manage weeds, however, the development of

herbicide resistance in weeds, environmental pollution and their adverse effects on biodiversity have led researchers to look for sustainable, biodegradable and eco-friendly alternatives with novel sites of action for target weeds. Essential oils offer great potential in this context. Hence, this study explores the effect of the essential oil of *Corymbia citriodora*, a lemon-scented eucalypt on the stress physiology of coffee senna, a noxious wasteland weed. The increased level of stress-related metabolites in treated plants suggests its possible use as a bioherbicide in the future.

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