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Inhibition of Peanut (*Arachis hypogaea* L.) Growth, Development, and Promotion of Root Nodulation Including Plant Nitrogen Uptake Triggered by Polyvinyl Chloride Microplastics

Udayshankar Halder, Chaithra Radharamanan, Karthick Venkatesan, and Siddhuraju Perumal*

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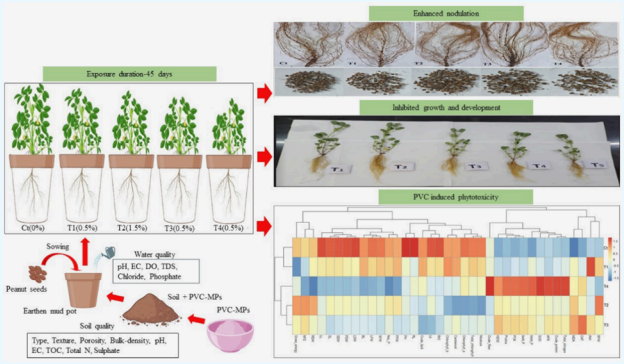
ABSTRACT: Agroecosystem sustainability and global food security may be threatened by the widespread presence and distribution of microplastics (MPs). This study investigates the impact of polyvinyl chloride (PVC) microplastics with four different dosages (0.5, 1.5, 2.5, and 3.5%) on the growth, development, and nitrogen uptake of peanut (*Arachis hypogaea* L.), a legume that forms symbiotic relationships with nitrogen-fixing root nodules. Oxidative stress was indicated by increases in the activity of hydrogen peroxide, proline, superoxide dismutase, peroxidase, and ascorbate peroxidase of 54.3, 72.93, 135.74, 41.59, and 44.59%, respectively, for the 3.5% dose (T4) and malondialdehyde and catalase of 23.7 and 17.52%, respectively, for the 2.5% dose (T3) over the control. Peanut seedlings' growth and development were inhibited through the suppression of chlorophyll a (30.92%), chlorophyll b (36.36%), and carotenoid (25.65%) for treatment 2 (T2) and plant height (19.52% for T4), plant dry weight (46.09%), leaf number (18.86%), and branch length (59.37%) for T4. However, root nodule number, weight, and plant N content promoted 30.19−72.32, 55.88−141.16, and 1.46−7.01%, respectively, from control to T4, which may be an adaptive mechanism for legumes to overcome N deficiency through the morphological and physiological adjustments in the stressed conditions. The study outcomes may provide worthy implications for correctly managing peanut crops in PVC MP-contaminated soil, which will ensure food security and ecosystem sustainability.



INTRODUCTION

Microplastics (MPs), the most omnipresent synthetic polymeric matrix of increasing eco-toxicological emerging contaminants of serious global environmental concern,[1−3] have regular or irregular shapes including fragments, fibers, pellets, spheres, films, and foams[4] and sizes ranging from 1 μm to 5 mm.[5] Based on formation, MPs are classified as primary MPs, manufactured for commercial use like exfoliating beads, particles in cosmetic and medical products, toothpaste, and industrial abrasives,[6] and secondary MPs, formed when larger plastics are fragmented via weathering caused by environmental factors and anthropogenic activities.[3,6,7] MPs have been detected in all environmental matrices from the Antarctic to the Arctic and from the highest Tibetan Plateau to the deepest Mariana Trench, even in human thrombi, breast milk, and the feces of newborn infants.[2,4] Terrestrial soil MPs are found to be 4−23 times greater than in aquatic ecosystems,[6] and the estimated concentrations in natural and agricultural soil range from 50 to 130 and 50−18760 items/kg, respectively,[8,9] accumulating via plastic mulching, sewage sludge amendment, sewage sludge disposal, wastewater disposal, atmospheric deposition, greenhouse materials, etc.[1,3,4]

MP dispersion and interaction in soil ecosystems depend on polymer types, size, shape, charge, concentration, large specific surface area, hydrophobicity, chemical inertia, structural characteristics, released additives, persistence, and environmental factors.[1,10−13] Soil physicochemical characteristics such as porosity, pH, water holding capacity, water permeability, organic carbon, aeration, and bulk density are altered due to MP contamination, leading to an imbalance in ecological functions.[13−16] Contradictory alterations, including decreases,[11] increases,[17] and even nonsignificant[18] activities of soil enzymes critical for nutrient cycling and indicators of soil pollution, are done by the presence of MPs in soil.[16] Soil organisms, including earthworms and nematodes, growth, weight, feeding behavior, activities, gut microbiota, immune systems, and reproduction, have been adversely affected by MP

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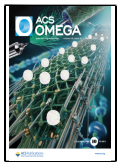

Table 1. Effects of PVC MPs on Morphological Traits of the Peanut Plant[a]

parameters	Ct	T1	T2	T3	T4
plant height (cm)	74.74 ± 11.35a	63.11 ± 3.87b	61.80 ± 5.11b	60.15 ± 7.21b	60.70 ± 5.08b
shoot length (cm)	34.91 ± 2.91a	31.82 ± 1.86b	31.05 ± 3.75bc	28.75 ± 1.43cd	27.80 ± 2.61d
leaf length (cm)	10.98 ± 1.25a	10.34 ± 1.18ab	10.00 ± 0.50ab	10.04 ± 0.35ab	9.66 ± 0.35b
root length (cm)	39.83 ± 12.71a	31.29 ± 3.92b	30.75 ± 4.30b	31.40 ± 7.01b	32.44 ± 5.95b
no. of leaves per plant	21.20 ± 1.64a	20.60 ± 2.70a	20.00 ± 1.22a	19.00 ± 1.58ab	17.20 ± 1.78b
branch length (cm)	20.75 ± 2.51a	19.41 ± 2.88a	14.36 ± 1.77b	12.13 ± 1.99c	8.43 ± 2.65d
plant fresh weight (g)	9.34 ± 1.40a	9.19 ± 1.51a	8.40 ± 0.81ab	6.87 ± 0.72bc	5.84 ± 1.26c
plant dry weight (g)	2.56 ± 0.35a	1.94 ± 0.30b	1.87 ± 0.15b	1.62 ± 0.10bc	1.38 ± 0.22c
leaf fresh weight (g)	4.97 ± 1.25a	4.34 ± 0.81ab	3.74 ± 0.40bc	3.30 ± 0.45bc	2.77 ± 0.51c
leaf dry weight (g)	1.34 ± 0.31a	0.92 ± 0.16b	0.86 ± 0.08bc	0.74 ± 0.10bc	0.62 ± 0.11c
shoot fresh weight (g)	3.01 ± 0.58a	2.73 ± 0.72ab	2.26 ± 0.30bc	1.96 ± 0.13c	1.75 ± 0.33c
shoot dry weight (g)	0.75 ± 0.16a	0.59 ± 0.15b	0.53 ± 0.07bc	0.45 ± 0.05bc	0.39 ± 0.07c
root fresh weight (g)	1.36 ± 0.49b	2.11 ± 0.39a	2.41 ± 0.24a	1.81 ± 0.39ab	1.31 ± 0.61b
root dry weight (g)	0.46 ± 0.09ab	0.43 ± 0.07ab	0.47 ± 0.04a	0.42 ± 0.04ab	0.35 ± 0.07b
root to shoot ratio	1.74 ± 0.68a	1.42 ± 0.51ab	1.11 ± 0.18b	1.06 ± 0.21b	1.14 ± 0.23b

[a]Each value is expressed as mean ± standard deviation. The letters a−d show a significant ($P < 0.05$) difference between treatments and the control. Ct—control, T1—treatment 1 (0.5%), T2—treatment 2 (1.5%), T3—treatment 3 (2.5%), and T4—treatment 4 (3.5%).

exposure and ingestion.[1,19,20] In the case of microorganisms like bacteria, including Rhizobiales,[21] plastic degraders, pathogenic[22] and fungal community structure, relative abundance, and activity are influenced selectively, either reduced[11,18] or enriched[16,23] via the exposure of MPs acting as an important source of carbon, providing a distinct niche for some microbial varieties.[24]

Ultimately, plants as primary ecosystem producers are greatly influenced by MP exposure directly[10,25] or indirectly through the changes in the soil's physicochemical and biological properties due to MP contamination.[11,15,21] Recent studies have highlighted a series of important underlying mechanisms, including physical blockage of pores in cell walls of seed, root, stem, and leaf stomata;[7,26] mechanical damage of cells;[27,28] inhibition of water and nutrient uptake;[23,29] imbibition of water;[30] impairment of the metabolic pathway;[31] alteration in photosynthetic activity;[15,18] changes in nodulation;[20] modification in the structure of DNA or gene;[26] and production of excess reactive oxygen species (ROS)[12,17] through which plant growth, development, performance,[28,30,32] physiology and biochemistry,[15,33] biomass allocation and accumulation,[10,17] biomass nutrient content, crop yields, and crop quality and nutrient content[25,29,34] are affected. Varied and contradictory impacts ranging from negative[20,31] to neutral,[7,23] even positive,[18,22] were also observed depending on plant species, including crops critical for maintaining food security, ecosystem stability, and climate feedback.[32,35] To understand MP−plant interactions properly, more plant species need to be studied, considering better-associated parameters including polymer types, size, concentration, and charge.[10,23,35]

Peanut (*Arachis hypogaea* L.) as an oilseed, food, and feed legume crop of tropical and semiarid tropical countries is highly significant in biological nitrogen fixation (100−152 kg/h N) even in response to abiotic stress and is largely consumed globally due to its high content of fat, protein, dietary fiber, vitamins, minerals, and antioxidants.[36,37] The peanut plant's vegetative growth and N uptake were reduced by 1% (w/w) polypropylene (PP) and rubber crumb (RC) MPs through damaging root cells and altering N cycling in soil.[38] High-density polyethylene (HDPE), polystyrene (PS), and polylactic acid (PLA) showed no phytotoxic effects on plant biomass but adverse impacts on the plant N content of peanuts, while PLA enhanced root nodulation by enriching symbiotic N-fixer Rhizobiales.[16] Another study also reported that the peanut plant's height, culm diameter, root and total biomass, PSII photochemical quantum yield (Fv/Fm), hundred-grain weight, and soluble sugar were reduced by PP MPs, but chlorophyll content was increased by polyester MPs.[13]

To date, only one scientific study has been done to understand polyvinyl chloride (PVC) MP effects on legume plants, where a substantial decrease in photosynthetic rate in soybean was observed by PVC MPs than PE-MPs.[39] However, PVC, as the most toxic one among PP, PE, polyethylene terephthalate (PET), and PVC[40] covering nearly 46% of the total global plastic production, is considered one of the top three plastics[41] commonly found in farmland soil.[42] In this present study, PVC MPs were selected to investigate their effects on peanut plants. We hypothesized that PVC MPs can have a significant negative dose-dependent influence on peanut plant growth and development. To verify our hypothesis, the main objectives of this study were to evaluate the effects of PVC MPs on physiological and biochemical traits, nutritional quality, and stress-causing mechanisms of peanut plants that might help the management of peanut crop cultivation in MP-contaminated soil, ensuring food security.

MATERIALS AND METHODS

Soil, PVC MPs, and Peanut Seeds. The test soil (upper 10−15 cm) was reddish brown, loamy, and sandy and was collected via random sampling from an agricultural land for multiple cropping, such as tomato, chili, and different bean practices without plastic mulching. After the removal of plant and animal residues, stones, and other debris, fresh soils were air-dried and sieved through a 2 mm sieve for the study. Soil physicochemical parameters were also determined to know its suitability for plant growth (Table 1). 98% pure dry white powder form (average size 200 μm, using scanning electron microscopy—Icon Analytical/Fei; Quanta 200) of PVC MPs was purchased from Arihant Solvents and Chemicals, Coimbatore, Tamil Nadu, India. Peanut "CO-6" seeds were procured from the Oilseed Department of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

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Table 2. Effects of PVC MPs on Aboveground Biomass of Peanut Plant[a]

parameters	control	T1	T2	T3	T4
moisture content	8.35 ± 2.97a	8.02 ± 1.52a	7.08 ± 2.10a	7.57 ± 0.96a	7.71 ± 1.49a
ash content	9.68 ± 0.66a	10.59 ± 0.80a	9.80 ± 0.14a	10.17 ± 0.37a	9.80 ± 0.22a
total nitrogen	3.42 ± 0.12b	3.47 ± 0.03b	3.49 ± 0.02b	3.53 ± 0.02b	3.66 ± 0.06a
crude protein	21.41 ± 0.79b	21.64 ± 0.27b	21.83 ± 0.15b	22.10 ± 0.17b	22.89 ± 0.38a
crude fiber	21.11 ± 1.59a	21.12 ± 1.10a	21.03 ± 0.42a	21.07 ± 1.78a	21.54 ± 0.85a
crude lipid	6.93 ± 0.99a	6.84 ± 0.64a	6.07 ± 0.88a	5.85 ± 0.56a	6.37 ± 0.42a
carbohydrate (NFE)	40.84 ± 3.79a	39.80 ± 1.33a	41.25 ± 1.15a	40.78 ± 2.31a	39.37 ± 1.24a
gross energy (kj/kg)	1134.78 ± 38.10a	1119.91 ± 11.45a	1136.80 ± 8.64a	1130.56 ± 33.12a	1127.37 ± 15.13a

[a]Each value is expressed as mean ± standard deviation. The letters a and b show a significant ($P < 0.05$) difference between treatments and the control. Ct—control, T1—treatment 1 (0.5%), T2—treatment 2 (1.5%), and T3—treatment.

Experimental Setup. A pot experiment was conducted in the greenhouse (temperature 20−34 °C, humidity 54−66%, light hours 12−12.5 h) built on the terrace of the Department of Environmental Sciences under natural conditions. PVC MPs were applied in four different doses (0.5, 1.5, 2.5, and 3.5% w/w by weight of dry soil) based on the occurrence of MPs in agricultural soil[43] along with a control treatment (fresh soil only). The soil-MP-homogenized mixture was prepared in a big iron container manually; 5 kg was transferred into each earthen pot (five treatments and four replicates for each treatment, resulting in a total of 20 pots) a week before to allow interactions between the soil microbiome, soil, and MPs.

Surface sterilization of uniformly and healthily selected seeds (43 mg average weight) was done with 70% v/v ethanol for 5 min and washed three times thoroughly via double-distilled water (ddH_2O). Overnight soaked and imbibed seeds were placed on wetted filter paper on Petri plates for 12 h. After the moisture content level of the soil was set at 60% of field water capacity, five seeds were sowed at a depth of 1.5−2 cm in each pot, and 100% germination was observed within a week. Daily 100, 200, and 250 mL of tap water were irrigated to seedlings up to 15, 30, and 45 days, respectively. Physicochemical parameters of the irrigated water were also studied to know its suitability for plant growth and development (Table 2). There was no application of external fertilizers (organic and chemical). The pots were placed randomly and rearranged once every 5 days. After 45 days of exposure, the plants were destructively sampled at the flowering stage by separating and thoroughly washing each plant's roots, shoots, and leaves with ddH_2O.

Measurement of Morphological Traits. On the day of harvest, each plant's height (PH), shoot length (SL), leaf length (LL), branch length (BL), and root length (RL) were measured by a steel meter scale. The number of leaves per plant (NoL_P) and number of nodules per plant (NoN_P) were counted manually. The plant fresh weight (PFW), leaf fresh weight (LFW), shoot fresh weight (SFW), root fresh weight (RFW), and weight of nodules per plant (WoN_P) were recorded using an analytical balance model (CPA224S, Sartorius AG, Germany). After drying in an oven at 80 °C, the plant dry weight (PDW), leaf dry weight (LDW), shoot dry weight (SDW), and root dry weight (RDW) were studied again by using the same balance. Dry biomass was ground in powder form and stored for further analysis. Root to shoot ratio (RtS) represents the ratio of root biomass to shoot biomass, which was calculated from seedlings shoot biomass/root biomass.

Estimation of Photosynthetic Pigments. To determine chlorophyll a and chlorophyll b, total chlorophyll, and carotenoid content, fresh leaves (500 mg) were homogenized with 80% acetone (5 mL) and centrifuged at 7000 rpm and 4 °C for 20 min repeatedly until colorless residues appeared. The absorbance of the collected supernatant (100 mL by adding 80% acetone) was read at 470, 645, and 663 nm for calculation.[44]

Assay of Leghemoglobin (Lb) Content. The root nodule's Lb content was assayed by the cyanmethemoglobin protocol with slight modifications. Frozen nodules (400 mg) of 21.85% moisture content were crushed with 3 mL of Drabkin's solution, centrifuged at 7000 rpm and 4 °C for 15 min, and repeated two more times. The absorbance of combined extracts (8 mL via adding Drabkin's solution) was read at 540 nm. The Lb content calculation was done against a standard curve of diluted standard cyanmethemoglobin.[45]

Determination of Proximate Composition. The moisture content was read using an MA 35 moisture analyzer (Sartorius AG, Germany) at 105 °C. The micro-Kjeldahl method was employed to determine total nitrogen (N), while a 6.25 nitrogen-protein conversion factor was used for crude protein (N × 6.25) estimation.[46] Crude lipid (Soxhlet extraction), crude fiber, and ash content were also assessed using gravimetric methods described in the Association of Official Analytical Chemists.[47] The carbohydrate/nitrogen free extract (NFE) content was calculated by subtracting percentile ash, crude protein, lipid, and fiber from 100. The addition of multiplied values of the percentage of crude protein, lipid, and carbohydrate by 16.7, 13.7, and 16.7, respectively, was used to calculate gross energy (kJ/kg).[48] The proximate composition was expressed as g/100 g dry matter (DM).

Proline, Lipid Peroxidation (MDA), and Hydrogen Peroxide (H_2O_2) Estimation. Fresh leaves (0.5 g) were homogenized with 3% sulfosalicylic acid (10 mL) and centrifuged at 10,000 rpm and 4 °C for 10 min. The 6 mL mixture (2 mL of supernatant, 2 mL of glacial acetic acid, and 2 mL of acidic ninhydrin) was placed in a water bath at 100 °C for 1 h. After the reaction had been completed in an ice bath, toluene (4 mL) was added and vortexed for 15 s. The absorbance of the toluene phase dissociated from the aqueous phase in the dark after 20 min was read at 520 nm. Proline content was assessed from a standard curve of proline.[49]

Samples of the fresh leaf (0.5 g) with 0.1% trichloroacetic acid (5 mL) were homogenized and centrifuged at 7000 rpm and 4 °C for 15 min to extract the supernatant used in the assessment of MDA and H_2O_2. A 5 mL portion of a mixture (supernatant 1 mL, 0.5% thiobarbituric acid 2 mL, 20% trichloroacetic acid 2 mL) was incubated in a water bath at 95 °C for 25 min and cooled in an ice bath. The absorbance was measured at 532 and 600 nm to estimate the MDA content

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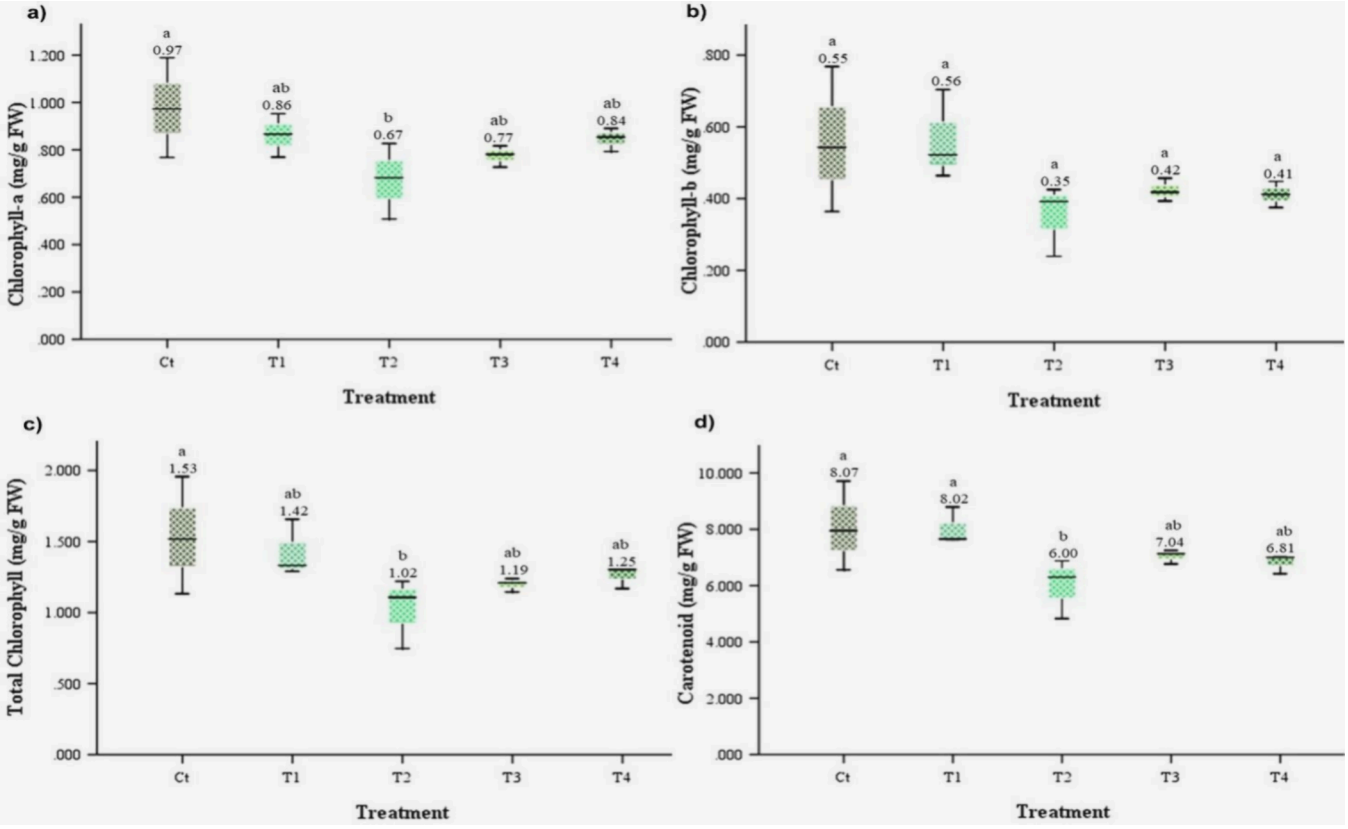

Figure 1. Effects of PVC MPs on the photosynthetic pigments. (a) Chlorophyll a, (b) chlorophyll b, (c) total chlorophyll, and (d) carotenoid. Each value is expressed as mean ± standard deviation. The letters a and b show a significant ($P < 0.05$) difference between treatments and the control. Ct—control, T1—treatment 1 (0.5%), T2—treatment 2 (1.5%), T3—treatment 3 (2.5%), and T4—treatment 4 (3.5%).

using the molar extinction coefficient of 155 mM cm^{-1}.[50] To determine H_2O_2 content, a 2 mL reaction mixture (0.5 mL of supernatant, 1 mL of 1 M potassium iodide, and 0.5 mL of sodium phosphate buffer pH 7) was incubated for 1 h in darkness. The absorbance was recorded at 390 nm and used to calculate the H_2O_2 content against a calibration curve of H_2O_2.[51]

Extraction and Determination of Antioxidant Enzyme Activity. To extract antioxidant enzymes, fresh leaves (0.5 g) with 5 mL of 50 mM sodium phosphate buffer (pH 7) were homogenized and centrifuged at 10,000 rpm and 4 °C for 15 min. The supernatant was used to assess antioxidant enzyme activity.[52]

To assess peroxidase (POX) activity, enzyme extract (0.1 mL) was added to a 3.5 mL reaction mixture (3 mL of buffered pyrogallol consisting of 0.05 M pyrogallol in 0.1 M phosphate buffer pH 7.0, 0.5 mL 1% H_2O_2). The change in optical density was measured at 430 nm every 30 s for 2 min. The POX activity was estimated using an oxidized pyrogallol extinction coefficient (4.5 L/mol).[53] 3% H_2O_2 (800 μL) was added to the assay mixture (200 μL of enzyme extract, 1200 μL of 0.1 mM ethylenediaminetetraacetic acid in 0.05 M sodium phosphate buffer pH 7.0, and 800 μL of 0.5 mM ascorbic acid) to start the reaction. The absorbance decrease was read at 290 nm every 30 s for 3 min. An extinction coefficient (ε = 2.8 mM^{-1} cm^{-1}) was used to calculate the ascorbate peroxidase (APX) activity.[54]

Enzyme extract (0.2 mL) was added to a mixture (1.5 mL of 100 mM sodium phosphate buffer pH 7.0, 0.4 mL of distilled water, and 1 mL of 30 mM H_2O_2), and the decrease in absorbance was recorded at 240 nm every 1 min for 3 min. The extinction coefficient of H_2O_2 (39.4 mM^{-1} cm^{-1}) was used for the assessment of catalase (CAT) activity.[55] In the assay mixture of 1.6 mL (500 μL of phosphate buffer solution pH 7.3, 200 μL of 20 mM methionine, 100 μL of 2 mM nitroblue tetrazolium, 200 μL of 0.2% Triton-X, 500 μL of ddH_2O, and 100 μL of enzyme extract), 100 μL of 6 mM riboflavin was added and heated under a 15 W fluorescent lamp for 15 min. After finishing the reaction in the dark, the absorbance was observed for samples with enzyme extract and blank without enzyme extract. The superoxide dismutase (SOD) activity was estimated by subtracting the sample value from the blank value, and one unit of enzymatic activity was considered the quantity of enzyme required for a 50% color decrease.[56] The absorbance of different wavelengths was read using a UV−visible spectrophotometer model UV-1800 240 V (Shimadzu Corporation, Kyoto Japan).

Extraction of PVC MPs. Saturated sodium chloride (NaCl) solution (density of 1.2 g/mL) was added to a beaker containing the soil sample. After 10 min of magnetic stirring at high rpm, the solution was allowed to settle for 6 h. A vacuum filter apparatus was used to filter the collected supernatant portion of the solution. MPs were separated from inorganic and organic debris with the help of both sieving and manual separation from air-dried residue found on filter paper.[57]

Determination of Surface Morphology and Functional Groups of PVC MPs. The surface morphology of both virgin and extracted PVC MPs from soil after harvest was determined using scanning electron microscopy (SEM) (Icon Analytical/Fei; Quanta 200) with energy-dispersive spectral

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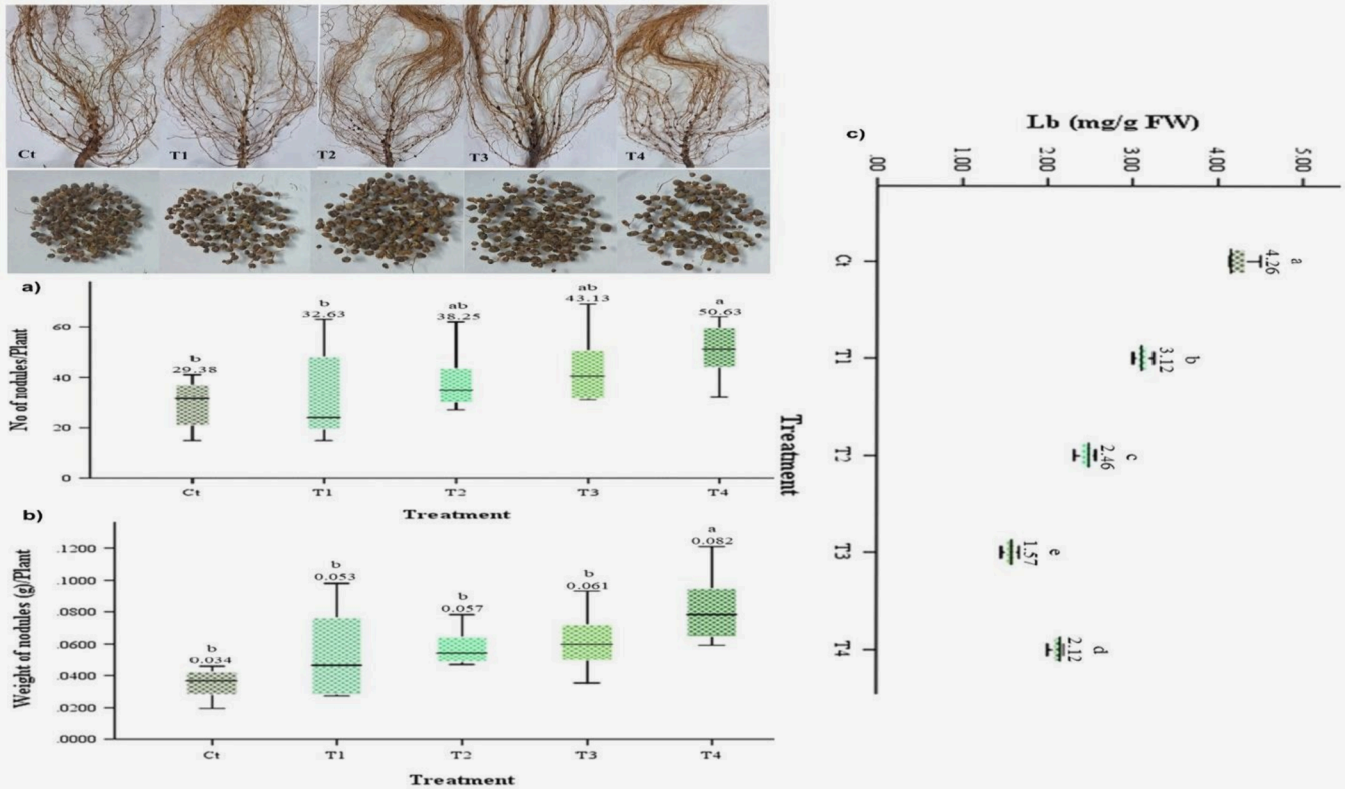

Figure 2. Effects of PVC MPs on nodulation. (a) Number of nodules/plant. (b) Weight of nodules/plant. (c) Lb. Each value is expressed as mean ± standard deviation. The letters a−e show a significant ($P < 0.05$) difference between treatments and the control. Ct—control, T1—treatment 1 (0.5%), T2—treatment 2 (1.5%), T3—treatment 3 (2.5%), and T4—treatment 4 (3.5%).

analysis (Icon Analytical/Edax; Genesis XM4) and X-ray diffraction (Spectris Technologies/PANalytical X'Pert PRO). For the determination of chemical structure, Fourier transform infrared (FTIR) spectroscopy (FT/IR-4700 type A; F066661788) was used.

Data Analysis. Kolmogorov−Smirnov (upper-a) and Shapiro−Wilk tests were employed to confirm the normal distribution assumption. Screening data for homogeneity of variance was done using Levene's test. Data transformation was done for data variables that violated normal distribution. The results were expressed as the mean ± standard deviation of the replicates. Evaluations of peanut growth parameters, Lb, photosynthetic pigments, proximate composition, and enzyme activity of different PVC MP treatments in our pot experiment were conducted via one-way ANOVA; parameters that were significantly affected ($p < 0.05$) by PVC MP addition were separated using a post-hoc test (Duncan's multiple comparisons) in SPSS v25 (IBM Corporation, New York, USA) and MS Excel. During sample processing, reagent blanks were run along with each batch of samples to check for background contamination. Principal component analysis (PCA), correlation matrix (CM), and heatmap were performed using RStudio-2024.04.2-764 by incorporating related software packages including ggcorrplot, ggplot2, factoextra, ggbiplot, and pheatmap.

RESULTS

Effects of PVC MPs on Morphological Traits. Compared with the control, significant ($p < 0.05$) induced variations were observed on morphological traits of plants due to the addition of PVC MPs (Table 1). PH [15.56−19.52%], SL [8.85−20.36%], LL [5.82−12.02%], PDW [24.21−46.09%], LFW [12.67−44.26%], LDW [31.34−53.73%], SFW [9.30−41.86%], and SDW [25.33−48%] were significantly reduced along with the increase in doses compared to the control to T4. A similar significant suppression trend except the highest (39.08%) for the 2.5% dose [T3] was also noticed between treatment groups in the case of RtS. NoL_P depicted a sequential diminution in treatment groups from 2.83 to 18.86% including a significant decline for 2.5% [T3] and 3.5% doses [T4]. BL and PFW reduced significantly by 30.79−59.37 and 10.06−37.47% for the 1.5% [T2] and 3.5% doses [T4], respectively. RL showed a decrease in treatment groups significantly but the lowest (18.55%) and the highest (22.79%) for the 3.5 and 1.5% doses, respectively. RFW showed significant promotions of 33.08, 55.14, and 77.20% for the 2.5, 0.5, and 1.5% doses, respectively, but a nonsignificant reduction of 3.67% for the 3.5% dose. However, RDW diminished significantly in treatment groups except for a nonsignificant increase for T2.

Effects of PVC MPs on Photosynthetic Pigments. In comparison to the control, significant ($p < 0.05$) declines were visualized in the content of photosynthetic pigments including chlorophyll a and chlorophyll b, total chlorophyll, and carotenoid of peanut leaf (Figure 1). Chlorophyll a and total chlorophyll depicted a significant suppression of 11.34−30.92 and 7.18−33.33%, respectively, between treatment groups, while the highest diminution for 1.5% dose (Figure 1a,c). However, chlorophyll b indicated a nonsignificant increase and decrease of 1.81 and 23.63−36.36% for the 0.5% dose and the 1.5−3.5% doses, respectively, and the highest cut-down for 1.5% dose (Figure 1b). In the case of carotenoid, a reduction

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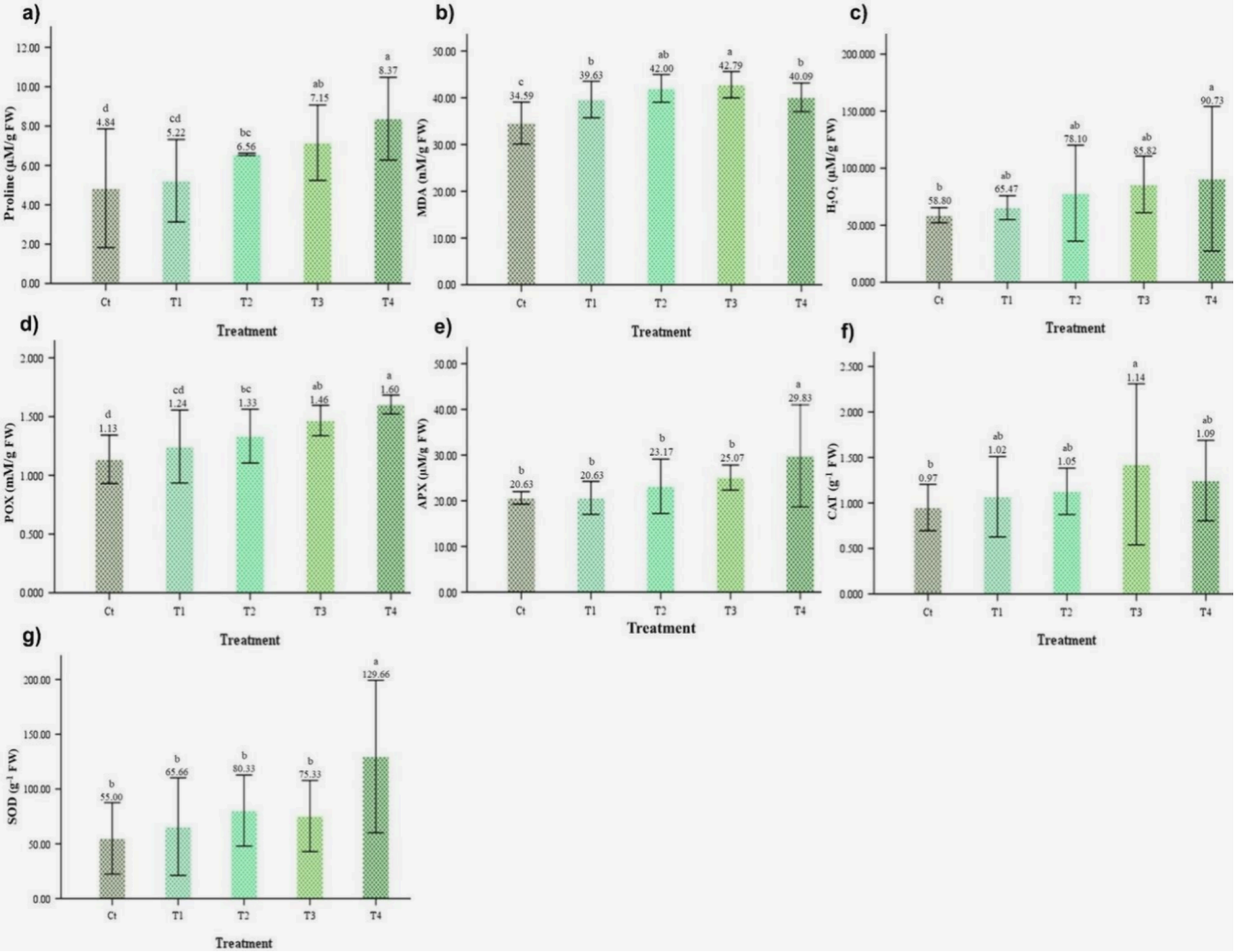

Figure 3. Effects of PVC MPs on nonenzymatic and enzymatic activities. (a) Proline, (b) MDA, (c) H_2O_2, (d) POX, (e) APX, (f) CAT, and (g) SOD. Each value is expressed as mean ± standard deviation. The letters a−g show a significant ($P < 0.05$) difference between treatments and the control. Ct—control, T1—treatment 1 (0.5%), T2—treatment 2 (1.5%), T3—treatment 3 (2.5%), and T4—treatment 4 (3.5%).

(0.61−25.65%) was also observed between treatment groups but significantly for 1.5−3.5% doses along with the highest decrease for the 1.5% dose (Figure 1d).

Effects of PVC MPs on Nodulation. Root nodulation showed a significant ($p < 0.05$) promotion throughout the treatment groups relative to the control (Figure 2). A significant improvement of 55.88−141.16% for WoN_P was recorded with an increase in the dose of PVC MPs between treatment groups (Figure 2b). Similarly, NoN_P also indicated a significant augmentation from 30.19 to 72.32% for the 1.5, 2.5, and 3.5% doses (Figure 2a). Root nodule Lb content showed a significant ($p < 0.05$) diminution but not sequential throughout the treatment groups relative to the control (Figure 2c). The decline was of 26.76, 42.25, 63.14, and 50.23% for the 0.5, 1.5, 2.5, and 3.5% doses, respectively.

Effects of PVC MPs on Proximate Composition. The proximate composition of the plant's aboveground biomass depicted mostly nonsignificant ($p < 0.05$) variations among treatment groups except for total nitrogen and crude protein than the control (Table 2). Moisture content lessened nonsignificantly between treatment groups from 3.95 to 15.2%, where the 1.5% dose showed the highest reduction. Ash content depicted a nonsignificant increase of 1.23−9.4% throughout the treatment groups, while the highest was for the 0.5% dose. A sequential promotion of 1.46−7.01 and 1.07−6.91% for total nitrogen and crude protein, respectively, was found throughout the treatment groups, while significant augmentation was observed only for the highest dose (3.5%). Crude fiber indicated nonsignificant changes, including an increase of 0.37 and 0.18% for the 1.5% and 2.5% doses and a reduction of 0.04 and 2.03% for the 0.5% and 3.5% doses, respectively. A nonsignificant drop of 1.29−15.58% was found for crude lipids between treatment groups, and the highest cut-down was for the 2.5% dose. Similarly, a nonsignificant suppression of 0.14−3.59 and 0.37−1.31% was recorded for NFE and gross energy, respectively, except for an improvement of 1% (NFE) and 0.17% (gross energy) for the 1.5% dose.

Effects of PVC MPs on Proline, MDA, and H_2O_2. Significant ($p < 0.05$) promotion of proline, MDA, and H_2O_2 content was observed between treatment sets relative to the control (Figure 3a−c). Both proline and H_2O_2 indicated a sequential significant dose-dependent augmentation throughout the treatment groups from 7.85 to 72.93 and 6.67−54.3%, respectively (Figure 3a and Figure 3c, respectively). A similar significant increasing trend from 14.57 to 23.7% was also found

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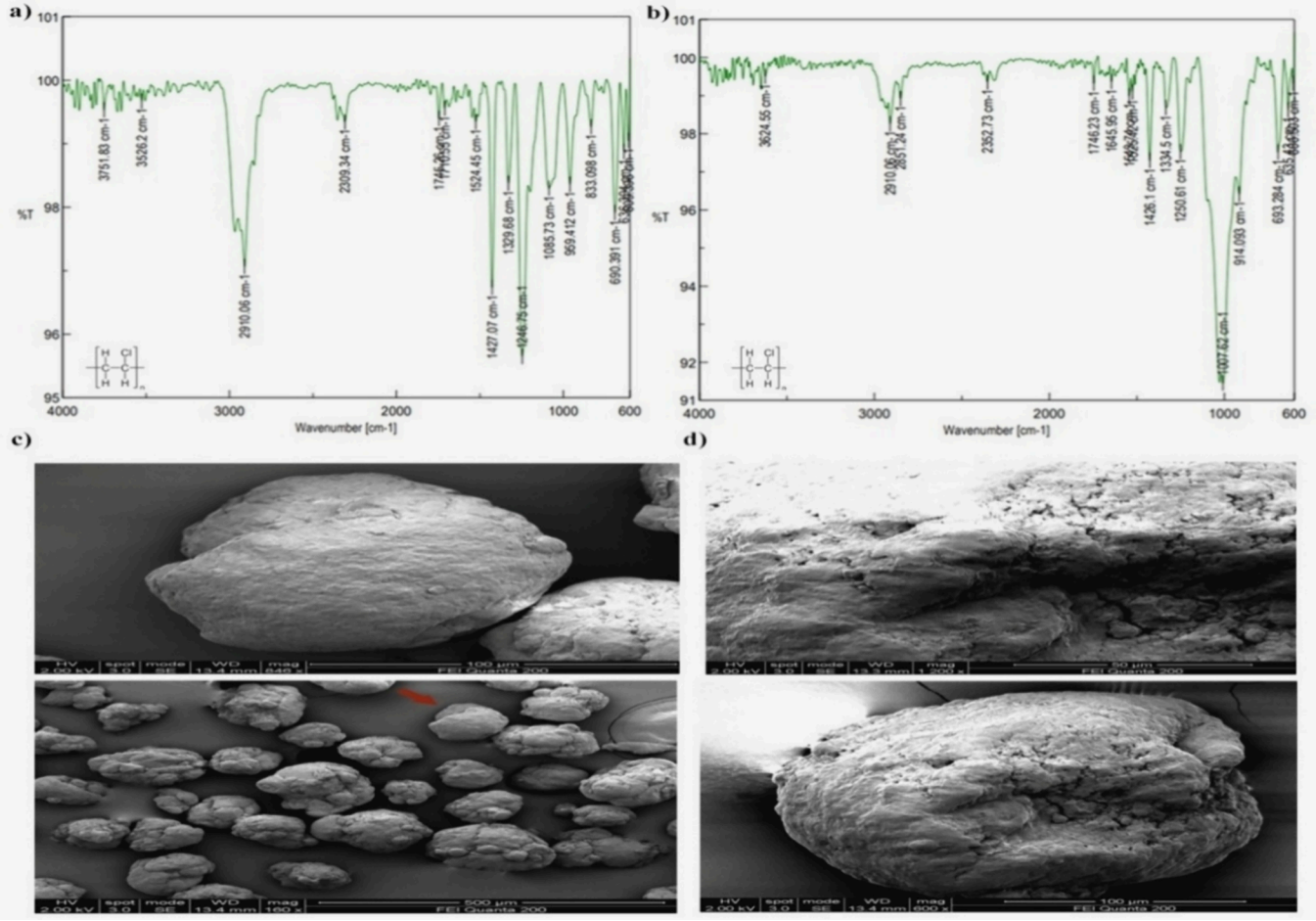

Figure 4. FTIR spectra of (a) virgin and (b) extracted PVC MPs. SEM images of (c) virgin and (d) extracted PVC MPs.

for MDA in the treatment groups where the highest rise was at the 2.5% dose (Figure 3b).

Effects of PVC MPs on Antioxidant Enzyme Activity. Antioxidant enzymes, including POX, APX, CAT, and SOD activities of leaves, were significantly ($p < 0.05$) augmented between all the treatment groups compared with the control (Figure 3d−g). A significant sequential dose-dependent improvement in POX activity was observed from 9.73 to 41.59% throughout the treatments (Figure 3d). The control and 0.5% doses showed the same activity, and 1.5, 2.5, and 3.5% doses depicted 12.31, 21.52, and 44.59% increases in APX activity, respectively, but substantial promotion was only in the 3.5% dose (Figure 3e). CAT activity of all treatment sets indicated a significant rise from 5.15 to 17.52%, and the 2.5% dose showed the highest (Figure 3f). A significant augmentation (135.74%) was found in the 3.5% dose, whereas a nonsignificant development of 19.38−46.05% was recorded in other treatments for SOD activity (Figure 3g).

Effects on Surface Morphology and Chemical Structure of PVC MPs. The surface morphology of recovered PVC MPs (Figure 4d) depicted a very minor crack appearance compared to that of the virgin one (Figure 4c). FTIR spectra of PVC MPs (virgin extracted from soil after harvesting) showed several very weak absorption peaks (Figure 4a,b). The bands at 3751.83, 3624.55, and 3526.2 cm^{-1} were attributed to the nonbonded OH stretch of CH_2.[58] The absorption peaks at 2910.06 and 2851.24 cm^{-1} were assigned to the axial deformation of the C−H group of CH_2 via stretching.[59] C═N stretching of nitriles was related to peaks at 2309.34 and 2352.73 cm^{-1}. Due to C═O stretching, spectra showed peaks at 1746.23, 1745.26, 1710.55, and 1645.95 cm^{-1}.[58] In addition, the C−O stretching vibration was related to peaks at 1085.73 and 1007.62 cm^{-1}.[60] Deformation of the C−H group from CH_2-Cl was identified by peaks at 1543.74, 1525.42, 1524.45, 1329.68, and 1334.5 cm^{-1}.[61,62] Spectra showed bands at 693.28, 690.39, 635.43, 606.5, 636.39, and 609.39 cm^{-1} attributed to stretching vibration of the C−Cl group.[59,61] Other peaks corresponded to CH bending (1426.1 and 1427.07 cm^{-1}), rocking (1250.61 and 1246.75 cm^{-1}), and wagging (959.41 and 914.09 cm^{-1}) modes in the spectra.[59] The peak length at 2910.06 cm^{-1} was reduced, but a long band at 1007.62 cm^{-1} was observed for the spectrum of extracted PVC MPs compared to the virgin one.

PCA, CM, and Heatmap. PCA, CM, and a hierarchical clustered heatmap of the relative abundance of variables based on variables measured and doses of PVC MPs were employed to identify a coordinated response of peanut plant growth (Figure 5). PCA was used to determine the explanatory variable (the control and doses of PVC MPs—0.5, 1.5, 2.5, and 3.5%) effects on response variables. Similarities and differences between response variables were also identified. In PCA, PC1 (eigenvalue −2.55) and PC2 (eigenvalue −6.82) explained 69.1 and 18.5%, respectively, of the total variance of 87.6%. According to PC1, the 3.5% dose indicated a large positive value and was highly associated with POX, APX, SOD, proline, H_2O_2, total nitrogen, crude protein, crude fiber, NoN_P, and

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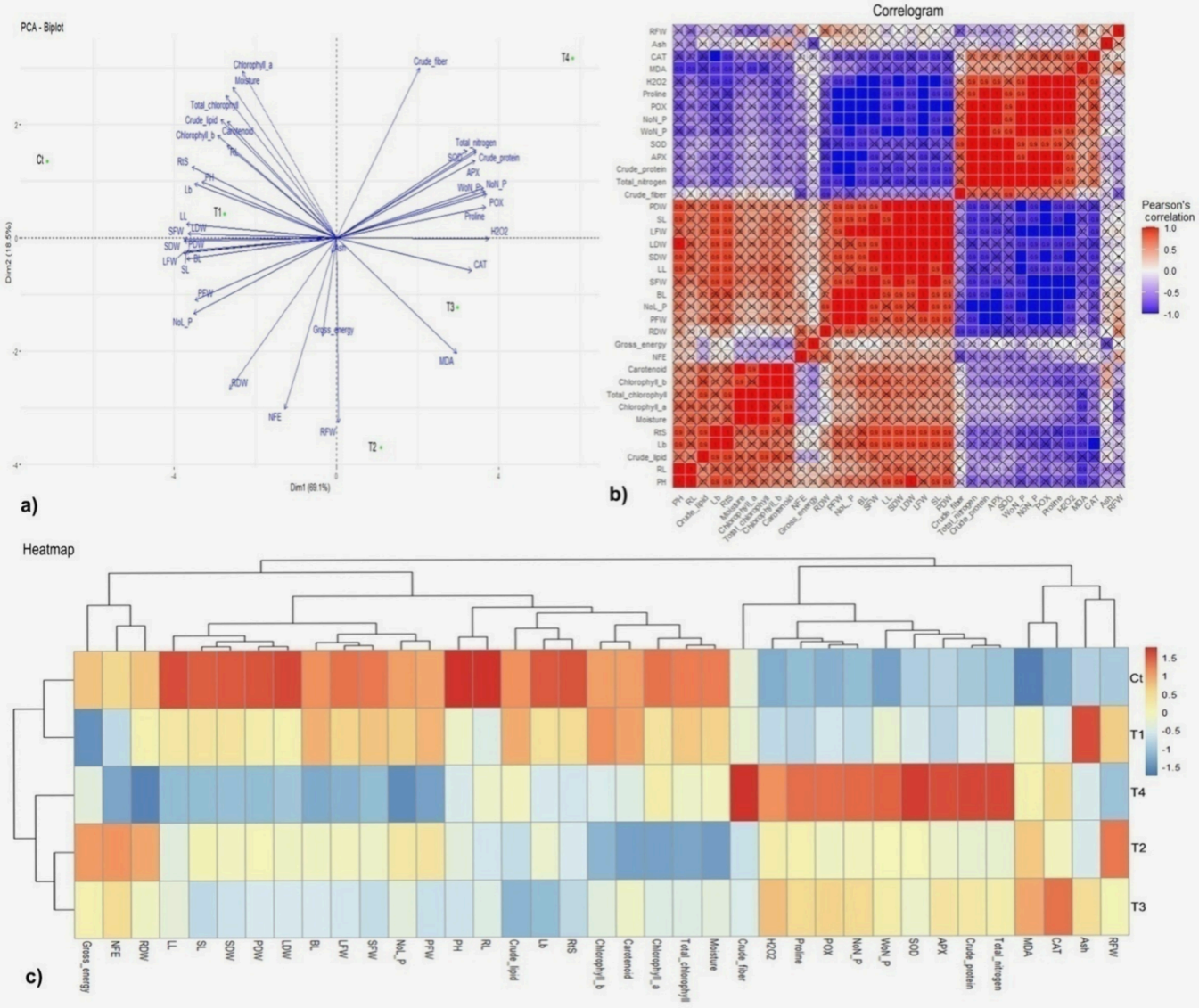

Figure 5. Relationship between variables and different doses (Ct—control, T1—treatment 1 (0.5%), T2—treatment 2 (1.5%), T3—treatment 3 (2.5%), T4—treatment 4 (3.5%)) of PVC MPs. (a) PCA, (b) CM or correlogram, and (c) heatmap of the relative abundance of the measured variables.

WoN_P. A moderate positive value for the 2.5% dose positioned very close to CAT and MDA indicated a close association. Similarly, RFW, RDW, NFE, and gross energy showed a good association with the 1.5% dose. On the contrary, the control and 0.5% dose depicted high and moderate negative values, respectively, and were connected accordingly with PH, SL, RL, NoL_P, PFW, SFW, LFW, BL, PDW, SDW, LDW, RtS, chlorophyll a and chlorophyll b, total chlorophyll, carotenoid, crude lipid, moisture, and Lb. Ash was close to the 0.5% dose and highly connected to it (Figure 5a).

Hierarchical cluster heatmap CM was employed to identify significant ($p < 0.05$) correlations, either positive or negative, between the variables measured, especially growth parameters and stress indicator variables, including proline, MDA, H2O2, POX, APX, CAT, and SOD. To identify significant correlations along with color gradients, correlation coefficient values were added. Insignificant ones were marked by a dotted line cross sign to segregate significant correlations. Due to the application of hierarchical clustering, both significant and insignificant correlations were clustered. Growth parameters including PH, RL, Lb, RtS, SFW, SDW, LFW, SL, chlorophyll a, PFW, NoL_P, BL, LL, LDW, and PDW depicted a significant negative correlation with one or more stress indicator variables, while total nitrogen, crude protein, NoN_P, WoN_P, and crude fiber showed a significant positive correlation with one or more stress indicator variables (Figure 5b).

A heatmap of the relative abundance of measured variables was employed to cluster significantly ($p < 0.05$) correlated variables distributed in a dose-dependent manner. Five rows indicated that the control and four treatment groups were clustered into three independent clusters, namely, (1) control (Ct) and T1 (0.5%); (2) T2 (1.5%) and T3 (2.5%); and (3) T4 (3.5%). Total nitrogen, crude protein, APX, SOD, POX, proline, H_2O_2, WoN_P, and NoN_P were clustered together showing an increasing trend in a dose-dependent manner. Similarly, MDA and CAT also pointed to an augmented aptitude, but the highest abundance was in the 2.5% dose. PFW, NoL_P, SFW, LFW, BL, LDW, PDW, SDW, SL, and LL formed a cluster of decreasing trends along with dose

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promotion. Another important cluster was developed where moisture, total chlorophyll, chlorophyll a, chlorophyll b, carotenoid, RtS, Lb, RL, PH, and crude fiber depicted a decreasing tendency. However, the reduction propensity did not follow the dose rise accordingly, accompanied by the relative abundance of the lowest and the second lowest at 1.5 and 2.5% doses, respectively (Figure 5c).

DISCUSSION

Phenotypes including PH, SL, LL, RL, NoL_P, BL, PFW, PDW, LFW, LDW, SFW, SDW, RFW, RDW, and RtS represent plant growth and development and can also be used to determine plant health and growth rate for understanding the direct effects of MPs on the plant.[17,63] In this study, all of the visual traits except RFW showed a significant decline in treatment sets compared to the control either in a dose-dependent or mixing manner. In the case of RFW, the control, 2.5%, and 3.5% doses showed significantly lower values than 0.5 and 1.5% doses (Table 1). Recent studies also indicated similar outcomes regarding different morphological characteristics related to plant growth and development. A 30-day exposure to PVC MPs suppressed the stem length, root length, and fresh and dry weights of *Brassica rapa*.[40] The lettuce plant's size decreased visually, but root length ($p < 0.05$) and shoot, root, and total fresh weight ($p < 0.01$) diminished significantly compared to the control due to 0.1 g L^{-1} PVC MP addition.[12] Plant height, culm diameter, total biomass, and root biomass of peanut plants tended to decline by PP-MPs.[13] 1% w/w PP-MPs and RC-MPs also inhibited the vegetative growth of peanut plants significantly.[38] Compared to shoot biomass (in terms of both length and weight), root biomass showed a less negative effect even with significant promotion of RFW for 0.5 and 1.5% doses. Similar impacts of PVC MPs were reported on shoot and root biomass of *Zea mays*.[17] Several mechanisms can initiate the inhibition of peanut plant growth and development. Peanut plant growth and development can be prevented indirectly by PVC MPs due to the changes in the overall environment of water and nutrient availability and transfer through the alteration of physicochemical parameters including water holding capacity, pH, bulk density, and soil organic and microbial biomass carbon.[11,13,15] As an indirect suppression mechanism, PVC MPs can also change the ecological environment required for proper plant growth and development through the perturbation of enzyme activity (critical for nutrient cycling) and bacterial and fungal community percentage and abundance.[11] Physical blockage of cell wall pores and cell-to-cell connections by PVC MPs can prevent water and nutrient uptake leading to direct suppression of peanut plant growth and development.[12,26] Oxidative stress can cause mechanical damage to root cells resulting in impaired water and nutrient transfer due to MPs, which is also considered a direct inhibition process for plant growth and development.[26,38] MP-induced reduction of the photosynthesis rate and efficiency may have directly promoted negative effects on the performance of plants.[33,39] MP-induced impairment of phytohormones, e.g., indole-3-acetic acid, critical for regulating plant growth and development and combating biotic and abiotic stress homeostasis can disrupt crosstalk between phytohormone signaling. As a result, altered signal perception, transduction, and mediation of stress response may prevent the growth and development of plants as a direct negative impact-causing mechanism.[64]

Photosynthetic characteristics and activity are considered important indicators to assess MP-induced toxicity leading to changes in plant physiological parameters.[65] Photosynthetic pigments especially chlorophyll dominantly present in plants play a crucial and central role in light absorption during photosynthesis.[66] Chlorophyll a and total chlorophyll indicated a significant reduction between treatment sets, and carotenoids showed a significant reduction at 1.5, 2.5, and 3.5% doses than the control. At the same time, chlorophyll b tended to decline throughout all treatments but nonsignificantly. The selective highest suppression of all photosynthetic pigments for the 1.5% dose indicated the most severe toxic dose. Similarly, a significant reduction of chlorophyll a and b and total chlorophyll content in mug beans due to PS-MPs compared to the control is observed.[34] Moreover, 1% w/w PP-MPs and RC-MPs caused a significant decrease in chlorophyll a and b while RC alone induced a significant cut-down in carotenoid content of peanut than the control.[26] Blockage of cell-to-cell connections or cell wall pores due to MP exposure can prevent plant nutrient absorption and transportation, creating inhibition of pigment expression.[67] In addition, MP-induced accumulation of ROS can create oxidative stress, which in turn can destroy chlorophyll structures, damage the chloroplasts, block chlorophyll biosynthesis, accelerate chlorophyll decomposition, and suppress photosynthesis.[26,68]

Root nodules are a special organ of legume plants where symbiotic biological nitrogen fixers called bacteroids fix atmospheric nitrogen. MPs can promote symbiotic biological nitrogen fixation by enhancing nodule formation.[23] The outcomes of the current study showed a significant augmentation of NoN_P and WoN_P along with the progress of the PVC MP dose compared to the control. Similarly, a significantly higher specific root nodule in common beans was reported due to ≥1.0% low-density polyethylene (LDPE) and all doses of bio-MPs compared to the control and 0.5% LDPE-MPs.[20] MP-induced great enrichment of symbiotic rhizoboales can promote the formation of root nodules.[16,21,23] Lb content correlates positively with nodulation, nodule tissue development, and root development.[18] However, an interesting outcome was visualized in our study in the case of the Lb content. The level of Lb showed a significant suppression between treatments relative to the control, which was the opposite trend from that of NoN_P and WoN_P indicated. A recent study also reported similar observations where higher doses of PS MPs except for 0.5% demonstrated a corresponding decline in the Lb content of cowpea root nodules.[18] Accumulation of millimolar Lb content in the cytoplasm of infected plant cells prior to nitrogen fixation buffers free oxygen in the nanomolar range to avoid the inactivation of oxygen-sensitive nitrogenase but maintain enough high oxygen flux for bacteroid respiration.[69] The relative abundance of Lb content in nodules can vary substantially depending on the rhizosphere partial oxygen pressure (pO_2) expressed on a plant or nodule weight basis. The Lb content labels in nodules can show higher values when cultured in lower rhizosphere pO_2 compared to higher ones.[70] MPs can promote air permeability or the flow of oxygen in soil, which in turn can increase rhizosphere pO_2 resulting in a lower label of Lb content in root nodules.[14]

Nutrients and calorific value evaluation play significant roles in understanding the nutritional and energy values of plant species using proximate composition assessment.[71] The proximate composition depicted a significant promotion of

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plant total nitrogen and crude protein content between treatments compared to the control. Soybean plant N uptake was augmented by PP and polyethylene MPs while growth and development were suppressed.[23] Similarly, higher values of total dissolved N, dissolved organic N, protein, and amino acid content in pea plants were found due to PS MP exposure between treatment groups while productivity of plants decreased.[34]

Remarkable oxidative stress marker ROS (peroxide radicals, hydroxyl radicals, and H_2O_2) are produced in plants along with a dynamic balance during normal metabolism.[7] However, exposure to abiotic stress (MPs) greatly perturbs the dynamic balance due to accumulated ROS.[72] The current study observed a significant dose-dependent promotion of the H_2O_2 content in peanut leaf due to PVC MPs relative to the control. Similar augmentation of H_2O_2 content in sweet potato was reported due to PVC MPs.[73] Highly accumulated ROS including H_2O_2 leads to oxidative damage to the cells[74] considered one of the main cytological effects mostly used to evaluate the ecotoxicity of various environmental contaminants.[75] Plants have adopted a highly concerted antioxidant machinery system including nonenzymatic and enzymatic antioxidants to prevent ROS-induced oxidative damage.[76] MP-induced ROS accumulation initiates the activity of antioxidant enzymes (i.e., proline, MDA, SOD, CAT, POX, and APX) in plants to alleviate oxidative damage through the scavenging of surplus ROS.[77] Proline, an important osmolyte during abiotic stress, is a potent nonenzymatic antioxidant against oxidative stress.[78] A substantial elevation of proline content throughout the treatment sets compared to the control was observed in peanut leaf. The findings are similar to a recent study where 12 μm NH2-PS-MPs promoted proline content in *Cicer arietinum*. This dose-dependent promoted proline activity can indicate the neutralization of excessive oxidative stress as a ROS scavenger rather than an osmoprotectant.[10] MDA as a major product of lipid peroxidation indicates the degree of membrane damage due to oxidative stress.[79] Relative to the control, the MDA content was promoted significantly in all sets of treatment. However, a reduction was observed in the 3.5% dose after a sequential increase from the 0.5 to 2.5% dose. Several recent studies also observed significant augmentation of MDA content in plants due to MP stress.[12,26,39,73] Membrane damage due to PVC MP-induced ROS can result in increased MDA production in peanut leaves.[10] The reduction in the 3.5% dose after a sequential increase can be an indication of stress over the peanut plant's threshold of tolerance, which in turn leads to the development of ROS and oxidative damage to cells.[80]

SOD, a ubiquitous enzymatic antioxidant metalloenzyme in aerobic organisms, acts as the first line of defense against ROS in enzymatic antioxidant processes.[77] Along with isoenzymes, it can dismutase excessive oxygen free radicals into H_2O_2, which can be converted in the form of H_2O and O_2 by CAT and POX.[79] The SOD activity of the current study depicted a gradual increase in all the sets of treatments, while a significant promotion for the 3.5% dose only compared with the control. Significant elevation of SOD activity due to MPs was also reported in several recent studies.[7,12,38,39] Due to the external stress of MPs, an augmented intracellular oxygen free radical expression can be the reason for high SOD activity in the higher doses of PVC MPs.[81] As a tetrameric, heme-containing, H_2O_2 oxidoreductase (dismutation of H_2O_2 into water and O_2), CAT plays a crucial role in plant metabolism, defense, and signal protection.[82] The findings showed a similar trend for CAT as in the MDA content. All of the treated sets depicted a significant rise rather than the control. After a sequential increase from the 0.5 to 2.5% doses, a reduction appeared for the 3.5% dose. The development of CAT activity due to MP-induced stress was also reported in recent studies.[7,17,26,38] This decrease or inhibition of CAT activity like MDA in the 3.5% dose can also be an indication of ROS development and oxidative damage of peanut leaves.[80] POX (ubiquitous in plants) represents one of the most crucial enzymes in the ascorbate−glutathione−reductase cycle and plays a significant role in the plant stress defense system.[73,83] A substantial dose-dependent elevation in POX activity was noticed relative to the control. The findings of the recent literature also indicated a similar augmentation trend in POX activity due to MP stress.[26,28] This significant sequential increase between the treated sets can be an intimation of direct scavenge of H_2O_2 by converting them into H_2O and O_2 in peanut leaves.[83] APX significant for the collection and detoxification of H_2O_2 acts as the final active enzyme in both Mahler and glutathione−ascorbate cycles.[84] This enzyme prevents oxidative stress by utilizing various functions including an enzyme cofactor, a donor, or an acceptor in transmitting plasma membrane electrons or chloroplasts.[85] Compared to the control, a dose-dependent elevation was found in APX activity, but the significant rise was in the 3.5% dose only. The substantial promotion of APX activity can be a sign of PVC MP-induced oxidative stress prevention by the redox cell modulation through the implementation of Mahler and glutathione−ascorbate cycles.[86] The higher antioxidant enzyme activities (proline, MDA, SOD, CAT, POX, and APX) can be an indication of improved PVC MP stress tolerance of peanut plants by scavenging ROS.[87]

Environmental factors and MP physicochemical qualities can affect the degradation of MPs in various ways when contacted with soil and water. Total mineralization of MPs and transformation into smaller molecules like oligomers and monomers can be done by microorganisms or free radicals.[88] In addition, the surface morphology of MPs and its breakdown to a smaller size can also be done by root exudates, MP concentration, and hydrolysis.[89,90] The appearance of small cracks on the surface and minor variation in FTIR absorption spectra of extracted PVC MPs indicated the very beginning of degradation (Figure 4). This beginning of PVC MP degradation can be initiated by the action of bioenzymes.[39]

So, stress indicator variables as an indicator of oxidative stress were found to be the most critical factors behind the varied response of peanut plant growth under different doses of PVC MPs (Figure 5). The higher doses of PVC MPs can induce significant promotion of stress indicator variables (proline, H_2O_2, MDA, SOD, CAT, POX, and APX), which in turn can significantly inhibit PH, PFW, SFW, LFW, SDW, PDW, LDW, SL, LL, BL, RtS, NoL_P, and chlorophyll a leading to reduced peanut plant growth and performance.[12,17] A significant negative correlation between chlorophyll a and MDA can be an indication for the reduction of chlorophyll-a expression due to lipid peroxidation-related membrane damage, which ultimately can reduce photosynthesis leading to inhibition of plant growth (Figure 5b).[38,39] However, dose-dependent significant elevation of NoN_P, WoN_P, total nitrogen, and crude protein and their substantial positive correlation with stress indicator variables except for CAT and MDA can be a sign of positive effects of PVC MPs on peanut

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plant's nitrogen availability by symbiotic nitrogen fixation and nutritional quality as an adaptive mechanism to get over nitrogen scarcity caused by PVC MPs or by other means (Figure 5b,c).[20,34] Better nodulation can be a possible reason behind the alleviated negative effects along with the significant promotion of RFW for 0.5 and 1.5% doses on root biomass compared to shoot biomass due to the positive correlation between nodulation and root development.[17]

The current study did not consider interactions between PVC MPs and soil physicochemical parameters and soil microorganisms. The uptake and translocation of PVC MPs and the molecular mechanisms through which growth parameters of peanut plants were influenced need to be addressed. In addition, the study only assessed the effects of PVC MPs on peanut plants up to the flowering stage.

CONCLUSIONS

The study shows that peanut plants (*Arachis hypogaea* L.) were severely inhibited in their growth and development by poly(vinyl chloride) (PVC) MPs, as indicated by decreased biomass, plant height, and general vitality. Furthermore, although PVC MPs had a detrimental effect on plant development, they seemed to encourage root nodulation, a crucial step in nitrogen fixation, which may have an effect on nitrogen intake. These findings demonstrate the intricate and dual impacts of MP pollution on plant physiology, whereby alterations in nutrient absorption systems coexist with growth suppression. According to the research, MPs have the potential to upset agricultural systems by changing nutrient dynamics and plant growth. To further understand the wider environmental and agricultural implications of MP pollution, future studies should examine the long-term effects of PVC MPs on crop production and soil health, as well as the underlying mechanisms driving these interactions. In addition, in-depth field studies should focus on the remediation of MP contaminants in the soil through microbial degradation and the application of biochars prepared from agricultural plant residues.

ASSOCIATED CONTENT

Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supporting Information files. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c00063.

Methodology for the determination of soil and water physicochemical parameters and its results (PDF)

AUTHOR INFORMATION

Corresponding Author

Siddhuraju Perumal − *Bioresource Technology Laboratory, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore, Tennessee 641046, India;* orcid.org/0000-0002-3663-4182; Email: psiddhuraju@buc.edu.in

Authors

Udayshankar Halder − *Bioresource Technology Laboratory, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore, Tennessee 641046, India*

Chaithra Radharamanan − *Bioresource Technology Laboratory, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore, Tennessee 641046, India*

Karthick Venkatesan − *Bioresource Technology Laboratory, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore, Tennessee 641046, India*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.5c00063

Author Contributions

Siddhuraju Perumal: conceptualization, methodology, supervision, review, and editing. Udaysankar Halder: investigation, methodology, formal analysis, data analysis, data curation, visualization, writing—original draft, review, and editing. Chaithra Radharamanan: investigation, methodology, data analysis, data curation, writing, review and editing, and formal analysis. Karthick Venkatesan: writing and editing.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

MPs	microplastics
PVC	polyvinyl chloride
ROS	reactive oxygen species
PP	polypropylene
RC	rubber crumb
HDPE	high-density polyethylene
PS	polystyrene
PLA	polylactic acid
PET	polyethylene terephthalate
TOC	total organic carbon
EC	electrical conductivity
DO	dissolved oxygen
PH	plant height
SL	shoot length
LL	leaf length
RL	root length
NoL_P	no. of leaves per plant
BL	branch length
PFW	plant fresh weight
PDW	plant dry weight
LFW	leaf fresh weigh
LDW	leaf dry weight
SFW	shoot fresh weight
SDW	shoot dry weight
RFW	root fresh weight

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RDW	root dry weight
NoN_P	no. of nodules per plant
WoN_P	weight of nodules per plant
RtS	root to shoot ratio
Lb	leghemoglobin
NFE	nitrogen free extract
DM	dry matter
MDA	malondialdehyde
H_2O_2	hydrogen peroxide
POX	peroxidase
APX	ascorbate peroxidase
CAT	catalase
SOD	superoxide dismutase
SEM	scanning electron microscopy
FTIR	Fourier transform infrared
PCA	principal component analysis
CM	correlation matrix
pO_2	partial oxygen pressure

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