

RESEARCH

Open Access



Role of multi-walled carbon nanotubes as a growth regulator for *Basella alba* (Malabar spinach) plant and its soil microbiota

Garima Singh, Sayantan Ghosh, B. Dinakar and Bismita Nayak*

Abstract

The effect of pure multi-walled carbon nanotubes (MWCNTs) was studied on the development and morphology of the *Basella alba* plant. The plants were treated with varying sonicated concentrations of MWCNTs. The parameters taken into consideration were germination percentage of seeds, protein content in the plant, estimation of chlorophyll concentration, and the effect on the soil microbial community after treatment with MWCNTs. A boost in vigour index was recorded with the $200 \mu\text{g ml}^{-1}$ concentration of MWCNTs. Increment in other parameters like protein content, chlorophyll concentration, and microbial community in soil samples have also been observed. All parameters showed higher efficiency in a concentration-dependent manner of MWCNTs compared to control by testing the significance of results through statistical one-way ANOVA analysis. The uptake of MWCNTs by plants was confirmed by SEM–EDX analysis of treated and control leaf tissue sections. This study concludes that MWCNTs exhibit significant growth effects with no toxicity to *Basella alba*.

Keywords: Nano-biotechnology, Multi-walled carbon nanotubes, *Basella alba*, Soil microbiota, Growth regulator

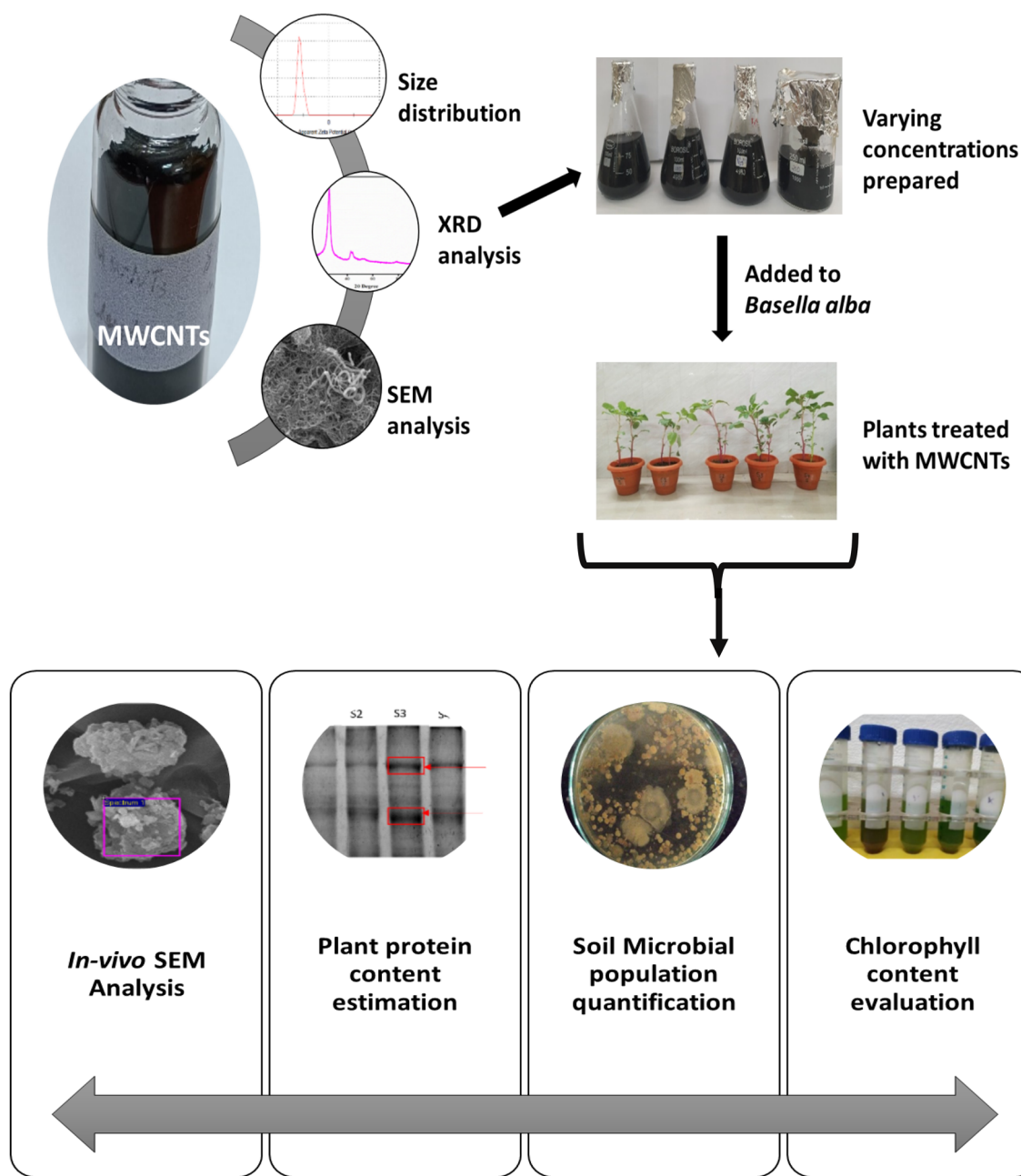
*Correspondence: nayakb@nitrrkl.ac.in

Department of Life Science, National Institute of Technology Rourkela,
Rourkela, Odisha 769008, India



© The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Graphical Abstract



Introduction

Nanomaterials find a vast application in agriculture biotechnology. As the name suggests, the size of a nanomaterial falls in a range of less than a few nanometres, which helps deliver various pesticides, fertilizers, and growth factors in a controlled and sustainable manner. [1]. Since the discovery of the carbon

nanotubes (CNTs) in 1991 by Iijima et al. [2], it has gained immense acceptance in terms of its application in different fields due to its size and outstanding chemical, physical, and mechanical properties (lightweight, stability, high conductivity, strength, and thermal properties). These extraordinary properties of CNTs have found various applications in the electronics industry,

biomedical research, agriculture, animal production, healthcare, and anti-microbial activity [3–5]. These carbon nanotubes are of two kinds—single-walled and multiple-walled. Single-walled ones have a single sheet of graphene rolled up to form a cylindrical shape, while MWCNTs have multiple graphene layers rolled up to form a concentric hexagonal shape when the transverse section is viewed. In the near future, demand for food is going to increase, and to meet the food productivity, the application of CNTs into agriculture is very much evident. It is a realistic alternative as we have a finite supply of natural resources like land, water, and soil fertility. The advancement of nanotechnology in agriculture helps increase food production through precision farming, which is principally a crop management process and goes by the name of satellite farming or specific crop management (SSCM) [6]. The available literature has made it evident that CNTs can fetch into the plant cell through the cell wall smoothly as their size ranges from 5 to 100 nm [7] [8]. Nanoparticles less than 5 nm have a better chance of penetrating through the cell wall efficiently [9]. This application paved a new path for employing them in treatment along with the pesticides or fertilizers at a particular time and location as directed in precision farming. Nanoparticle application in agricultural research has initiated and disclosed some immense increment in plants' growth. These Nanotubes' application in agriculture has shown a positive mark as crop growth regulators [10–14]. Nanoparticles treatment of crops has a history of being a decent replacement for fertilizers to upturn plants' productivity [15]. The oxidized-multiwalled carbon nanotubes (O-MWCNTs) have enhanced the root length and height of the wheat plants, while the untreated MWCNTs promoted early germination and growth of the wheat plant [16]. On the other hand, the original, i.e. untreated MWCNTs have increased the growth and productivity of the tomato plants [17]. It is documented that CNTs increase the root growth in onion and cucumber [18]. Lin et al. have reported that carbon nanomaterial is easily uptaken and translocated in rice plants [19]. Another case of increased germination rate has been stated in the spinach (*Spinacia oleracea*) seeds when they were treated with a blend of nano-TiO₂ and water [20]. A synthetic apatite prepared along with the phosphorous nanoparticles has worked as an upgraded fertilizer for soya bean (*Glycine max*) [10]. Iron oxide nanoparticles also find an application as a fertilizer for the peanut plant (*Arachis hypogaea*) [21]. MWCNTs have a variable impact on different plant types and their diverse parameters, and the most insensitive plant species to this nanoparticle is Timothy grass [22]. On the other hand, an increase in concentrations of MWCNTs

has completely halted the growth of *Phaseolus vulgaris* (commonly known as bush bean) [23]. Some functionalized MWCNTs have even been documented as being cytotoxic for selective microbial populations as they can either lyse the bacterial cell wall or inhibit protein synthesis by obstructing Nucleic acid synthesis, thereby keeping the pathogenic microbes at bay [24, 25]. The anti-bacterial efficacy of MWCNTs has a limitation of being concentration and time-dependent when adsorbed with AOT-surfactant [26]. Apart from being anti-bacterial, anti-fungal properties were also found in surface-modified MWCNTs as they have been relatively active against the pathogenic fungus (*Fusarium graminearum*) [27]. The possible benefit of plant nano-biotechnology is to develop pest-resistant plants, reduce chemical fertilizers use, increase production yield, and reduce labour cost.

The lacunae with the reference studies mentioned above are that they have tested the efficacy of functionalized nanotube and on plant's physiological parameter. No study has offered an extensive study on the in vivo state of non-functionalized MWCNTs, its effect on soil microbiota, and the impact of varying concentrations of MWCNTs for different parameters. In addition, functionalized MWCNTs have been reported as being more genotoxic than a pure form of MWCNTs [28]; hence in our study, we have tried to fill the above-mentioned research gaps by using the native form of MWCNTs and looking into the overall plant yield.

MWCNTs do not fit the template "One size fits all", each vegetation species has a widely divergent response to the presence of MWCNTs, which needs to be looked into dedicatedly. While being the chief source of antioxidants, minerals, and vitamins, vegetables have always been the primary food item consumed in the daily human diet. The parameters kept in mind while choosing a vegetable plant for our study were easy seasonal growth, less space requirement, and a short harvesting period. A locally available seasonal plant, *Basella alba*, had been chosen, as it cleared our requirement criteria for the study. *Basella alba* of the *Amaranthaceae* family is a seasonal winter plant, hence capable of thriving in the month of January–February in the south-eastern parts of India [29]. This dioecious plant proliferates from mild to harsh winter climates.

This plant, which contains a high amount of protein and iron, has not yet been the subject of any research. Therefore, we studied how MWCNTs affect the growth of *Basella alba* plants and its microbial population in the rhizosphere. So, before treating the plants with the MWCNTs, we have characterized the MWCNTs sample with different analytical techniques like X-ray diffraction to determine the sample's crystalline structure [30,

31]. From our research, we are delivering evidence of increased plant growth, protein content, and chlorophyll concentration in the *Basella alba* plant after treatment with MWCNTs at gradually increasing concentrations, i.e. $50 \mu\text{g ml}^{-1}$, $100 \mu\text{g ml}^{-1}$, $150 \mu\text{g ml}^{-1}$, and $200 \mu\text{g ml}^{-1}$ supplemented along with the water. Soil microbiota being the primary driving force in assisting the plants to absorb essential nutrients and symbiotically, they get plant waste products as their food source [32]. Keeping this in mind, we have also checked how MWCNTs' presence affected the soil microbial population after a long time of exposure to this MWCNTs treatment.

Materials and methods

MWCNTs sample preparation

MWCNTs were synthesized by following the protocol described by Couteau et al. As a carbon source, acetylene was used, and the catalyst support used for this process was Fe, Co/CaCO₃. MWCNTs were synthesized using the traditional catalytic chemical vapour deposition (CCVD) method using a rotary oven for acetylene decomposition at 700–730 °C with constant N₂ flow. For purification, the final product was stirred with 10% HCl for 45 min, followed by gradual washing in dilute HCl and subsequently with double distilled water until it resulted in a neutral pH [33]. The acid aids in removing both the catalyst support as well as the metallic particles. This MWCNTs sample was characterized to ensure that the synthesized MWCNTs sample is of pure, pristine form. For characterization, MWCNTs sample was subjected to dynamic light scattering and zeta potential to define the diameter of the sample and its stability in the solvent used, respectively. Scanning electron microscopy (SEM) and field emission scanning electron microscopy (FE-SEM) were performed to check micromorphology of the MWCNTs [34] and UV–Vis spectroscopy to check the absorption spectrum of MWCNTs sample [35]. After the successful characterization, treatment was given to the plant sample.

X-ray diffraction

For X-ray diffraction (Ultima IV model Rigaku X-ray diffractometer) of MWCNTs, the sample was taken up in an appropriate quantity into the slide cavity and flattened evenly to study the crystalline structure of MWCNTs sample. The crystalline analysis was performed using angle 2θ value between 20° and 80° along with the step size of 0.05 s and a scan rate of 5° per minute.

Field emission scanning electron microscopy (FE-SEM)

For FE-SEM analysis, the MWCNTs sample was prepared in double-distilled water as a solvent, and this

solution was sonicated with the help of Labsonic® Sartorius stedim probe sonicator for 5 min. The prepared FE-SEM sample was then dropped on a small square-shaped glass piece (size 1 mm*1 mm) and dried at room temperature overnight in a sterile environment. This sample glass plate was later coated with carbon for better conductivity, and the sample was then visualized under FE-SEM NOVA NANOSEM450 at different magnifications and locations for fine images of MWCNTs.

UV–Vis spectroscopy

The MWCNTs were dispersed in double-distilled water and sonicated using Labsonic® Sartorius stedim probe sonicator (Type: BBI-8535027) for 30 min for UV–Vis spectrophotometer (Cary 100, Agilent technology, Singapore) observation. The MWCNTs absorption was measured to lie between 200 and 500 nm wavelength [36].

Dynamic light scattering (DLS) and zeta potential

To determine the size and surface charge of the MWCNTs, the sample was sonicated in a double-distilled water solvent using Labsonic® Sartorius stedim probe sonicator for 10 min. As per the instruction manual of the zeta-sizer, a concentration of 0.1 mg/ml was taken to perform this protocol [37]. The same sonicated MWCNTs sample was taken for the zeta and DLS (Malvern Zeta sizer Nano zS90) examination and repeated eight times to confirm the size and surface charge. This analyzer can accurately calculate the zeta potential after obtaining electrophoretic mobility of the nanoparticle in the solvent using Smoluchowski's equation [38].

Procurement of plant seeds

To identify the in vivo effect of MWCNTs on the growth of plants, *Basella alba* was chosen for this study as the favourable climatic condition requirement of this plant could be easily fulfilled at Rourkela (Odisha, India). These plant seeds were purchased from a local vendor in the Rourkela market. Plant seeds were soaked for 48 h with double-distilled water before sowing in the soil. The seeds were pre-soaked to receive all the seeds in sprouted condition. Only the sprouted seeds were selected to sow in the soil-filled pot so that when we treated these with MWCNTs, all of the sample plants were at the same growth stage.

Preparation of dispersed MWCNTs solution

The treatment solution was prepared by taking MWCNTs at different concentrations ($50 \mu\text{g ml}^{-1}$, $100 \mu\text{g ml}^{-1}$, $150 \mu\text{g ml}^{-1}$, and $200 \mu\text{g ml}^{-1}$) in double-distilled water. Each MWCNTs treatment solution was sonicated for

10 min using a Sartorius Stedim probe sonicator (Type: BBI-853502). The MWCNTs treatment sample with $50 \mu\text{g ml}^{-1}$, $100 \mu\text{g ml}^{-1}$, $150 \mu\text{g ml}^{-1}$, and $200 \mu\text{g ml}^{-1}$ of different concentrations was given to S1, S2, S3, and S4 labelled pots, respectively, through the usual watering process. Each plant was treated with 5 ml of MWCNTs solution per week, and the treatment was continued for four consecutive weeks before harvesting these plants [36].

Phenotypical analysis

Basella alba seeds were soaked in double-distilled water for 2 days with a regular change of double-distilled water every 12 h. After the sprouting of seeds vigour index and germination percentage were calculated for all the samples, including control. These sprouted seeds were later transferred into soil-filled pots and labelled as control (treated with plain water), S1 (treated with $50 \mu\text{g ml}^{-1}$ concentration of MWCNTs), S2 (treated with $100 \mu\text{g ml}^{-1}$ concentration of MWCNTs), S3 (treated with $150 \mu\text{g ml}^{-1}$ concentration of MWCNTs) and S4 (treated with $200 \mu\text{g ml}^{-1}$ concentration of MWCNTs). These plant samples were exposed to 8 h of sunlight daily and rest in the dark. After 6 days of sowing the sprouted seeds, the plantlets emerged above the soil surface. The treatment of MWCNTs was given with a time gap of 1 week. The control and other samples of plants were duplicated to rule out any false positives for better reproducible results. Before harvesting the plants from pots, the number of leaves was counted from each sample pot for the phenotypical assessment. After these plants were harvested, each sample's height and the number of leaves were counted to the phenotypical growth and vigour index [39, 40]:

$$\text{Vigour index} = (\text{root length} + \text{shoot length}) \\ * \text{seed germination percentage.}$$

Protein content estimation

To estimate the changes in the protein concentration of individual plant samples, the SDS-PAGE experiment was performed six times. Five grammes of fresh plant leaves were collected from each sample pot using the digital weighing balance. These fresh leaves were wrapped with transparent plastic and stored in an ice tray for the next 15 min. Leaves were ground with 1X PBS in mortar and pestle. This sample paste was collected in 2 ml tubes and centrifuged at 10,000 rpm (rotation per minute), 4°C for 10 min. This process was repeated until a clear

supernatant was obtained and later subjected to SDS-PAGE analysis [41].

SDS-PAGE

All five samples of plant leaves were subjected to protein content analysis. For SDS-PAGE, the usual standard laboratory protocol was followed, 5% stacking and 10% resolving gel were prepared, and Gel electrophoresis was carried out using GeNei® vertical mini dual gel system-05-02 SDS-PAGE system.

Chlorophyll extraction and estimation

From each sample (C, S1, S2, S3, and S4), 500 μg of leaf sample was collected and ground using 2 ml acetone in mortar–pestle on an ice tray. After grinding, the sample solution was later transferred to 25 ml acetone. Extracted chlorophyll sample was taken for UV–Vis spectroscopy analysis [35]. The chlorophyll extraction experiment and its analysis were performed in 6 duplicates.

Leaf tissue fixation for SEM–EDX study

A small section of leaf part has been removed from each plant sample (C, S1, S2, S3, and S4) using a sterile dissection blade. These plant sections were dehydrated and formalin-fixed using the standard plant tissue fixation protocol [42, 43]. After fixation, the leaves sections were dehydrated in serially graded ethanol (30%–100%) and left to dry at room temperature overnight. These sections were then sputter-coated with platinum to be viewed under SEM.

Impact of MWCNTs on soil microbiota

Before harvesting plants from pots, 5 g of soil sample was collected from the vicinity of each sample (C, S1, S2, S3, S4) plant's roots. The soil samples were serially diluted to 10^{-9} before spreading on nutrient agar media. After plating, these culture plates were incubated at 37°C for 48 h, and the observation was recorded at 24 h. This experiment has facilitated us to determine the effect of MWCNTs on the microbial population present in the rhizosphere of each sample plant. Soil microbiota was collected before the supplementation of MWCNTs, and directly treated with nanotubes to study its effect on microbial growth of soil samples. This experiment was performed in duplicates and repeated four times.

Statistical analysis

The data are expressed as mean $\pm$ SEM and examined through the one-way analysis of variance (ANOVA) test and Levene's test for equality of variance, using statistical software SPSS (IBM®, Version 24). The $p \leq 0.05$ is considered significant for all the cases.

Results

Structural characterization of MWCNTs

Dynamic light scattering and zeta potential of MWCNTs

DLS scanning size distribution Fig. 1a of MWCNTs was observed to be of diameter 37.84 nm which can be taken up efficiently by the plant cells [7], and the surface charge Fig. 1b was observed to be -55.5 mV which specified that nanotubes were highly stable in double-distilled water after sonication with noteworthy diameter for uptake by plants.

X-ray diffraction (X-RD) analysis of MWCNTs

Usually, the XRD examination of MWCNTs reflects a few extra peaks. “This peak family is generated because of atomic planes declining where 3D structure with regular stacking nanotube layers has to be established” [44]. Figure 2 depicts the crystallinity of MWCNTs. The most substantial diffraction peak was witnessed at the angle (2θ) of 25.903° , which can be attributed to the C (002) reflection of the hexagonal graphite structure of MWCNTs. The other characteristic diffraction peaks of graphite were at 2θ of about 43.23, 53.13, 77.92 in accordance with C (002), C (004), and C (110), and all the peaks were in reference to JCPDS file number 74–1602. This X-RD graph was plotted with Xpert high score.

SEM and FE-SEM characterization analysis of MWCNTs

To understand the sample surface morphology, SEM and FE-SEM analyses were performed. MWCNTs

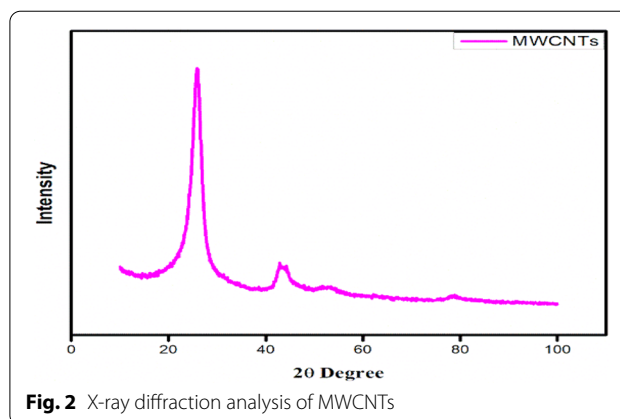


Fig. 2 X-ray diffraction analysis of MWCNTs

sample was dispersed in double-distilled water to visualize under the FE-SEM and SEM to observe carbon nanotubes' morphological structures and size distribution. Since most of the biological cell structures lie in the range of nanometres, the size and structure of CNTs must comply with them. Figure 3a is a SEM image analysis after magnification up to 15000X. The SEM image presented the micromorphology of MWCNTs as long tubes and aggregated clusters. The same water-dispersed MWCNTs sample was visualized under FE-SEM, presenting better image resolution and focus than the SEM. The photograph of FE-SEM Fig. 3b shows the micromorphology of individual MWCNTs with high resolution and focus at 100000X magnification.

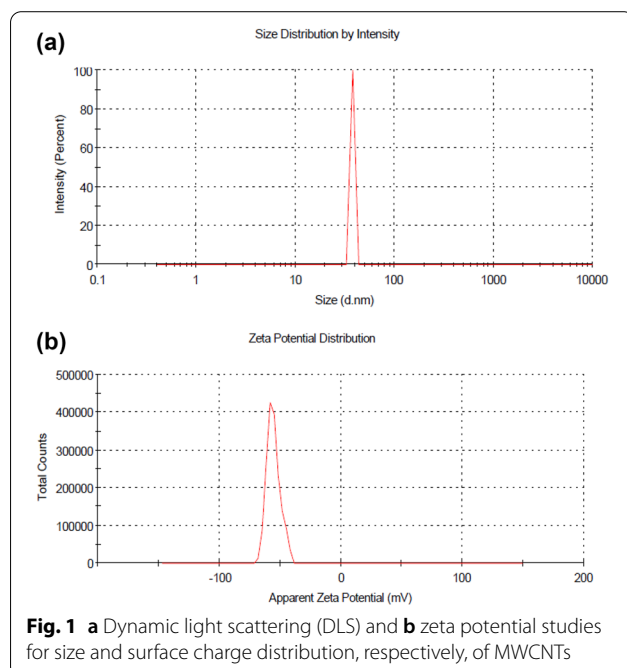


Fig. 1 a Dynamic light scattering (DLS) and b zeta potential studies for size and surface charge distribution, respectively, of MWCNTs

UV-visible spectroscopy

The absorption range of pure MWCNTs falls between 250–260 nm [32]. From the UV-Vis spectroscopy characterization, it has been shown that the characteristic existence of absorption peak (Fig. 4) at 258 nm which aligns with the characteristic absorption peak range of pure MWCNTs.

Phenotypic analysis of *Basella alba* after MWCNT treatment

The plant pots (Fig. 5b C (Control), S1, S2, S3, and S4) were treated with different concentrations $0 \mu\text{g ml}^{-1}$ (control), $50 \mu\text{g ml}^{-1}$, $100 \mu\text{g ml}^{-1}$, $150 \mu\text{g ml}^{-1}$, and $200 \mu\text{g ml}^{-1}$ of MWCNTs treatment sample, respectively.

Germination percentage

It was observed that out of 30 *Basella alba* plant seeds, all seeds that were soaked in double-distilled water

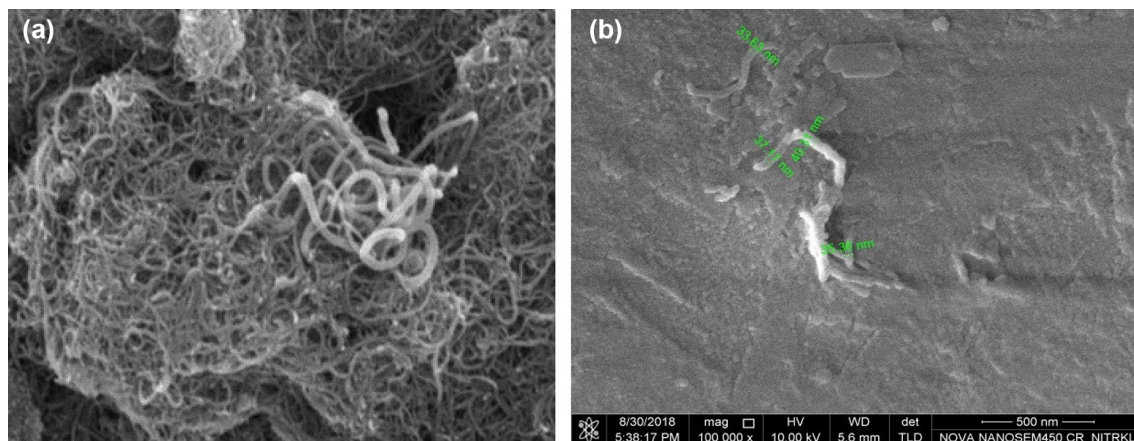


Fig. 3 MWCNTs viewed under **a** SEM with magnification of 15000X, **b** FE-SEM with magnification of 100000X

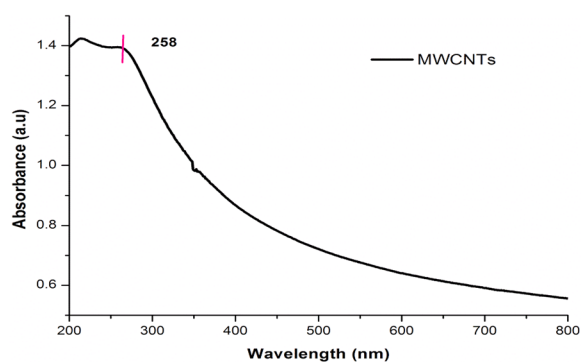


Fig. 4 UV-Vis spectroscopy observation of MWCNTs

were germinated successfully, and the germination percentage was calculated to be 100%.

Vigour index calculation

Comparative analysis of root and stem was performed after harvesting from pots. Figure 5a graph depicts the highest mean length of the root system, which was found to be 8.9 cm in the S3 sample treated with $150 \mu\text{g ml}^{-1}$, and the highest mean of the shoot length was found to be 48 cm in the S4 sample which was treated with $200 \mu\text{g ml}^{-1}$ concentration of MWCNTs. Statistically significant difference was found between the control group and S4 sample group; however, no such

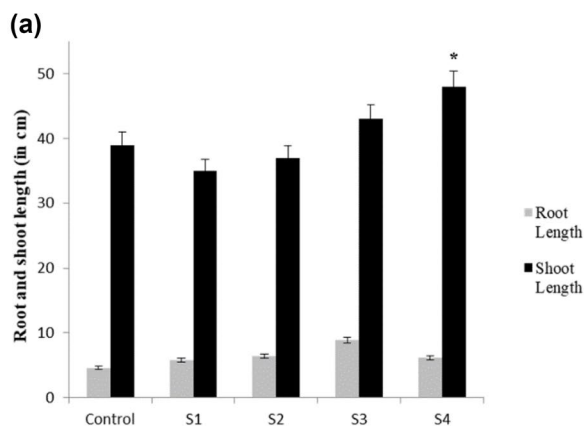


Fig. 5 **a** Comparative growth analysis of *Basella alba* plant after 47th day, data represented as mean $\pm$ SEM, $*p \leq 0.05$ as compared to the control. **b** Plants' height on 47th day before harvesting from the pots

significant difference was found between S1, S2 and S3 with a $p \geq 0.05$. When the S1 sample was treated with a concentration of 50 g ml^{-1} MWCNTs, the root and shoot lengths were seen to be shorter. *Basella alba* is an annual crop with a short harvesting period and without a long taproot system. In comparison to the control plant sample, plant shoot length increased and root length decreased as the MWCNTs treatment concentration increased.

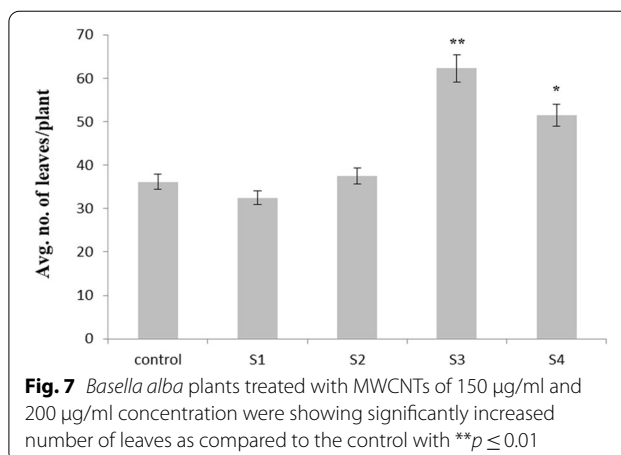
Vigour index

The highest vigour index was observed to be at 4390.2 in the S4 sample (treated with $200 \text{ } \mu\text{g ml}^{-1}$ concentration of MWCNTs) (Fig. 6), which was statistically significant, giving $p = 0.001$ while being compared to the control, S1 and S2 sample, while no significant difference was found between S3 and S4 as declared by $p \geq 0.05$. Treated plant samples showed substantial vigour index. From this study, it can be inferred that the plant samples supplemented with a higher concentration ranging from $150 \text{ } \mu\text{g ml}^{-1}$ to $200 \text{ } \mu\text{g ml}^{-1}$ of MWCNTs can grow in sub-optimum conditions very well, in comparison to the control.

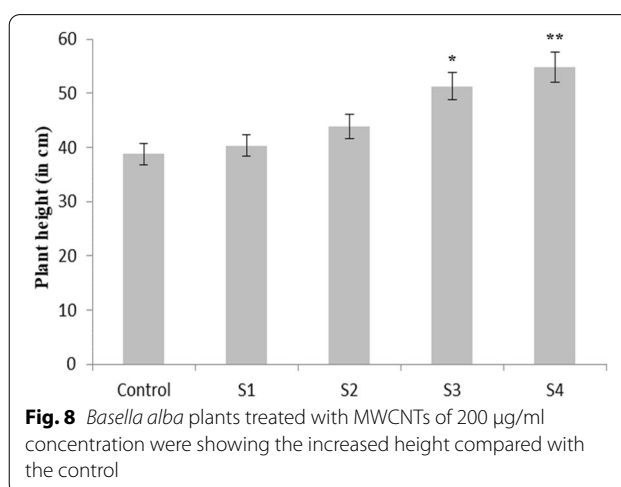
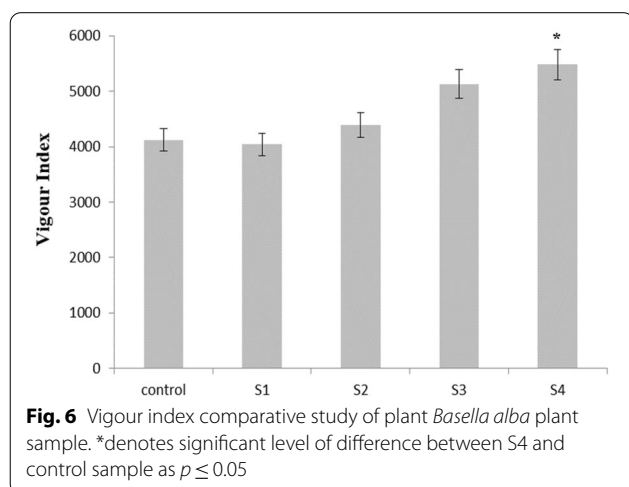
Phenotypical analysis—leaf count

The phenotypical analysis offers statistical data regarding morphological changes that occurred in the sample plants. This study was done to determine the impact of MWCNTs on the growth of *Basella alba* plant leaf samples after the treatment. Before harvesting, the number of leaves was counted in all the sample plants, and their average was considered for the study.

From the above data (Fig. 7), it was evident that the plants treated with a higher concentration of MWCNTs



have shown good and healthy growth compared to the control sample. The plants that were treated with the $50 \text{ } \mu\text{g ml}^{-1}$ showed low leaf count, and the plants treated with $100 \text{ } \mu\text{g ml}^{-1}$ showed a similar number of leaves as the control *Basella alba* plant sample. As compared to the control, these two samples (S1 and S2) have not shown statistically significant differences, hence raising the $p \geq 0.05$. While the plants treated with a higher concentration of MWCNTs of $150 \text{ } \mu\text{g ml}^{-1}$ and $200 \text{ } \mu\text{g ml}^{-1}$ were shown to have the highest mean number of leaves in comparison with the control *Basella alba* plant sample, $**p = 0.00078$. From the phenotypical analysis, a promising increment in the growth of the average number of plant leaves has been seen in the treated samples with high MWCNTs concentration. Both $150 \text{ } \mu\text{g ml}^{-1}$ and $200 \text{ } \mu\text{g ml}^{-1}$ concentrations of MWCNTs were found to be effective for obtaining better output in leaf production.



Phenotypical analysis—average height of the plants

Available statistical data on the height of the plant sample treated with a low concentration, i.e. $50 \mu\text{g ml}^{-1}$ and $100 \mu\text{g ml}^{-1}$ of MWCNTs sample, have shown a lower mean height (Fig. 8). While the plant samples treated with $150 \mu\text{g ml}^{-1}$ and $200 \mu\text{g ml}^{-1}$ have shown increased plant height as compared to the control plant sample. Hence, maximum plant growth has been observed in the samples which were treated with a higher concentration of $150 \mu\text{g ml}^{-1}$ and $200 \mu\text{g ml}^{-1}$ MWCNTs.

Upon comparison between S4, S3, and the control, the p-value obtained was 0.013724, while the comparison between the control group and S1 and S2 sample groups did not show any statistically significant increment in plant height. From the above study, it was affirmed that *Basella alba* plant height observation is significant and true to conclude that MWCNTs is showing its impact on the height of S3 and S4 treated *Basella alba* plant sample.

SDS-PAGE analysis

Positive results have been achieved regarding protein content in MWCNTs-treated *Basella alba* plant leaves. In the SDS gel, each well was filled with different plant protein samples (C, S1, S2, S3, and S4) of *Basella alba* plants. The loaded protein samples confirmed that dark and thick bands at molecular weights 42 kDa and 18 kDa of plant protein samples were obtained in plants treated with a high concentration of MWCNTs. From the SDS-PAGE gel image (Fig. 9) it is evident that the sample loaded in the S3 well showed bright and thick bands

compared to the control plant protein sample. Here in the S1 sample well, all protein bands are very light and thin, indicating less protein content. This concludes that plants treated with a lower concentration of MWCNTs had lesser protein content than the treated plant sample. $150 \mu\text{g ml}^{-1}$ concentration of MWCNTs has been proven to increase the protein concentration in *Basella alba* plants.

Chlorophyll content estimation analysis by UV-visible spectroscopy

The chlorophyll extraction from *Basella alba* plant samples of C, S1, S2, S3, and S4 has been done using the acetone method. Leaf extractions samples were taken for UV-Vis spectroscopy analysis to identify chlorophyll concentration present in each plant sample. The absorption spectroscopic scan was performed between 600 and 800 nm, expecting sharp peaks in the chlorophyll absorption wavelength range. The absorption spectroscopy results disclosed that all sharp peaks were observed between 650 and 675 nm wavelength, which is the absorption spectrum of chlorophyll pigment, and it has been demonstrated in the above graph (Fig. 10) obtained from UV-visible spectroscopy. Comparing the *Basella alba* plants treated with 200 g ml^{-1} MWCNTs to the control, it was found that they exhibited the maximum absorption peaks. Hence it can be established that treatment with a $200 \mu\text{g ml}^{-1}$ concentration of MWCNTs resulted in an elevated chlorophyll pigment concentration. The biochemical pathway and the underlying

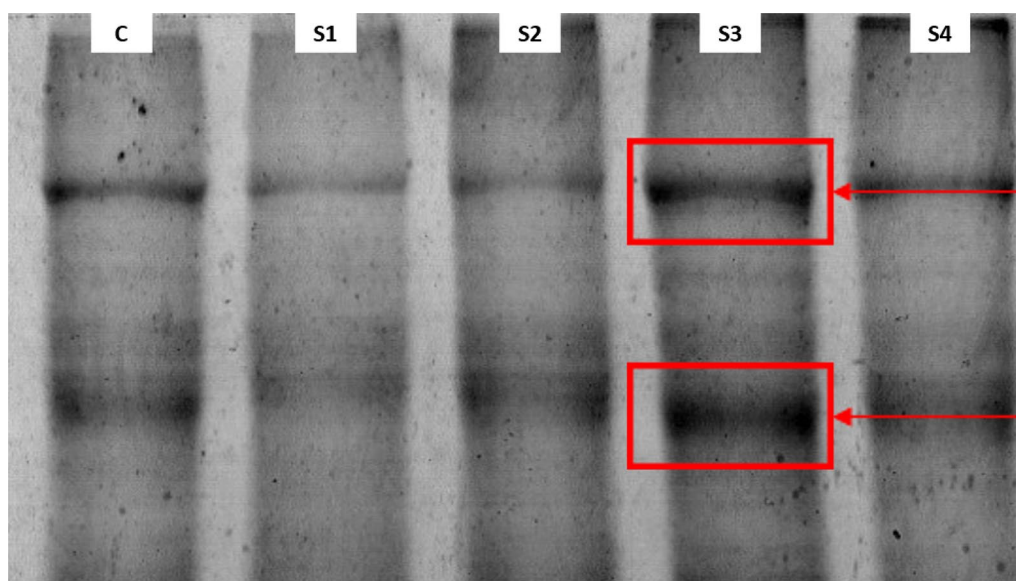
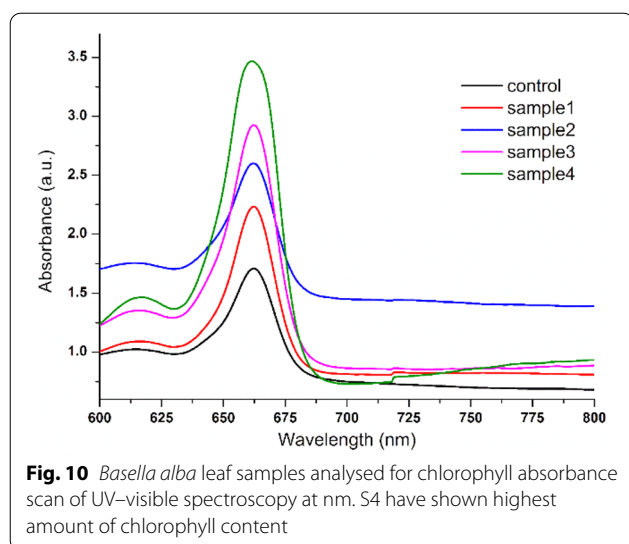


Fig. 9 SDS-PAGE gel of *Basella alba* plant leaves proving S3 has more amount of protein of same molecular weight 42 kDa and 18 kDa as compared to other plants treated with varying MWCNTs concentration



mechanism involved in increased chlorophyll content are yet to be ventured.

SEM-EDX (scanning electron microscopy–energy dispersive X-ray diffraction)

Basella alba leaf was investigated for the presence of MWCNTs, and certain other elements in the control leaf sample and MWCNTs-treated leaf sample. EDX has been used to examine the elemental composition present in the samples. To ensure that MWCNTs were uptaken by the plants, EDX spectra are considered. In SEM analysis, the sample's surface morphology has been considered for further analysis. *Basella alba* plant leaf samples were investigated with EDX analysis to obtain the carbon weight percentage in control, and MWCNTs-treated plant leaf samples. The elemental scan using EDX declared clearly that the control carbon weight percentage is much less than treated *Basella alba* plant leaves (Fig. 11). The observed carbon weight percentage in control *Basella alba* plant leaves is 49.85, and the treated carbon weight percentage in *Basella alba* plant leaves is 65.45. The SEM imaging disclosed that MWCNTs are absorbed and accumulated in the form of clumps in *Basella alba* leaves. The SEM-EDX spectra from control and treated *Basella alba* plant leaves have shown the expected results for the absorption of MWCNTs.

Study of soil microbiota after MWCNT supplementation Impact of MWCNTs on soil microbiota

For plants to absorb nutrients, they must first be processed into their elemental form, predominantly by soil bacteria. This symbiotic interaction between microorganisms and plants must be strengthened for enhanced growth parameter results. To determine the effect of

MWCNTs on the microorganisms population in the rhizosphere, we undertook a study through the plating method.

The microbial growth was at a slow pace after incubation of 24 h. However, after 48 h of incubation, a considerable boost in microbial growth was noticed as the treatment concentration was increased. The highest number of microbial colonies were observed in *Basella alba* plant-soil S4, which was treated with $200 \mu\text{g ml}^{-1}$ of MWCNTs, while the lowest number of colonies were observed in the control soil sample (Fig. 12). This response of the soil microbial culture plating revealed that MWCNTs are boosting the microbial community development in the soil microbiota surrounding the rhizosphere. The additional carbon source provided by MWCNTs for these microbial strains is responsible for the enhanced soil microbiota population.

Discussion

MWCNTs have many economically and physiologically beneficial impacts on different plants; however, they have not been extensively explored. In this study, the artificially synthesized MWCNTs have been characterized to possess a small diameter size ranging from 5 to 100 nm, which makes it easier for uptake by the plant [45]. While other physical characterizations like zeta potential, XRD, SEM analysis, and UV-visible spectrometric studies have suggested that it is suitable for in vivo studies in agricultural crops. The carbon nanotubes' dispersion and agglomeration can create nanomaterial toxicity in the physiological system [46]; therefore, it becomes essential to determine the surface charge to estimate the dispersion efficacy in a particular solvent and the size of the MWCNTs in that physiological solution. The characterization of the synthesized MWCNTs revealed that the pure form of MWCNTs had a 37.84 nm diameter and -55.5 mV surface charge and retained a hexagonal crystalline structure. This hexagonal structure is a transverse section that forms when graphene sheets fold up to form long, tube-like structures [47]. According to the image analysis performed using both the SEM and FE-SEM techniques, the MWCNTs sample possessed the morphology of a long tube-like structure with a diameter within a few nm ranges, which is one of the distinctive structural configurations of MWCNTs [48]. The structural analysis of MWCNTs was to certify the synthesized sample's purity by measuring its diameter, stability, crystallinity, and micromorphology. The experiment's findings indicated that the MWCNTs were in their pure state and easily absorbable by the plant root system when supplemented with water.

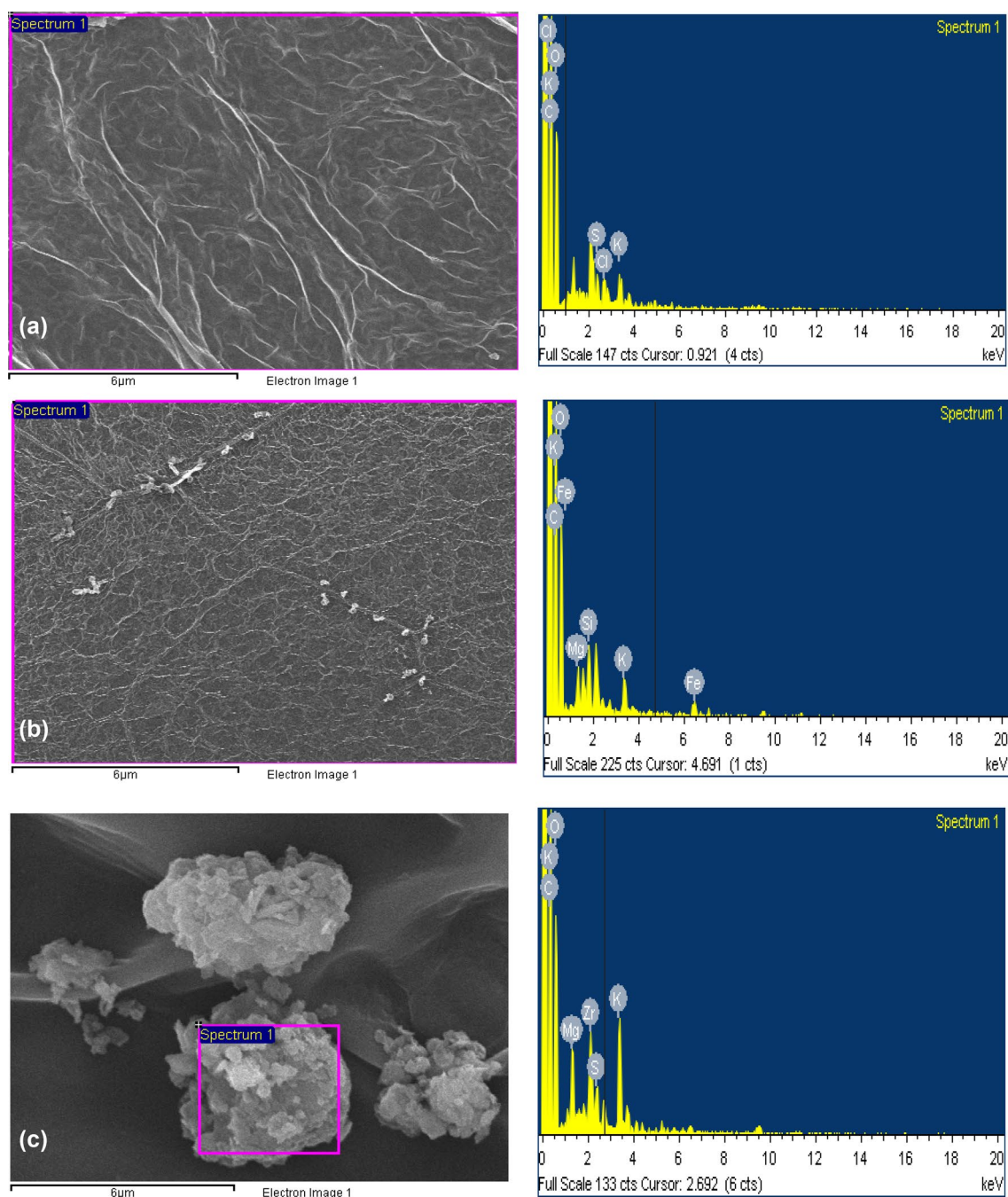
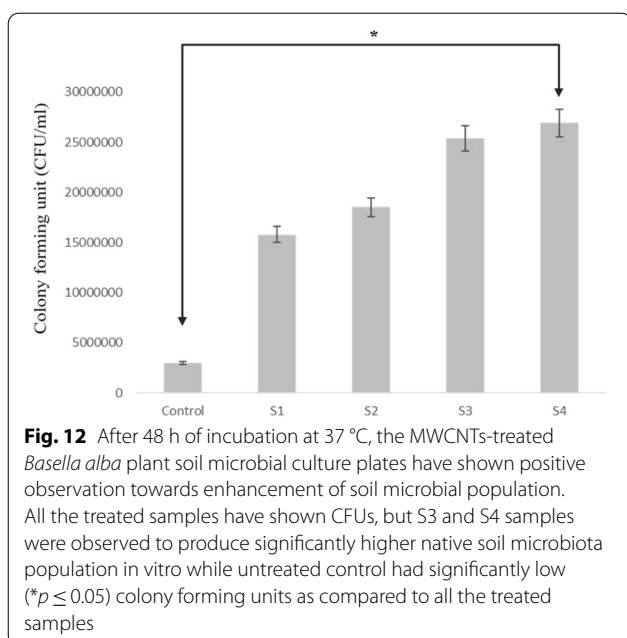


Fig. 11 SEM and EDX analysis confirms the presence of micro as well as macro nutrients in the samples and carbon has been found uniformly in **a** control **b** S4 sample and **c** magnified section of a MWCNT clump from image (b). As visible from SEM images of leaf sections, the control leaf has a smooth morphology without any clump-like structure, while MWCNT treated leaf is showing the clumps and its EDX spectra are also demonstrate increased weight percentage of carbon as the leaves have successfully uptaken the MWCNTs

Since MWCNTs has previously shown its growth regulation effect on various flora like broccoli [49], rice [50], and periwinkle [51] to name a few; hence, for further in vivo analysis, the *Basella alba* plant was chosen

to evaluate the effect of MWCNTs on plant growth and overall yield. Our research has convincingly demonstrated that MWCNTs have a substantial influence on total plant growth and yield, as observed in the instance



of the *Basella alba* plant. $150 \mu\text{g ml}^{-1}$ and $200 \mu\text{g ml}^{-1}$ concentration of MWCNTs have improved overall plant performance compared to the control. Here, we have examined the effect of MWCNTs on different growth parameters, the protein content of plants, and their soil microbiota. It is crucial to ascertain whether the treatment with MWCNTs had any effect vigour index as it estimates overall plant yield at the ecophysiological level [52]. The phenotypical study after treatment with MWCNTs suggested that the highest vigour index enables these plants to grow in sub-optimum conditions with a significant germination rate. The plant height and leaves' count can offer insight on MWCNTs' impact only after the plant is fully grown and ready to harvest. However, the vigour index offers information on the germination and viability of seeds in the field under sub-optimum conditions long before the plant begins to grow. The higher concentration of MWCNTs, i.e. $150 \mu\text{g ml}^{-1}$ and $200 \mu\text{g ml}^{-1}$ treatment, has produced a significantly higher vigour index in plant samples than the control and the other lower concentration (S1 and S2) counterparts. SEM–EDX investigation of *Basella alba* leaf has provided evidence to confirm the absorption and translocation of MWCNTs from soil to *Basella alba* plant leaf.

As the edible part of this plant is its leaves, we have focussed on its growth after MWCNTs treatment. The plant samples' average number of leaves and the average height showed that the samples treated with MWCNTs had better outcomes. The images from SEM–EDX (Fig. 12) exhibited the elemental percentage and absorbed MWCNTs clumps in the treated samples. The

treated leaf sample conveyed the highest weight percentage of carbon source than the control plant leaf section using EDX. Further zoomed-in images from SEM have revealed the morphology of absorbed MWCNTs in the form of aggregated clumps. The effect of MWCNTs being absorbed by plants and transported to leaves has eventually been seen in the analysis of protein and chlorophyll concentration. Looking at the increment in chlorophyll and protein content, it can be inferred that MWCNTs played a role at the morphological and molecular levels. $200 \mu\text{g ml}^{-1}$ concentration of MWCNTs was effective for incrementing the protein concentration and chlorophyll pigment content. UV–Vis spectroscopy data indicate that the photosynthesis rate could be higher in MWCNTs-treated plants, which led to an increase in protein content. As we all know, microbes present in the rhizosphere are responsible for cycling nutrients in the soil, creating crucial mutualistic relationships, providing energy in the absence of sunlight, and many more vital roles in the ecological web [53]. Micro-organisms around the plants' roots utilize carbon as their food source while decomposing organic matter and releasing an excess of nutrients in the soil for plants to uptake. Therefore an increased microbial population in the rhizosphere, in the MWCNTs-treated plants is a positive indication of overall growth and long-term survival. All the above examinations established that the plant parameters taken under this study had responded consistently to the increased concentrations of MWCNTs treatment. To summarize our findings, we can safely say that MWCNTs have a promising application as a growth regulator for *Basella alba*, at both physiological and molecular levels.

Conclusion

In conclusion, we have demonstrated that adding MWCNTs impacted the phenotype of the *Basella alba* plant. From our study, it can be established that a higher concentration of MWCNTs positively impacts plants' morphological and molecular level growth along with the enhancement of the soil microbes present in the rhizosphere. Hence, we can conclude that even without functionalization, MWCNT can act as an excellent carbon source that directly correlates to plant growth. This finding offers fresh insights into the technological potential for using MWCNTs as growth regulators in the agriculture sector. Accordingly, applying carbon nanotubes to speed up plant development can open up new opportunities for various fields, from biofuel crops to plants cultivated in space. Among future applications of MWCNTs, it can safely be used as an additional plant growth promoter and growth regulator to curb the need for food sustainability. It is suggested to conduct comprehensive research on the effects and assessment of the toxicity of

carbon nanotubes employed as plant growth regulators at all ecosystem levels, including microorganisms, animals, and humans.

Acknowledgements

The authors acknowledge the National Institute of Rourkela for providing laboratory, and other necessary infrastructure for carrying out the research.

Author contributions

GS designed the study and curated the protocols; SG performed experiments and analysed the data; BD analysed the data and wrote the first draft of the manuscript; BN designed and supervised the research and prepared the final draft of manuscript. All authors read and approved the final manuscript.

Funding

Not applicable.

Availability of data and materials

All the data of this study are included in this file.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors give their full consent for this research work to be published.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Received: 30 May 2022 Accepted: 3 September 2022

Published online: 06 October 2022

References

- Srilatha B. Nanotechnology in agriculture. *J Nanomed Nanotechnol*. 2011;2:1–5.
- Iijima S. Carbon nanotubes: past, present, and future. *Phys B Condens Matter*. 2002;323:1–5.
- Tomova A, Gentile G, Grozdanov A, Errico ME, Paunovic P, Avella M, et al. Functionalization and characterization of MWCNT produced by different methods. *Acta Phys Pol A*. 2016;129:405–8.
- Odom TW, Huang J-L, Lieber CM. Single-walled carbon nanotubes: from fundamental studies to new device concepts. *Ann N Y Acad Sci*. 2002;960:203–15.
- Al-Jumaili A, Alancherry S, Bazaka K, Jacob M. Review on the antimicrobial properties of carbon nanostructures. *Materials (Basel)*. 2017;10:1066.
- Ditta A. How helpful is nanotechnology in agriculture? *Adv Nat Sci Nanosci Nanotechnol*. 2012;3:1–10.
- Canas J, Long M, Nation S, Vadan R, Dai L, Luo M, et al. Effects of functionalized and non-functionalized single-walled carbon nanotubes on root elongation of select crop species. *Environ Toxicol Chem*. 2008;27:1922–31.
- Kang S, Herzberg M, Rodrigues DF, Elimelech M. Antibacterial effects of carbon nanotubes: size does matter. *Langmuir*. 2008;24:6409–13. <https://doi.org/10.1021/la800951v>.
- Salata OV. Applications of nanoparticles in biology and medicine. *J Nano-biotechnol*. 2004;2:1–6.
- Liu R, Lal R. Synthetic apatite nanoparticles as a phosphorus fertilizer for soybean (*Glycine max*). *Sci Rep*. 2014;4:5686.
- Huang X, Wang D, Hu L, Song J, Chen Y. Preparation of a novel anti-bacterial coating precursor and its anti-bacterial mechanism. *Appl Surf Sci*. 2019;465:478–85. <https://doi.org/10.1016/j.apsusc.2018.09.160>.
- Guastello SJ. Nonlinear dynamical models in psychology are widespread and testable. *Am Psychol*. 2014;69:628–9. <https://doi.org/10.1002/jfsa.8818>.
- Diallo M, Duncan J, Savage N, Street A, Sustich R. Nanotechnology applications for clean water. 1st ed. Amsterdam: Elsevier; 2014. <https://doi.org/10.1016/B978-1-4557-3116-9.00043-3>.
- Shen C-X, Zhang Q-F, Li J, Bi F-C, Yao N. Induction of programmed cell death in *Arabidopsis* and rice by single-wall carbon nanotubes. *Am J Bot*. 2010;97:1602–9. <https://doi.org/10.3732/ajb.1000073>.
- Taha RA. Nano carbon applications for plant. *Adv Plants Agric Res*. 2016;5:483–4.
- Stampoulis D, Sinha SK, White JC. Assay-dependent phytotoxicity of nanoparticles to plants. *Environ Sci Technol*. 2009;43:9473–9.
- Deshmukh SA, Gaikwad DK. A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of *Basella alba* (*Basellaceae*). *J Appl Pharm Sci*. 2014;4:153–65.
- Bamidele O, Akinnuga AM, Olorunfemi JO, Odetola OA, Oparaji CK, Ezeigbo N. Effects of aqueous extract of *Basella alba* leaves on haematological and biochemical parameters in albino rats. *African J Biotechnol*. 2010;9:6952–5.
- Lin S, Reppert J, Hu Q, Hudson JS, Reid ML, Ratnikova TA, et al. uptake, translocation, and transmission of carbon nanomaterials in rice plants. *Small*. 2009;5:1128–32.
- Zheng L, Hong F, Lu S, Liu C. Effect of Nano-TiO₂ on strength of naturally aged seeds and growth of Spinach. *Biol Trace Elem Res*. 2005. <https://doi.org/10.1385/BTER:104:1:083>.
- Rui M, Ma C, Hao Y, Guo J, Rui Y, Tang X, et al. Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). *Front Plant Sci*. 2016;7:1–10. <https://doi.org/10.3389/fpls.2016.00815/abstract>.
- Gala-Czekaj D, Bocianowski J, Radkowski A, Radkowska I, Gala-Czekaj D. Effect of multi-walled carbon nanotubes on the germination and growth characteristics of three fodder grasses in vitro and in chernozem soil. *J Elem*. 2017;23:21–33.
- Keita K, Okafor F, Nyochembeng L, Overton A, Sripathi VR, Odutola J. Plant and microbial growth responses to multi-walled carbon nanotubes. *J Nanosci Curr Res*. 2018;03:1–7.
- Dodd IC, Ruiz-lozano JM. Microbial enhancement of crop resource use efficiency. *Curr Opin Biotechnol*. 2012;23:236–42. <https://doi.org/10.1016/j.copbio.2011.09.005>.
- Yah CS, Simate GS. Nanoparticles as potential new generation broad spectrum anti-microbial agents. *DARU J Pharm Sci*. 2015. <https://doi.org/10.1186/s40199-015-0125-6>.
- Bai Y, Park IS, Lee SJ, Wen PS, Bae TS, Lee MH. Effect of AOT-assisted multi-walled carbon nanotubes on anti-bacterial activity. *Colloids Surf B Biointerfaces*. 2012;89:101–7. <https://doi.org/10.1016/j.colsurfb.2011.09.001>.
- Wang X, Zhou Z, Chen F. Surface modification of carbon nanotubes with an enhanced anti-fungal activity for the control of plant fungal pathogen. *Materials (Basel)*. 2017;10:1375.
- Patlolla AK, Hussain SM, Schlager JJ, Patlolla S, Tchounwou PB. Comparative study of the clastogenicity of functionalized and non-functionalized multiwalled carbon nanotubes in bone marrow cells of Swiss-Webster mice. *Environ Toxicol*. 2010;25:608–21.
- Saroj V, Rao P, Rao S, Krunal S. Pharmacognostical study of *Basella alba* stem. *Int J Res Pharm Biol Sci*. 2012;3:1093–4.
- Sartory D, Grobbelaar J. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia*. 1984;187:176–87.
- Cullity B, Stock S. Elements of X-ray Diffraction. New York: Prentice-Hall; 2001.
- Jiang L, Gao L, Sun J. Production of aqueous colloidal dispersions of carbon nanotubes. *J Colloid Interface Sci*. 2003;260:89–94.
- Couteau E, Hernadi K, Seo JW, Thiën-Nga L, Mikó C, Gaál R, et al. CVD synthesis of high-purity multiwalled carbon nanotubes using CaCO₃ catalyst support for large-scale production. *Chem Phys Lett*. 2003;378:9–17.
- Brodusch N, Demers H, Gauvin R. Field emission scanning electron microscopy. Berlin: Springer; 2018.
- Perkampus H-H. UV-VIS spectroscopy and its applications. Berlin: Springer; 1992.
- Khodakovskaya MV, Kim BS, Kim JN, Alimohammadi M, Dervishi E, Mustafa T, et al. Carbon nanotubes as plant growth regulators: effects

- on tomato growth, reproductive system, and soil microbial community. *Small*. 2013;9:115–23.
37. Hirsch P. Zeta potential analysis of nanoparticles. *NanoComposix*. 2012. p. 204. <https://nanocomposix.com/products/zeta-potential-nanoparticle-analysis?variant=14138179780#target>
 38. de la Cruz EF, Zheng Y, Torres E, Li W, Song W, Burugapalli K. Zeta potential of modified multi-walled carbon nanotubes in presence of poly (vinyl alcohol) hydrogel. *Int J Electrochem Sci*. 2012;7:3577–90.
 39. Kaushik N. Effect of capsule maturity on germination and seedling vigour in *Jatropha curcas*. *Seed Sci Technol*. 2003;31:449–54.
 40. Panse BVG, Sukhatme PKN. Published online July, 1956. 1956;1956.
 41. Laing W, Christeller J. Extraction of proteins from plant tissues. *Curr Protoc Protein Sci*. 2004. <https://doi.org/10.1002/0471140864.ps0407s38>.
 42. De la Calle I, Menta M, Séby F. Current trends and challenges in sample preparation for metallic nanoparticles analysis in daily products and environmental samples: a review. *Spectrochim Acta Part B*. 2016;125:66–96. <https://doi.org/10.1016/j.sab.2016.09.007>.
 43. Talbot MJ, White RG. Methanol fixation of plant tissue for scanning electron microscopy improves preservation of tissue morphology and dimensions. *Plant Methods*. 2013;9:1–7.
 44. Das R, Hamid S, Ali M, Ramakrishna S, Yongzhi W. Carbon nanotubes characterization by X-ray powder diffraction—a review. *Curr Nanosci*. 2014;11:23–35.
 45. Seddighinia FS, Iranbakhsh A, Oraghi Ardebili Z, Nejad Satari T, Soleimanpour S. Seed priming with cold plasma and multi-walled carbon nanotubes modified growth, tissue differentiation, anatomy, and yield in bitter melon (*Momordica charantia*). *J Plant Growth Regul*. 2020;39:87–98. <https://doi.org/10.1007/s00344-019-09965-2>.
 46. Zeinabad HA, Zarrabian A, Saboury AA, Alizadeh AM, Falahati M. Interaction of single and multi wall carbon nanotubes with the biological systems: tau protein and PC12 cells as targets. *Sci Rep*. 2016;6:26508. <https://doi.org/10.1038/srep26508>.
 47. Kushwaha SKS, Ghoshal S, Rai AK, Singh S. Carbon nanotubes as a novel drug delivery system for anticancer therapy: a review. *Brazilian J Pharm Sci*. 2013;49:629–43.
 48. Kukovecz Á, Kozma G, Kónya Z. Multi-walled carbon nanotubes. In: Vajtai R, editor. *Springer handb nanomater*. Berlin: Springer; 2013. p. 147–88.
 49. Martínez-Ballesta MC, Zapata L, Chalbi N, Carvajal M. Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. *J Nanobiotechnol*. 2016. <https://doi.org/10.1186/s12951-016-0199-4>.
 50. Joshi A, Sharma L, Kaur S, Dharamvir K, Nayyar H, Verma G. Plant nanobiogenic effect of multi-walled carbon nanotubes on growth, anatomy, yield and grain composition of rice. *Bionanoscience*. 2020;10:430–45. <https://doi.org/10.1007/s12668-020-00725-1>.
 51. Ghasempour M, Iranbakhsh A, Ebadi M, Oraghi AZ. Multi-walled carbon nanotubes improved growth, anatomy, physiology, secondary metabolism, and callus performance in *Catharanthus roseus*: an in vitro study. *3 Biotech*. 2019;9:404. <https://doi.org/10.1007/s13205-019-1934-y>.
 52. Finch-Savage WE, Bassel GW. Seed vigour and crop establishment: extending performance beyond adaptation. *J Exp Bot*. 2016;67:567–91. <https://doi.org/10.1093/jxb/erv490>.
 53. Li J, Wang C, Liang W, Liu S. Rhizosphere microbiome: the emerging barrier in plant-pathogen interactions. *Front Microbiol*. 2021. <https://doi.org/10.3389/fmicb.2021.772420/full>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)