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REVIEW

Interaction of carbon nanotubes with plant system: a review

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Abstract
Recent years have witnessed remarkable development in the field of nanotechnology and it has been affirmed that carbon-based nanomaterials have wide applications in agriculture, industrial, biomedical and environmental sectors. Due to distinctive physicochemical properties of the carbon nanotubes (CNTs), they have been extensively utilized in plant science as a growth promoter, and thus, could be a boon for biomass production of agricultural products. Studies suggest that CNTs help increase the plant's ability to absorb water and essential nutrients, thereby increasing growth. Apart from this, CNTs have been scrutinized for their utilization in genetic engineering for the delivery of genes, proteins or drugs. However, the literature discloses mixed effects of CNTs exposure on plants like in inducing oxidative stress by generating reactive oxygen species (ROS). Moreover, studies concerning CNTs interaction with plant system is at a nascent stage and needs further investigations to explore the mechanisms influencing the growth and toxicity in plants. Therefore, this review attempts to highlight the current literature on CNTs (including both single walled and multi walled) exposure on plants. It also explores unresolved challenges, as well as recommendations to ensure sustainable development of CNTs while minimizing any possible adverse health impacts.

Keywords Carbon nanotubes · Single walled · Multi walled · Plant growth · Agricultural application

1 Introduction

Nanotechnology, a new emerging field of science, permits advanced research in several areas. The use of nanoscience for the production of engineered nanomaterials like CNTs is a scientific breakthrough and could open up novel applications in the field of biotechnology, agriculture and others [1]. CNTs are hollow cylinders of carbon atoms that appear like rolled tubes of graphite formed in large bundles of single or multiple sheets of graphene to give single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs), respectively. It is a honeycomb lattice rolled on to itself, with lengths ranging from several hundred nanometers to several micrometers and diameters of a few nanometers (SWCNTs) to dozens of nanometers (MWCNTs) [2]. They are a large group of carbon-based, tube-like nanomaterials, which not only differ in length and the number of layers they consist of but also vary in types of impurity, contents and surface modification. CNTs have become increasingly popular due to unique features such as dimension, structure and topology. They have become one of the most studied and exploited engineered nanomaterials due to their outstanding electronic, mechanical, optical and structural properties. The applications of CNTs include biomedicine, nanoelectronics, bioengineering and mechanical engineering. Currently, the use of CNTs has been further extended to health care and agriculture to improve the quality of life. It can also be used in solving the environmental problems such as air, water and soil pollution where remediation technologies are limited [3, 4]. Some scientists observed that the L-cysteine moiety in L-glutathione is responsible for the susceptibility to oxidation by metallic impurities present in the carbon nanotubes. Their results These results assessed the toxicity of carbon-nanotube materials [5].

Research and development in the field of agriculture is very essential because most of the living beings depend on it. Currently, there is tremendous research interest in

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nanotechnology-based enhancement of agricultural products [6]. Carbon nanotubes can act as regulators of seed germination and plant growth. Report on MWCNTs has the ability to enhance the growth of tobacco cell culture (55–64%). They found that a correlation between the activation of cells growth exposed to MWCNTs [7]. Major emphasis of these studies is to enhance agricultural outputs, detection of diseases and their remediation processes [8]. Although researchers are keen to develop new techniques that could be suitable for plants to boost their native functions, they are also trying to increase the efficiency of plants to uptake more nutrients from the soil. This will ultimately lead to enhancement in the overall increase of biomass and fruitition of the plants. In the agricultural sector, CNTs might have properties that are effective to crops which aids in, controlling the release of chemical fertilizers and support the nutrients that regulate plant growth which may enhance target activity [6, 9]. In this respect, CNTs have a large potential to provide an opportunity for researchers of plant science and other field, to develop new tools for incorporation into plants that could augment existing functions and include new ones.

In recent years, CNTs have gained interest due to their possible applications in regulating plant growth [10]. Some researchers have investigated the effects of CNTs on plants and achieved significantly higher germination rates for seeds that contain CNTs [11]. It is suggested that CNTs help increase the plant's ability to uptake water, which abets wholesome growth of the plant, including the roots and shoots, and proliferates branching. Plants have produced two times more flowers and fruits when grown in soil supplemented with CNTs [12]. Studies propose that CNTs can enhance the growth of plants and have an effect on the expression of genes that are essential for cell division and plant development [13]. These studies highlight the potential to use CNTs, and nanomaterials to enhance plant functions.

On the contrary, inhibitory effects of CNTs were also reported in a few literatures [14, 15]. The effect of CNTs on plants is mainly influenced by different plant species, their growth stage and the nature of the CNTs used. Also, their property to penetrate the cell wall and their interactions with intracellular structures owing to their small size and high surface area contribute to potential cellular and genetic toxicity by the induction of oxidative stress. Studies on the cytotoxicity of CNTs include a decrease in cell viability, potential cytotoxicity in *Arabidopsis,* delayed flowering, reduction in yield and cell death due to apoptosis in rice, reduction in root length in lettuce, wilting and curling of leaves, and pigment loss in red spinach [15]. Cell aggregation, condensation of chromatins, plasma membrane deposition, generation of ROS and DNA damage are some other cellular changes among them.

Several works also highlighted the effects of CNTs on the microbial diversity and their threat to the useful microbial population [16]. Under high concentrations, CNTs significantly affect nutrient retention, decrease enzyme activity and microbial biomass, and degrade enzymes which are responsible for microbial biomass [17]. In a study [18], it was reported that CNTs may exhibit antimicrobial activity when they come in direct contact with bacterial cells, which leads to membrane mutilation and generation of oxidative stress.

Although the major objective of the use of CNTs is to increase agricultural productivity, concerns about possible side effects in ecosystems, human health, and agricultural industries need to be addressed.

2 Classification and characterization of carbon nanotubes

2.1 Classification

On the basis of structural composition, CNTs are mainly classified into three groups: stacked-cup carbon nanotubes (SCCNTs), MWCNTs and SWCNTs [19]. Jackson et al., [20] classified carbon nanotubes into three categories: MWCNTs, double-walled carbon nanotubes (DWCNTs) and SWCNTs. Their functional character changes with the change in structure and symmetry. Structures of carbon nanotubes are unique; they are hollow cylinders of graphite and possess hexagonally arranged carbon rings. Their end contains hexavalent arched structure while they are capped with the pentavalent ring [21]. They possess high tensile strength which resembles the property of graphene. As reported, CNTs remains stable even at very high temperatures and maximize vibrational entropy [22].

SWCNTs have a diameter in the range of 0.4–3 nm and their length ranges in micrometer [23]. They are hexagonally arranged in a bundle and form crystal-like structures [23, 24]. They can be differentiated into different forms in accordance with the type of wrapping, chirality, zigzag, and armchair nature [23]. Properties of SWCNTs are almost the same when compared with MWCNTs except for its high tensile strength [25].

MWCNTs contain many concentric hollow cylinders with an interlayer spacing of 0.34–0.39 nm [23, 26]. The decrease in inner wall diameter depends on wall layers, that is inner wall varies from 0.4 mm to a few nm, while the outer one ranges from 2 to 30 nm [23]. The endings of MWCNTs are closed with dome-shaped half fullerene capping. They are arranged in a way that one carbon nanotube lies another. The diameter of the inner carbon nanotubes is generally smaller than the outer one. This arrangement is also known as a Russian doll arrangement. Yet another arrangement is Parchment, in which one CNT is surrounded or rolled by multiple copies of CNT. Findings also suggest that the outer wall protects the inner wall from chemical reactions [23].

2.2 Characterization

The synthesis procedures available for CNTs are chemical vapour deposition (CVD) [27], arc discharge (AD) [28] and laser ablation (LA) [29] of which CVD is the most popular technique because of well-aligned structure and the desired orientation of the layers [30]. Other synthesis methods for CNTs are also described in the literature [31–33]. The produced CNTs are impure due to the presence of different undesired byproducts (fullerenes, carbonaceous residues, amorphous carbon and catalyst impurities). This led to the intensification of research on the purification of the synthesized CNTs [34–39]. Conversely, in this modern era of highly innovative technology driven processes, it remains a challenge to synthesize high purity CNTs specific in length and diameter.

Since the available synthesis procedures for CNTs do not ensure the homogeneity of the product in reference to their length and diameter, numerous efforts have been made to characterize CNTs obtained from different synthesis procedures. It should be noted that the toxicity of nanoparticles significantly depends on their morphology [40]. Due to the very small size of the CNTs highly sophisticated techniques are employed to assess their characteristics and morphology. Moreover, their bundle and aggregate structure make their use difficult. For application purposes, it must be dispersed either in water or in any another solvent to enhance its property. This can be achieved through the process of oxidation, sonication, centrifugation and ultrasonication combined with dispersing agents. As the concentration of CNTs in the initial phase is altered during the disaggregation processes and the final concentration in the liquid phase is lower than that initially used for preparation. It is important to study the characterization to determine the concentration of the dispersed CNTs in water. The techniques used to achieve the goal are outlined in Table 1, that can be broadly classified into four categories: microscopic and diffraction, spectroscopic, thermal, and separation techniques (Table 2; Fig. 1).

2.3 Role of CNTs in plant growth

Interaction of CNTs with plants is a complex phenomenon which causes several physiological and morphological changes in the plant species [58]. These changes are influenced by the size, concentration, type of CNTs as well as type of plant species and life stages. Several researchers attempted to study the uptake and translocation of the CNTs in plants and put forward a strategy dependent on the ratio of the size of the CNTs to the pore size of the cell wall. Methods like ultrasonic-assisted chemical oxidative cutting, the introduction of carboxylic groups on CNTs, to make it more soluble, are also used to increase the uptake by plants (Figs. 2, 3).

2.4 Effect of single-walled carbon nanotubes (SWCNTs)

In recent studies, increasing evidences suggest that SWCNTs increase plant growth and development. According to Yan et al. [59] SWCNTs have the ability to accelerate the seminal root growth in maize plants by influencing the gene expression. Similar studies on rice seedlings revealed that SWCNTs promoted growth by upregulating the expression of genes related to root growth [60]. Results also suggested a direct correlation between leaf development and the expression of the related genes in response to the CNTs. The positive effects of CNTs were also observed on the vegetative growth of the plant including increased leaf growth, chlorophyll contents and enhanced photosynthetic rates. Canas et al. [61] studied the effect of exposure of functionalized and non-functionalized SWCNTs on root elongation of six crop species that included cabbage, carrot, cucumber, lettuce, onion and tomato. Enhanced root elongation and increase in root number were reported in onion and cucumber plants.

Table 1 Characterization of carbon nanotubes using different analytical techniques

Characterization Technique	Goal
Microscopy and Diffraction Techniques [41–44]	Morphological analysis of internal structure (diameter, number of layers and distance between them), Morphological analysis of bulk samples
Spectroscopic Techniques [45–51]	Purity and presence of by-products, diameter distribution, (nm) chirality, Purity, functionalization by attaching functional groups to the sidewall, Dispersion efficiency, diameter and length distribution, purity, Size, dispersion efficiency, (n, m) chirality, Elemental composition, functionalization (covalent and non-covalent)
Thermal Techniques [52–54]	Purity and presence of by-products, quality control of synthesis and manufacture processes
Separation Techniques [55–57]	Purification, separation by size (length, diameter and cross-section) Fractionation by size (length) Separation by chirality, electronic type, length and enantiomeric identity

Table 2 Effects of MWCNTs on different plant species

Plant species	Concentration	Effect	References
Wheat, maize, peanut and garlic	50 µg/mL	Increase in root and shoot length Increase in biomass However, low concentrations of oxidized MWCNTs were more effective	[7]
Maize	20 mg/L	Enhanced germination rate at low concentrations; Improved water absorption, nutrient uptake (essential nutrients like Ca and Fe) and biomass of plant	[6]
Tomato	40 µg/mL	Increased growth, biomass, and uptake of nutrient elements (K, Ca, Fe, Mn and Zn)	[75]
Barley, soybean and corn	50–200 mg/L	Increased seed germination and growth, hence overall biomass of the plant; increased root length	[74]
Rice	5–20 mg/L	Penetrates cell wall and cell membrane, promotes seedling growth, and increases endogenous level of phytohormone	[60]
Mustard	2.3–46 mg/mL	Increased moisture content of seeds and water absorption	[76]
Common gram	6 mg/L	Increases root and shoot length and water uptake	[73]
Cabbage	10–60 mg/L	Increased growth under salt stress conditions; Enhanced aquaporin transduction	[71]
Date palm	0.05–0.1 mg/L	Increased callus fresh weight; increased germination rate, shoot length, leaf numbers, root number and length; Enhanced mineral uptake	[77]
Tobacco	0.1–500 mg/L	Increased growth rate and facilitate water transport	[65]

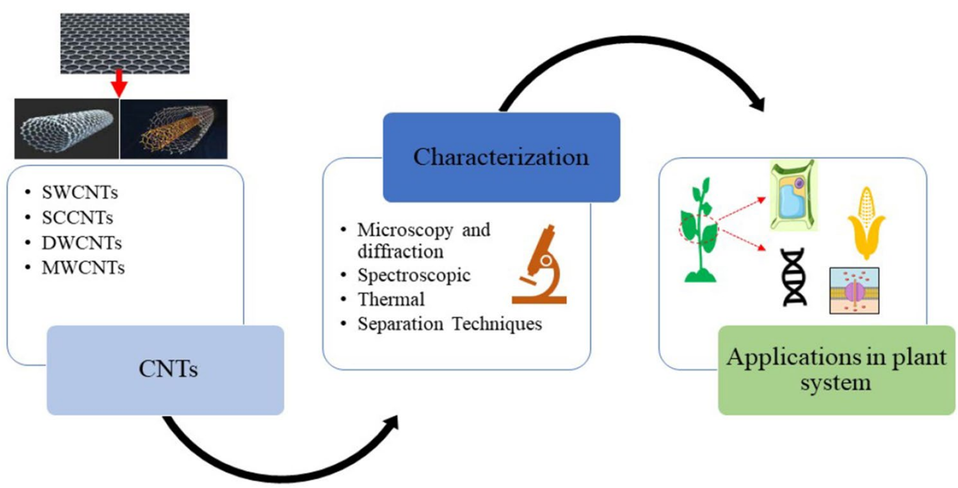

Fig. 1 Schematic diagram showing CNTs; their characterization and applications in plant system

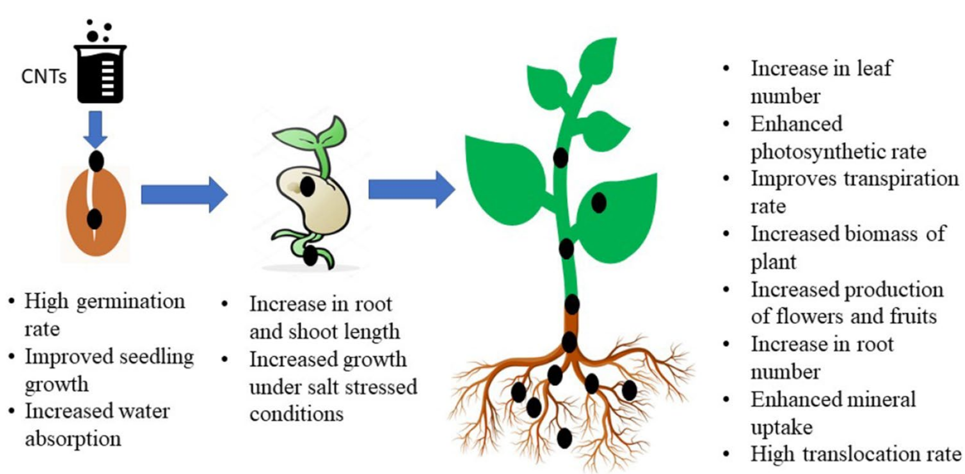

Fig. 2 Schematic diagram showing the role of CNTs in the plant at various stages

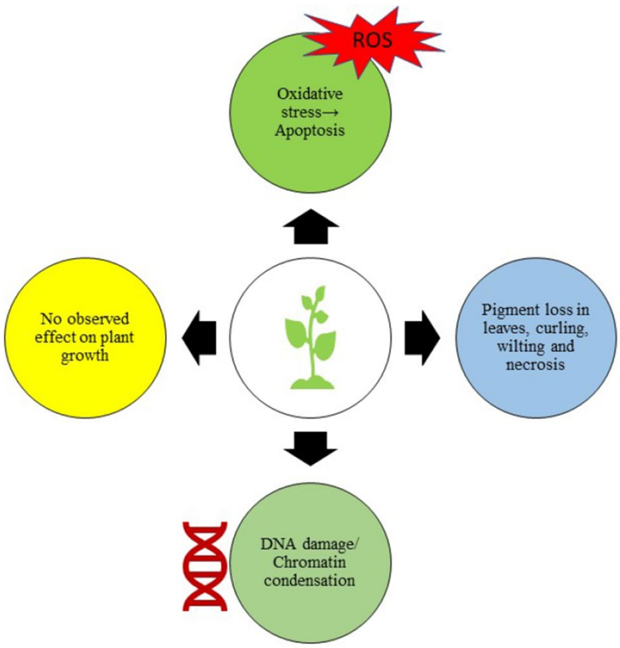

Fig. 3 Implications of CNTs in the plant system

A study on seed germination and seedling growth of salvia, pepper and tall fescue revealed that SWCNTs increased seed germination rates in the plants and the best SWCNT concentrations for seed germination and seedling growth for salvia and tall fescue were at 30 mg/L of SWCNT and at 10 mg/L for pepper [62]. The outcome of yet another study confirmed, that the CNTs (10–40 mg/L) improved the germination rate of tomato and onion seedlings. Further, there was increase in the dry weight of tomato and radish shoots. This study also revealed that the effectiveness of CNTs may depend on the concentration of CNTs and particular plant species [63].

Similar studies [64] on *Rubus adenotrichos,* using SWCNTs-COOH and SWCNTs-Fe resulted in elongation of roots and shoots, and enhanced cell metabolism. Khodakovskaya et al., [65] confirmed the increase in biomass in tobacco plants when treated with SWCNTs. In a separate study Mohammad et al., [66] used SWCNTs functionalized with quantum dots (QDs) on tomato seeds and found that addition of CNTs helped in increasing the chlorophyll content, and the total weight and height of the root/shoot system.

The SWCNTs also influences the reproductive growth in plants which are highlighted by the study on tomato plants by significantly increasing the flower and fruit formation. Other effects were increased in plant height, chlorophyll content and the total weight of the root system [12].

A study investigated, the role of SWCNTs under drought stress on *Hyoscyamus niger* seeds and it was found that SWCNTs helped in providing resistance against drought stress by enhancing water uptake and activating the plant defense system [67]. Another study on SWCNTs treated soybean seeds showed increased tolerance of seeds to drought stress. Apart from this, there was increase in the activity of catalase, superoxide dismutase etc. which may play an important role under stress conditions [68].

Due to the unique properties of SWCNTs, it can easily penetrate the cell wall and deliver chemicals to cells. This aspect was further explored to facilitate the delivery of DNA to the cells. Experiments performed by Corredor et al. [69] in *Tobacco* and *Catharanthus* cells evidenced that the SWCNTs translocate through the cell wall and the cell membrane. Wu et al., [70] investigated the cellular delivery of DNA by SWCNTs and observed that they were protected from enzymatic cleavage. They targeted a specific mRNA, which increased the capability of self-delivery and intracellular biostability when compared to free DNA probes.

2.5 Effect of multi-walled carbon nanotubes (MWCNTs)

Research on MWCNTs role in the increase of plant growth, seed germination, biomass etc. has been underway since the last decade. Even the role of CNTs in stress conditions was explored highlighting the fact that MWCNTs can enter into the seeds of broccoli and facilitate water uptake in plants thereby increasing the growth [71]. Further, it was observed that MWCNTs induced changes in lipid composition, rigidity and permeability of the root plasma membranes and ultimately, increased aquaporin transduction under salt-stressed conditions [72]. Furthermore, a study revealed the non-toxic nature of water-soluble CNTs that helped in enhancing the growth of roots, shoots and branching in gram plant (*Cicer arietinum*) [73]. This property of the CNTs may prove useful in optimum utilization of water in areas face water scarcity.

Research was also carried out on the effects of MWCNTs on seed germination, growth and the development of three important crops (barley, soybean, corn). Reverse transcription polymerase chain reaction (RT-PCR) analysis revealed that the expression of genes encoding water channel proteins increased in soybean, corn, and barley seeds coated with MWCNTs compared with control [74]. A similar study on wheat, maize, peanut and garlic depicted the beneficial effects of functionalized MWCNTs (in a dose-dependent manner) on root and shoot growth, biomass and number of leaves in all the plant species [7]. The ability of MWCNTs to penetrate the seat coat by forming new pores helped in water uptake resulting in the increase of the germination rate. Additionally, it has been also observed that CNTs have the capacity to penetrate plant seed coat [11]. The germination rate and plant growth were found to be significantly higher for seeds that germinated on medium containing CNTs (1040 g/mL) compared to control.

Tiwari et al., [75] investigated the effect of MWCNTs on tomato seedlings and found that at the concentration of 40 µg/mL, absorption of essential nutrient elements increased, which resulted in enhanced growth and biomass of the plant. In another study on tomato, it was found that the production of flowers and fruits were twice as compared to control [12]. Study on maize plant [3], demonstrated the effect of pristine MWCNTs which includes enhanced uptake of water and essential nutrients leading to increase in biomass of the plant. This property of the CNTs may prove to be beneficial for plants growing in nutrient-deficient soil.

Uptake and accumulation of MWCNTs can alter the morphology and biochemical characteristics of *Onobrychis arenaria* seedlings. Effect of the engineered nanomaterial containing MWCNTs (concentration 100 µg/mL and 1000 µg/mL), on the growth of *O. arenaria* seedlings suggested that MWCNTs have a capacity to penetrate the cell walls as they accumulate in roots and translocate to the leaves [78].

Larue et al. [79] studied the impact of CNTs on seed germination, root elongation, plant biomass, evapotranspiration, chlorophyll in wheat and rapeseeds. It was observed that less than 0.005% of the applied MWCNT dose is taken up by plant roots and translocated to the leaves. Further, there was no observed effect on photosynthetic activity or oxidative stress in plant.

A study on the effect of MWCNTs on germination and seedling growth of *Cichorium intybus* L inferred that even though, there was no observed effect on seed germination or plant growth, there was increase in weight of seeds treated with MWCNTs [80].

2.6 Implications in plant system due to use of CNTs

The toxicity of CNTs in plants has been highlighted in several studies. However, few researchers reported that interaction of CNTs with plants manifested toxicity effects, although other studies pointed at the positive correlation or no effect in plants.

Several studies revealed various factors viz. concentration of CNTs, particle size (surface area), plant type, growth stage, growth media etc. that may contribute to toxicity in plants [81, 82]. Further, characterizing CNTs is an essential component of ecotoxicity testing and can include evaluating the starting materials, its characteristics during ecotoxicity tests and the extent to which the CNTs altered during the test. Investigations comparing the toxicity of CNTs to bulk hard carbons should consider potential differences in surface area as a possible variable to explain differences in toxicity. CNT is transpiring novel nano-carbon fertilizer in the agricultural field, but at the same time it can cause deleterious effect on soil microbial density, composition, and population.

A study involving the MWCNT and cotton cellulose nanofibers toxicological effects on freshwater green microalgae *Chlorella vulgaris* was conducted [83]. It was observed that the uptake of MWCNT and cotton CNF by *C. vulgaris* led to reductions on algal growth and cell viability [84–86].

Wang et al. [87] observed that the carboxylated multiwalled carbon nanotubes cause biochemical and subcellular damages in leaves of broad bean (*Vicia faba* L.) seedlings under combined stress of lead and cadmium. The study mentioned the deleterious effects i.e. oxidative damage in the leaves due to combined use of MWCNTs-COOH with Pb and Cd while no such effect was observed in the leaves treated with MWCNTs-COOH or Pb and Cd individually. This study concluded that the combination of MWCNTs-COOH with heavy metals may cause phytotoxicity and hence, health risks among individuals.

The application of SWCNTs to rice resulted in the delayed flowering and decreased yield of this essential crop [88] whereas, a study presented a genotoxic potential in *Arabidopsis* [15] protoplasts relative to their concentration and size. Other cellular changes observed due to SWCNTs application were cell aggregation, condensation of chromatins, plasma membrane deposition and H_2O_2 accumulation in rice and *Arabidopsis*. A similar study was also carried out in *Arabidopsis* using MWCNTs which resulted in reduced cell viabilities and dry weight decreased cell chlorophyll content, and superoxide dismutase activities.

Upon their (MWCNTs) treatment to *Amaranthus tricolor*, several morphological and physiological changes were observed in the treated leaves like removal in red pigment of leaf, necrosis, curling, and wilting. The effects were not only limited to leaves there was concentration-dependent reduction in root-shoot height, root-shoot weight, and leaf numbers. Also, cell function was impaired due to enhanced electrolytic damage, generation of ROS and increased apoptosis [14]. Researchers reported reduced germination percentage in *Cichorium intybus* [80], reduced biomass in *Zucchini* [89] decrease in root lengths in lettuce plants [90] and DNA damage in *Allium cepa* [91].

Several significant negative effects related to translocation and uptake were reported in different plant species when treated with MWCNTs. Reduction in growth, uptake and translocation was observed in the soybean plants when treated with MWCNTs, 10–50 mg/L [92]. In a study the impact of MWCNTs and accumulation behavior of contaminants in mustard plants were observed. The study found that the permeability and transportability of MWCNTs were intact in mature mustard plants while there was an enhancement in contaminant accumulation [93]. Miralles et al., [94] explained the toxicity and uptake of industrial-grade MWCNTs and their impurities in Alfalfa and Wheat. They found phytotoxicity in both seed germination and seedling growth. Similar studies highlighted the fact that CNTs were acting as contaminant carriers and eventually, translocating the contaminants within edible parts of the crops.

In a study [95], suspension rice cells were used with MWCNTs (Size:10–30 nm and concentration: 20,40 mg/L) that led to several negative effects like condensed chromatin, detachment of cell membrane from cell wall, generation of ROS and decreased cell viability and leading to cell death. In yet, another study the toxic effects of MWCNTs on rice cells were reported [96]. Scanning electron microscope results revealed that cell density decreased with increase in MWCNT concentration. It was concluded that the MWCNTs have direct contact with rice cells and produce an unfavourable effect on rice growth.

Stampoulis et al. [89] focused on the assay-dependent phytotoxicity of nanoparticles on plants. The study investigated the application of nanomaterials viz. MWCNTs, Ag, Cu, ZnO, Si and their response towards seed germination, root elongation, biomass, etc. The hydroponic assay trial showed that after 15 days, the biomass of plants exposed to MWCNTs was reduced to 60–70%.

In addition to this, no observed growth effects on seed germination were reported by few researchers in several plant species like *Alfalfa,* wheat [94], mustard, black lentil [97], barley, maize, soybean [74] and lettuce [98]. Another, study confirmed that there was no effect of MWCNTs on seed germination on different plant species (rape, radish, ryegrass, lettuce, corn and cucumber) [90]. Others emphasized the accumulation of CNTs in various parts of the plant, changes in gene expression and their involvement in various biochemical changes as discussed earlier.

Therefore, with the development and application of CNTs in the plant system the potential hazards related to this is receiving greater attention [40]. However, this field needs to be explored further prior to arriving at any conclusions about the effects of CNTs. Currently, studies are sparsely related to the mechanisms of CNTs toxicity in plants and hence, more focused approach is needed.

3 Conclusion and future prospects

This review explored recent developments in plant science that focuses on the role of CNTs in plant growth and development and also on plant mechanism. Nanomaterials, like CNTs, due to several advantageous properties i.e. small size, high surface area, ability to penetrate cell wall etc. provide promising possibilities for future studies. On the one hand, they show a great potential to enhance seed germination, plant development and various plant physiological processes. On the other hand, several studies also reported the deleterious effects of CNTs in the plant system. Currently, studies concerning CNTs interaction with plant system is at a nascent stage and needs further investigations to explore the mechanisms influencing the growth and toxicity in plants. There are several gaps in our present knowledge about CNTs than there are certainties and more research is needed in the following proposed areas:

- Investigations concerning internal mechanisms (toxicity and genotoxicity) in plant system.
- Outcome of CNTs in the plant system and their role in the food chain.
- Identification of target plants and detailed analysis of cytotoxicity: toxicity study covering the life cycle of the plant.
- Toxicity studies related to soil media and microbial population.
- Need to explore other toxicity indicators in plants for instance biological markers etc.
- At the genetic level, extensive study related to the expression of genes.
- Thorough risk assessment of all nanomaterial-integrated products.
- Phytoremediation.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no competing interest.

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