Chapter 5
Toxicological Impact of Carbon Nanomaterials on Plants

Prakash M. Gopalakrishnan Nair

Abstract The fast growth of nanotechnology has resulted in the production and use of engineered nanoparticles with unique physical and chemical properties in various fields. The increased utilization of engineered nanoparticles enhances the risks associated with their release into the environment. The smaller size and modified physico-chemical properties raise concerns about their entry and adverse effects in plants. For instance, studies have shown that nanomaterials can be absorbed and translocated within plants. Since plants represent a major component of the ecosystem, the accumulation of engineered nanoparticles in plants is a threat to plants and the food chain.

This chapter reviews phytotoxic effect of carbon nanomaterials under *in vitro* and in vivo exposure conditions. Carbon nanomaterials are widely incorporated in commercial products used in agriculture. Recent studies have been conducted to test the toxic effects of carbon nanomaterials either alone or in combination with other chemicals in plants. Results reveal that the effect of carbon nanomaterials in plants are intricate and challenging and vary between different plant species, type of the nanomaterial and concentrations tested. Carbon nanomaterials were evidenced to penetrate through seed coats, enter into the plant cells and translocate into different plant parts. Exposure to carbon nanomaterials decreases seed germination, root growth and changes the roots architecture. Carbon nanomaterials inhibits seedling growth and changes morphological, physiological, biochemical, molecular, nutritional and genetic levels in plants. Modulation in the expression of genes related to cell division and plant development were also reported.

Keywords Plants • Engineered nanoparticles • Carbon nanomaterials • Phytotoxicity

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5.1 Introduction

The beginning of the "nano-era" in early twentieth century revolutionized the nanotechnology industry with the production of a variety of engineered nanoparticles (Roco 2003). The engineered nanoparticles are broadly classified into four categories based on their composition (1) carbon-based materials (2) metal-based nanoparticles (3) dendrimers and (4) bio-inorganic complexes having one (nanolayers), two (nanowires and nanotubes) or three dimensions (quantum dots, metal nanoparticles and fullerenes) on the nanoscale (US EPA 2007). The unique physico-chemical characteristics of engineered nanoparticles vary based on their shape, surface composition and size and are used as free nanoparticles or incorporated in to different products. The engineered nanoparticles are used in several applications viz. biomedical imaging for diagnosis, drug and gene delivery, pharmaceuticals, cosmetics, fuel additives and electronics.

The engineered nanoparticles enter into the environment through emission from manufacturing processes or through various anthropogenic activities. Waste discharges from waste water treatment plants and the application of sewage sludge in agricultural fields results in the deposition of engineered nanoparticles in agricultural soils (Gottschalk et al. 2009; Westerhoff et al. 2011). According to the available datas 50, 55 and 100% of treated wastewater sludge or bio-solids are used for agriculture use in Australia, USA and Switzerland respectively (Gottschalk et al. 2009). As per the reports, soils, sediments and landfills are the sinks of approximately 80% of the carbon nanomaterials released to the environment (Keller et al. 2013). This has raised concerns regarding the high risk of carbon nanomaterials to agricultural regions (Keller and Lazareva 2014).

Nanotechnology offers many uses in agriculture and due to their ever increasing use, the potential risks remain unclear. In agriculture, the engineered nanoparticles are incorporated in nano-agrochemicals (nano-pesticides and nano-fertilizers), nano-biosensors and nano-biocomposites (Adhikari et al. 2012; Gopal et al. 2012; Sekhon 2014). Apart from the direct risks to plants, since plants belongs to the first trophic level of the terrestrial food chain, exposure to engineered nanoparticles will have significant implications on human health and environment (Klaine et al. 2008;

Ma et al. 2010; Rico et al. 2011). Therefore, it has become essential to conduct risk assessments which will be useful for the safer use of nanotechnology (Tolaymat et al. 2015). The initial studies on the effect of engineered nanoparticles on germination and seedling growth in plants was reported in soybean (Lin and Xing 2007). Later on, several morphological, physiological and genetical changes have been reported in plants exposed to different types of engineered nanoparticles (Reddy et al. 2016). The impact and efficacy of engineered nanoparticles depends on their composition, physico-chemical properties, size, concentration as well as plant species (Ma et al. 2010; Khodakovskaya et al. 2012; Dasgupta et al. 2015, 2016, 2017; Shukla et al. 2017; Walia et al. 2017; Siripireddy et al. 2017; Maddinedi et al. 2015, 2017; Tammina et al. 2017; Ranjan and Ramalingam 2016; Dasgupta and Ramalingam 2016; Sannapaneni et al. 2016; Ranjan et al. 2014, 2016; Jain et al. 2016). This review covers the effects of carbon nanomaterials on *in vitro* cultures, seed germination, growth, mixture toxicity with other chemicals and the possible mechanisms of toxicity.

5.2 Carbon Nanomaterials and Their Applications

The detection of carbon-based nanomaterials started with the identification of "buckminsterfullerene" or the "buckyball" (Kroto et al. 1985), followed by carbon nanotubes (fullerene derivatives) (Lijima 1991) and graphene (Klaine et al. 2008). Presently due to their special optical, mechanical, electrical and thermal properties, carbon nanomaterials are increasingly used in several applications (Hurt et al. 2006; Srivastava et al. 2015). The carbon nanomaterials contains materials of diverse structure and size such as fullerenes, nano-onions, nano-cones, nano-horns, carbon dots, carbon nanotubes, nano-beads, nano-fibers, nano-diamonds and graphene (Sharon et al. 2010; Chai et al. 2013).

Fullerenes are hollow spheres (cage like structure) with a hexagonal network of carbon atoms (Chichiriccò and Poma 2015). For example, the fullerene C_{60} consists of 60 carbon atoms located at the vertices of twenty hexagons and twelve pentagons (Yadav and Kumar 2008). The fullerenes are also produced as higher mass with different geometric structures, such as, C_{70}, C_{76}, C_{78} and C_{80} (Kikuchi et al. 1992) and are extensively used in lubricants, electronics, cosmetics, fuel cells and in dietary supplements (Loutfy et al. 2002).

The carbon nanotubes are cylindrical structures with open or closed ends and are mainly categorized into single walled carbon nanotubes (having an outer diameter of 0.8–2 nm), and multi-walled carbon nanotubes (having outer diameter of 5–20 nm) depending on the number of rolled graphene layers (De Volder et al. 2013). Carbon nanotubes are used in electronic devices, paper batteries, cables and wires, field emission devices, transistors, electrical circuits, composite materials, absorbent for pollutant removal from water and in biomedical imaging (De Heer et al. 1995; Yao et al. 1999; Fuhrer et al. 2000; Rueckes et al. 2000; Franklin et al. 2001; Kemp et al. 2003; Jerosz et al. 2011; Islam et al. 2015).

Graphene is one atom thick planar sheet of sp^2-bonded carbon atoms packed in a hexagonal honeycomb crystal lattice, having two dimensions (Georgakilas et al.

2015). Graphene has several applications in electronics, biochemical sensors and in solar cells (Choi et al. 2010). Grapheme oxide is obtained by oxidation of graphite and has several applications in biomedical fields, such as drug delivery, cancer photothermal therapy, tissue engineering, bio-sensing and biological imaging (Sun et al. 2008; Zhang et al. 2011a,b; Sheng et al. 2013).

The carbon nanomaterials are utilized in various environmental applications such as in solar cells, for the production of renewable energy, soil remediation, contaminant degradation and in the detection as sensors for pollutants (Mauter and Elimelech 2008; Rasool and Lee 2015). In agriculture, carbon based nanomaterials contributes to approximately 40% of the total engineered nanoparticles used and are mainly used either as additives or as active components (Gogos et al. 2012). For example, fungicides encapsulated in multiwalled carbon nanotubes were more toxic to *Alternaria alternate* compared to bulk pesticides which were not capsulated (Sarlak et al. 2014). In the case of fertilizer application, for slow and efficient release, encapsulation with graphene oxide films was found to be effective (Zhang et al. 2014). For example, Zhang et al. (2014) reported that encapsulation of potassium nitrate in graphene oxide prolonged the release into the soil thereby making the availability of potassium nitrate more efficiently to the plants. Carbon nanomaterials could be used as additives for the development of efficient fungicides due to antifungal properties (Wang et al. 2014). In nano-biotechnology areas, the ability of carbon nanomaterials to penetrate and enter into cells could be used for the purpose of delivery of DNA molecules (Liu et al. 2009a, b; Burlaka et al. 2015). The various applications of carbon nanomaterials in different areas are shown in Fig. 5.1.

5.3 Phytotoxic Effect of Carbon Nanomaterials on Plants

The effects of carbon nanomaterials in plants were mainly studied on their effect on seed germination, plant growth and development. Only few studies have been reported on the toxicity of carbon nanomaterials in combination with other chemicals and on the mechanism of toxicity in plants. According to the available reports, the responses of carbon nanomaterials in plants varied based on the plants species, concentration tested and the stage of development. The reported effects of carbon nanomarials in plants are summarized in Fig. 5.2.

5.3.1 Penetration of Carbon Nanomaterials in Plants

The ability of different types of carbon nanomaterials to penetrate in to the plants has been reported from several studies. Lin et al. (2009a, b) investigated the uptake and translocation of carbon nanomaterials in rice plants (*Oryza sativa* L.). They found that fullerene C_{70} could be easily taken up by roots and transported to shoots.

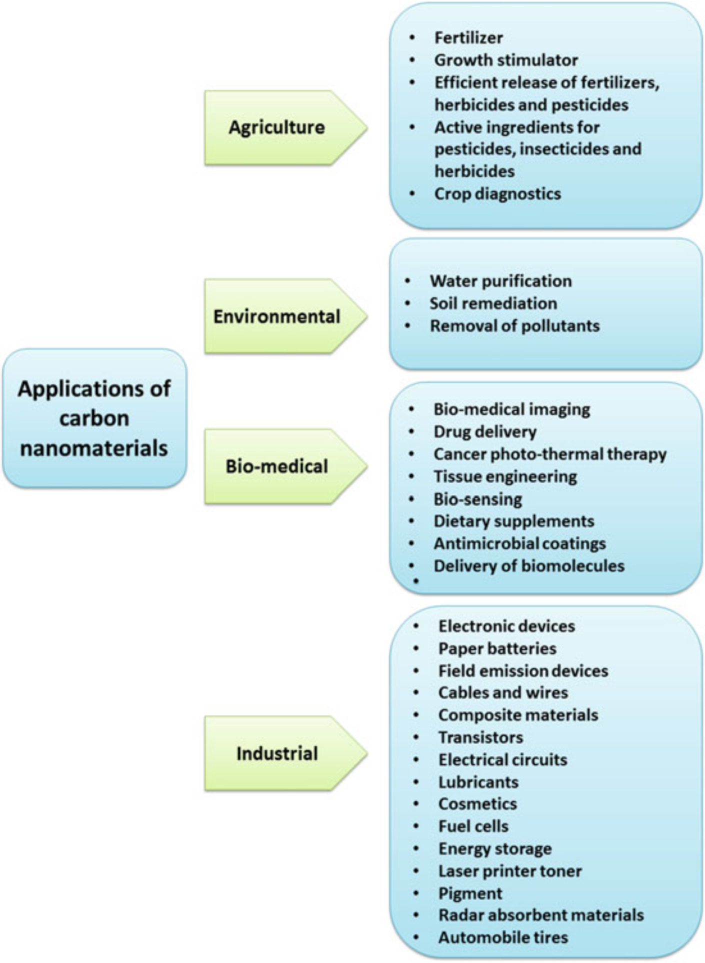

Fig. 5.1 The application of carbon nanomaterials for various uses in agriculture, environmental, bio-medical and industrial areas

It was also observed that C_{70} could be potentially transported downward from leaves to roots through phloem if C_{70} entered into plants through plant leaves. According to reports, the main pathway for the uptake of C_{60} fullerene to the plant from the soil is through the roots. Avanasi et al. (2014) reported that the highest accumulation of fullerene C_{60} (40–47%) occurred in the roots, followed by tuber (22–23%), stem (12–16%) and leaves (18–22%). Husen and Siddiqi (2014) observed that small sized carbon nanomaterials assimilated to the spaced areas after being transported through the capillary system and larger ones accumulated in the narrow passages thereby blocking the nutrient flow.

Wang et al. (2016) investigated the bio-accumulation of fullerenol (water-soluble derivative of fullerene carbon nanomaterial) nanoparticles in wheat using

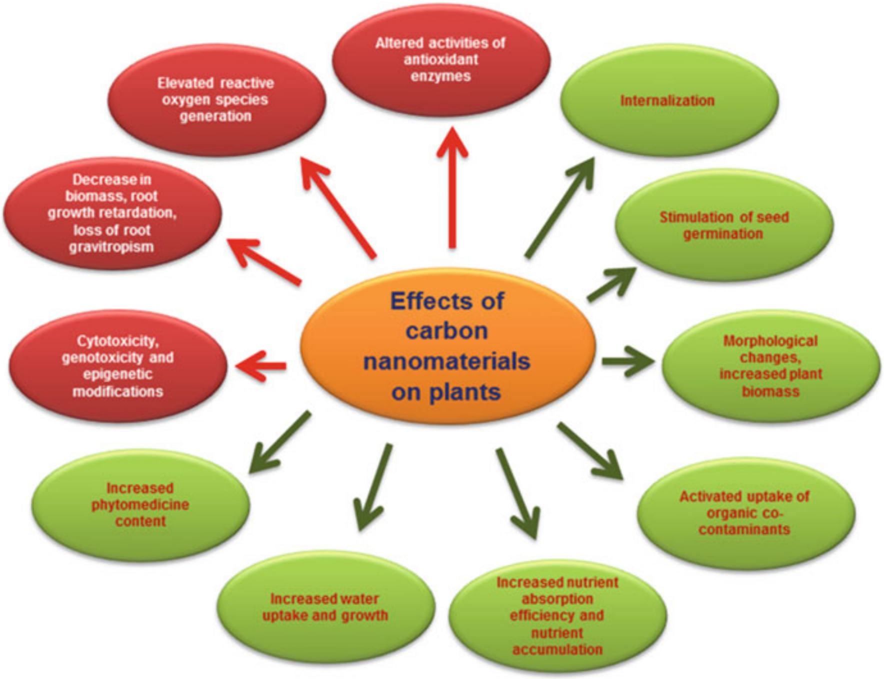

Fig. 5.2 Schematic representation showing various effects of carbon nanomaterials in plants at morphological, physiological, genetical and molecular levels

^{13}C-labelling techniques. The maximum bio-accumulation was observed in roots. Prolonged exposure with lower concentrations of fullerenol showed significant increase of ^{13}C content in roots and higher concentrations (10 µg/mL) suppressed the bio-accumulation. Only limited translocation of fullerenol from roots to stems and leaves was observed. Scanning electron microscopy analysis confirmed the presence of fullerenol nanoparticles in roots, with smaller particles being found in the vascular cylinder area of roots.

Samaj et al. (2004) demonstrated that single walled carbon nanotubes of length less than 500 nm labeled with fluorescein isothiocyanate penetrated the cell wall of the living plants by endocytosis. The results showed that though fluorescein isothiocyanate alone is not easily taken up by the plants, both of them jointly facilitated the absorption and penetration of carbon nanomaterials. In another study, Liu et al. (2009a, b) demonstrated the cellular uptake of both single walled carbon nanotubes and fluorescein isothiocyanate and single walled carbon nanotubes and DNA conjugates, demonstrating that single walled carbon nanotubes hold great promise as nano-transporters for walled plant cells. The penetration of chemically shortened single walled carbon nanotubes into the cell wall and cell membrane of tobacco (*Nicotiana tabacum*) and periwinkle (*Catharanthus roseus*) has also been reported (Liu et al. 2009a, b; Serag et al. 2011, 2012a, b, c).

Smirnova et al. (2011) demonstrated the presence of multi walled carbon nanotubes from Taunit inside the cells and tissues of seedling roots and leaves. The results showed the ability of multi walled carbon nanotubes to penetrate and accumulate inside the roots as well as their ability to be transported to the seedling leaves.

Ghosh et al. (2011) reported the internalization of multi walled carbon nanotubes inside the plant cells resulting in chromosomal aberrations, DNA fragmentation and apoptosis in Allium root cells. Wang et al. (2012a, b) revealed that wheat seedlings exposed to o- multi walled carbon nanotubes penetrated the cell wall and entered the cytoplasm through the roots. Larue et al. (2012) observed the uptake and translocation of^{14}C-radio labeled multi walled carbon nanotubes through the roots to the leaves in wheat and rape seed. Lahiani et al. (2013) reported the penetration and presence of multi walled carbon nanotubes to the seed coats of barley, soybean and corn. Yan et al. (2016) reported that exposure to multi walled carbon nanotubes in rice resulted in the penetration to the cell walls of roots of rice seedlings.

Zhai et al. (2015) studied the vegetative uptake of differentially charged multi walled carbon nanotubes viz. neutral pristine multi walled carbon nanotubes, positively charged multi walled carbon nanotubes-NH_2 and negatively charged multi walled carbon nanotubes-COOH in model food chain plants viz. maize (*Zea mays*) and soybean (*Glycine max*). The aim of the study was to find the effect of cellular, charge and size selectivity on the uptake differentially charged multi walled carbon nanotubes on food crops. They observed that the multi walled carbon nanotubes were directly taken-up through the roots and translocated to stems and leaves of maize and soybean plants. Accumulation of multi walled carbon nanotubes was observed in the xylem and phloem cells and also in the cytoplasm, cell wall, cell membrane, chloroplast and mitochondria using transmission electron microscope studies. Overall the study showed that the uptake and translocation of different types of multi walled carbon nanotubes were based on the cellular, charge and size in maize and soybean.

The post uptake behavior of carbon nanotubes inside the plant cells was studied by Serag et al. (2012a, b, c). It was observed that the plant cells differentiating into tracheary elements incorporated the cup-stacked carbon nanotubes into cell structure via. oxidative cross-linking of monolignols to the nanotubes surface during lignin biosynthesis. In a later study, Serag et al. (2013) reported that the diameter and length of single walled carbon nanotubes are the major restraining features for their effective penetration into the plant cell wall. Zhao et al. (2015) reported the accumulation of grapheme oxide in root hair and root parenchyma cells of *A. thaliana*. However, no translocation of grapheme oxide from the roots to the stem or leaves was observed.

Due to their smaller size and altered physical, chemical and structural properties, the absorption and translocation of different types of carbon nanomaterials raises serious concerns about their toxic effects on plants and also on the environment since plants represent the interface between the environment and biosphere.

5.3.2 In vitro *Exposure Studies of Carbon Nanomaterials*

The vulnerability of carbon nanomaterials on plant cells under *in vitro* cell culture conditions has been investigated by few studies. Liu et al. (2013) studied the effects of water-soluble carboxy fullerenes [ws-C_{70}; $C_{70}(C(COOH)_2)2–4$] in tobacco BY-2 cells (*Nicotiana tobacum*, cv. Bright Yellow). The results showed cell boundary disruption and growth inhibition, possibly due to the adsorption of ws-C_{70} to the plant cell wall through hydrostatic interaction with the carboxylic groups of fullerenes. Shen et al. (2010) showed that exposure to single walled carbon nanotubes exerted adverse effects on Arabidopsis and rice leaf protoplasts through oxidative stress, leading to programmed cell death or apoptosis, DNA damage and chromatin condensation. Dang et al. (2012) reported that exposure to water-soluble single walled carbon nanotubes and fullerene $C_{70}(C(COOH)_2)4–8$ on *Nicotiana tabacum* BY-2 cells caused shrunken morphology and lower proliferation rates. An increase in reactive oxygen species generation, abnormal mitochondrial distribution, decreased mitochondrial activity and impaired actin cytoskeleton arrangement was observed. However, no cytotoxicity was observed after treatment with either single walled carbon nanotubes or $C_{70}(C(COOH)_2)4–8$. In comparison, exposure to $C_{70}(C(COOH)_2)4–8$ caused more serious adverse effects on BY-2 cells. Tan et al. (2009) also reported that rice (*Oryza sativa* L.) suspension cells treated with multi walled carbon nanotubes enhanced the reactive oxygen species generation and decreased the cell viability.

The toxic effects of graphene was studied in *A. thaliana* (Columbia ecotype) T_{87} cell suspensions using different toxicological end points viz. morphology, mitochondrial dysfunction, reactive oxygen species generation and also the translocation of graphene inside the cells (Begum and Fugetsu 2013). Transmission electron microscopy analysis revealed the uptake of graphene nanoparticles in to the cells probably through endocytosis. Graphene exposure also caused morphological changes, fragmented nuclei, membrane damage and mitochondrial dysfunction in exposed cells. The results showed that graphene induced cell death in *A. thaliana* T_{87} cells through mitochondrial damage mediated by reactive oxygen species generation.

5.3.3 *Phytotoxic Effects of Fullerenes*

Investigations on the inhibitory effect of water-soluble fullerene C_{70} $(C(COOH)_2)$ 4–8 at the cellular level has been studied by Liu et al. (2010) using the transgenic seedling lines expressing fluorescent makers. Root growth retardation and loss of root gravitropism was observed and fluorescence imaging revealed the abnormalities of root tips in hormone distribution, cell division, microtubule organization and mitochondrial activity. Jiang et al. (2014) observed that rice seeds germinated in the presence of 150 µg/mL of carbon nanotubes decreased the root length, root activity, and stem length of the germinated seedlings of rice. The results showed the toxicity of higher concentrations of carbon nanotubes on seed germination and root growth. Mushtaq (2011) investigated the inhibitory effects of carbon nanoparticles (size 30–50 nm; 0 to 5000 µg/mL) on seed germination and root elongation of cucumber (*Cucumis sativus*). Inhibitory effect was more obvious in root growth than the seed germination percentages.

Liu et al. (2013) provided the first direct evidence for the change of plant cell wall composition under fullerene treatment using atomic force microscopy ligand-receptor binding force measurement to the living plant cell. They studied the changes of tobacco plant cell wall (*Nicotiana tobacum* L. cv. Bright Yellow) after treatment with water-soluble carboxy fullerenes ($C_{70}(C(COOH)_2)(2–4)$). It was observed that, the $C_{70}(C(COOH)_2)(2–4)$ were adsorbed to the cell wall which led to the disruption of cell wall and membrane leading to cell growth inhibition. A time and dose dependent increase of glycosyl residue on the cell wall accompanied by enhanced reactive oxygen species generation was observed.

5.3.4 Effects of Single Walled Carbon Nanotubes

The species dependent toxicity of single walled carbon nanotubes was studied by Canas et al. (2008). It was observed that exposure to single walled carbon nanotubes significantly affected root elongation of tomato, cabbage, carrot and lettuce. Tomato (*Lycopersicon esculentum*) showed the highest degree of sensitivity to single walled carbon nanotubes. The results showed that functionalized single walled carbon nanotubes demonstrated different toxic behaviors and are less toxic than non-functionalized single walled carbon nanotubes. This work highlighted the importance of investigating the surface properties of carbon nanotubes in determining their phytotoxicity. Pourkhaloee et al. (2011) reported that in salvia (*S. macrosiphon*), pepper (*C. annuum*) and tall fescue (*F. arundinacea*) exposure to higher concentrations of single walled carbon nanotubes affected the development of seedlings.

Yan et al. (2013) reported that exposure to single walled carbon nanotubes in maize seedlings caused inhibition of root hair growth. Gene transcription analysis showed that exposure to single walled carbon nanotubes decreased the root hair associated gene (*RTH1*, *RTH3*) expression. Treatment with single walled carbon nanotubes up-regulated the expression of epigenetic modification enzyme genes resulting in the global deacetylation of histone H3. It was concluded that exposure to single walled carbon nanotubes increased the histone deacetylation as a result of accumulation of the nanoparticles in the root apex.

5.3.5 Toxic Effects of Multi Walled Carbon Nanotubes

The germination rate of maize and rye grass decreased after exposure to 2000 mg/L of multi walled carbon nanotubes (Lin and Xing 2007). Stampoulis et al. (2009) investigated the effect of multi walled carbon nanotubes exposure under hydroponic conditions on zucchini. Treatment with 1000 mg/L for 15 days resulted in a 60% biomass reduction compared to control and bulk carbon. Ghodake et al. (2010) reported that exposure to 40 μg/mL of multi walled carbon nanotubes reduced root length and number of root hairs in *Brassica juncea* and *Phaseolus mungo*. Phytotoxicity studies using soil and in vitro conditions in young seedlings of *Parodia ayopayana* exposed to single walled and multi walled carbon nanotubes were conducted by Basiuk et al. (2013). The plant height, width and total root lengths were affected after 22 weeks of growth in soil and 16 weeks under in vitro conditions. The stronger effect was observed under in vitro conditions. Exposure to single walled carbon nanotubes caused higher effects as compared to multi walled carbon nanotubes. Reduction in primary spine length and under developed micrometer-sized secondary spines was observed. This gave evidences for the strong and systemic phytotoxic effect of multi walled carbon nanotubes on plant growth and development.

Mondal et al. (2011) revealed the dose dependent toxicity of multi walled carbon nanotubes in mustard, where oxidized-multi walled carbon nanotubes exerted higher negative effects than pristine multi walled carbon nanotubes. At higher exposure concentrations, both pristine and oxidized-multi walled carbon nanotubes caused toxicity, reducing germination by 4.4% and 7.6% and dry biomass by 1.6 and 2.2-fold respectively. Zhai et al. (2015) reported that exposure to differently charged multi walled carbon nanotubes viz. neutral pristine multi walled carbon nanotubes, positively charged multi walled carbon nanotube-NH_2 and negatively charged multi walled carbon nanotube-COOH inhibited the growth of soybean.

The importance of cyto-genotoxic effects of multi walled carbon nanotubes in the plant system and the importance of epigenetic studies on nanoparticle toxicity was investigated by Ghosh et al. (2015). They investigated the cytotoxic, genotoxic and epigenetic effect of multi walled carbon nanotubes in *Allium cepa*. Uptake of multi walled carbon nanotubes into the root cells significantly altered the cellular morphology, compromised the membrane integrity and mitochondrial function. Induction of DNA damage, micronucleus formation and chromosome aberration was also observed. Apoptotic cell death was observed as indicated by formation of inter nucleosomal fragments. The cyto-genotoxic effects were also confirmed by the accumulation of cells in the sub-G0 phase of the cell cycle and an increase in CpG methylation using the isoschizomers MspI/HpaII. Significant increase in the levels of 5-methyl-deoxy-cytidine was revealed by High Performance Liquid Chromatography analysis of DNA samples.

Apart from studying the toxic effects of multi walled carbon nanotubes in plants, few studies have been conducted to understand their mixture toxicity along with other environmental pollutants. The implications of the application of multi walled carbon nanotubes alone or in combination with pesticides for their use in agriculture, especially in leafy vegetables were studied by Hamdi et al. (2015). They evaluated the effect of non-functionalized and amino-functionalized multi walled carbon nanotubes as well as the presence of multi walled carbon nanotubes on the coexistent pesticide accumulation in lettuce (*Lactuca sativa* L.). The phytotoxicity of multi walled carbon nanotubes during germination and growth of lettuce seeds was monitored after sowing into 1000 mg/L of multi walled carbon nanotubes-amended vermiculite. The seedlings were subsequently exposed to 100 ng/L of chlordane (cis-chlordane, trans-chlordane and trans-non achlor and p, p'-DDE in the irrigation solution for a 19-days during the growth period. No significant influence on seed germination and plant growth was found after multi walled carbon nanotubes and pesticide exposure. However, the presence and type of multi walled carbon nanotubes significantly influenced pesticide contents in the seedlings. In roots and shoots, exposure to the non-functionalized multi walled carbon nanotubes decreased the root and shoot pesticide content by 88% and 78%, respectively. Exposure to amino-functionalized multi walled carbon nanotubes decreased the pesticide content by 57% in the roots and 23% in the shoots. However, the presence of humic acid completely reversed the reduction in the accumulation of pesticides induced by amino-functionalized multi walled carbon nanotubes probably due to the strong competition over adsorption sites on the multi walled carbon nanotubes.

To determine the combined effect of multi walled carbon nanotubes and heavy metals on agricultural crops, Wang et al. (2014) studied the effects of carboxylated multi walled carbon nanotubes (2.5, 5, and 10 mg/L) and their combination with 20 μM lead and 5 μM cadmium (shortened as Pb + Cd) in *Vicia faba* L. seedlings. It was observed that exposure to multi walled carbon nanotubes disturbed the nutrient element homeostasis, induced oxidative stress and damaged the leaves. Exposure to combination of carboxylated multi walled carbon nanotubes with Pb + Cd resulted in an increase in the content of Pb and Cd and decrease in oxidative damages as compared to treatments with carboxylated multi walled carbon nanotubes or Pb + Cd alone. The results showed that exposure to carboxylated multi walled carbon nanotubes caused oxidative stress and biochemical and subcellular damages as a result of treatment with Pb + Cd in the leaves. The results obtained from the investigation suggested that the continuous release of carboxylated multi walled carbon nanotubes into the environment may result in phytotoxicity and aggravate the ecological risks due to their combination with heavy metals.

The systemic toxicity and potential influence of multi walled carbon nanotubes was evaluated in red spinach by Begum and Fugetsu (2012). After 15 days of exposure under hydroponic conditions, the multi walled carbon nanotubes exposed plants showed inhibition of growth, cell death, and changes in root and leaf morphology. Reactive oxygen species generation and cytotoxicity were greatly increased in red spinach 15 days post-exposure to multi walled carbon nanotubes. However, supplementation of ascorbic acid reversed the adverse effects of multi walled carbon nanotubes exposure. It was concluded that excess reactive oxygen species generation and oxidative stress are the primary mechanism of toxicity of multi walled carbon nanotubes in red spinach.

The effect of multi walled carbon nanotubes in the presence and absence of NaCl was studied in broccoli (Martı́nez-Ballesta et al. 2016). Uptake and higher accumulation of multi walled carbon nanotubes was observed in cells demonstrating that multi walled carbon nanotubes can enter into the cells of adult plants under salt stress. Increased water uptake, growth and assimilation of CO_2 were observed in plants treated with multi walled carbon nanotubes. The authors hypothesized that assimilation of multi walled carbon nanotubes promoted more-favorable energetic forces and enhanced net assimilation of CO_2. Multi walled carbon nanotubes also enhanced aquaporin transduction which improved water uptake and transport, alleviating the negative effects of salt stress probably due to changes on plasma membrane properties of the cell wall.

5.3.6 Phytotoxic Effects of Graphene Nanomaterials

Few studies have been conducted to understand the effect of graphene nanomaterials on plants. Begum et al. (2011) investigated the interactions of graphene and its derivatives on root and shoot growth, biomass, shape, cell death and reactive oxygen species generation in cabbage, tomato, red spinach and lettuce. Morphological and physiological analyses indicated that exposure to graphene significantly inhibited plant growth and reduced the biomass. A dose dependent reduction in the number and size of leaves were observed in graphene-treated plants. A concentration-dependent increase in reactive oxygen species generation and cell death was observed in cabbage, tomato and red spinach indicating that the toxic effects were mediated through oxidative stress. Morphological analysis indicated that the epidermis of graphene treated roots of tomato and red spinach was loosely or completely detached.

Anjum et al. (2013) evaluated the effect of graphene oxide on the fava bean (*Vicia faba*) glutathione redox system, a major determinant of cellular redox homeostasis. A concentration dependent stress-response as well as decreased oxidative enzyme activity was observed. In a later study, Anjum et al. (2014) investigated the effect of single-bilayer grapheme oxide sheet in germinating faba bean seedlings to study their impact and potential mechanisms of toxicity. The results showed a concentration dependent decrease in the growth parameters and the activity of ascorbate peroxidase (*APX*) and catalase (*CAT*) enzymes and the levels of electrolyte leakage, H_2O_2 generation, lipid peroxidation and protein oxidation increased. Liu et al. (2015a, b) studied the effects of different concentrations of graphene on the germination and growth of rice seeds. Delay on the germination rate of rice seeds was observed with increasing graphene concentration. Inhibition of radicle and plumule growth and changes in the root length, stem length, adventitious number, root fresh weight, fresh weight of over ground part and root cap ratio of rice seedlings was also observed.

Short term and long term exposure studies were conducted in wheat (*Triticum aestivum* L.) to evaluate the phytotoxic effects of graphene on its growth and nutritional levels (Zhang et al. 2016). The plants were exposed different concentrations i.e. 250, 500, 1000 and 1500 mg/L of graphene under hydroponic culture conditions for 48 h and 30 days. Significant improvement in root elongation was observed but the root hair production was impaired. The phytotoxic effects were correlated with graphene induced-oxidative stress indicated by enhanced lipid peroxidation and antioxidant enzyme activity. Long term exposure to graphene resulted in reduction in shoot biomass, chlorophyll content, PSII activity and levels of nutrient elements viz. N, K, Ca, Mg, Fe, Zn and Cu. The results indicated that exposure to graphene inhibited plant growth and photosynthesis and caused an imbalance of nutrient homeostasis. The authors concluded that graphene has growth-limiting effects on plants, including root hair reduction, oxidative burst, inhibition of photosynthesis and nutritional disorder.

To understand the effects of graphene oxide on plant growth and development, *B. napus* cv. Zhongshuang 11 was treated with various concentrations of graphene oxide (Cheng et al. 2016). Treatment with 25–100 mg/L of graphene oxide resulted in shorter seminal root length compared to controls. Decrease in fresh root weight was observed when treated with 50–100 mg/L of graphene oxide. No significant change in lipid peroxidation as indicated by malondialdehyde content was observed. An increase in transcript levels of genes involved in absicic acid (ABA) biosynthesis (NCED, AAO and ZEP) and indole 3 acetic acid (IAA) biosynthesis (*ARF2*, *ARF8*, *IAA2* and *IAA3*) was observed after treatment with 50 mg/L of graphene oxide. However, an inhibition of transcript levels of IAA4 and IAA7 was observed. As compared with the control, exposure to graphene oxide treatment resulted in a higher ABA and lower IAA content. Overall, the results indicated that exposure to graphene oxide modulated the root growth of *B. napus* and affected ABA and IAA biosynthesis and concentration.

In a co-exposure study with graphene oxide and arsenate [As(V)], Hu et al. (2014) found that 0.1–10 mg/L of graphene oxide exposure enhanced the adverse effects of As (V) in wheat seedlings. They revealed that exposure to grapheme oxide greatly amplified the phytotoxicity of arsenic (As) in wheat causing a decrease in biomass and root numbers. Graphene oxide also triggered damage to cellular structures and enhanced the uptake of graphene oxide and arsenate. Co-transport of graphene oxide-loading arsenic (As) and transformation of As (V) to high-toxicity As (III) by graphene oxide were observed. The generation of dimethyl arsinate, produced from the detoxification of inorganic arsenic, was inhibited by graphene oxide in plants. Significant reduction in the fresh mass, shoot length and chlorophyll content was also observed in treated plants. In addition, the activity of peroxidase and superoxide dismutase, likely biomarkers for stress response, were increased in a concentration-dependent manner. Graphene oxide also regulated phosphate transporter gene expression and arsenate reductase activity to influence the uptake and transformation of As, respectively. The authors concluded that the indirect nanotoxicity of graphene oxide should be carefully considered in food safety. The results obtained from studies conducted on the effect of different types of carbon nanomaterials on various types of plants species has been concluded in Table 5.1.

Table 5.1 The phytotoxic effects of different carbon nanomaterials on different plant species is summarized in the table

Nanoparticle(s)	Plant	Toxic growth effects	Reference(s)
C_{60} fullerenes	Corn, soybean	Reduced biomass	Torre-Roche et al. (2013)
Functionalized carbon nanotube	Lettuce	Reduced root length	Cañas et al. (2008)
ws-C_{70}	Tobacco BY-2 cells	Cell boundary disruption and growth inhibition	Liu et al. (2013)
SWCNTs	Rice	Delayed flowering, decreased yield	Lin et al. (2009a, b)
Non-functionalized and functionalized SWCNTs	Carrot, cabbage, cucumber, lettuce, onion, tomato	Functionalized SWCNTs inhibited root growth in tomato. Non-functionalized SWCNTs inhibited lettuce root growth	Ca~nas et al. (2008)
MWCNTs	Rice cells	Increased ROS generation and decreased cell viability	Tan et al. (2009)
MWCNTs	Rice	Delayed flowering and seed setting. Reduced seed weight	Lin et al. (2009a, b)
MWCNTs	Zucchini	Negatively affected biomass production and transpiration	Stampoulis et al. (2009)
MWCNTs	Wheat	Enhanced the uptake of phenantrene to the living cells	Wild and Jones (2009)
MWCNTs	Garden cress, sorghum, tomato, radish, cucumber	Influenced seed germination and root growth depending of the type of sewage sludge	Oleszczuk et al. (2011)
MWCNTs	Onion	Chromosomal aberrations, DNA fragmentation and apoptosis in root cells	Ghosh et al. (2011)
MWCNTs	Onobrychis	Enhanced the POD activity	Smirnova et al. (2012)
MWCNTs	Lettuce	Reduced root length	Lin and Xing (2007)
MWCNTs	Rice	Chromatin condensed inside the cytoplasm and caused cell death, plasma membrane detachment from cell wall and cell shrinkage	Tan et al. (2009)
Oxidized MWCNTs	Mustard	Shorter germination time, enhanced root growth, and seedling stem development	Mondal et al. (2011)
CNTs	Red spinach	Growth inhibition, changes to tissue structure	Begum and Fugetsu (2012)
GO	Faba bean	Concentration dependent decrease in oxidative enzyme activity.	Anjum et al. (2014)
Single bilayer GO Sheet	Faba bean	Stress in plant development and growth. Reduction on peroxidase enzyme activity	Anjum et al. (2014)

(continued)

Table 5.1 (continued)

Nanoparticle(s)	Plant	Toxic growth effects	Reference(s)
Graphene	Arabidopsis	Fragmented nuclei, membrane damage and mitochondrial dysfunction	Begum and Fugetsu (2013)
Water-soluble graphene oxide (ws-GO)	Lettuce, cabbage, red spinach, tomato	Reduced plant growth, biomass, the number and size of leaves, increased ROS along with necrotic symptoms	Begum et al. (2011)

sWCNTs single walled carbon nanotubes, *MWCNTs* multi walled carbon nanotubes, *CNTs* carbon nanotubes, *GO* graphene oxide

Species names: – Corn – *Zea mays*; Soybean – *Glycine max*; Lettuce – *Lactuca sativa*; Rice – *Oryza sativa*; Carrot – *Daucus carota*; Cabbage – *Brassica oleracea*; Cucumber – *Cucumis sativus*; Onion – *Allium cepa*; Tomato – *Solanum lycopersicum*; Zucchini – *Cucurbita pepo*; Wheat – *Triticum aestivum*; Garden cress – *Lepidium sativum*; Sorghum – *Sorghum bicolor*; Tomato – *Solanum lycopersicum*; Radish – *Raphanus sativus*; Onobrychis – *Onobrychis arenaria*; Mustard – *Brassica juncea*; Red spinach – *Amaranthus dubius*; Faba bean – *Vicia faba*; Arabidopsis – *Arabidopsis thaliana*

Overall, analysis of the available reports indicate that the ability of different types of carbon nanomaterials to enter into plant cells and translocate to various parts poise different types of risks both in terms of plant health and as well as raises environmental issues. Following exposure, the different types of carbon nanomaterials were able to penetrate and enter through the seed coats, decreased seed germination, root growth and elongation. For example, from the studies it was observed that multiwalled carbon nanotubes were able to penetrate into the root system and translocated to the leaves and the fruits. The carbon nanomaterials were also able to modulate the expression of genes related to root development and also affect the synthesis of hormones related to root growth and development. The entry of carbon nanotubes to the plant tracheary elements may affect plant defense responses as well as wood development. Apart from this, mixture toxicity studies have found that carbon nanomaterials will enhance the toxicity and uptake of other environmental pollutants in plants.

5.4 Conclusions and Future Work

The application of carbon nanomaterials are growing. However, the production and release of different types of nanomaterials possessing various properties have complicated the evaluation of different types of carbon nanomaterials on plants. Due to safety considerations, several studies are being conducted to evaluate the effects of different types of carbon nanomaterials either alone or in combination with other environmental pollutants and heavy metals in plants. Results from the available studies indicate that exposure to different types of carbon nanomaterials caused toxic responses in plants and are depended on the concentration, exposure media and plant species. However, one limitation is that most of the studies were conducted under *in vitro* or controlled growth conditions thereby making it difficult to predict the effect of carbon nanomaterials under natural field conditions. Therefore, further studies need to done to understand the toxic effects of various carbon nanomaterials under natural conditions in plants.

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