Phytotoxicity of nanoparticles in agricultural crops

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1 author:

Parthasarathi Theivasigamani
Ben-Gurion University of the Negev

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T. Parthasarathi
Department of Crop Physiology
Tamil Nadu Agricultural University
Coimbatore - 641003. Tamil Nadu, India
agrisarathi@yahoo.com

Abstract— **Nanoagriculture is a viable technology by using nanomaterials under various purposes such as nano biocides, seed germination, genetic material transfer in agricultural crops. Nanoparticles were toxic to the plant, human and environment. The nanoparticles are move to the plant by various mechanisms by root uptake, cuticular translocation. The Phytotoxic effect of nanoparticles were reduced root length, shoot length, biomass production, increased genetic material damage, agglomeration observed by the increasing nanoparticle concentration. By reduce the effect of nano material in cultivable crops; prior invitro analysis reduces the phytotoxic effect. Standardization of safe concentration of each nano material at varying concentration and its physiological, biochemical response to agricultural crops should be studied are used to reduce the phytotoxicity to the sustainable safe environment and sustainable for second green revolution in the future.**

Keywords-component; NPs (Nanoparticles); nanotubes; Phytotoxicity; biocide; Agglomeration

I. Introduction

The nanoagriculture is using nanotechnology to boost the productivity of plants for food, fuel and other uses that scientists are reporting a huge gap in knowledge about the effects of nanoparticles on corn, tomatoes, rice and other food crops. The uptake and build-up of nanoparticles varies, and these factors largely depend on the type of plant and the size and chemical composition of the nanoparticles. Some plants can take-up and accumulate nanoparticles. But it is unclear whether this poses a problem for plants or for the animals (like humans) that eat them. "The knowledge on plant toxicity of [nanomaterials] is at the foundation stage," the article states, noting that the emerging field of nanoecotoxicology is starting to tackle this topic [1].

In the agricultural applications, nanotech research and development is likely to facilitate and frame the next stage of development of genetically modified crops, animal production inputs, biocides and precision farming techniques. While nano-chemical pesticides are already in use and it may be commercialized soon. So the interaction of plant cell with the nanoparticles results in modification of plant gene expression and associated biological pathways, which ultimately affect plant growth and development [2]. Nanoparticles, with their ultra-small size, specific shape, geometric structure, and unique properties, may have the potential for increased toxicity level [3]. Nanoparticles can drastically modify their physico-chemical properties compared to particles of bulk size. The effects of nanoparticles on different plant species can vary greatly with plant growth stages and method and duration of exposure and depend also on nanoparticle size, concentration, chemical composition, surface structure, solubility, shape, and aggregation [4]. Carbon nanoparticles can penetrate plant cells [5,6] and induce Phytotoxicity at high doses [7,8], leading to conclude that certain carbon nanoparticles are not 100% safe.

Plants have been used as indicators in studies on mutagenesis in higher eukaryotes. The increasing interest in the fate of the environment and the effects of nanomaterials on the environment needs to be addressed by adequate nano-toxicity research for plants that are ecologically important [9-11]. Plants may be involved in the path for the transport of NPs and could be a cause for their bioaccumulation into the food chain [12]. A limited number of phytotoxicity reports showed both the positive and the negative effects of NPs on higher plants [13-15]. Positive effects existed with the multi-walled carbon nanotubes through germination and seedling growth of *Brassica juncea* and *Phaseolus mungo* [13]. The seed germination and root growth of some plant species were inhibited by the zinc and zinc oxide NPs [8,13]. Some rare earth oxide NPs severely inhibited the root elongation of higher plant species [16].

The phytotoxicity on development of *Arabidopsis thaliana* by four different metal oxide nanoparticles namely, aluminum oxide (nAl_2O_3), silicon dioxide ($nSiO_2$), magnetite (nFe_3O_4) and zinc oxide (nZnO) were studied earlier. Toxic effects by ZnO were more in seed germinations, root elongations and number of leaves than other particles [17]. Effect was more as exposure time increases resulting significant inhibitions [18]. Effects of silver nanoparticles results reductions in biomass and transpirations rate than bulk silver.

Interestingly, it was found that during penetrations of particles inside the roots cell, they damaged the cell wall as well as vacuoles to enter. It may be due to the penetrations of

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large particles entering through small pores of cell walls. Research of uptake, toxicity and effects of nanoparticles in higher plant needs quick attentions.

II. ROLE OF NANOPARTICLES ON AGRICULTURAL CROPS

The use of nanostructures with unusual novel properties in agriculture [19] and for technological applications has been an active and exciting area of research in recent years. The nanoparticles, with their ultra-small size, specific shape, geometric structure, and unique properties, may have the potential for increased toxicity [3]. Nanoparticles can drastically modify their physico-chemical properties compared to particles of bulk size [4].

The agrochemicals are conventionally applied to crops by spraying and/or broadcasting. Nano-encapsulated agrochemicals should be designed in such a way that they possess all necessary properties such as effective concentration (with high solubility, stability and effectiveness), time controlled release in response to certain stimuli, enhanced targeted activity and less ecotoxicity with safe and easy mode of delivery thus avoiding repeated application [20,21]. The control of parasitic weeds with nanocapsulated herbicides thereby reducing the phytotoxicity of herbicides on crops is a best example [22]. Properly functionalized nanocapsules provide better penetration through cuticle and allow slow and controlled release of active ingredients on reaching the target weed. Properly functionalised lipophilic nanosilica gets absorbed into the cuticular lipids of insects by physiosorption and damages the protective wax layer [made of various fatty acids and lipids that acts as an effective barrier for the evaporation of water] and induces death by desiccation [23,24].

Nanotechnology is introducing a new array of potentially more toxic pesticides, plant growth regulators and chemical fertilisers than those in current use at a time when we should be increasing our support for more sustainable food systems (Table 1). Some of the first nano agrochemicals in development are nano-reformulations of existing pesticides, fungicides, plant, soil and seed treatments [19,20,25]. An exception is Syngenta, the world's largest agrochemical company, which has been selling its nano-formulated "Primo MAXX" plant growth regulator for several years. Primo MAXX is marketed as a "micro-emulsion" concentrate [26]. Nanobiotechnology offers a new set of tools to manipulate the genes using nanoparticles, nanofibres and nanocapsules [27,28] crop engineering and environmental monitoring. Reports came on the integration of carbon nanofibres, which are surface modified with plasmid DNA with viable cells for controlled biochemical manipulations in cells [29].

Table 1. Nano agrochemicals under development around the world

Type of product	Product name & manufacturer	Nano content	Purpose
"Super" combined fertiliser and pesticide [30]	Pakistan-US Science and Technology Cooperative Program	Nanoclay capsule contains growth stimulants and biocontrol agents	Because it can be designed for slow release of active ingredients, treatment requires only one application over the life of the crop
Herbicide [31]	Tamil Nadu Agricultural University (India) and Technologico de Monterry (Mexico)	Nano-formulated	Designed to attack the seed coating of weeds, destroy soil seed banks and prevent weed germination
Pesticides, including herbicides [32]	Australian Commonwealth Scientific and Industrial Research Organization	Nano-encapsulated active ingredients	Very small size of nanocapsules increases their potency and may enable targeted release of active ingredients

The successful delivery and integration of plasmid DNA was confirmed from the gene expression. This process has similarity with microinjection method of gene delivery [33,34] and hence possible with the plant cells in which the treated cells could be regenerated into whole plant that would express the introduced trait. The application of fluorescent labeled starch-nanoparticles as plant transgenic vehicle was reported in which the nanoparticle biomaterial was designed in such a way that it bind and transport genes across the cell wall of plant cells by inducing instantaneous pore channels in cell wall, cell membrane and nuclear membrane with the help of ultrasound [35].

The uptake efficiency and effects of various nanoparticles on the growth and metabolic functions vary differently among plants [2]. The nano materials are used to increase the seed germination of agricultural crops (Fig 1). The comparative study of carbon nanomaterials (such as single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotube (MWCNTs), carbon buckyballs, etc.) also found increased applications in the field of agriculture and food. The effects of both SWCNTs [in purchased form and purified form] and MWCNTs on the germination of rice seeds and observed an enhanced germination for seeds germinated in the presence of nanotubes.

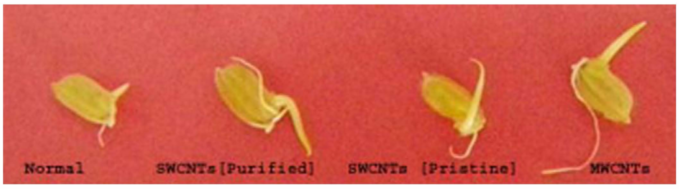

Figure 1. Effects of different carbon nanotubes on the germination of rice seeds. Both SWCNTs (single-walled carbon nanotubes) and MWCNTs (multi-walled carbon nanotube) are added to plant growth media @ 30 g/ml of the media. Enhanced germination was observed for seeds germinated in presence of carbon nanotubes compared to the control (Picture reproduced from [2]).

The distribution of properly functionalized nanoparticles throughout the plant vascular system and guides them to targeted sites, these nanoparticles can be successfully used to unload chemicals (fungicides, insecticides, etc.), or other substances (plant hormones, elicitors, nucleic acids) into localized areas of plant tissues. This could help to carry on several studies at physiological, biochemical and genetic levels. Plant mediated synthesis of metal nanoparticles provide a safe synthesis route with better control over morphology of nanoparticles.

Since nanoparticles are introduced into the soil as a result of human activities, among the many fields that nanotechnology takes into consideration, it is also important to recall the analyses of the connections among nanoparticles, plants and soil where plants live and grow up. A mesoporous silica nanoparticle system that can transport DNA and chemicals into isolated plant cells (protoplasts from *Nicotiana tabacum* culture) and intact leaves (*Zea mays* young embryos) [36]. According to [37] analysed the influence of metal nanoparticles on the soil microbial community and germination of *Lactuca* seeds. The results showed a insignificant influence of the nanoparticles in the soil on the number of colony forming units confirming the results of [38,39]. On the contrary metal nanoparticles influence the growth of *Lactuca* seeds, this influence was tested by measuring the length of the root and shoot of the plant after 15 days of incubation. An increase if the shoot/root ratio compared to that of the control was evidenced.

III. MOVEMENT OF NANOMATERIALS IN PLANT

Before understand the possible benefits of applying nanotechnology to agriculture, the first step should be to analyze penetration and transport of nanoparticles in plants. Movement of the nanoparticles was detected at different levels: chains of nanoparticle-aggregates carrying cells were apparent close to the application point, when such application was made by ‘injection’ of the nanoparticle suspension into the pith cavity of the stem, suggesting the flux of nanoparticles from one cell to another. The nanoparticles are capable of penetrating through the leaf cuticle and into the cell cytoplasm.

Plants provide a potential pathway for the transport of nanoparticles to the environment and serve as an important route for their bioaccumulation into food chain. Plant cell wall acts as a barrier for easy entry of any external agent including nanoparticles into plant cells. The sieving properties are determined by pore diameter of cell wall ranging from 5 to 20nm [40]. Only nanoparticles or nanoparticle aggregates with diameter less than the pore diameter of the cell wall could easily pass through and reach the plasma membrane [41,42]. There is also a chance for enlargement of pores or induction of new cell wall pores upon interaction with engineered nanoparticles, which in turn enhance nanoparticle uptake. They may also cross the membrane using embedded transport carrier proteins or through ion channels. In the cytoplasm, the nanoparticles may bind with different cytoplasmic organelles and interfere with the metabolic processes at that site [43]. When nanoparticles are applied on leaf surfaces, they enter through the stomatal openings or through the bases of trichomes and then translocated to various tissues [44,45]. However, accumulation of nanoparticles on photosynthetic surface cause foliar heating which results in alterations to gas exchange due to stomatal obstruction that produce changes in various physiological and cellular functions of plants [46].

The application of microscopy tools and techniques at different level of resolution to visualize and track the transport and deposition of nanoparticles inside the plants. Nanoparticles tagged to agrochemicals or to other substances could reduce the injury to plant tissues and the amount of chemicals released into the environment; a certain contact is however unavoidable, due to the strong interaction of plants with soil growth substrates. The carbon-coated magnetic nanoparticles (carbon encapsulation provides biocompatibility and a large adsorption surface) in living plant as *Cucurbita pepo* and the results showed the presence of nanoparticles both in the extracellular space and within some cells [47].

One of the pathways was also reported where particle size of 20 nm silver nanoparticles may be transported inside the cells through plasmadesmata [16]. Particles must be entered through cell wall and plasma membrane of root cells. Xylem is one of the main passages of uptake and transportations to shoot and leaves of plant. Pores size of cell wall was in the range of 3-8 nm, smaller than engineered nanoparticles [48] (Carpita and Gibeaut 1993).

Penetration rates of foliar applied polar solutes are highly variable and the mechanism is not yet fully understood, [45] investigated in *Allium porrum* and *Vicia faba* size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. So the nanoparticle pathway in leaf follow the stomatal pathway that differ fundamentally from the cuticolar foliar uptake pathway. The limits of uptake and the distribution of silver nanoparticles in *Brassica juncea* and *Medicago sativa*. In contrast to *Brassica juncea*, *Medicago sativa* showed an increase in metal uptake with a corresponding increase in the substrate of metal concentration and exposure time [49]. The silver nanoparticles were located in the nucleus and applying the definition of [50] suggested that both *Medicago sativa* and *Brassica juncea* were hyperaccumulators of silver.

Due to the ability of specific plant species to hyperaccumulate NPs without apparent physiological damages, at least in particular experimental conditions, plants may represent from one hand a potential transport pathway of NPs in the environment, from the other, in specific cases, a cost-effective alternative to clean up NPs contamination. Besides *Medicag*o and *Brassica*, it is noteworthy to recall *Cucurbita maxima* and its capability to take up a significant amount of magnetite nanoparticles from liquid growth medium and to accumulate them within roots and leaves [12].

Atmospheric particulate matter deposition on the leaves leads to remarkable alteration in the transpiration rates, thermal balance and photosynthesis. The structural features of leaf of *Byrsonima sericea* and *Psidium guineense* such peltate trichomes and hypodermis probably formed a barrier reducing the penetration of metal ions into the mesophyll as observed by the lower iron leaf content and iron accumulation in trichomes. [51] analysed the uptake of Palladium (Pd) by *Hordeum vulgare* and the behaviour of Pd nanoparticles in nutrient solutions used to grow plants. Smaller and larger Pd particles were comparatively assessed and the results showed that Pd uptake, via the roots, depends on its particle diameter. Smaller Pd particles cause stress effects in leaves at low concentration in nutrient solution. The *Cucurbita maxima* growing in an aqueous medium containing magnetite nanoparticles can absorb, move and accumulate the particles in the plant tissues, on the contrary *Phaseolus limensis* is not able to absorb and move particles [12]. Therefore different plants have different response to the same nanoparticles.

The change on the cytoplasm of the cells was accompanied by the fact that the cell-to-cell movement of the particles in regions with a high density of aggregates seems to direct them to the exterior of the organism, what points to a physiological response from the plant to the intracellular presence of nanoparticle aggregates. Despite the obvious advance that supposes the possibility of the application of nanoparticles on agronomical applications, it is also clear that there are many aspects in the protocols for detection of the nanoparticles and for their infiltration into the plant than can be improved [47]. The distribution of nanoparticles is still very limited, and most of the intracellular translocation of nanoparticles takes place near the application point, and much more efficiently when it is effectuated by injection. The most interesting way of application, the pulverization, has given only very limited results, with presence of intracellular nanoparticles but just in the first cellular layer, the epidermis. The long-range transport has still a low efficiency.

IV. PHYTOTOXIC EFFECT OF NANOPARTICLES IN PLANTS

The nanoparticles in plants caused a severe effect on seed germination; plant biomass (Fig 2) and root shoot elongation. High concentration nanoparticle stock may enhance agglomeration, which in turn lowers the concentration of "free" nanoparticles [52].

The phytotoxicity of five types of nanoparticles, namely multi-wall carbon nanotubes (MWCNT), metallic nanoparticles made from aluminum and zinc, and metal oxides (Al_2O_3 and ZnO) with six different plant species, namely radish, rape, ryegrass, lettuce, corn and cucumber [13]. The germination rate was only affected by Zn and ZnO-particles, and only in rye grass and corn, respectively. Effect of phytotoxicity of nanoparticles on seed germination and root growth in cultivable plants studied earlier [53].

Nano-TiO_2 [anatase] improved plant growth by enhanced nitrogen metabolism (Yang et al., 2006) that promotes the absorption of nitrate in spinach and, accelerating conversion of inorganic nitrogen into organic nitrogen, thereby increasing the fresh weights and dry weights. The zinc oxide NPs affected both the cellular and the chromosomal facets; this indicated their seriously hazardous nature. Nano-sized cobalt and zinc oxide particles are able to permeate *A. cepa* roots and affect the roots' elongation, metabolism, and genetic materials. Cobalt oxide NPs could block the water channels through adsorption and the zinc oxide NPs possibly penetrate

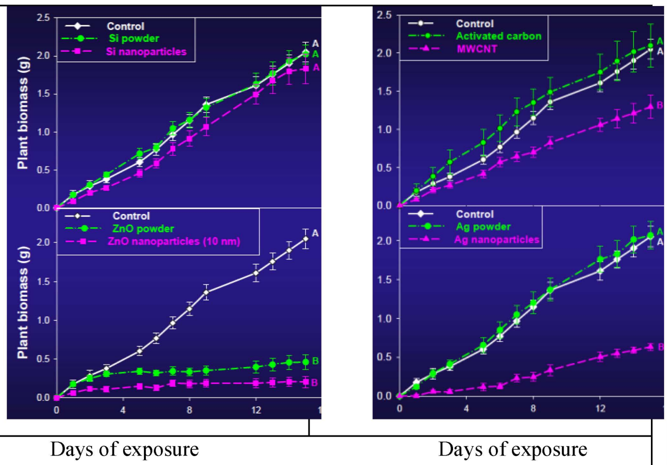

Figure 2. Effect of Si or Si nanoparticles (left top), ZnO powder or ZnO nanoparticles (left bottom), activated carbon or MWCNTs (right top), Ag powder or nanoparticles (right bottom) on zucchini biomass under hydroponic conditions (Picture reproduced from [15].

radically into onion roots and spoil the whole cellular metabolism and stages of cell division (Fig 3).

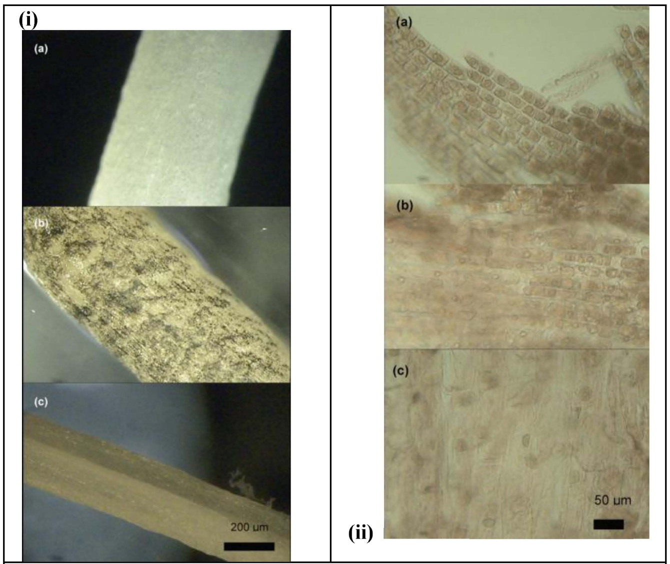

Figure 3. (i) Adsorption, morphological phenotype and (ii) Microscopic cellular phenotype of A. cepa roots: without NPs (a), with 5 gml^{-1} cobalt oxide (b) and with 5 gml^{-1} zinc oxide NPs (c). (Picture reproduced from [8]).

In addition, the zinc oxide NPs affected both the cellular and the chromosomal facets; this indicated their seriously hazardous nature [8]. So the different flora and fauna responds differently to the nanomaterials and hence, it is necessary to evaluate the safe effective concentration of each group of nanoparticles before their application that reduce the risks of ecotoxicity to a great extend.

Zinc Oxide Nanoparticle on Phytotoxicity

Phytotoxicity of nano-Zn and nano-ZnO was evident. Seed germination of ryegrass and corn was inhibited by nano-Zn and nano-ZnO, respectively. Their suspensions significantly inhibited root growth of corn and practically terminated root development of the other five plant species whose root length at the end of experiment was unable to be accurately measured with a ruler. The root growth was almost halted by seed soaking and incubation in the suspensions of nano-Zn and nano-ZnO. Root growth of radish, rape and ryegrass was nearly terminated under soaking in water, then incubation in suspension, while roots grew relatively well under soaking in suspension, then incubation in water though the root development of the three plant species was significantly inhibited by nano-Zn, and, that of ryegrass by nano-ZnO [56]. Five concentration points of 0, 1, 2, 3 and 4 Zn^{2+} mg/L were made from ZnSO4•7H2O to investigate the phytotoxicity of Zn^{2+}. No significant effect on the root of radish, rape and ryegrass from these Zn^{2+} concentrations was observed [57]. But the yellow and withered shoots at higher Zn^{2+} concentrations indicates that Zn^{2+} might be more toxic to the ryegrass than nano-ZnO (Fig 4). It was concluded, the phytotoxicity of nano-ZnO could not mainly come from its dissolution at root surface or inside root tissue. Root uptake and phytotoxicity of ZnO nanoparticles was also reported by [57]. The toxicity of ZnO nanoparticles and Zn^{2+} ions to the ryegrass seedlings were evident and increased with increasing concentration of both ZnO nanoparticles and Zn2+, which could be easily observed by visual examination.

The mechanism of the nanotoxic effects of zinc and zinc oxide nanoparticles in their experiments also remains unknown. "It may be attributed to two different modes of action": 1) a chemical toxicity based on the chemical composition, e.g., release of (toxic) ions; and 2) stress or stimuli caused by the surface, size and/or shape of the particles (Fig 4). Solubility of oxide nanoparticles greatly affected the cell culture response.

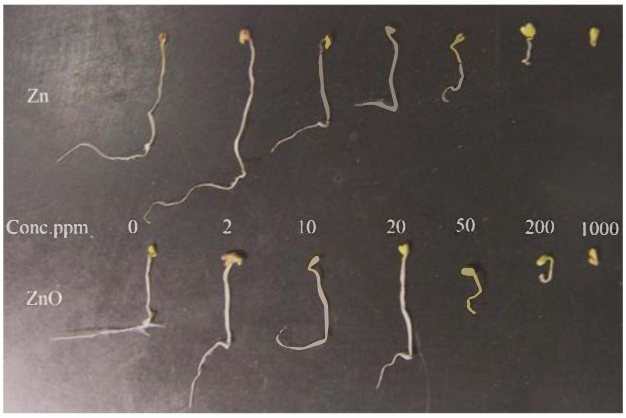

Figure 4. Root growth inhibition of rape seeds by zinc and zinc oxide nanoparticles. (Image: Dr. Baoshan Xing/University of Massachusetts)

In summary of ZnO nanoparticles were found able to concentrate in the rhizosphere, enter the root cells, and inhibit seedling growth; the phytotoxicity of ZnO nanoparticles could not primarily come from their dissolution in the bulk nutrient solution or the rhizosphere.

Carbon Nanoparticles on Phytotoxicity

Two forms of carbon nanoparticles are, CNTs (Carbon nano Tubes) and graphene, have similar chemical composition and crystalline structure. Based on this fact, a reasonable inference is that the two would be similar in many ways, such as biological activity. There is evidence that CNTs could translocate to systemic sites (roots, leaves, and fruits) and engage in a strong interaction with the cells of tomato seedlings, resulting in significant changes in total gene expression in roots, leaves, and fruits [58] and exerting toxic effects [58, 59].

The effect of four carbon materials (single-, multiwall CNTs, few-layer graphene materials and activated carbon) on the seedling stage of tomato at 50 µg⁄mL. Under such low-concentration conditions, metals play an essential role in the development of plants at the limit of plant tolerance. If an excess of metals is absorbed by plants, toxic effects can manifest, including growth reduction and abnormalities in cell division [60], possibly because of the excess metals acting as cofactors for enzymes involved in the formation of intermediate metabolites. In zucchini plants, no negative effects were observed on seed germination and root elongation in the tested range of MWCNTs whereas a decrease in the biomass of plants was observed during further growth in the presence of SWCNTs [61]. Hence the response of plants to nanomaterials varies with the type of plant species, their growth stages and the nature of nanomaterials since the studies showed contradictory effects of even the same nanomaterial in different plants at different developmental stages. Sayes and his worker in lab *et al*., (2006) carbon nanoparticle functionalization led to a remarkable decline in toxic effects.

CNTs are known to have phytotoxic effects in plant cells because of aggregation [61] causing cell death and accumulated ROS in a dose-dependent manner [62]. Graphene may have the ability to generate ROS production, based on the similarities of some of the properties of graphene sheets and CNTs. Intracellular ROS might have a crucial role in induction of cell death induced by graphene. It has been reported that the accumulation of ROS causes cell death, which is demonstrated by electrolyte leakage from cells [63]. The root surface area of cabbage was significantly increased by graphene treatment, and it may be that an excess of graphene resulted in swelling in *Origanum vulgare* by [64] and in cowpea by [65]. CNTs are also known to have phytotoxic effects in plant cells because of aggregation [61], causing cell death and accumulated ROS in a dose-dependent manner [62]. There is evidence that CNTs could translocate to systemic sites (roots, leaves, and fruits) and engage in a strong interaction with the cells of tomato seedlings, resulting in significant changes in total gene expression in roots, leaves, and fruits [6] and exerting toxic effects [7]. Considering these aspects, unexpected to find toxic effects of graphene on terrestrial plant species, in tomato, cabbage, and red spinach. The similar growth pattern was observed in tomato, cabbage, and red spinach by using graphene nano particles (Fig 5). At a higher graphene concentration (1000 mg/L), the root hair growth of cabbage and red spinach was decreased compared to control plants.

Concentration of graphene (G) in growth medium

Figure 4. Effect of graphene (G) on growth and development of cabbage, tomato, and red spinach seedlings. Tomato, Cabbage and Red spinach seedlings were hydroponically grown in Hoagland media for 20 days with and without graphene with concentration (0, 500, 1000, 2000 mg/L), respectively (Picture reproduced from [66]).

Overproduction of ROS induced by graphene could be responsible for significant plant growth inhibition and biomass reduction [4] reported that the production of ROS could be a key factor in the toxicological effects of nanostructure materials. Observation of accumulation ROS production by means of H_2O_2 visualization along with visible signs of necrotic damage lesions and evidence of a massive electrolyte leakage all indicated an oxidative stress mechanism mediated through the necrotic pathway, which requires further study. Evaluation of graphene toxicity to targets on terrestrial plant species and applying a prolonged exposure period with different concentrations to measure any potential risk.

Silver Nanoparticles

The toxic effect of silver nanoparticles inside the cellular mechanisms of rice crop revealed that silver nano particle causes severe damage in metabolism. Slight variations in intensity of *Oryza sativa* roots confirm involvement of functional group such as carboxyl, Amine, hydroxyl etc in nanoparticle binding. Transmission Electron Microscope (TEM) images of rice roots revealed that various particle sizes deposited inside the root cells. The penetrations of particles inside the roots cell, that damaged the cell wall as well as vacuoles to enter. It was due to the penetrations of large particles entering through small pores of cell walls [67]. Silver NPs have been reported to disrupt the root tip cells of *A. cepa* and thus damage the cell division process by causing the formation of chromatin bridge, stickiness, and cell disintegration [68].

Gold Nanoparticle on Phytotoxicity

Potential applications of gold nanoparticles in biomedicine include chemical sensing and imaging applications [69,70]. While bulk gold has been deemed "safe", nanoscale particles of gold need to be examined for biocompatibility and environmental impact if they are to be manufactured on a large scale for *in vivo* usage [71,72]. Several groups have examined the cellular uptake and cellular toxicity (cytotoxicity) of gold nanoparticles.

Studies on the seed germination and root growth of zucchini plants in hydroponic solution amended with Ag NPs showed no negative effects whereas a decrease in plant biomass and transpiration was observed on prolonging their growth in presence of Ag NPs [15]. The cytotoxic and genotoxic impacts of Ag NPs were studied using root tips of onion. Results of Ag NPs impaired the stages of cell division and caused cell disintegration [68]. All such studies throw light to the need for a more cytotoxic and genotoxic evaluations by considering the properties of nanoparticles, their uptake, translocation and distribution in different plant tissues.

Alumina Nanoparticle on Phytotoxicity

The first report of negative effects of nanoparticles on plants at relatively low dosage was by [73]. Phytotoxicity of uncoated and phenanthrene-coated nanoscale alumina and concluded that uncoated alumina particles in 2 mg L^{-1} concentration inhibited the root elongation of corn, cucumber, soybean, cabbage and carrot. It was commented that the toxic effect might not be nanospecific but as well be due to dissolution of aluminium [74].

Effects of submicron alumina particles were investigated to evaluate the chemical material of Al_2O_3 can impose toxic effect on the seedling root growth. Reports of negative effects, ("Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles") that showed that aluminum oxide nanoparticles, commonly found in everything from cosmetics to environmental catalysts that reduce pollution, can stunt root growth in five plant species (corn, cucumber, soybean, carrot and cabbage), although preliminary findings suggest extremely high concentrations of such particles are necessary for such damage. The presence of nano alumin particles did not have a negative effect on the growth of *Phaseolus vulgaris* and *Lolium perenne* in the tested concentration range. Aluminum concentration in rye grass was increased 2.5-fold above control tests whereas no uptake of aluminum was observed in kidney beans which inferred the difference in uptake and distribution efficiency of even the same kind of nanoparticles by different plants.

Titanium dioxide (TiO_2) Nanoparticle on Phytotoxicity

The Titanium dioxide nanoparticles may produce reactive oxygen species upon interaction with the organisms or with ultraviolet radiation [75]. The acute toxic effects of manufactured TiO_2 nanoparticles were low. The effects did not follow a clear dose-effect relationship, probably due to the formation of aggregates and subsequent sedimentation. The aggregates were similar in size for both diameters, but more aggregates were formed with the larger particles, and in the presence of Willow tree roots. Genomic DNA quantification was performed in root tips after seven days of treatment in cucumber (*Cucumis sativus*) showed that, plants treated at the highest TiO_2 NP concentrations (2000 and 4000 mg/L) had a decrease in genomic DNA compared to controls. The toxic effect of TiO_2 nanoparticles is probably not given by the released titanium ions from TiO_2 particles, which was experimentally proved by limited dissolution of Ti from a TiO_2 sample [9]. According to the literature review, the exact mechanism of the toxic effect of TiO_2 nanoparticles is still unknown.

Magnetic Nanoparticle on Phytotoxicity

The influence of magnetic nanoparticles coated with tetramethylammonium hydroxide (TMA-OH) (as a stabilizing agent) on the growth of maize plants in early growth stages was studied [76]. 'Chlorophyll a' level was increased at low ferrofluid concentrations while at higher concentrations it was inhibited. The water based magnetic fluid obtained by coating magnetic nanoparticles with perchloric acid was added to germinated maize seeds [77]. A slight inhibitory effect was observed on the growth of the plantlets that led to brown spots on leaves at higher volume fractions of magnetic fluid. The excess iron treatment generated some oxidative stress, which in turn affected photosynthesis and led to decreased metabolic process rates. The oxidative stress induced by the ferrofluid concentration on living plant tissues [78]. Exposure to electromagnetic field produced some local heating on the applied region due to the electromagnetic field energy absorbed by internalized magnetic nanoparticles in plant tissues that in turn affect the redox reactions involved in photosynthesis process.

Copper Nanoparticle on Phytotoxicity

The toxicity and bioavailability of copper nanoparticles to the plants *Phaseolus radiatus* and *Triticum aestivum* employing plant agar test as growth substrate for homogeneous exposure of nanoparticles. The growth rates of both plants were inhibited and as result of exposure to nanoparticles and the seedling lengths of tested species were negatively related to the exposure concentration of nanoparticles. Wheat crop showed a greater accumulation of Cu NSPs in its roots due to root morphology. Bioavailability was estimated by calculating the bioaccumulation factor defined as Cu NSPs concentration in the plants divided by the Cu NSPs concentration in the growth media. Growth inhibition of a seedling exposed to different concentrations of Cu nanoparticles on *Phaseolus radiatus* was more sensitive than *T. aestivum*. A cupric ion released from Cu nanoparticles had negligible effects in the concentration ranges of the present study, and the apparent toxicity clearly resulted from Cu nanoparticles. Bioaccumulation increased with increasing concentration of Cu nanoparticles, and agglomeration of particles [79].

Bioaccumulation of NPs increased with its concentration in growth media and their bioavailability to the test plants was estimated by calculating the bioaccumulation factor. This study demonstrated plant agar test as a good protocol to test phytotoxicity of nanoparticles, which is hardly water-soluble. Also, studies on the effects of Cu NPs on the growth of zucchini plants showed reduced length of emerging roots [7]. However, the germination of lettuce seeds in the presence of Cu NPs showed an increase in shoot to root ratio compared to control plants [37].

The effect of nano particle toxicity to the plants and food crops are evident. The clear impact on crop growth, root length, shoot length, biomass accumulation, germination are observed from the previous experimental results (Table 2). The residue analysis of crop plants or food products on nano particle contamination before the commercialization of technology might increase the toxicity to the plant and human community as well as hazard to environment also [80].

Table 2. Phytotoxic effect of nano particles in crops

Nanoparti-cle	Crop	Phytotoxic Effects Observed	Reference
Zinc (Zn^{2+} & ZnO)	Radish, Rapegrass & Ryegrass	Inhibition of root growth and Decrease in shoot biomass	[57]
	Onion	Affect cellular metabolism - cell division	[8]
Gold (Ag)	Zucchini	Cellular toxicity, Decrease in plant biomass and Transpiration	[15]
Silver	Onion	Cytotoxic & Genotoxic impacts in root tip	[68]
	Rice	Severe damage in metabolism, Damaged cell wall and vacuoles	[67]
Carbon Nano Tubes (CNT)	Tomato	Accumulation of ROS causes cell death, Change in gene expression in roots, leaves and fruits	[58,59]
Nanoparti-cle	**Crop**	**Phytotoxic Effects Observed**	**Reference**
Graphene	Tomato, Cabbage & Spinach	Plant growth inhibition and biomass reduction	[4,66]
Alumina	Cow pea	Swelling of roots	[65]
Titanium di Oxide (TiO_2)	Corn, Soybean, Cabbage & Carrot	Inhibited the root elongation	[74]
	Cucumber	Decrease in genomic DNA content	[9]
Copper (Cu)	Wheat, onion	Inhibition of growth rates, Greater accumulation of NSPs in root	[8,79]
	Mung bean	Inhibition of growth rates	[8]
Magnetic	Zucchini	Reduced length of emerging roots	[7]
	Lettuce	Increase in shoot : root ratio	[37]
Magnetic	Maize	Oxidative stress in leaf cells, Reduced photosynthesis led to decreased metabolic rates and Decreased chlorophyll a content	[77]

Based on the analysis the nanoparticles were having wider area of opportunities in agricultural application in future research studies. The phytotoxicity of nano particle impact in crops still unclear to scientists. So further research on nano phytotoxicity should be considered, because we depend food for agricultural crops. So to reduce the effect of nano material in crop plants, prior invitro analysis on nano particle toxicity reduces the phytotoxicity effect to crops [81]. So the standardization of correct dose / recommendation of each nano material at varying concentration and its physiological, biochemical aspects on agricultural crops should be studied were used to reduce the phytotoxicity to the sustainable safe environment and sustainable for second green revolution in the future.

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