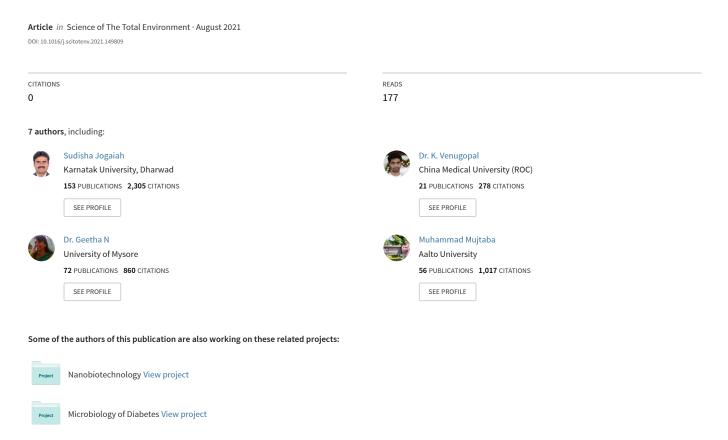
# Phytotoxicological effects of engineered nanoparticles: An emerging nanotoxicology



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# Phytotoxicological effects of engineered nanoparticles: An emerging nanotoxicology



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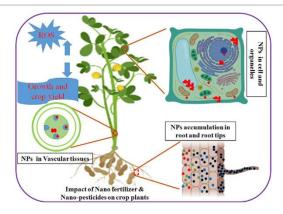
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#### HIGHLIGHTS

#### Metal based 3D nanostructures are widely used as photocatalysts in modern agriculture.

- Cellular magnifications of metal-based NPs induce cytotoxicity via over production of ROS.
- Carbon nanomaterials are strong adsorbents or nanocarriers of pesticides and fertilizers
- ENPs can penetrate cell membranes and interfere with mitochondria electron transport.
- ENPs can alter the DNA sequence (lesions) that lead to changes in functional mRNA.

#### GRAPHICAL ABSTRACT



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#### ABSTRACT

Recent innovations in the field of nanoscience and technology and its proficiency as a part of inter-disciplinary science has set an eclectic display in innumerable branches of science, a majority in aliened health science of human and agriculture. Modern agricultural practices have been shifting towards the implementation of nanotechnology-based solutions to combat various emerging problems ranging from safe delivery of nutrients to sustainable approaches for plant protection. In these processes, engineered nanoparticles (ENPs) are widely used as nanocarriers (to deliver nutrients and pesticides) due to their high permeability, efficacy, biocompatibility, and biodegradability properties. Even though the constructive nature of nanoparticles (NPs), nanomaterials (NMs), and other modified or ENPs towards sustainable development in agriculture is referenced, the darker side i.e., eco-toxicological effects is still not covered to a larger extent. The overwhelming usage of these trending NMs has led to continuous persistence in the ecosystem, and their interface with the biotic and abiotic community, degradation lanes and intervention, which might lead to certain beneficial or malefic effects. Metal oxide NPs and polymeric NPs (Alginate, chitosan, and polyethylene glycol) are the most used ENPs, which are posing the nature of beneficial as well as environmentally concerning hazardous materials depending upon their fate and persistence in the ecosystem. The cautious usage of NMs in a scientific way is most essential to harness beneficial

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aspects of NMs in the field of agriculture whilst minimizing the eco-toxicological effects. The current review is focused on the toxicological effects of various NMs on plant physiology and health. It details interactions of plant intracellular components between applied/persistent NMs, which have brought out drastic changes in seed germination, crop productivity, direct and indirect interaction at the enzymatic as well as nuclear levels. In conclusion, ENPs can pose as genotoxicants that may alter the plant phenotype if not administered appropriately.

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#### 1. Introduction

Nanoparticles (NPs) are materials, which can occur naturally in the form of minerals, clay, and products from microbes (Rastogi et al., 2017). The reactivity of NPs with biomolecules is influenced by several factors like particle size, shape, purity, solubility, surface polarity, core composition, solubility, and stability as well as manufacturing methods (Teske and Detweiler, 2015; Wang et al., 2016; Geetha et al., 2021). However, in the last few decades, only the engineered nanoparticles (ENPs) were acknowledged as a material with two measurements ranging from 1 nm to 100 nm (Ma et al., 2010; Maurer-Jones et al., 2013). ENPs have been widely used as a carrier of agrochemicals like pesticides (Kong et al., 2021), fertilizers (Bratovcic et al., 2021; Seleiman et al., 2021), fungicides which can act as anti-microbial agents that protects crop plants from phytopathogenic agents (Jogaiah et al., 2007; El Hadrami et al., 2010; Divya et al., 2017). Engineered nanoparticles (ENPs) are divided into zero-valent metals or metallic NPs (ZVi/ MNPs), carbon nanotubes (CNTs), carbon quantum dots (CQDs), polymeric NPs (PNPs), mesoporous NPs and scaffold NPs (Fig. 1) (Handy et al., 2008).

Zero-valent iron (Zvi) NPs possesses a large surface area and reactivity. Zvi NP's are popularly used in environmental applications such as remediation (Ponder et al., 2000; Ponder et al., 2001). Nanoscale iron particles are effectively used for the transformation and detoxification of chlorinated organic solvents, organochlorine, and polychlorinated pesticides (Zhang, 2003). Metal oxide NPs like titanium dioxide (TiO<sub>2</sub>), zinc oxide (ZnO) are important for heterogeneous catalysis (Theerthagiri et al., 2019). Zeolite has a 3D crystalline microporous alumino-silicates used as a natural adsorbent to remove excess pesticide and also for the slow release of nutrients and fertilizers in agriculture (Tsintskaladze et al., 2016; dos Santos Pereira et al., 2021). Nanoceria, a water-based synthetic nanocrystalline CeO<sub>2</sub>, used for the

degradation of toxic organophosphate pesticides (chemical warfare) e.g. parathion methyl (Janoš et al., 2016; Tolasz et al., 2020). Recently, metal containing organic frameworks (MOFs) is used in photocatalytic and sonophotocatalytic reactions for organic pollutants (e.g. pesticides) removal from the environment (Theerthagiri et al., 2020; Theerthagiri et al., 2021).

Carbon nanomaterials (CNMs) include activated carbon (AC), graphene or graphene oxide (GO), carbon nanotubes (CNTs), carbon nano-onions (CNOs), fullerenes, fullerols, carbon quantum dots (CQDs), and carbon nanohorns (CNHs). AC and GO are well known adsorbents widely used to trap organic pollutants (e.g. pesticides removal resins) or inorganic pollutants (e.g. toxic metal trappers) from air, aqueous and solid phase. In addition, they have been used as nanocarriers of pesticides or fertilizers in agriculture (Saxena et al., 2020). Graphenebased photocatalysts have been developed and effectively used in the degradation of environmental pollutants. For example the synthetic 3D graphene-based TiO2-AgPO4 materials have strong capacity of adsorption and photocatalytic degradation of pesticides and dyes (Nyankson et al., 2021). Generally, CQDs/QDs are one of the most promising core ENPs (Fig. 1), which controls the optical properties and protects them from oxidation (Dabbousi et al., 1997). CQDs are water-soluble, chemically inert, less toxic, and show good biocompatibility. CQDs can be prepared from bio-waste materials at a low cost (Krishna et al., 2021) and can be used as fertilizer (Peralta-Videa et al., 2020). For example, Romaine lettuce was hydroponically cultivated at different concentrations of pollen-derived CDs that enhanced crop yield (Zheng et al., 2017). CDs are fluoroscence, and optically active elements and exhibiting photocatalytic activity with the ability to transform the alcohol pollutants (e.g. Benzyle alcohol to benzaldehyde) to their corresponding aldehyde forms (Li et al., 2014). In fact, metal oxides and CQDs both produce photocatalytic activity, but in the case of hybrid composite of these two NPs photocatalytic activity is known to

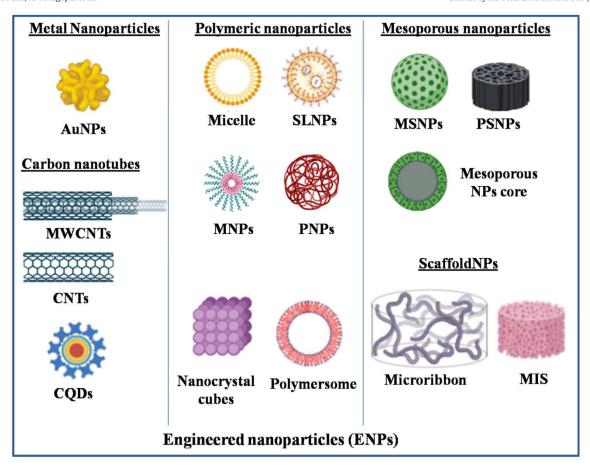


Fig. 1. Structure and shapes of different types of nanoparticles.

increase (e.g. in CQDs/TiO $_2$  and CQDs/ZnO composites photocatalytic activities of TiO $_2$  and ZnO) by 12.7 times and nine times, respectively (Deng et al., 2021; Maddu et al., 2021). In modern agriculture, optically active CDs are widely used for screening and quatification of pesticides pollution as well as other environmental pollutants (Liu et al., 2020; Tafreshi et al., 2020).

Unlike CQDs, carbon nanotubes (CNTs) are also promising NMs widely used in nano sciences and agriculture fields (Bratovcic et al., 2021). They consist of single-walled (SWNTs), surface functionalized single walled CNTs (sfSWCNTs), and multi-walled CNTs (MWCNTs). Carbonaceous nanoparticles or carbon nanotubes (CNTs) consist of fullerenes and nanotubes; which are the most abundant ENPs (Ma et al., 2010). Recently, CNTs-based NMs have been attracted by agricultural chemists and agrochemical industries due to their optical activity, biocompatibility, non-toxicity, biodegradability, and also containing free electrons (Patel et al., 2020). CNTs are hydrophobic in nature, they form strong π-π, hydrogen bonding, electrostatic, and covalent interactions with organic pollutants in both solid and aqueous phases. These strong interactions suggest that CNTs can be used as strong adsorbent (Song et al., 2014). CNTs are water soluble that are used in agriculture for growth stimulation, upon treatment enhance overall growth of common gram plant (Cicer arietinum) (Tripathi et al., 2011). SWCNTs have capability to penetrate plant cell wall and cell membranes of intact plant cells. Due to this penetration, SWCNTs are prominently used as a nano transporters for the delivery of nutrients, pesticides and biomolecules (Liu et al., 2009). MWCNTs have ability to increase growth (up to 55-66% than control) of tobacco cell cultures by the regulation of cell division (CyB) and cell wall extension (NtLRX) genes in tobacco (Khodakovskaya et al., 2012). Presence of CNTs in the soil can induce or stimulates metabolism of plants that promote overall growth (Taha, 2016), biomass, and yield (Kizilbash et al., 2020).

Polymeric nanoparticles (PNPs) are non-toxic, bio-based, and biodegradable, which are considered as superior nanocarriers. Alginate (ALG), chitosan (CS), tripolyphosphate (TPP), and poly (ethylene glycol) methyl ether-block- lactide-co-glycolide (mPEG-PLGA) are some of the commonly used PNPs in different application areas (Campos et al., 2014; Kashyap et al., 2015; Tong et al., 2017; Jogaiah et al., 2020). The ALG/CS and CS/TPP nanocarrier systems containing gibberellic acids (GA<sub>3</sub>), which provided growth enhancement in tomato plant with 4-fold increase in fruit production (Pereira et al., 2019). PNPs are also used as pesticide delivery systems and metolachlor (Tong et al., 2017). For example, the application of chitosan NPs loaded with nitrogen, phosphate, and potassium (Chitosan-NPK) avoiding the direct interaction with soil systems. Wheat plant growth and yields were increased in the chitosan-NPK nano-fertilizer treated as compared to control (Abdel-Aziz, 2019). The biodegradable nanocarriers like micelles and liposomes are lipid-based and natural, which have been recommended for drug delivery studies due to their high biocompatibility (Jarai et al., 2019).

Mesoporous nanoparticles (MSNs) are chemically and thermally stable exhibiting tunable pore size (tunable from 2 to 10 nm in diameter). MSNs have been used as an ideal nanocarrier for various molecules. For example, in the gene gun system, DNA-coated gold microparticles are used as bullets for the bombardment of plant cells and tissues to achieve gene transformation. The honeycomb mesoporous silica NP system can be used to deliver DNA and chemicals into isolated plant cells and intact leaves (Torney et al., 2007). Typically, synthetic polymer and natural biopolymers are used for three-dimensional (3D) scaffold nanomaterial and hydrogel preparation in tissue engineering and regeneration technology (Chocholata et al., 2019; Pina et al., 2019). The plant polysaccharides like starch, cellulose, pectin (Adrian et al., 2019), xylan (Beckers et al., 2020) and lignin (Weiss et al., 2020) are routinely

used as biopolymers for different applications such as the delivery of agrochemicals (Iravani and Varma, 2019).

Plants are primary producers that play an important role in an ecological system (Patlolla et al., 2012). Currently, various ENPs are widely used in the plant sciences to improve crop yield but it brings some defects to the environment (Cañas et al., 2008; Nandini et al., 2020). Releasing a huge amount of ENPs into the environment is an inevitable predicament. The NPs alter the mobility of the plants' cells through physical, chemical, and biological transformations that causes threat to the eco-systems (Lee et al., 2013a). NPs may accumulate in plants to an higher-level and also can enter into the food chain and cause adverse effects in several organisms (Patlolla et al., 2012). Moreover, plant cells interact with ENPs and induce cell disrupts and leads to cytotoxicity (Wang et al., 2008). NPs induce phytotoxic, cytotoxic, and genotoxic defects in plants led to decreased plant growth, seedling growth rate, slow germination, and root elongation (Wang et al., 2012).

Phytotoxicity alludes to abandons in plant growth, seed germination, and root extension (Brunner et al., 2006). Genotoxicity in plants can initiate harm to the hereditary material and can prompt mutagenicity and cancer-causing nature (Kang et al., 2008). Hence, the researchers have many questions in their minds about the risks and benefits of NPs (Raskar and Lawre, 2013). This review is meant to collect the phytotoxic effects of various ENPs that have been proposed as effective agrochemical delivery systems.

# 2. Current status of nanoparticles application in the agriculture sector

The agriculture sector is facing many challenges such as rapid changes in climate (i.e. drought stress cold stress, floods etc.), soil erosion, reduced soil fertility, nutrients (i.e. macro and microelements) deficiency, overuses of synthetic chemical fertilizer and pesticides and

load of heavy metals in the soil (Pandey, 2018). To overcome these problems, nanotechnology has been contributed in developing sustainable agriculture techniques to improve crop yield and restoration of soil fertility or quality (Parisi et al., 2015; Usman et al., 2020; Bhavya et al., 2021). Nanotechnology is a new tool that has been practiced in agrofood sectors to enhance quality and crop yield at low cost. For example, controlled delivery of synthetic nano pesticides or nano fertilizers composites, transport of genetic material and developing nano biosensors for rapid detection of pathogens, and other biotic and abiotic stress facts (Acharya and Pal, 2020; Singh and Sengar, 2020). In the agriculture sector, NP-based nutrients delivery methods (Fig. 2) boost the crop production and reduces the wastage of fertilizers as well as decrease synthetic chemicals contamination in soil, air and aquatic environments (Fellet et al., 2021). Currently, research and development (R&D) on NPs have been increased throughout the globe. Out of 195 countries in the worldwide, India has been contributed a high percentage of R&D activities on NPs and their applications in the agriculture sector (Fig. 3).

#### 3. Phytotoxicity of nanoparticles (NPs)

Even though the application of NMs and NPs are vast for agriculture purpose, type of materials used, the size, time interval and mode of application and other factors of NMs/NPs are contributing towards various adverse toxic effects on plants ranging from disturbance in cell cycle, nucleotide damage, early growth of seedlings, growth inhibition, activation of stress induced signaling pathway, etc. (Cañas et al., 2008; Hao et al., 2018). Various types of NPs have been proposed as nutrient and pesticide delivery agents in plants. Phytotoxicity includes inhibition of germination, changes in root and shoots biomass growth, ROS generation leading to oxidative stress and structural development of the plant tissues. The various effects of NMs or NPs on plants physiology has been summarized (Table 1). AgNPs have been the most studied

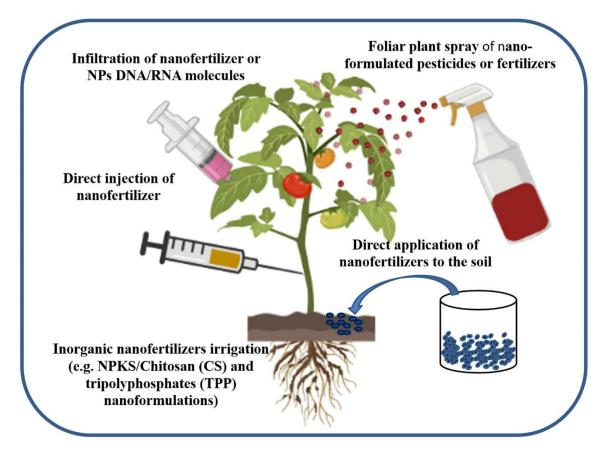


Fig. 2. Pictorial representation of different methods for the delivery of nano pesticides or nano fertilizers to the soil or crop plants.

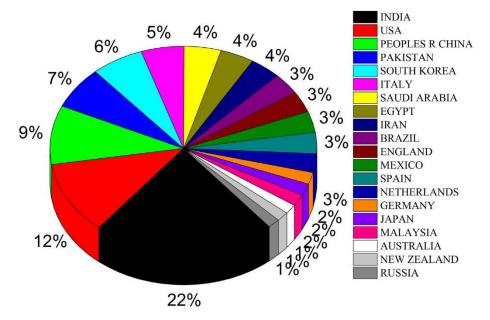


Fig. 3. Global research and developmental activities on NPs synthesis and application of NPs in the agriculture sectors world-wide (Data source web of science from 2010 to 2021).

NPs and their genotoxicity, phytotoxicity has been observed in *Vicia faba* (Patlolla et al., 2012), *Phaseolus radiates* (Lee et al., 2012), Soybean and rice (Li et al., 2017). The effect of AgNPs on stress quantity and distribution in *Egeria densa* and *Junus effuses* (Yuan et al., 2018) and tobacco seedlings (Sabo-Attwood et al., 2012) have been reported. Similarly, the effects of other NPs like TiO<sub>2</sub>NPs on onion seed germination (Raskar and Lawre, 2013) and *Nicotiana tabacum* (Ghosh et al., 2010). Moreover, the negative impacts of different NMs are

presented in (Fig. 4). Besides, the stage-specific effects of NPs on plants are discussed below.

#### 3.1. Metal-containing nanoparticles

#### 3.1.1. Silver nanoparticles (AgNPs)

Nanoparticles (NPs) have impressive considerations of late because of their variety of properties and applications in biotechnology (Patlolla

Table 1
Effects of nanomaterials (NMs) or nanoparticles (NPs) on plant physiology.

S. no	Nanoparticle	Plant name	Toxicity	Effects	References
1	AgNPs	Vicia faba	Genotoxicity	Impairing the stages of cell division	(Patlolla et al., 2012)
2	AgNPs	Phaseolus radiatus, Sorghum bicolor	Phytotoxicity	Affect seedling growth	(Lee et al., 2012)
3	AgNPs	Arabidopsis thaliana	Accumulation, and Phytotoxicity	Dose-dependent germination	(Geisler-Lee et al., 2013)
4	AgNPs	Egeria densa, Juncus effuses	Stress quantity	Inducing stress on the plant	(Yuan et al., 2018)
5	AgNPs	Soybean, rice	Accumulation and Phytotoxicity	Inhibit the growth rate	(Li et al., 2017)
6	AgNPs	Tobacco seedlings	Distribution and toxicity	Aggregation occurs within the root	(Sabo-Attwood et al., 2012)
7	TiO <sub>2</sub> NPs	Onion seeds	Seed germination and early seedling growth	More than 40 micro gm/L affect seedling growth	(Raskar and Lawre, 2013)
8	Single-walled fCNT and CNT	Cabbage, Carrot, Cucumber, Lettuce, Onion and Tomato	Root elongation and phytotoxicity	Crop species were affected by both f CNT and CNT except cabbage and carrot	(Cañas et al., 2008)
9	Metal oxide NPs and metal ions (Zn, Cu, Ce)	Carrot	Accumulation of metals	Dietary intake of metal components leads to chronic toxicity	(Ebbs et al., 2016)
10	CuO <sub>2</sub> NPs	Radish, Perennial ryegrass, Annual rye-grass	Damage of DNA	Affect plant viability	(Atha et al., 2012)
11	CuO <sub>2</sub>	Elsholtzia slplendens	Phytotoxicity and accumulation	Dose-dependent bioaccumulation	(Shi et al., 2014)
12	MWCNT, Ag, Cu, ZnO, Si	Cucurbita pepo	Phytotoxicity	Decrease biomass and transpiration	(Hawthorne et al., 2012)
13	CeO <sub>2</sub> NPs	Brassica rapa	Physiological and Biochemical response	Affect the size and growth stages	(Ma et al., 2016)
14	ZnO and CuO	Buckwheat (Fagopyrum esculentum)	Genotoxicity	Affect seedling growth	(Lee et al., 2013b)
15	AuNPs	Aquatic microsomes	Aquatic system	AuNPs affects phytotoxicity on aquatic system	(Ostroumov et al., 2014)
16	CeO <sub>2</sub> , TiO <sub>2</sub>	Hordeum vulgare	Phytotoxicity and Genotoxicity	Shortage of root elongation	(Mattiello et al., 2015)
17	TiO <sub>2</sub>	Allium cepa, Nicotiana tabacum	Genotoxicity	DNA damage	(Ghosh et al., 2010)
18	LacCo <sub>3</sub>	Cucumber	Phytotoxicity and Biotransformation	Accumulation of La on-cucumber roots	(Ma et al., 2011)
19	MWCNT, Aluminium, Alumina, Zinc, Zinc oxide	Radish, grape, Corn, ryegrass, Lettuce, Cucumber	Phytotoxicity	The plants mediate dose- dependent response on NPs	(Lin and Xing, 2007)
20	Mesoporous carbon NPs	Oryza sativa	Phytotoxicity	Dose-dependent phytotoxicity	(Hao et al., 2018)

Action	Stage 1 Seedling	Stage 2 Germination	Stage 3 plant Growth
Free from NPs		7	
Exposure with NPs			
Accumulation of NPs			

Fig. 4. Impact of nanoparticles (NPs) on various stages of plant growth.

et al., 2012). Despite the fast advancements and an early acknowledgement of nanobiotechnology, the likely unfavourable impacts in people and non-people biota and the biological system are yet to be completely investigated (Chen and Schluesener, 2008). In recent years, AgNPs are most commonly used, particularly in medical wound dressing and food services. They have been attracting much interest due to their antimicrobial activities like anti-bacterial and biocidal properties (Kumari et al., 2009). The bioaccumulation and phytotoxicity of AgNPs on rice were investigated by (Nair and Chung, 2014a). In rice (Oryza sativa L), a significant reduction in root elongation of the seedling, shoot and the chlorophyll, carotenoids levels were decreased when exposed to 0.5 mg/L and 1 mg/L of AgNPs for one week. Moreover, seedlings exposed to 0.5 mg/L of AgNPs significantly increased the production of H<sub>2</sub>O<sub>2</sub> in both shoots and roots. Reactive oxygen species (ROS) levels were also increased, which leads to cell death in root tips (Nair and Chung, 2014a). The size distribution and bioaccumulation tests of the AgNPs confirm that the Ag-containing particles were accumulated in the plant's roots, and this inhibits the seedling growth of the plants (Patlolla et al., 2012; Stegemeier et al., 2015). These results explain that AgNPs express the defective scenarios in the environment (Li et al., 2017). AgNPs may enter plant frameworks, and many with intracellular parts impeding the phases of the cell division (Arora et al., 2008). AgNPs could interact with the intracellular parts of the plant and lead to cell damage, water imbalance, and decreased photosynthesis (Cekic et al., 2017; Moteriya and Chanda, 2017; Abdelsalam et al., 2018). There was an increase in the frequencies of chromosomal aberrations (Patlolla et al., 2012) and micronucleus induction in the root-tip of Vicia faba that are widely used for monitoring air pollution (Vannini et al., 2014) and for screening environmental chemicals for their genotoxic effects (Patlolla et al., 2012).

Arabidopsis thaliana is a small and long-day flowering plant having a short life cycle of 6-8 days being a well-defined model organism in plant biology studies (Ke et al., 2018). AgNPs are not affected by the seed germination of Zucchini (Stampoulis et al., 2009), ryegrass, barley and flax (El-Temsah and Joner, 2012). Similarly, the seed germination of *A. thaliana* was also not affected by AgNPs in hydroponic media, but AgNPs treated seedlings had shorter roots than control and the tips of their roots became brown colored in all experimental concentrations of AgNPs rather than Ag<sup>+</sup> treated plants (Geisler-Lee et al., 2013). Lee assessed the effect of citrate-balanced out AgNPs at three levels: physiological phytotoxicity, sub-cell transport and cell gathering in *A. thaliana*.

The root elongation was reduced for the plants exposed to AgNPs as compared to Ag+. The transport of AgNPs in root tip cells and intracellular transport of AgNPs was observed in the 1-2 mm of a root tip in both individual and aggregated clumps (Geisler-Lee et al., 2013). Phytotoxicity revealed that the seed germination in the hydroponic conditions was not affected compared to the untreated controls (Wiechers and Musee, 2010). Accumulation of AgNPs in *A. thaliana* seedlings shows the absorption of AgNPs in particle or ion form (Moteriya and Chanda, 2017).

The crop plants strongly react with their terrestrial environments and atmospheric conditions and are expected to exert adverse effects during exposure to NPs (Lee et al., 2012). AgNPs have a significant effect on plant's yield, for example, *Phaseolus radiatus* and *Sorghum bicolor* productivity decreased (Salama, 2012; Almutairi and Alharbi, 2015). The growth-dependent establishment in nanotoxicology reveals that NPs were well dispersed in the test medium. The seedling development of test species was unfavorably influenced by an introduction to AgNPs (Lin and Xing, 2007). The soil study denotes that the growth rate of the crop plants was not affected by impediment within the concentrations tested in comparison to differed agar dissolved silver ion effects. This assessment has been ascribed to the diminished toxicity of AgNPs to the plants in the soil medium (Lee et al., 2012).

The AgNPs induce increased chromosomal aberrations, oxidative damage, and decreased seed germination during the over-exposure period (Yuan et al., 2018). The phytotoxicity of AgNPs to the aquatic plants such as *Egeria densa* and *Juncus effuse* were investigated. Measuring the physiological and enzymatic responses to exposure with AgNPs was assessed using peroxidase activity, superoxide dismutase, malondialdehyde enzyme activities, and chlorophyll content (Bao et al., 2016). It was revealed that the AgNPs induce biochemical stress and the enzymatic responses on the aquatic plants. The test results showed that the ENPs induced enzymatic stress response to submerged macrophytes (Liu et al., 2018).

#### 3.1.2. Copper oxide nanoparticles (CuONPs)

CuONPs are used in various applications such as in ceramics, bioactive coatings, air and liquid filtrations, skincare products, inks and lubricant oils as wells electronic. The bioaccumulation and translocation of CuONPs increased in mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*) seedlings increased when exposed to CuONPs (Lee et al., 2013a). However, the exposure of *Arabidopsis thaliana* to different

contentions of CuONPs (2, 5, 10, 20, 50 and 100 mg/L) induces a significant decrease of total chlorophyll content and reduction in root elongation. Nevertheless, anthocyanin levels, proline lipid peroxidations levels were increasing upon exposure to overdoses i.e., 5, 10, 20, 50 and 100 mg/L of CuONPs (Nair and Chung, 2014b). In addition, an environmental research group have evaluated that the accumulation and phytotoxicity of CuONPs in Elsholtzia splendens under hydroponic conditions (Shi et al., 2014). The seed germination rate and root elongation results of the plants show that the seed germination was not significantly affected by copper oxide bulk particles, and soluble copper (Liu et al., 2018). But the seedling growth of the plant was strongly inhibited by CuONPs (Gojon et al., 2009). After exposure of 14 days, the total Cu content was investigated in the roots, stems, and leaves of the plant. Results revealed dose-dependent bioaccumulation in the plant parts (Nelson et al., 2011). The DNA damage in agricultural and grassland plants have been induced by copper oxide NPs and significant accumulation of oxidatively modified mutagenic DNA lesions (Xiong et al., 2017) and strong plant growth inhibition (Wang et al., 2014) was observed for radish, perennial ryegrass, and annual ryegrass under laboratory conditions (Larue et al., 2014).

#### 3.1.3. Cerium oxide nanoparticles (CeO<sub>2</sub>NPs)

CeO<sub>2</sub>NPs have been incorporated into many commercial products. Thus, their potential release into the environment through the use and disposal of these products has caused serious concerns in terms of the long-term impact on plants (Ma et al., 2010). The potentially different impact of CeO<sub>2</sub>NPs and their bulk counterparts on plants is also unclear. Zhao et al. (2004) have reported the physiological and biochemical adjustments in Brassia Rapa, which is in growing conditions by continued irrigation with solutions containing different concentrations of CeO2NPs. Plants exposed to high concentrations of CeO<sub>2</sub>NPs from 10 and 100 mg/L enhanced plant biomass by 28% and 35% respectively. This activity was not affected by either size of CeO<sub>2</sub> throughout the life cycle of Brassia rapa. Altogether the study demonstrated that plant responses to CeO2 exposure varied with the particle size and the growth stages of plants (Xiong et al., 2017). Corn plants (Zea mays) exposed to CeO<sub>2</sub>NPs for 3 weeks show high concentrations of H<sub>2</sub>O<sub>2</sub> in the phloem, xylem and epidermal cells of the shoot (Zhao et al., 2012). The plants treated with 800 to 4000 mg/L CeO<sub>2</sub>NPs triggering the up regulation of catalase and heat shock protein (HSP70). From these observations, it is believed that the increased enzyme activity and HSP70 proteins are due to induced reaction against CeO<sub>2</sub>NPs (Siddiqi and Husen, 2017).

#### 3.1.4. Gold nanoparticles (AuNPs)

Gold nanoparticles (AuNPs) are non-toxic carriers are one of the most attractive MNPs that have been used in various fields. The biodistribution, uptake, and toxicity of AuNPs increased in tobacco plants (Nicotiana Xanth) while exposed to gold nanoparticles. For this reason, synchrotron-based X-beam micron examination with X-beam ingestion and high-resolution electron microscopy tests were explored (Gojon et al., 2009). Results revealed that the gold NPs reach the vascular system in the plant through the root. The aggregate bodies were identified inside the root cell cytoplasm. The outcomes clarify the possible scope of AuNPs to enter plants through a size-subordinate system to the cells and tissues of the plant and can cause biotoxicity (Sabo-Attwood et al., 2012). Uptake and distribution of AuNPs depended on surface charges. For example, the positively charged AuNPs were readily taken up by Radish and ryegrass roots, while the negatively charged AuNPs were translocated into shoot from the roots in rice and pumpkin (Zhu et al., 2012). In seedlings of brush bean (*Phaseolus vulgaris*), the concentration of H<sub>2</sub>O<sub>2</sub> was increased with increasing the concentration of surface coated AuNPs (25, 50, and 100 mg/L). From these experimental results, the AuNPs uptake is surface charged dependent that effect on plant physiological process including redox homeostasis of plants (Ma and Quah, 2016).

#### 3.1.5. Lanthanide metal oxide nanoparticles (La<sub>2</sub>O<sub>3</sub>NPs)

Lanthanide metal oxides are one of the most significant ENPs (Cui and Hope, 2015). The rare earth oxide NPs have a huge scope of application in different files due to their refractivity of optical fibers, agriculture films, semiconductors, and electroforming electronic materials (Balusamy et al., 2015; Sisler et al., 2016). Due to the extensive application of La<sub>2</sub>O<sub>3</sub>NPs are releasing into the environment. Plants are significant components of the ecosystem, which serves as a carrier of NPs from the environment to the food chain by bioaccumulation mechanism (Li et al., 2014). The La<sub>2</sub>O<sub>3</sub>NPs strictly inhibit the root elongation in seven higher plants like radish, rape, tomato, lettuce, wheat, cabbage, and cucumber (Ma et al., 2010). Moreover, maize exposed to La2O3NPs (5 mL/L) for two weeks decreased the shoot, root biomass, and total chlorophyll content (Liu et al., 2018). Barrena evaluated the phytotoxicity of lanthanum oxide (La2O3) NPs on cucumber (Cucumis sativus) and determined its distribution and biotransformation in roots (Barrena et al., 2009). Seed germination and growth rate of cucumber revealed the dose-dependent relationship of La<sub>2</sub>O<sub>3</sub> nanoparticles and LaCl<sub>3</sub> to the root elongation of cucumber (Ma et al., 2011). After treating with La<sub>2</sub>O<sub>3</sub> nanoparticles, most of La with the composition of LaPo<sub>4</sub> was deposited at the intracellular spaces and middle lamella of cucumber roots that lead to phytotoxicity in the cucumber plant (Ma et al., 2011).

### 3.1.6. Zinc oxide nanoparticles (ZnONPs)

Many soil-borne and air-borne NPs can enter the plants, which are the primary producers in the food chain (Atha et al., 2012). Seed germination was not influenced aside from the hindrance of nanoscale zinc on ryegrass and zinc oxide (ZnO) on corn at 2000 mg/L (Stampoulis et al., 2009). Among NPs and plants, the inhibition of root growth is greatly varied. A 50% inhibitory focuses (IC50) on nano-Zn and nano-ZnO was assessed to be almost 50 mg/L for radish and about 20 mg/L for grape and ryegrass (Sabir et al., 2014). The phytotoxic and genotoxic effects of ZnONPs on buckwheat (Fagopyrum esculentum) seedling were investigated. The inhibition of root growth and biomass at the tested concentrations of NP suspensions and dissolved free ion suspensions were compared. Localization of NPs inside the root epidermis and changes in root morphological features were observed (Lee et al., 2013b). By random amplified polymorphic DNA assays the comparative effect of ZnONPs on DNA stability was reported. It indicated alternate DNA polymorphisms at 2000 mg/kg of ZnONPs, contrasted with the controls. The genotoxic effects of ZnONPs at physiological and molecular levels in buckwheat significantly affected the genetic stability of the plant (Lee et al., 2013a).

### 3.1.7. Titanium dioxide nanoparticles (TiO<sub>2</sub>NPs)

Every year, 3000 tons of TiO<sub>2</sub>NPs have been produced (Keller and Lazareva, 2013) and 50% of which is used in personal care products (Weir et al., 2012). Early phytotoxic and genotoxic effects of TiO<sub>2</sub>NPs were investigated by Taylor and Mushtaq in seedlings of Hordeum vulgare. Caryopses were taken and maintained in a Petri dish at 21 °C for three days (Taylor and Mushtaq, 2011). The percentage of germination was calculated as the ratio of germinated seeds out of the total seeds of each Petri dish. The second arrangement of caryopses was treated for seven days in a similar condition for the assessment of root lengthening (Wang et al., 2012). Results demonstrated that the highest concentration of NPs did not affect the germination and root elongation of caryopses. Even though early germination was not influenced by CeO<sub>2</sub> and TiO<sub>2</sub> suspensions, the grouping of Ce and Ti in the seedling parts and root shoot movement demonstrated portion subordinate reactions. TiO<sub>2</sub>NPs have high soundness and are seen as naturally cordial and these NPs have been used in the decomposition of phytotoxic compounds (Raskar and Lawre, 2013). The TiO<sub>2</sub>NPs were treated with onion seeds to consider their impact on seed germination and early seedling development. These results stated that the seed growth and seed germinations are not affected in presence of TiO<sub>2</sub>NPs, which promotes the seed germination until 40  $\mu$ g/mL<sup>-1</sup> concentrations (Ghosh et al., 2010). Eventually, 20, 30, and 40  $\mu$ g/mL<sup>-1</sup> concentrations of TiO<sub>2</sub>NPs significantly increased the total seedling length. Whereas, a reduction in the seedling growth was noticed at 50  $\mu$ g/mL<sup>-1</sup> (Raskar and Lawre, 2013).

#### 3.2. Mesoporous carbon nanoparticles and their phytotoxicity

Phytotoxicity is also known as plant injury is defined as detrimental effects on various physiological processes that may be occurred when exposed to chemicals to control plant pests, synthetic fertilizer to regulate plant growth. Yuan research group noticed the poisonous effects of mesoporous carbon NPs (MCN) in rice (*Oryza sativa*) seedlings. The seedlings are treated with two types of MCNs (10 mg/L) for 20 days not showing any impact on the root length (Yuan et al., 2018). The effects of the MCNs suppressed the seedling growth of the plants exposed to 50 mg/L and 150 mg/L. From these results, the phytotoxicity of MCNs on rice is dose-dependent (Hao et al., 2018). The root weight of the plant is significantly reduced while treated with MCNs. These results illustrate the potential risk of MCNs on crop plants (Hao et al., 2018).

#### 3.2.1. Carbonaceous nanoparticles (CNTs)

Single-walled carbon nanotubes (CNTs) having colossal advantages for humans as well as plants (Zhao et al., 2004). The impacts of functionalized and non-functionalized carbon nanotubes were assessed in six different routine vegetable salads such as cabbage, carrot, cucumber, lettuce, onion, and tomato were taken for the examination of singlewalled CNTs on root extension and their yield (Cañas et al., 2008). SEM (scanning electron microscopy) was used for the evaluation of the potential uptake of CNTs (Cañas et al., 2008; Khodakovskaya et al., 2009). In general, CNTs are highly hydrophobic and can create barriers in the cell walls leading to damage of plant cells. Besides the root length was mostly affected by non-functionalized CNTs as compared to functionalized CNTs. Non-functionalized CNTs restrained root lengthening in tomatoes and improved the root elongation of onion and cucumber (Wang et al., 2012). Functionalized CNTs repressed the root elongation of lettuce, and the underlying foundations of cabbage and carrots were not influenced (Templeton et al., 2006). Root elongation of cabbage and carrot was not affected by the presence of either FCNT or CNT (Cañas et al., 2008).

#### 3.3. Polymeric nanoparticles (PNPs)

Polymeric NPs are non-toxic, natural, and biodegradable and can be employed as good nanocarriers for agrochemicals, plant nutrients, phytohormones and other active compounds. However, the application of PNPs at higher concentrations shows phytotoxicity. For example, CS/TPPNPs  $(1.33 \times 10^{10})$  caused complete inhibition of germination and negatively affected the initial growth of *Zea mays*, *Brassica rapa* and *Pisum sativum* (Nakasato et al., 2017). In a similar study, the application of nano-chitosan at toxic doses of bulk  $(5, 10, \text{ and } 20 \text{ mgL}^{-1})$  vividly triggered the cessation of plant growth and development. The enzymatic assays revealed activation of phenylalanine ammonia-lyase after supplementing the mentioned NPs in lethal quantities. The study revealed that the enormous variances among triggering and toxic concentrations of the supplements could be ascribed to the physicochemical modifications of nano-polymers (Asgari-Targhi et al., 2018).

The usage of chemicals during the synthesis of raw materials of polymeric nanoparticles like DEP in Cellulose acetate contributes towards the adverse effect on plants under certain environmental conditions leading to chlorosis in leaves (Krizek and Mirecki, 2004). The studies on micro-plastics and its toxicity effects on plant have revealed its dynamic interactions with various membranes of cellular and subcellular organelles attributing towards the effective changes on the porosity mechanism (Maity and Pramanick, 2020). Further, the phytotoxic effects of polymeric NPs on plant growth and development have been

summarized in the (Table 2). The toxic effects of some polymeric NPs like nano-chitosan, nanoplastics, Arabic gum etc., on plant species like *Capsiccum annuum*, *Lycopersicon eculentum* and some other crop plants have also been reported (Behboudi et al., 2017; Asgari-Targhi et al., 2018; Taban et al., 2020; Maity and Pramanick, 2020) (Table 2).

#### 4. Cytotoxicity

Cytotoxicity is a measure subordinate phytotoxicity of NPs on plant physiology. The effects of NMs such as multi-walled carbon nanotubes (MWCNTs), Ag, Cu, ZnO, Si, Au, and their corresponding bulk counterparts on seed germination, root elongation, and biomass of Cucurbita pepo (Zucchini) were demonstrated (Handy et al., 2008). Plants were exposed to MWCNTs and AgNPs for 15-day in hydroponic media, which significantly induces the reduction in total biomass (Wang et al., 2008). The biomass and transpiration rate have been decreased in Zucchini exposed to AgNPs bulk powder at 0-1000 mg/mL for 17 days. Introduction to AgNPs at 500 and 100 mg/L came about in 57% and 41% decreases in plant biomass and transpiration, respectively as compared to controls to the plant (Stampoulis et al., 2009). Cytotoxicity of AuNPs depends on the NPs size instead of surface chemistry (Pan et al., 2007; Lin et al., 2010). 5 mg/L of AuNPs did not show any impact on the physiological process in the bush bean, while the accumulation of positively charged AuNPs was significantly increased in root tissues than neutral and negatively charged AuNPs. The accumulated positively charged AuNPs induced ROS synthesis that enhances cellular oxidative stress (Ma and Quah, 2016). Plants were grown in hydroponic solution containing 100 mg/L CuNPs, which reduced root length emergence by 77% and 64% relative to unamended controls and seeds exposed to bulk Cu powder, but seed germination was unaffected by any of the treatments (Ghosh et al., 2016) (Fig. 5).

#### 5. Genotoxicity of ENPs

DNA plays a central role to transfer genetic information from one generation to another generation as well as it is a primary library for various RNAs and proteins. Recently ENPs induces DNA lesions causing genotoxicity (Carriere et al., 2017). The mechanism of NPs genotoxicity is classified into two categories: direct and indirect genotoxicity (Karami Mehrian and De Lima, 2016). In direct genotoxicity, NPs damage the DNA by direct interaction with genetic material (chromosomes) by mechanical or chemical bonding that may alter the physical properties and may be induced breaks in the chromosome (Singh et al., 2009; Wang et al., 2013). The NPs like TiO<sub>2</sub>NPs (Shukla et al., 2011), AgNPs (Asharani et al., 2009; Hackenberg et al., 2011a), and ZnONPs (Hackenberg et al., 2011b) have been found in the cell nucleus. For example, the genotoxicity of TiO2NPs has been determined at two trophic level plants, the genotoxicity of TiO2NPs can be assessed by classical genotoxic at the endpoints by comet assay and DNA laddering experiments (Kang et al., 2008). The DNA damaging potential of TiO<sub>2</sub>NPs of Allium cepa and Nicotiana tabacum was evaluated by comet assay (Kim et al., 2011). It revealed a dose-dependent response in A. cepa and an increase in extend of DNA damage in N. tabacum. The DNA laddering reveals that the DNA damage was observed at a treatment concentration of 4 nM (Ghosh et al., 2010).

In the case of indirect genotoxicity, DNA damage may arise because of intermediate biomolecules like proteins interaction with NPs and reactive oxygen species (ROS) (Fig. 6). In an indirect DNA damage mechanism, when the plants are exposed to NPs, they can interact with proteins that are involved in DNA replication, repair system, and mitotic division. Several studies showed metal-based nanoparticles (MNPs) like Ag, Au, ZnO, TiO<sub>2</sub>, Cu, Pt and ZnNPs are primarily induced oxidative damage by ROS production (Magdolenova et al., 2014; Mahaye et al., 2017). Excess generation of ROS regulates the various intracellular signaling cascades as secondary messengers, transcription factors, phosphatases, and protein kinases (Cheng and Song, 2006). They also

**Table 2**Phytotoxic effects of polymeric nanoparticles on plant growth and development.

Polymeric nanoparticle	Plant	Toxic effect	Reference
Nano-chitosan	Capsicum annuum	Cessation of plant growth and development	(Asgari-Targhi et al., 2018)
Chitosan/tripolyphosphate	Zea mays, Brassica rapa and Pisum sativum	Complete inhibition of germination	(Nakasato et al., 2017)
Chitosan and SiO <sub>2</sub> Nanoparticles	Triticum aestivum L., Hordeum vulgare L.	Effect on germination, pick value	(Behboudi et al., 2017)
Arabic gum/gelatin, apple pectin,	Lycopersicon esculentum Mill. Amaranthus	Toxicity injuries	(Taban et al., 2020)
gelatin	retroflexus L		
Cellulose acetate	Cucumber plants	Stunted plants and showed marginal chlorosis	(Krizek and Mirecki, 2004)
Micro/nanoplastics	Soil and plants	Inhibit plant growth, seed germination and gene expression	(Maity and Pramanick, 2020)
Hydroxy-aluminium polymer	Oryza saliva L.cv. Chiyohonami	Inhibited root elongation	(Saigusa et al., 1995)

inhibit oxidative defense enzyme activities e.g., the depletion of glutathione and inactivation of glutathione reductase and superoxide dismutase by the interaction of silicon carbide NPs (Barillet et al., 2010). Besides, many reports proved that the NPs interact with nuclear proteins, which are essential for genome expression and mitotic spindle formation. For instant  $\rm TiO_2NPs$  interact with disturbing the protein kinases (PLK1), which are the cell cycle checking proteins to initiate DNA replication and mitotic cell division (Huang et al., 2009).

#### 6. Conclusions and future outlook

The innovations in agricultural nanoscience has forecasted potential benefits of employing NPs, NMs, ENPs and nano polymers as various nano formulations for smart agricultural practices. The deliberate introduction of NMs for agricultural practices is resulting in unintended health outcomes along with environmental hazards. There is very limited knowledge concerning the biosafety of NMs, the adverse effects caused by them by appropriate usage, fate, and acquired biological reactivity once applied into the soil ecosystem, which drags further scientific inputs to assess possible nano-agricultural hazards. Plant species are broadly utilized for checking air contamination and for screening natural synthetic compounds for their genotoxic impacts. The developing

open discussion on the harmfulness and ecological effects of applications of NMs to plants has not yet been altogether developed. The high impacts of the NMs on plant systems have huge toxic effects on the morphology, biochemical and genetic modifications in numerous crops. The phytotoxicical effects include seed germination inhibition, differences in shoot and root biomass, genotoxicity and cytotoxicity leading to oxidative burst in plant tissues, which are as discussed in the current review. NPs in the dirt can be particularly essential to comprehend the earth-bound poisonousness of NMs. There are some studies on seed germination aspects, which were able to detect significant differences in germination of seeds caused by certain nanotubes. The toxicological effects of the applied NMs are determined by physicochemical characteristics, and by the experimental designs.

In addition, the exposure time over the plant, the stage-specific effects on plant when it comes in contact with NMs, the methods of introduction of the NMs, which leads to inappropriate infiltering leading to accumulation of NMs in toxic levels. Enhanced input of NMs into agricultural crops pose number of challenges concerning the fate and transportation of these NMs in the plant system. Even though there are techniques and assays, which confirm the toxic effects of NMs on plant biota, there is a requirement to develop, implement and standardize in silico methods (simulation models) in plant systems at the initial

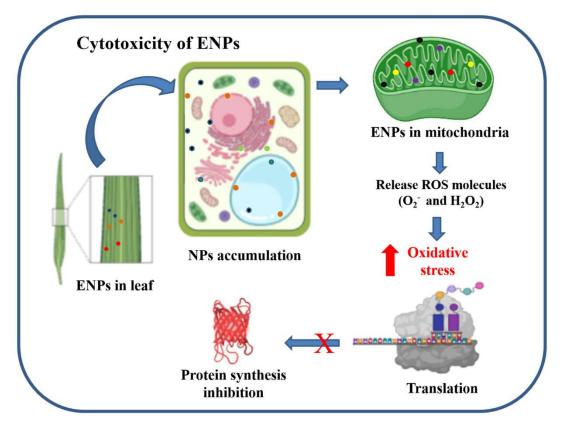


Fig. 5. Schematic representation of cytotoxicity of engineered nanoparticles (ENPs).

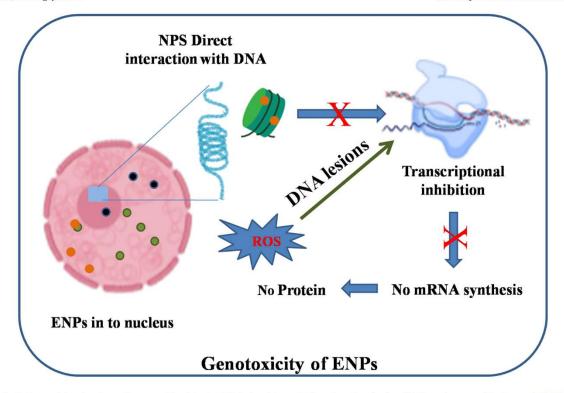


Fig. 6. Genotoxicity of engineered nanoparticles either by DNA lesions (due to ROS) or direct inactivation of DNA repair system (Carriere et al., 2017).

stages of experiments. Certain NPs like TiO<sub>2</sub>NPs, AgNPs, and ZnONPs have been found in the cell nucleus and observed to cause genotoxicity at two trophic level plants, which were assessed by classical genotoxic at the endpoints by comet assay and DNA laddering experiments. Hence, it is necessary to standardize the parameters like the size of NM/P's, concentration, dosage, time interval regarding the application at the field levels comprising the contemplation of environmental conditions. This will further help in the development of techniques to determine the potential toxicity of CNTs, functionalized carbon nanotubes (fCNTs), and other ENPs to plants. The optimal doses of polymeric NPs for plant applications need attention as there is an existing huge research gap in this area.

Polymers such as chitosan, cellulose, and alginate can be efficiently utilized in some major applications (controlled release of phytohormones agrochemicals, macro/micronutrients, etc.) of plants. Various risks resulting from Polymeric NM exposure should be tested using a suitably tailored life-cycle outlook. Focus should be driven towards toxicological research to define hazards caused by inappropriate usage of NMs and address the levels of exposure of the life cycle of nano-enabled products, and, various physico-chemical features affecting nanomaterial toxicity have to be assessed for their probable relations with agro-system coformulants. These intriguing issues should be addressed to extend ethical regulatory responsibilities for the genuine applications of green nanotechnology for sustainable agriculture and ecosystem.

#### **Declaration of competing interest**

The authors declared that they don't have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Any conflict of interest in this manuscript.

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