ARTICLE / INVESTIGACIÓN

Plants anatomically engineered by nanomaterials

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Abstract: Anatomical characteristics are essential in determining the stress that affects plants. In addition, they provided a piece of evidence for environmental pollution. The increasing use of nanomaterials (EnNos) in industries, medicine, agriculture, and all fields. Nanomaterials also have many uses as a new science; they have toxic effects that have not been studied well. Therefore, this research was interested in recording recent studies on (EnNos) and their impact on the anatomical characteristics of plants. Moreover, the possibility of using anatomical characteristics as evidence of nano contamination (nanotoxicity) in plants comprises a crucial living component of the ecosystem. Studies on the effect of EnNos (carbon) on plant anatomy indicated that excess EnNos content affects the anatomical structure of the plant from the vital structures of the root, stem and leaves. Toxicological effect on xylem and phylum vessels from toxicological studies to date, Toxicological effects on EnNos of various kinds can be toxic if they are not bound to a substrate or freely circulating in living systems. Different types of EnNos, behavior, and plant capacity generate different paths. Moreover, different, or even conflicting, conclusions have been drawn from most studies on the interactions of EnNos with plants. Therefore, this paper comprehensively reviews studies on different types of carbon EnNos and their interactions with different plant species at the anatomical responses.

Key words: Anatomical characteristics, nanomaterials, nanotoxicity, Fullerene and Carbon Nanotubes.

Introduction

The Term Of Nanotechnology, Nanotechnology means the generation, processing and diffusion of nanomaterials (Nos), a wide range of applications¹. It became essential for industrial growth in all countries². (Nos) are known as nanotechnology-derived products³. The increase of nanoparticles in recent years and their use in different ways⁴. Stimulating by Nanomaterials, the (EnNos) development in agricultural applications led to the development of genetically modified crops (GMCs), livestock production, biopesticides, and tissue culture⁶. Because they are low-cost, field application techniques¹⁻⁵. Studies have proven that nano-fertilizers stimulate seed germination in addition to enhancing production. Researchers report a significant knowledge gap about the effects of (NOs) on food crops such as vegetables and grains7. Accumulation and uptake depend on plant type, chemical composition, and size of (EnNos)8. Some plants are capable of absorbing and accumulating stimuli (Nos). Plant cell interaction with (Nos) alters plant gene expression and associated biological pathways, ultimately influencing plant anatomy⁹. The effects of (Nos) on different plant species can vary significantly with different plant shapes, methods and duration of exposure and depend on (Nos) shape, size, chemical composition, concentration, and solubility¹⁰. More studies are needed on the potential risks of using (Nos) and their potential adverse effects¹¹. Research indicates that (Nos) are highly toxic to aquatic life, bacteria, and human cells at the nanoscale; according to particle physics and studies of micro-atmospheric pollutants, even usually benign substances may become dangerous. Size range of nano-

materials that remain suspended for days to weeks in the air and are respired in plant respiratory system¹²; therefore, of particular concern is the ability of nanoparticles to be directly taken up by individual cells and cell nuclei. Bioaccumulation of (Nos) important concern¹⁰⁻¹². Since the physical characteristics of the endoscopic variants are not good, it is difficult to give a definitive opinion on the health and environmental risks¹³⁻¹⁶. Therefore, plant cells are determined by the interactions and modified plant gene expression (Nos). They have linked biological pathways, which ultimately influence seedling growth and development. This is due to different effects on seedling growth due to the unique properties of (Nos) that can modify its physical and chemical properties, depending on the surface area and quantum properties¹⁷⁻²⁰. wherefore, (Nos) must have all properties such as effective concentration with high potency, stability and solubility, and time-controlled release in response to specific stimuli21-25. The vast majority of the research work done for each product (Nos) and product in agriculture is from it to investigate its stimulating potential before it is considered safe²⁶.

Materials and methods

Stimulating By Ennos On Plant Anatomy

The plants interacting with (Nos) is possible and increased with increasing application and variety of instruments and goods. A plant can accumulate toxic elements inside

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tissues usually, and intolerant species suffer effects that can lead to death²⁷⁻³⁰. as well as absorbs essential and nonessential elements, which may induce a certain concentration³¹. (Nos) can enter the plant body directly or indirectly, or by pollutants in the air, water or soil, which leads to the transfer of (Nos) to the food chains through food. This route is primarily responsible for the transfer of nanotoxicity to the plant-related food chain³²⁻³⁶. Research studies on selenium have found that selenium-laden plants can supply selenium deficiency in ruminants and other animals, even in very narrow and low toxicity and deficiency states³⁷⁻³⁹. Other studies have focused on this aspect, including radish (Raphanus sativus), corn (Zea mays), lettuce (Lactuca sativa), cucumber (Cucumis sativus), ra pe (Brassica napus), and many more¹⁹. Most studies have focused on Nos' uptake, accumulation, transport and biotransformation because biodegradation is difficult to trace in living organisms.

Results

Plant Anatomy Response By Carbon Based

Carbon-based (Nos) is total production that led to release in living systems and greater possibilities of adverse environmental effects 41. fullerene C70, fullerol (C60(OH)20), and carbon nanotubes are most studied materials. All of them are highly hydrophobic and able to aggregate; it could accumulate in the living system42. This led to an increased capability to interact with many organic substances. Some plants can uptake mechanisms and accumulation (Nos). Appropriately functionalized (Nos) provided better penetration through the cuticle. This, in turn, allows for a slow and governed discharge of active ingredients on reaching the target weed. For instance, appropriately functionalized lipophilic nano silica gets absorbed into the cuticular lipids of insects by physisorption, damages the protective wax layer, and induces death by desiccation43-45.

Plant Anatomy Response By Fullerene

The individual fullerenes nanoparticle is believed to enter the plant roots through osmotic pressure, capillary forces, and pores in the cell walls by the intercellular plasmodesmata or using the significantly regulated symplastic routes 46. Only the fullerene particles with a diameter of less than the pore diameter of the cell wall could pass through and reach the plasma membrane 47.

Plant Anatomy Response By Fullerol

The small size and hydrophobicity properties inducing a permeability of fuller through the cell wall pores in the plant cell suspension leads to minimal uptake of the (Nos) 48. Consequently, the fuller has accumulated at the interface between the cell wall and plasma membrane 49. This accumulation also occurred between adjacent epidermal cell walls, showing its apoplectic mode of transport in the plant tissues 50-52.

Plant Anatomy Response By Carbon Nanotubes

Carbon nanotubes (CNTs) may possibly have single or multiple carbons layers established in a cylinder⁵³⁻⁵⁵. CNTs behave as fibers, with their properties very different from bulk carbon or graphite⁵⁶. Thus, CNTs possess excellent tensile strength and are possibly the strongest, smallest fiber known⁵⁷⁻⁵⁹. Most studies are increasingly carried out to ob-

tain the uptake and transport mechanism of carbon-based (Nos) into intact plant cells⁶⁰. CNTs have phytotoxic effects on plant cells due to aggregation and cause cell death in a dose-dependent manner⁶¹. Cell death is demonstrated by electrolyte leakage and the swelling of the cell plant. Theoretically, a single-wall carbon nanotube is too large to penetrate the cell wall. However, the evidence of an endocytosis-like structure of the plasma membrane in an Arabidopsis thaliana leaf cell indicates the existence of carbon nanotubes. It is exceptionally relevant to guide additional studies with other edible plants⁶²⁻⁶⁴. Then, researches with cell suspensions of Nicotiana tabacum cv. Bright Yellow found that the water-soluble single-wall carbon nanotube with a length of less than 500nm has penetrated the intact cell wall and membrane over fluidic phase endocytosis65,66. Due to their small size, carbon nanotubes tend to interact with the polysaccharides and proteins in the cell wall and elicit hypersensitive retorts mimicking plant pathogens, leading to cell mortality⁸⁰⁻⁸³. This is supported by recognizing monovalent interactions involving rice mortality and carbon nanotubes. Thus, CNTs could improve root growth of cucumbers (Cucumis sativus), onions (Allium cepa), and nanotube sheets formed by both functionalized-CNTs and no functionalized CNTs on root surfaces, but none entered the roots^{67,68}.

Plant Anatomy Response By Mwcnts

Multiwall carbon nanotubes (MWCNTs) are 1mm long and 20 nm in diameter⁶⁹. MWCNTs are taken up by the seeds and roots system by creating new pores and water uptake to develop tomato seedlings⁷⁰. MWCNTs are visualized to be on the root surface before eventually piercing the epidermal and root hair cell walls and cap of the seedlings³¹. Furthermore, some studies described that MWCNTs permeate tomato seeds and boost the germination rate by improving the seed water uptake71,72. Other researchers pointed out that the cell walls of rice cell suspension restrict the entry of the MWCNTs into the cellular cytoplasm, forming black clumps that strongly wrap around and associate with the cells³². The presence of the clumps, with an increase in the concentration of carbon (Nos), would increase in size and number³¹. This hypersensitive response is thought to prevent the entry of MWCNTs through the plant cell walls³². For example, the seeds treated with MWCNTs showed a few aggregate nanotubes in the vascular system and none in the tissues.

Plant Anatomy Response By Swcnts

The dimension of a typical single-walled carbon nanotube (SWCNT) is about 1 to 2 nm in diameter and 0.1 µm in length⁷³. Some studies indicate that carbon-based (Nos) surface modifications increased its widespread, dispensability, and water column stability^{74,75}. Contrarily, no uptake of SWCNTs and its functionalized roots of cucumber seedlings are found after treatment for 84 h. In the form of a nanotube sheet, the SWCNTs were found to adhere to the external surface of the primary and secondary roots⁷⁶. However, current results are insufficient to determine the translocation of SWCNTs from the root systems to the aerial parts of the plant⁷⁷.

Plant Anatomy Response By Graphene

Graphene is an inert carbon with a two-dimensional crystalline shape with a strong bond of a single carbon atom layer. Studies indicated that at concentrations higher than (1000 mg/L), spinach and red cabbage root hair growth de-

creased compared to the control plants, although it was inactive⁷⁸. This may be due to the accumulation of free radicals, especially H2O2, which increases with increasing concentration and duration of exposure. It also increased necrosis and permeability of organisms, causing significant electrolyte leakage (oxidative stress)²⁸. For example, the intracellular reduction-oxidation system probably has an essential function in the Induction of cell death induced by graphene⁷⁹. It described the accumulation of graphene as leading to cell death, shown by electrolyte leakage from cells⁸⁰. Via graphene treatment, the root surface area of cabbage significantly improved, and it may be that an excess of graphene resulted in swelling in Origanum vulgare and Origanum⁸⁰⁻⁸³. Graphene is known to induce phytotoxic effects in plant cells due to its accumulation mechanism. This causes cell death and accumulation in a dose-dependent manner⁸⁴.

A similar growth pattern was observed in tomato, cabbage, and red spinach using graphene (Nos) in (38). At higher concentrations of graphene (1000mgL⁻¹), the root hair growth of red spinach and cabbage compared to control plants was reduced⁸⁵. Overproduction associated with the accumulation induced by graphene could produce substantial Plant anatomy inhibition, and biomass reduction reported that the production of accumulation could be a primary factor in the toxicological effects of nanostructured materials⁴⁰. Declaration of accumulation production by means of H₂O₂ visualization, 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^{89,90}. The assessment of graphene-stimulated targets terrestrial plant species⁸⁶⁻⁸⁸. It applies a prolonged exposure period with different concentrations to measure potential risks. I will quote my master's research findings to support the findings of this article.

Effect of ZnO NPs on anatomical trials of studied wild plants

Response of studied wild plants leave cross-section to ZnO NPs treatments

The Scanning electron microscope (SEM) photograph analysis of the studied wild plants' leave cross-section revealed an anatomical structure typical of Dicotyledonous in the control variant (Figure 1). The structure of the leaf tissue was characterized by an ordered organization and the cell's uniform localization in the chlorenchyma of the leaf. The division of mesophyll cells into palisade and spongy parenchyma was remotely traced⁹¹⁻⁹³. The parenchyma cells are located in several rows between the upper and lower epidermal layers, characterized by the round form. As mentioned earlier, an insignificant proportion of tightly contacting cells and an extensive intercellular space were established for the cells of parenchyma spatial organization. The vascular bundles comprise about four sectors, the xylem, phloem, parenchyma and bundle sheath (Figure 1- 12). However, there were anatomical differences in the dimensions of the bundles in different treatments. These differences were selected for comparison (Figure 1-12).

Response of Peganum harmala leave cross-section

The (SEM) photograph of Peganum harmala plants leave cross-section revealed in the control variant (Figure 1). A uniform localization in the leaf's parenchyma characterized the leaf tissue's structure. The round form characterized the cells of the parenchyma. The vascular bundles comprise about four sectors, the xylem, phloem, parenchyma and bundle sheath (Figure 1).

Treatment of 150 mg/L showed a noticeable increase in the leaf cross section due to the increase in the layers of parenchyma. The vascular bindle increased in number, especially the vessels of xylem and phloem (Figure 2).

P eganum harmala leave cross-section with 300 mg/l treatments showed decreased parenchyma cell size. The vascular bindle decreased in number, especially the vessels of xylem (Figure 3).

Figure 4 at the end of the experiment showed decay in the parenchyma tissue, vascular bundle and increased epidermis shrivel of Peganum harmala leave cross-section with 600 mg/l treatments.

Response of Portulaca oleracea leave cross-section

The (SEM) photograph of Portulaca oleracea plants leave a cross-section with control treatments (Figure 5). The structure of the parenchyma of the leaf consists of several layers. The cells of the parenchyma were round form. The vascular bundles comprise the xylem, phloem, and parenchyma (Figure 5).

The Portulaca oleracea leave cross-section with 150 mg/l treatments appeared normal leave surface and increased cell wall thickness. The plants appeared smaller in vascular bundle diameter, increased in parenchyma cell size, and decreased in number (Figure 6).



Figure 1. Scanning electron microscope images of Peganum harmala leave cross-section under control treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.



Figure 2. Scanning electron microscope images of Peganum harmala leave cross-section with 150 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.



Figure 3. Scanning electron microscope images of Peganum harmala leave cross-section with 300 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.





Figure 4. Scanning electron microscope images of Peganum harmala leave cross-section with 600 mg/l treatments. A- epidermis, B-parenchyma, C- phloem, D-xylem.



Figure 5. Scanning electron microscope images of Portulaca oleracea leave cross-section under control treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.



Figure 6. Scanning electron microscope images of Portulaca oleracea leave cross-section with 150 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.

The Portulaca oleracea leave cross-section with 300 mg/l treatments appeared to shrivel in leave surface and increase cell walls thickness. The plants appeared smaller in vascular bundle diameter and increased in epidermis shrivel and started disintegration aerenchyma, which increased in size and decreased in number due to dissolved cell walls (Figure 7).

The Portulaca oleracea leave cross-section with 600 mg/l treatments appeared to fade in the leave epidermis cell with folded layer. The plants appeared smaller in vascular bundle diameter and decreased in number due to dissolved cell walls (Figure 8).

Response of Lepidium sativum leave cross-section

The (SEM) photograph of Lepidium sativum plants leave cross-section revealed in the control variant (Figure 9). A uniform localization in the leaf's parenchyma characterized the leaf tissue's structure. The round form characterized the cells of the parenchyma. The vascular bundles consist of about four sectors, the xylem, phloem, parenchyma and bundle sheath (Figure 9).

Lepidium sativum leave cross section with 150 mg/l

treatments showed accumulation of ZnO NPs particles on the surface of the plants. Epidermis of Lepidium sativum not arrangement with folded shape. Epidermis cells small and had different shape (Figure 10).

The Lepidium sativum leave cross-section with 300 mg/l treatments appeared Multi-layered epidermis and parenchyma with small cell size. The vascular cylinder occupied the central part of the cross-section and appeared to be few and of small diameter in xylem vessels. (Figure 11).

SEM images of various macroscopic magnification forces showing plants in 12 Figure. The plant appeared to decay in the parenchyma tissue with shriveling of the epidermis tissue and necrosis in vascular bundle due to the degradation of photosynthesis pigments and deposition of materials in the parenchyma tissue was observed by increasing the pigmentation of the cells and increasing the cell wall thickness (Figure 12).

Discussion

The only low surface friction of carbon nanotubes is required to assist the flow of organic substances into the



Figure 7. Scanning electron microscope images of Portulaca oleracea leave cross-section with 300 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.



Figure 8. Scanning electron microscope images of Portulaca oleracea leave cross-section with 600 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.

20 µm



Figure 9. Scanning electron microscope images of Lepidium sativum leave cross section under control treatments. Aepidermis , B- parenchyma, C- phloem, D- xylem.



Figure 10. Scanning electron microscope images of Lepidium sativum leave cross-section with 150 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.



Figure 11. Scanning electron microscope images of Lepidium sativum leave cross-section with 300 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.



Figure 12. Scanning electron microscope images of Lepidium sativum leaves cross section with 600 mg/l treatments. A- epidermis, B- parenchyma, C- phloem, D- xylem.

cytoplasm⁹⁴. The Fullerene aggregates were mainly present in or near the stem's vascular system and leaves, whereby the roots have been devoid of fullerene⁹⁵. The fullerene aggregation in leaves indicated that they followed the transmission route of nutrients and water through the xylem⁹⁶. It has been reported that the presence of fullerene in the form of black aggregates is more plentiful in seeds and roots compared to the leave sand stems for rice seeds²². There is proof that CNTs could translocate to systemic sites, such as fruits, leaves and roots, which could involve a strong interaction with the cells of the tomato seedling. This resulted in significant changes in whole fruits, leaves, and roots gene expression⁷⁹. Though CNTs were discovered to diminish root growth in tomato plants, recent works reported that CNTs penetrated tomato seed coats and significantly enhanced plant anatomy98.

Meanwhile, in the zucchini species, no adverse effects were noticed root elongation within the examined range of MWCNTs⁹⁹. There is proof that graphene could translocate to systemic sites, such as fruits, roots, and leaves, which interact strongly with tomato seedlings' cells, leading to substantial modifications in total gene expression in fruits, leaves, and roots and exerting toxic effects³⁸. With that, it is unexpected to find the toxic effects of graphene on terrestrial plant species in tomato, cabbage, and red spinach¹⁰⁰.

Anatomical indicators showed that the Peganum harmala plant showed the best response compared to the rest of the studied plants¹⁰¹, where the stomata remained open, and the xylem and phloem vessels were intact despite being damaged by an increase in the concentration of zinc nanoparticles¹⁰². The most studied plant affected was Lepidium sativum, where the stomata were closed and decreased in number, and damage to the vascular system⁵⁰.

The zinc nano ions entered the cell walls' structure and damaged the cellular organelles. The treatment of 600 mg/L harmed the vascular cylinder (xylem and phloem), causing a decrease in the size of the parenchyma cells. The epidermis is limp, and the number of stomata was decreased and smaller in size, which may lead to their complete closure¹⁰³.

These results agree with Nemček *et al.*⁵⁴ studies that reported a high content of Zn (1200 mg/kg dry weight) was determined in barley leaves grown in pots amended with 30 mmol/kg of Zn in the form of ZnSO4, whereas plants treated with ZnO (NPs) showed higher contents of Zn in the root tissues⁵⁵⁻⁵⁷. During the dissolution of ZnO in the root zone, nanosized particles were reported to be absorbed more rapidly than larger-sized particles, such as bulk ZnO particles. Near-edge X-ray absorption spectroscopy showed that ZnO (NPs) of 40 nm were dissolved by roots more quickly than their larger-size counterparts¹⁰⁴.

The results demonstrate that most Zn taken up was derived from Zn²⁺ released from ZnO (NPs), and Zn accumulated in the form of Zn phosphate. ZnO (NPs) were observed mainly in the epidermis, a small fraction of ZnO (NPs) were present in the cortex cells, and some further entered the vascular system through the sites of the plasmodesmata pore^{61,62}. However, small amounts of ZnO nanoparticle was observed to translocate to the vascular system, possibly due to the dissolution and transformation of ZnO (NPs) inside the plants¹⁰⁵⁻¹⁰⁷.

Conclusions

The extrapolation of the researcher's opinions into na-

notoxicity on plant anatomy led to the conclusion that exceeding the limits required for plant tolerance leads to harmful effects on cell walls, cell division, plant's ability to transport and vital activities, especially Transport units in plants (phloem and xylem) and thus affect negatively on growth. But the information so far is lacking about the permissible limits for accumulating (EnNos). This requires more research for each type of (EnNos) that differ in their physical and chemical properties and multiple uses. Plant species also differ in their response to nanotoxicity. Currently, the use of (EnNos) should be reduced to avoid its adverse effects on plants and the ecosystem.

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Institutional Review Board Statement

"Not applicable." for studies not involving humans or animals. You might also exclude this statement if the study did not involve humans or animals..

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Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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