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Review

# Research Progress on the Application of Carbon-Based Nanomaterials in Agriculture and Their Dual Effects

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## Abstract

As a significant branch of nanotechnology, carbon-based nanomaterials (CNMs) have garnered extensive attention for their broad application potential in agriculture, attributed to their unique structural and physicochemical properties. They are considered one of the important tools for promoting sustainable agricultural development. Among them, carbon nanotubes (CNTs), owing to their excellent mechanical properties, electrical characteristics, and high specific surface area, have recently attracted considerable interest in plant growth regulation and the development of agricultural inputs. This article systematically reviews the research progress of CNMs, especially CNTs, in agriculture. Firstly, it outlines the structural characteristics and physicochemical properties of different types of CNMs. Subsequently, from a plant physiological perspective, it focuses on analyzing their mechanisms of action in nutrient uptake, photosynthesis regulation, and antioxidant defense. Based on this, it summarizes the application progress of CNMs in plant growth promotion, nano-pesticide and fertilizer delivery, and precision agriculture sensing. Furthermore, this article emphasizes the dose-dependent biphasic effect (hormesis) of CNMs on plants: at low doses, they can promote growth and enhance stress resistance, whereas at high doses, they may induce oxidative stress, cellular damage, and photosynthesis inhibition. However, significant variations in responses exist depending on the material type, physicochemical properties, and plant species, and a unified understanding of the underlying mechanisms has not yet been established. Finally, this article discusses green synthesis strategies for CNMs and their potential ecological risks, and points out that future research should focus on key issues such as precise dose regulation, long-term environmental behavior, and multi-scale mechanism analysis. This review aims to provide a systematic reference for understanding CNMs-plant interactions and their safe application in agriculture.

**Keywords:** carbon-based nanomaterials; carbon nanotubes; agricultural application; plant growth; dual effects; nanotoxicity; green synthesis

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## 1. Introduction

Since the 21st century, persistent global population growth, escalating climate change, and the degradation of arable land resources have posed severe challenges to agricultural production systems. The Food and Agriculture Organization (FAO) of the United Nations projects that global food production must increase by approximately 60% by 2050 to meet the demands of population growth [1,2]. Yet this target is being pursued against a backdrop of diminishing resource availability: arable land per capita has declined by nearly 50% since the 1960s, and the overuse of synthetic inputs has driven widespread soil degradation, water eutrophication, biodiversity loss, and pesticide residue accumulation [3,4]. The tension between productivity imperatives and environmental

sustainability is not merely a policy challenge—it is a fundamental scientific problem that demands novel technological frameworks.

Nanotechnology has emerged as one such framework, offering a conceptually distinct approach to agricultural inputs. Nanomaterials (NMs), with their dimensions in the 1–100 nm range, exhibit quantum confinement effects, exceptionally high surface-area-to-volume ratios, and tunable surface reactivity that distinguish them from their bulk counterparts [5–8]. These properties underpin their proposed advantages in catalysis, targeted delivery, and real-time sensing. The resulting field of “nano-enabled agriculture” has attracted substantial research investment, yet it is important to acknowledge at the outset that the translation from laboratory promise to field-scale application remains incomplete, and the evidence base is characterized by significant heterogeneity and unresolved contradictions [9–12]. Cross-study comparisons are frequently confounded by differences in material synthesis routes, characterization standards, exposure conditions, and plant species—an inconsistency problem that this review treats not as a peripheral concern but as a central scientific challenge.

Among the numerous nanomaterials under investigation, carbon-based nanomaterials (CNMs) occupy a prominent position owing to their structural diversity, tunable physicochemical properties, and relatively well-characterized synthesis pathways [13,14]. The CNM family encompasses fullerenes (C<sub>60</sub>), carbon dots (CDs), carbon nanotubes (CNTs), and graphene and its derivatives (e.g., graphene oxide, GO), each with distinct geometries and surface chemistries that produce markedly different biological responses [15,16]. Carbon nanotubes, first reported by Iijima in 1991, have been particularly intensively studied due to their unique one-dimensional hollow structure, high aspect ratio, and exceptional electrical and thermal conductivity [17,18]. However, it is worth noting that even within the CNT category, single-walled (SWCNTs) and multi-walled (MWCNTs) variants, as well as functionalized versus pristine forms, can elicit qualitatively different plant responses—a distinction that is not always adequately controlled in the literature.

The reported effects of CNMs on plants span a remarkably wide spectrum. At low or optimized concentrations, CNMs have been shown to penetrate plant cell walls, activate aquaporins, accelerate seed germination, promote root development, and enhance nutrient uptake, with consequent improvements in crop yield and quality [19–21]. They also function as nanocarriers for targeted and slow-release delivery of pesticides and fertilizers, potentially reducing agrochemical losses and environmental contamination [22–24]. CNM-based nanosensors capable of real-time monitoring of soil nutrients, moisture, and plant stress signals (e.g., H<sub>2</sub>O<sub>2</sub>) represent an additional dimension of agricultural utility [25,26]. Taken together, these applications suggest a potentially transformative role for CNMs in sustainable agriculture.

However, the same physicochemical properties that confer these advantages also raise legitimate safety concerns. As CNM production and deployment increase, their release into soil–plant systems becomes inevitable, and the resulting interactions are complex and dose-dependent [27,28]. A growing body of evidence demonstrates that CNMs exhibit a characteristic “biphasic effect” or “hormesis”: stimulation at low concentrations and inhibition—or outright toxicity—at higher doses [29–31]. Critically, the concentration threshold separating these two regimes varies substantially depending on CNM type, surface functionalization, plant species, and environmental context, and no consensus threshold has been established. At suprathreshold concentrations, CNMs can trigger oxidative stress, membrane damage, chloroplast disruption, hormonal imbalance, and genotoxic effects including DNA methylation changes [32–34]. These findings carry direct implications for the design of safe application protocols and regulatory frameworks.

What makes this dual-effect phenomenon particularly challenging to interpret is the inconsistency of findings across the literature. Some studies report robust growth promotion even at relatively high concentrations, while others document phytotoxicity at doses well within the range considered “low” in other investigations. This inconsistency reflects genuine biological complexity and methodological heterogeneity that the field has not yet resolved. A rigorous review must therefore move beyond cataloguing reported effects and engage critically with the sources of this

variability, the mechanistic hypotheses that have been proposed to explain it, and the knowledge gaps that most urgently require attention.

This review aims to address these challenges by providing a critically integrated synthesis of recent research on CNMs in agriculture, with particular emphasis on CNTs. It systematically examines the classification and properties of CNMs, their mechanisms of action on plant physiological and biochemical processes, and their applications in growth promotion, pest and disease control, nano-fertilizer delivery, and precision sensing. Crucially, it devotes substantial attention to the dual effects of CNMs, the mechanistic basis of their dose-dependent toxicity, the key variables that modulate plant responses, and the inconsistencies that complicate cross-study interpretation. Finally, it evaluates green synthesis strategies and ecotoxicological risks, and identifies priority directions for future research. The overarching aim is to provide a theoretically grounded and critically reflective reference that supports both scientific progress and the responsible application of CNMs in sustainable agriculture.

## 2. Classification and Properties of Carbon-Based Nanomaterials

Carbon-based nanomaterials (CNMs) are a class of materials with carbon atoms as the primary framework, possessing at least one dimension in the nanoscale range (1-100 nm) [35]. The unique  $sp^2$  and  $sp^3$  hybridization capabilities of carbon atoms enable the formation of various allotropes, leading to nanomaterials with vastly different structures and diverse properties. Based on their dimensionality, CNMs can be mainly classified into the following categories:

**Zero-dimensional (0D) CNMs:** All dimensions are within the nanoscale. Typical representatives are Fullerenes and CDs. Fullerenes, particularly  $C_{60}$ , are closed cage-like structures formed by 60 carbon atoms bonded via  $sp^2$  hybridization, characterized by high symmetry and unique electron acceptor properties [36]. Carbon dots are a class of quasi-spherical fluorescent carbon nanoparticles, typically smaller than 10 nm, often composed of amorphous carbon or graphene quantum dots, exhibiting excellent photoluminescence, low toxicity, and good biocompatibility [37,38].

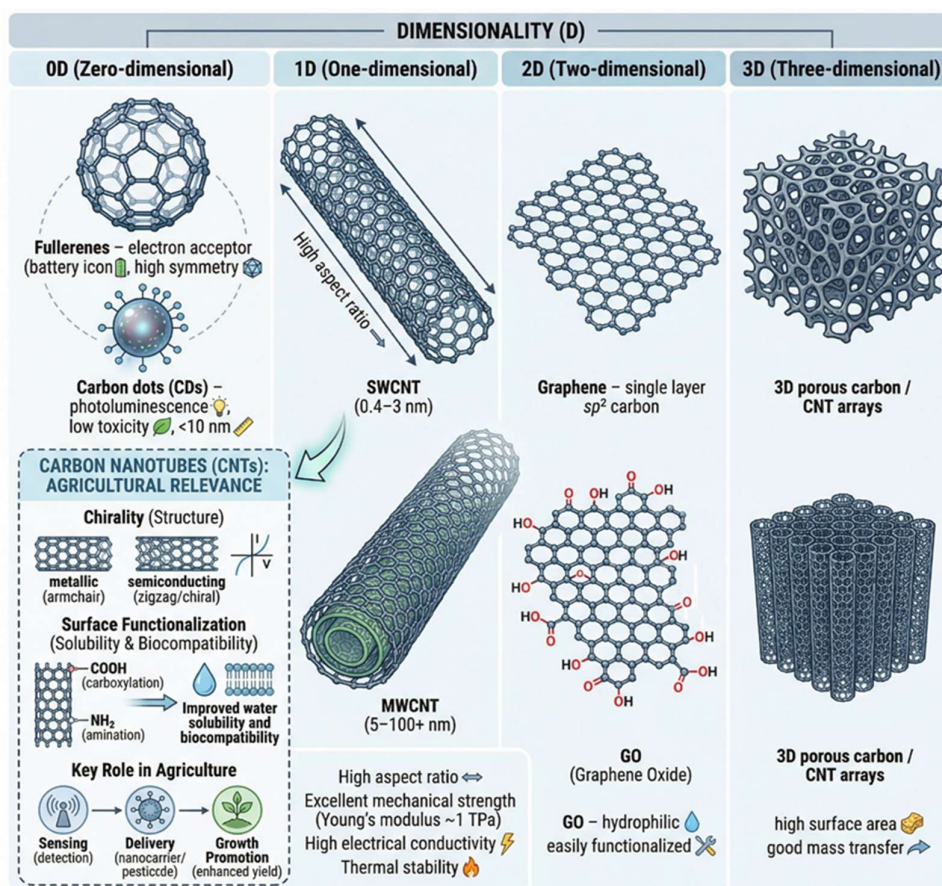
**One-dimensional (1D) CNMs:** Possess two dimensions at the nanoscale and one dimension at the macroscale. CNTs are the most typical representatives. CNTs can be considered as coaxial cylindrical structures formed by rolling up single or multiple layers of graphene sheets [39]. Depending on the number of graphene layers, CNTs are classified into Single-Walled Carbon Nanotubes (SWCNTs) and Multi-Walled Carbon Nanotubes (MWCNTs). SWCNTs typically have diameters of 0.4-3 nm, while MWCNTs can reach diameters of 5-100 nm or more [40]. CNTs are highly valued for their extremely high aspect ratio, excellent mechanical strength (Young's modulus up to 1 TPa), outstanding electrical conductivity, and thermal stability [41,42].

**Two-dimensional (2D) CNMs:** Only one dimension is at the nanoscale (thickness), while the other two dimensions can be at the micrometer or even macroscale. Graphene is a prominent representative of 2D CNMs, consisting of a single layer of carbon atoms densely packed in a  $sp^2$ -bonded hexagonal honeycomb planar structure, serving as the fundamental building block for other dimensional carbon materials [43]. GO is an important derivative of graphene, introduced with numerous oxygen-containing functional groups (e.g., hydroxyl, carboxyl, epoxy groups) onto the graphene sheets through chemical oxidation, imparting excellent hydrophilicity and ease of functionalization [44].

**Three-dimensional (3D) CNMs:** Complex macroscopic structures assembled from low-dimensional carbon nanostructures, such as three-dimensional porous carbon and carbon nanotube arrays, typically characterized by high specific surface area and good mass transfer properties [45].

Among the aforementioned CNMs, carbon nanotubes play a particularly crucial role in agricultural applications due to their unique structure and properties. The structural and performance differences of CNTs depend mainly on their chirality and number of layers. Chirality determines the electrical conductivity of CNTs, which can be metallic or semiconducting, laying the foundation for their application in electronic sensing [46]. The high specific surface area and hydrophobicity of CNTs facilitate the adsorption of organic molecules, but they also tend to

agglomerate in water, affecting their dispersibility and bioavailability. Therefore, surface functionalization of CNTs (e.g., carboxylation, amination) to improve their water solubility and biocompatibility is a key step for their application in agriculture [47,48].



**Figure 1.** Classification and physicochemical properties of carbon-based nanomaterials (CNMs). Schematic illustration of the classification of CNMs according to their dimensionality, including zero-dimensional (0D; fullerenes and carbon dots, CDs), one-dimensional (1D; carbon nanotubes, CNTs), two-dimensional (2D; graphene and graphene oxide, GO), and three-dimensional (3D; porous carbon structures and CNT arrays). The key structural features and functional properties of representative CNMs are highlighted. Carbon nanotubes are further categorized into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs), characterized by high aspect ratio, exceptional mechanical strength, electrical conductivity, and thermal stability. Surface functionalization (e.g., carboxylation and amination) are widely used to improve water solubility and biocompatibility. Their major roles in agricultural systems, including sensing, delivery, and growth promotion, are also summarized.

### 3. Mechanisms of Action of Carbon Nanomaterials in Plant Physiology

The interaction of CNMs with plant physiological processes operates across multiple scales—from molecular-level gene expression and ion transport to cellular membrane dynamics and organ-level growth responses—yet the mechanistic pathways connecting CNM physicochemical properties to specific biological outcomes remain incompletely characterized. While physical penetration of seed coats, activation of aquaporin-mediated water transport, modulation of reactive oxygen species (ROS) signaling, and transcriptional reprogramming have each been documented in specific experimental systems, the causal chains linking CNM surface chemistry to defined regulatory networks are poorly resolved, and findings from hydroponic or in vitro systems cannot be straightforwardly extrapolated to soil-grown crops. A rigorous understanding of these

mechanisms—including their species-specificity, dose-dependence, and sensitivity to environmental context—is therefore prerequisite not only for evaluating the potential agronomic benefits of CNMs, but equally for identifying the conditions under which these same mechanisms become drivers of phytotoxicity..

### 3.1. Mechanisms of Nutrient Uptake Promotion

CNMs can promote the uptake of water and mineral nutrients by plants through various means. Firstly, the nanoscale size of CNMs allows them to penetrate the seed coat or cell wall, potentially creating new pores in the cell wall physically or activating aquaporins, thereby significantly enhancing seed imbibition and water transport efficiency [49,50]. For instance, Khodakovskaya et al. found that MWCNTs could penetrate tomato seed coats and promote water uptake, thereby accelerating seed germination [51].

Secondly, CNMs can serve as carriers for nutrient elements, improving their availability and utilization efficiency. GO, due to its surface rich in negatively charged oxygen-containing functional groups, can combine with trace element cations (e.g.,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ) through electrostatic adsorption, forming "nano-nutrient" complexes and enabling slow release and targeted transport of nutrients [52]. Studies have shown that negatively charged MWCNTs can adsorb and slowly release trace elements such as copper (Cu) and zinc (Zn), promoting plant uptake and transport of these micronutrients [53,54]. Research on carbon dots (CDs) also indicates they can promote the absorption of macro- and micronutrients like iron (Fe), magnesium (Mg), and potassium (K), and regulate biomass accumulation through photosynthesis [55]. Hu et al. found that CDs significantly increased the content of elements such as Fe, Ca, Mg, and K in Chinese kale (*Brassica campestris* L.), ultimately promoting plant growth [56]. Furthermore, functionalized fullerenols have been shown to enhance the uptake of phosphorus (P) and potassium (K) in wheat under salt stress by regulating ion transport while reducing the accumulation of harmful sodium ions ( $\text{Na}^+$ ) [57,58].

### 3.2. Regulation of Photosynthesis

Photosynthesis is the core driver of plant growth. CNMs can influence photosynthetic efficiency through direct or indirect means. Firstly, CNMs can enter chloroplasts or act as "light converters" to enhance light energy utilization efficiency through their unique optical properties. For example, carbon dots can absorb ultraviolet light and emit blue light that can be efficiently absorbed by chlorophyll, thereby expanding the effective spectral range for photosynthesis and enhancing light capture and utilization [59,60]. Li et al.'s research confirmed that CDs with photoluminescent properties can significantly enhance the photosynthetic efficiency of plant leaves [61].

Secondly, CNMs can improve physiological indicators related to photosynthesis. Numerous studies have shown that treatment with CNMs can significantly increase leaf chlorophyll content, net photosynthetic rate ( $P_n$ ), stomatal conductance ( $G_s$ ), and electron transport efficiency of photosystem II (PSII) [62,63]. For instance, CDs derived from *Salvia miltiorrhiza* were found to increase chlorophyll content, net photosynthetic rate, and PSII quantum efficiency in lettuce under high-temperature stress [64]. Wang et al. also discovered that nitrogen-doped CDs could enhance PSII activity in maize, thereby improving its photosynthetic efficiency [65]. This regulatory effect may stem from CNMs' influence on stomatal movement; they can promote stomatal opening, increasing  $\text{CO}_2$  supply, while also enhancing light energy conversion efficiency by affecting components of the photosynthetic electron transport chain [66].

### 3.3. Enhancement of Antioxidant Defense and Stress Tolerance

When plants face adverse conditions (e.g., drought, salinity, high temperature, heavy metals), they accumulate excessive reactive oxygen species (ROS), leading to oxidative stress and cellular damage. CNMs can scavenge excess ROS by mimicking antioxidant enzymes ("nanozymes") or activating the plant's own antioxidant defense system, thereby enhancing plant stress tolerance

[67,68]. For example, fullerenols, known as efficient nano-antioxidants due to their potent free radical scavenging ability, can effectively alleviate oxidative damage in sugar beet and rapeseed under drought stress [69,70]. Shafiq et al. also found that fullereneol could enhance salt tolerance in wheat by increasing the activity of H<sub>2</sub>O<sub>2</sub>-scavenging enzymes [58,71]. CDs also exhibit excellent antioxidant activity, scavenging DPPH and hydroxyl radicals, and upregulating the activities of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), thereby reducing malondialdehyde (MDA) content and alleviating membrane lipid peroxidation damage [55,64,66]. This enhancement of antioxidant capacity enables plants to better cope with oxidative stress induced by heavy metals, salinity, and temperature extremes [72,73].

#### 4. Diversified Applications of Carbon-Based Nanomaterials in Agriculture

Owing to their positive regulatory effects on plant physiology, the application scope of CNMs in agriculture is increasingly broad, covering the entire process from seed germination and crop growth to pest control and post-harvest preservation.

##### 4.1. Plant Growth Promoters

CNMs can act as efficient plant growth regulators, promoting plant growth through seed priming or direct application to soil/foilage. Research indicates that low concentrations of CNTs (especially MWCNTs) can significantly enhance seed germination rates, root length, and seedling biomass in various crops, including tomato, rice, soybean, and wheat [19,51,74]. For example, MWCNT treatment significantly increased root and stem length in rice seedlings [75]. In field trials, tomato plants treated with MWCNTs produced twice as many flowers and fruits as untreated controls [76]. Graphene nanosheets have also been shown to promote the growth of pepper and eggplant, increasing fruit yield [77]. Bitter melon treated with fullereneol not only increased biomass by 54% but also boosted fruit yield by 128%, alongside significant increases in medicinal components such as cucurbitacin B and lycopene [78]. Additionally, carbon dots have been confirmed to significantly promote the growth and yield of peas, rice, and rapeseed [79,80].

##### 4.2. Nano-Pesticides and Plant Protection

CNMs exhibit significant potential in plant disease and pest management. They can act directly as antimicrobial/antifungal agents against pathogens or serve as efficient carriers for targeted delivery of pesticides, reducing pesticide application rates and environmental pollution. Studies have shown that SWCNTs, MWCNTs, GO, and fullerenes possess significant inhibitory effects against plant pathogenic fungi (e.g., *Fusarium graminearum*, *F. poae*) and bacteria (e.g., *Ralstonia solanacearum*, *Xanthomonas oryzae*) [81,82]. Their antimicrobial mechanisms primarily involve physical piercing of cell membranes via sharp edges, induction of oxidative stress, and interference with pathogen water and nutrient uptake [22]. Functionalized MWCNTs combined with pesticides form nanocomposites, such as MWCNT-fungicide complexes, exhibiting stronger effects against *Alternaria alternata* than the single agent [23]. More importantly, CNTs can serve as nanocarriers for double-stranded RNA (dsRNA), enabling spray-induced gene silencing (SIGS) technology to interfere with specific target genes, providing crops with sustained antiviral or antifungal protection [83,84]. For instance, chitosan-functionalized CNTs effectively deliver dsRNA, significantly enhancing rice resistance to stem borers [85]. Furthermore, GO-AgNPs nanocomposites exhibit significantly higher antifungal efficacy against *Fusarium graminearum* than pure AgNPs or GO alone [86].

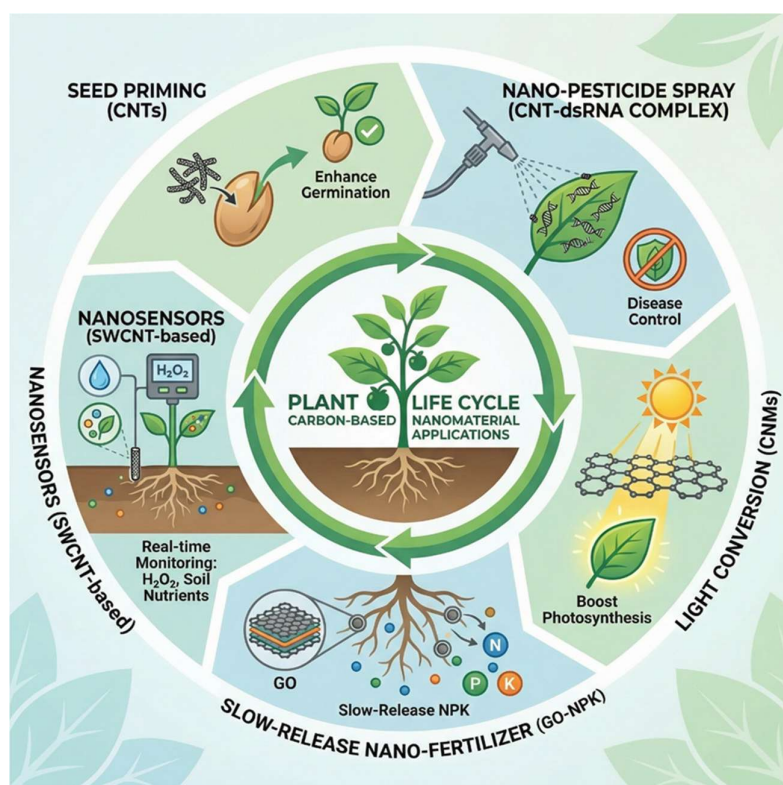
##### 4.3. Nano-Fertilizers and Slow-Release Systems

Traditional fertilizers suffer from extremely low utilization efficiency due to leaching and volatilization, leading not only to resource waste but also severe environmental pollution. CNMs can act as "nanocarriers" for nutrients, constructing slow- or controlled-release fertilizer systems to

enhance fertilizer use efficiency. GO, with its enormous specific surface area and abundant oxygen-containing functional groups, can effectively adsorb and load nitrogen, phosphorus, potassium, and micronutrients, forming slow-release fertilizers that extend the nutrient release period [52,87]. For example, encapsulating  $\text{KNO}_3$  in GO films allows for the slow release of potassium and nitrogen, better meeting the long-term needs of plants [88]. Carbon nanofibers (CNFs) have also been used as carriers for copper (Cu), which is taken up by roots and slowly released, improving Cu transport to shoots and significantly enhancing crop growth and protein content [53]. Additionally, porous carbon nanomaterials prepared from biomass waste (e.g., biogas plant residues), owing to their favorable pore structure and surface chemistry, show promise as potential slow-release fertilizer carriers [89].

#### 4.4. Sensing and Precision Agriculture

Real-time, non-destructive, high-throughput monitoring of plant and soil health is central to precision agriculture. CNMs, particularly CNTs, are ideal materials for constructing highly sensitive nanosensors due to their excellent electrochemical properties and ease of functionalization [90,91]. For example, near-infrared fluorescence sensors based on SWCNTs can monitor real-time  $\text{H}_2\text{O}_2$  signaling waves in plants induced by injury, pathogen infection, or stress, enabling non-destructive, dynamic monitoring of early plant stress [26,92]. By functionalizing SWCNTs to specifically recognize pathogen-secreted proteins (e.g., SDE1 protein of citrus Huanglongbing pathogen), highly sensitive and specific biosensors can be developed for early disease diagnosis [93]. Furthermore, CNT sensors are used for detecting pesticide residues. For instance, fluorescence sensors based on carbon dots and gold nanoparticles can rapidly detect organophosphorus pesticide residues like malathion in fruits and vegetables [94]. Biomass-derived carbon dots or graphene quantum dots can also be used for rapid, highly sensitive electrochemical detection of pesticides such as methyl parathion [95,96]. The development of these sensor technologies provides powerful technical support for achieving crop nutrition diagnosis, disease early warning, and environmental monitoring.



**Figure 2.** Applications of carbon-based nanomaterials (CNMs) throughout the plant life cycle. Overview of the multifunctional roles of carbon-based nanomaterials (CNMs) in different stages of the plant life cycle. CNMs

contribute to seed priming (enhanced germination), nano-enabled pesticide delivery (e.g., CNT–dsRNA complexes for disease control), and nanosensing (SWCNT-based sensors for real-time monitoring of hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, and soil nutrients). Additionally, CNMs facilitate slow-release nano-fertilizers (e.g., graphene oxide-based GO–NPK systems), improve photosynthetic efficiency via enhanced light conversion, and promote overall plant growth and productivity. These integrated applications highlight the potential of CNMs in precision agriculture and sustainable crop management.

## 5. Dual Effects and Toxicity Issues

Although CNMs demonstrate numerous benefits in agricultural applications, their impact on plants is not always positive. Numerous studies indicate that the effects of CNMs on plants exhibit a significant "biphasic effect," namely hormesis—stimulation at low doses and inhibition at high doses [29,30,97]. Yet this framing, while conceptually useful, risks oversimplifying a phenomenon that is far more variable and context-dependent than a universal dose-response curve implies. Reported effective concentration thresholds differ by orders of magnitude across studies and species, and the factors that determine where the inflection point falls—CNM type, surface charge, aggregation state, exposure route, plant developmental stage, and environmental matrix—interact in ways that current evidence cannot fully predict. Moreover, the majority of hormesis data derives from controlled hydroponic or *in vitro* systems, and whether these dose-response relationships hold under realistic soil-based agricultural conditions remains largely untested. A critical rather than merely descriptive engagement with this dual-effect phenomenon is therefore essential: the goal is not simply to acknowledge that CNMs can both help and harm, but to identify the mechanistic and contextual determinants of the stimulation-to-inhibition transition with sufficient precision to inform safe application guidelines.

### 5.1. Positive Effects

Carbon-based nanomaterials (CNMs) at low or optimized concentrations can effectively trigger plant defense and growth mechanisms, exhibiting various positive effects. Firstly, in promoting seed germination and seedling growth, CNMs can physically penetrate the seed coat, activate aquaporins, significantly enhance seed water uptake, thereby accelerating germination and boosting early seedling vigor [51,74]. Secondly, CNMs can significantly enhance plant nutrient uptake and transport; they can act as efficient nanocarriers, promoting the dissolution of mineral elements in soil and, through interaction with root surfaces, efficiently transport them into plants, thereby improving nutrient use efficiency [52,53,56]. At the photosynthetic level, CNMs can leverage their unique optical functions, for example, acting as light converters to transform ultraviolet light into usable blue light for plants, or enhancing chloroplast function, thereby improving light energy utilization and carbon assimilation, ultimately promoting photosynthetic product accumulation [61,64,65]. Concurrently, CNMs can activate the plant's antioxidant defense system; they can function as nanozymes or signaling molecules, inducing the activities of a series of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), effectively scavenging excess reactive oxygen species (ROS) and alleviating oxidative stress induced by adverse conditions [58,70,71]. At the molecular level, CNMs have been shown to modulate plant gene expression in ways that can support growth at low concentrations, including upregulation of aquaporin genes (e.g., NtPIP1), cell division markers (e.g., CycB), and cell-wall extension genes (e.g., NtLRX1) in tobacco cells exposed to MWCNTs [51,76]. However, this picture is considerably more complex than a simple activation of beneficial gene programs: transcriptomic profiling has revealed that surface chemistry is a primary determinant of gene regulatory outcomes, with certain functionalized CNTs (e.g., PEI-coated SWCNTs) inducing broad stress, immunity, and senescence programs rather than growth-promoting responses, while the same concentration of more chemically inert CNTs elicits only minor transcriptional changes. Species-specific differences further complicate generalization—charged MWCNTs that stimulate maize growth simultaneously inhibit soybean, reflecting divergent transcriptional and physiological responses that cannot be predicted from CNM properties alone.

These inconsistencies underscore that the gene regulatory effects of CNMs are not uniformly beneficial and that their direction and magnitude depend critically on CNM surface chemistry, plant species, and exposure conditions in ways that remain insufficiently characterized.

### 5.2. Negative Effects

When CNM concentrations exceed the tolerance threshold of a given plant system, the biological response undergoes a qualitative shift from promotion to inhibition—a transition that is well-documented but whose mechanistic basis and concentration boundaries remain incompletely characterized. Understanding the nature and determinants of this shift is essential for establishing safe application windows and for interpreting the contradictory outcomes that pervade the literature.

The most consistently reported negative effect of high-concentration CNM exposure is inhibition of seed germination and root elongation. Excessive CNM deposition can physically obstruct pores in root cell walls, interfere with normal water and nutrient uptake pathways, and reduce root length and overall biomass [98,99]. However, this inhibitory effect is not universal: the same MWCNT concentrations that suppress germination in one species may be stimulatory in another. Studies have reported that tomato seedlings exhibit sensitivity to SWCNTs at concentrations that elicit growth promotion in onion and cucumber [111]. This species-specificity is a critical and underappreciated source of variability in the literature, and its mechanistic basis—whether rooted in differences in cell wall composition, aquaporin expression, or ROS buffering capacity—remains to be systematically elucidated.

Beyond physical obstruction, excessive CNM accumulation induces oxidative stress through a well-characterized cascade: CNMs generate or catalyze the production of reactive oxygen species (ROS), which overwhelm the plant's antioxidant defenses and attack membrane lipids, triggering peroxidation, elevating malondialdehyde (MDA) content, disrupting membrane integrity, and causing electrolyte leakage [33,34,100]. In severe cases, this cascade culminates in cell death. What is less clearly resolved is the relative contribution of different ROS species and the extent to which the antioxidant response itself—initially protective—can become maladaptive at sustained high exposures. Meta-analytic evidence suggests that while antioxidant enzyme activities (SOD, POD, CAT) are broadly elevated by CNM treatment, the direction and magnitude of this response are highly concentration- and duration-dependent, and the relationship between enzyme induction and net oxidative damage is not linear.

Photosynthetic impairment represents another well-documented but mechanistically complex negative effect. High CNM concentrations have been shown to decrease chlorophyll content, impair PSII activity, and alter chloroplast ultrastructure—including thylakoid swelling and abnormal starch granule accumulation—thereby suppressing overall photosynthetic efficiency [101,102]. Notably, graphene and its derivatives appear to be particularly potent in this regard: multi-omics analyses have revealed that graphene derivatives induce oxidative damage, downregulate endocytosis and transmembrane transport proteins, and reprogram carbohydrate and amino acid metabolism in ways consistent with photosynthetic decline and metabolic stress. These molecular-level findings provide mechanistic context for the physiological observations, but they also highlight the fact that different CNM types—even within the carbon-based family—can operate through distinct toxicity pathways, a distinction that is frequently obscured in aggregate analyses.

Hormonal disruption constitutes a further dimension of CNM toxicity that has received comparatively limited systematic attention. High CNM concentrations can interfere with the synthesis and signal transduction of key phytohormones, including auxin (IAA) and abscisic acid (ABA), thereby disrupting normal developmental programs [103,104]. The mechanistic linkage between CNM exposure, ROS accumulation, and hormonal perturbation remains poorly characterized, representing a significant gap in current understanding.

Perhaps the most concerning—and least understood—negative effect is genotoxicity. Certain CNMs, particularly MWCNTs, have been shown to enter the plant cell nucleus, inducing DNA strand breaks, chromosomal aberrations, and altered DNA methylation patterns [105,106]. These epigenetic

changes are of particular concern because they may be heritable: C<sub>70</sub> fullerenes have been detected in second-generation seedlings, raising the possibility of multigenerational transmission of CNM-induced epigenetic alterations. The long-term implications of such changes for plant health, genetic stability, and food safety are currently unknown, and this represents one of the most urgent knowledge gaps in the field.

A critical synthesis of the negative effects literature reveals several recurring inconsistencies. First, the dose thresholds for toxicity vary by orders of magnitude across studies—from tens of mg·L<sup>-1</sup> in some hydroponic systems to thousands of mg·kg<sup>-1</sup> in soil-based experiments—making direct comparisons hazardous without careful attention to exposure medium and bioavailability. Second, the majority of phytotoxicity studies have been conducted under controlled laboratory conditions, often using hydroponic or agar-based systems that may not accurately reflect CNM behavior in natural soil environments, where aggregation, adsorption to organic matter, and microbial transformation can substantially alter bioavailability. Third, short-term exposure assays may underestimate chronic toxicity, while single-endpoint measurements fail to capture the systemic nature of CNM effects. These methodological limitations counsel caution in extrapolating laboratory results to field applications.

### 5.3. Toxicity Mechanisms and Influencing Factors

The phytotoxicity of CNMs is not attributable to a single mechanism but emerges from the interplay of multiple physicochemical and biological processes, the relative contributions of which vary with CNM type, concentration, and plant system. A mechanistically nuanced understanding of this complexity is essential for moving beyond descriptive toxicology toward predictive risk assessment.

At the molecular level, ROS-mediated oxidative stress is the most consistently implicated toxicity pathway. When the rate of ROS generation—whether through CNM surface reactivity, disruption of mitochondrial electron transport, or inhibition of antioxidant enzymes—exceeds the plant's scavenging capacity, a cascade of oxidative damage ensues, targeting proteins, lipids, and DNA [107]. However, it is important to distinguish between the initial ROS burst, which may function as a signaling event at low doses and activate adaptive responses, and the sustained ROS accumulation at high doses that overwhelms these responses and drives cellular damage. This distinction is mechanistically critical but is rarely captured in studies that measure ROS or antioxidant enzyme activity at a single time point. The temporal dynamics of the oxidative stress response—including the kinetics of enzyme induction, the rate of ROS clearance, and the point at which adaptive capacity is exceeded—represent a significant knowledge gap.

Physical interactions between CNMs and cellular membranes constitute a second, mechanistically distinct toxicity pathway. The sharp edges of CNTs and graphene sheets can physically pierce or disrupt cell wall and membrane structures, causing permeability changes and electrolyte leakage that are independent of ROS generation [22,108]. This physical membrane disruption may explain some of the toxicity observed even with relatively low concentrations of highly dispersed, surface-functionalized CNMs, where chemical reactivity is reduced but physical interactions with membranes are preserved. The relative importance of physical versus chemical toxicity mechanisms likely varies with CNM geometry and surface chemistry, but this question has not been systematically addressed across CNM types.

Beyond these two primary mechanisms, emerging multi-omics evidence points to additional pathways that are less well characterized but potentially important. CNM exposure—particularly by graphene derivatives—can downregulate endocytosis and transmembrane transport proteins, alter carbohydrate and amino acid metabolism, and reprogram stress-response gene networks in ways that extend well beyond simple oxidative damage. These findings suggest that CNMs can function as modulators of broader cellular signaling networks, with consequences for nutrient assimilation, energy metabolism, and developmental programming that are not captured by conventional toxicity

endpoints. The integration of transcriptomic, proteomic, and metabolomic approaches across multiple CNM types and plant species is therefore a priority for mechanistic research.

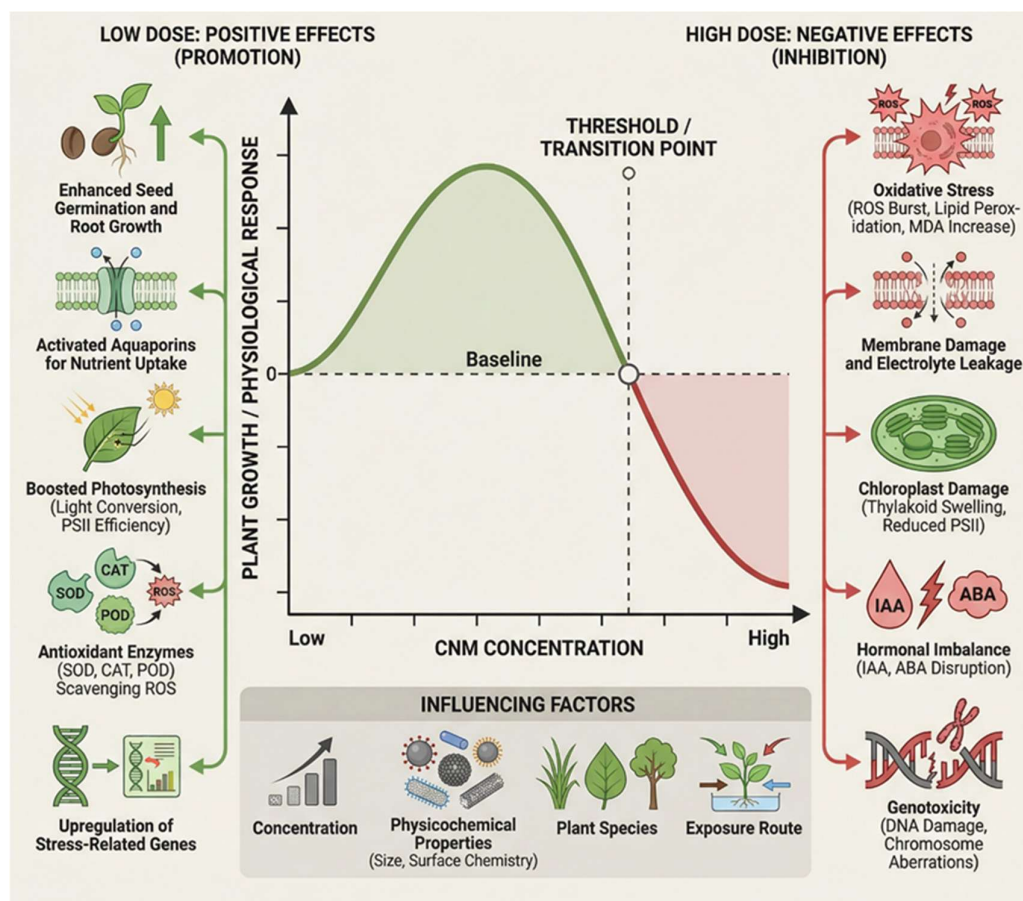
The expression of CNM toxicity is profoundly modulated by a set of interconnected factors, the interactions among which are not yet fully understood. Concentration remains the most critical determinant, but the relationship between applied concentration and biological effect is not simply monotonic: the hormesis curve implies that the same material can be beneficial at one concentration and harmful at another, and the position of the inflection point varies with CNM type, surface chemistry, and plant species [97]. This concentration-dependence is further complicated by the fact that applied concentration is not equivalent to bioavailable concentration, particularly in soil systems where CNM aggregation, adsorption to organic matter, and microbial transformation can substantially reduce the fraction available for plant uptake.

Physicochemical properties—including size, shape, surface chemistry, aggregation state, and purity—exert profound effects on CNM-plant interactions that are often underappreciated in studies that treat CNMs as homogeneous materials. Smaller CNTs may penetrate cell walls more readily, potentially enhancing both beneficial and toxic effects, while surface functionalization—particularly carboxylation and amination—generally reduces toxicity by improving dispersibility and reducing surface reactivity [47,109,110]. Crucially, the purity of CNM preparations is rarely rigorously controlled: residual metal catalysts (e.g., Ni, Co) used in CNT synthesis can themselves be phytotoxic, and their contribution to observed toxicity is frequently confounded with the effects of the carbon material itself—a systematic methodological problem that may account for some contradictions between studies using CNMs from different sources.

Plant species and developmental stage introduce a further layer of complexity that is well-documented but poorly mechanistically explained. The sensitivity of different species to CNMs varies substantially: tomatoes are sensitive to SWCNTs at concentrations that stimulate onion and cucumber growth, while seedlings are generally more vulnerable than mature plants [111]. These differences likely reflect variation in cell wall composition, root architecture, aquaporin expression patterns, and endogenous antioxidant capacity, but the specific molecular determinants of species-specific sensitivity have not been systematically characterized. This gap is particularly consequential for risk assessment, as it means that safety conclusions drawn from studies on one crop species cannot be straightforwardly generalized to others.

Exposure route and environmental conditions constitute the final, and arguably most practically important, category of modulating factors. Different application methods—root exposure, foliar spray, or seed priming—produce fundamentally different CNM uptake kinetics, tissue distribution patterns, and biological responses, yet these routes are rarely compared within the same experimental system [112]. Environmental factors, including soil pH, organic matter content, ionic strength, and microbial community composition, profoundly influence CNM aggregation state, surface chemistry, and bioavailability. CNTs have been shown to alter agrosystem multifunctionality—including soil microbial community structure and nutrient cycling processes—at concentrations that may not directly affect plant growth, suggesting that indirect ecological effects mediated through the soil microbiome may be as important as direct phytotoxic effects. These ecosystem-level interactions are largely absent from current risk assessments, which tend to focus on direct plant-CNM interactions under controlled conditions.

In summary, the toxicity of CNMs in agricultural systems is a multifactorial phenomenon that cannot be adequately characterized by any single mechanism or experimental approach. Progress in this area requires not only the expansion of mechanistic studies to include multi-omics and systems-level approaches, but also greater methodological standardization—particularly with respect to CNM characterization, exposure conditions, and the choice of toxicity endpoints—to enable the cross-study comparisons that are currently impeded by the heterogeneity of the literature.



**Figure 3.** Dose-dependent dual effects (hormesis) of carbon-based nanomaterials (CNMs) on plant systems. Conceptual model illustrating the dose-dependent biphasic (hormetic) effects of carbon-based nanomaterials (CNMs) on plant growth and physiological responses. At low concentrations, CNMs promote plant development by enhancing seed germination, activating aquaporins for nutrient uptake, improving photosynthetic efficiency (e.g., photosystem II, PSII), stimulating antioxidant defense systems (e.g., superoxide dismutase, SOD; catalase, CAT; peroxidase, POD), and upregulating stress-responsive genes. In contrast, high concentrations induce negative effects, including oxidative stress (reactive oxygen species, ROS burst and lipid peroxidation), membrane damage, chloroplast dysfunction, hormonal imbalance (e.g., indole-3-acetic acid, IAA; abscisic acid, ABA), and genotoxicity. The transition between beneficial and toxic effects depends on multiple factors, including CNM concentration, physicochemical properties (e.g., size and surface chemistry), plant species, and exposure pathways.

## 6. Sustainable Development and Green Synthesis

The dual-effect profile of CNMs—beneficial at low doses, potentially harmful at higher concentrations or with prolonged exposure—raises a fundamental question that has not been adequately addressed in the literature: can CNMs be deployed at agronomic scale in a manner that reliably captures their benefits while avoiding their risks? Answering this question requires not only a rigorous assessment of ecological and health risks, but also a critical evaluation of whether green synthesis strategies can meaningfully reduce the environmental footprint of CNM production. Neither of these questions has a definitive answer at present, and intellectual honesty demands that this uncertainty be explicitly acknowledged.

### 6.1. Environmental and Health Risks

The environmental risk profile of CNMs in agricultural systems is shaped by a complex interplay of release dynamics, environmental fate, and ecotoxicological effects that are only partially

characterized. CNMs released during production, application, or disposal can migrate through soil profiles, interact with soil organic matter and mineral surfaces, and undergo transformation processes—including oxidation, aggregation, and microbial modification—that alter their bioavailability and toxicity in ways that are difficult to predict from laboratory studies [28,113,114]. The behavior of CNMs in heterogeneous, dynamic soil environments differs substantially from their behavior in the controlled aqueous or agar-based systems used in most toxicology studies, and this gap between experimental conditions and field reality is a persistent limitation of current risk assessments.

Effects on non-target soil organisms represent a particularly undercharacterized dimension of CNM environmental risk. While direct phytotoxicity has been extensively studied, the impacts of CNMs on soil microbial communities, earthworms, nematodes, and aquatic organisms—which collectively underpin soil fertility and ecosystem functioning—have received comparatively little attention. Recent evidence suggests that CNTs can alter agrosystem multifunctionality, including microbial community composition and nutrient cycling processes, at concentrations within the range of agronomic application rates, suggesting that indirect ecological effects may be as consequential as direct plant toxicity. Similarly, the potential for CNMs to accumulate in edible plant tissues and enter the human food chain represents a health risk that has not been adequately quantified, particularly for nanoforms that are resistant to metabolic degradation.

A further concern that is frequently underemphasized is the contribution of residual synthesis impurities to observed toxicity. Many CNT preparations contain residual metal catalysts (e.g., Ni, Co, Fe) that are themselves toxic, and the failure to rigorously control for and characterize these impurities in ecotoxicological studies means that attributing observed effects specifically to the carbon nanomaterial rather than to its contaminants remains problematic [115]. This has substantive implications for risk assessment, because the toxicity of a purified, well-characterized CNM preparation may differ substantially from that of the crude material used in most agricultural studies. Comprehensive life cycle assessment (LCA) and ecotoxicological risk assessment frameworks that account for the full spectrum of CNM forms and impurities—not only the idealized pure materials studied in laboratory settings—are therefore a prerequisite for responsible commercialization.

## 6.2. Green Synthesis Pathways

Green synthesis strategies—utilizing renewable biomass resources as carbon precursors—have emerged as a promising approach to reducing the environmental footprint of CNM production, and the diversity of plant-derived materials that have been successfully employed is notable [116,117]. Plant-derived biomass (e.g., leaves, fruits, peels, seeds), rich in cellulose, hemicellulose, lignin, polysaccharides, and various secondary metabolites, serves as an ideal precursor for preparing CNMs [118,119]. For example, fluorescent carbon dots can be synthesized using plant extracts such as banana peels, green tea, chili peppers, and ginger via hydrothermal carbonization, microwave-assisted, or pyrolysis methods [38,120]. These plant-derived carbon dots not only exhibit good water solubility, photostability, and low toxicity but also possess naturally occurring surface functional groups, offering advantages in bioimaging and sensing applications [121]. Sonkar et al. prepared water-soluble carbon nano-onions (CNOs) via pyrolysis of wood wool and demonstrated their effectiveness in promoting the growth of gramineous plants [122]. Utilizing biomass waste (e.g., wheat straw, biogas residue) to prepare porous carbon materials not only addresses waste disposal issues but also yields high-performance carbon materials suitable for supercapacitors and slow-release fertilizer carriers [89,123].

However, a critical assessment of green synthesis must acknowledge several important limitations that are often downplayed in the literature. First, the physicochemical properties of biomass-derived CNMs—including size distribution, surface chemistry, and structural uniformity—are typically more variable than those of materials produced by conventional chemical methods, and this variability can translate into inconsistent biological effects. The “green” label should not be taken as synonymous with “safe”: biomass-derived CNMs can still exhibit dose-dependent toxicity, and

their environmental fate and ecotoxicological profile may differ from those of conventionally synthesized materials in ways that have not been systematically characterized [124]. Second, the scalability of green synthesis routes from laboratory to industrial scale remains largely undemonstrated, and the energy and resource inputs associated with large-scale biomass processing may offset some of the environmental benefits claimed for these approaches. Third, the diversity of biomass sources and synthesis conditions used in the literature makes cross-study comparisons extremely difficult, and standardized characterization protocols for biomass-derived CNMs are urgently needed.

This “nature-to-nature” synthesis strategy avoids the use of expensive reagents, toxic chemical reductants, and harsh reaction conditions, significantly reducing the production cost and environmental footprint of CNMs, offering a novel approach for their sustainable application in agriculture [124]. However, realizing these advantages at scale will require not only technical advances in synthesis and characterization, but also rigorous life cycle assessments that account for the full environmental costs of biomass sourcing, processing, and waste management.

## 7. Future Perspectives

The preceding sections have highlighted both the considerable promise of CNMs in agricultural applications and the significant scientific uncertainties that must be resolved before this promise can be responsibly realized at scale. The following research priorities are identified as the specific areas where the current evidence base is most deficient and where targeted investment is most likely to yield scientifically and practically consequential advances.

A. Resolving the Mechanistic Basis of Dose-Dependent Dual Effects: The hormetic dose-response of CNMs—stimulation at low doses, inhibition at high doses—is well-documented phenomenologically, but its mechanistic underpinnings remain insufficiently characterized. The critical need is not for more studies that document the existence of biphasic effects, but for mechanistic studies that identify the molecular switch points governing the transition from stimulation to inhibition. This requires integration of omics technologies (e.g., transcriptomics, proteomics, metabolomics) with time-resolved exposure experiments across multiple CNM types, concentrations, and plant species. Specifically, identifying the “critical threshold” at which ROS signaling transitions from adaptive to damaging, and how this threshold is modulated by CNM physicochemical properties and plant genotype, would provide a mechanistic foundation for safe-use guidelines that is currently lacking.

B. Standardization of CNM Characterization and Experimental Protocols: One of the most significant impediments to scientific progress in this field is the lack of standardized characterization and reporting protocols for CNMs used in biological studies. The current literature is characterized by substantial heterogeneity in CNM purity, size distribution, surface chemistry, aggregation state, and dosing units, making cross-study comparisons hazardous and meta-analyses unreliable. Establishing community standards for CNM characterization—including mandatory reporting of size distribution, surface area, functional group density, metal impurity content, and aggregation state in biological media—is a prerequisite for building a coherent and cumulative evidence base. Equally important is the standardization of exposure conditions, including the use of realistic soil-based systems alongside hydroponic controls, and the adoption of a common set of biological endpoints that capture both acute and chronic effects.

C. Long-Term Environmental Fate and Multigenerational Risk Assessment: The vast majority of existing studies have been conducted under controlled laboratory conditions over short time scales, providing limited information about the long-term environmental fate of CNMs in natural soil–plant systems. Field-scale, longitudinal studies are urgently needed to characterize CNM persistence, aging, transformation, and translocation to edible organs under realistic agronomic conditions. Of particular concern is the potential for multigenerational transmission of CNM-induced epigenetic changes: the detection of C<sub>70</sub> fullerenes in second-generation seedlings raises the possibility that CNM exposure could have heritable consequences for plant genetic stability and crop quality that would

not be detected in single-generation studies. Comprehensive risk assessment frameworks must therefore incorporate multigenerational exposure data, ecosystem-level effects on soil microbial communities and nutrient cycling, and food chain transfer assessments—none of which are currently available at the scale required for regulatory decision-making.

**D. Multifunctionalization and Smart Delivery Systems:** The most transformative near-term opportunity for CNMs in agriculture lies not in their use as individual agents but in the development of integrated, multifunctional nanosystems that combine growth promotion, targeted delivery, stress alleviation, and real-time sensing in a single platform. Future research should focus on developing “smart nano-enabled agricultural inputs” that integrate growth promotion, pest and disease control, stress alleviation, and sensing capabilities. For example, developing stimuli-responsive nanocarriers that release active ingredients in response to specific environmental signals (e.g., pH, enzymes, moisture) for on-demand, precise delivery. The realization of such systems requires advances in CNM surface functionalization chemistry, biocompatibility engineering, and in situ sensing integration that are at the frontier of current materials science.

**E. Scalable Production, Cost-Effectiveness, and Regulatory Frameworks:** The path from laboratory demonstration to commercial agricultural application requires progress on dimensions that are often neglected in research publications: scalable synthesis, cost-effectiveness, and regulatory compliance. Although green synthesis offers possibilities for low-cost CNM production, a gap remains between laboratory-scale synthesis and commercial large-scale production. Future development of scalable, stable, and economically feasible synthesis processes is needed. Concurrently, detailed cost-benefit analyses should be conducted to evaluate the economic advantages of CNMs in enhancing crop yields and reducing traditional agrochemical inputs, demonstrating their superiority over conventional technologies. As nano-enabled agricultural products increase, establishing and improving relevant regulations and standards systems is urgent. This includes standardizing definitions, characterization, safety evaluation, labeling, and traceability systems for CNMs. Simultaneously, strengthening science communication to enhance public scientific understanding of nano-enabled agricultural technologies and alleviate unnecessary concerns is crucial for promoting social acceptance and market application.

In conclusion, carbon-based nanomaterials, particularly carbon nanotubes, offer genuine and substantial opportunities to contribute to the goals of sustainable agriculture, but realizing this potential responsibly requires a more critical, mechanistically rigorous, and methodologically disciplined research agenda than has characterized much of the field to date. The inconsistencies and knowledge gaps identified in this review are not obstacles to be minimized but scientific problems to be solved—and their resolution will ultimately determine whether CNMs can transition from laboratory curiosity to transformative agricultural technology.

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