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## **EFFECT OF CARBON NANOPARTICLES ON CORN (*Zea mays* L.) SEED GERMINATION, GROWTH AND NUTRIENT UPTAKE**

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### **ABSTRACT**

The use of carbon nanoparticles (CNPs) in plant science and agricultural production has been on the rise in recent decades. In case of Carbon-based nanoparticles, carbon nanotubes (CNTs) have been used mostly in plant science. Carbon nanotubes (CNTs) provide a promising net effect in plant science due to their physiochemical properties and versatile applications processes. The CNTs improve the ability of plants to tolerate physiological stress by improving water dynamics and nutrient uptake as well as activating defense mechanisms against abiotic and biotic stresses. They can be taken up by roots and translocated within the plant, impacting water retention, nutrient assimilation, and photosynthesis. In this work, factory-synthesized multi-walled-CNTs (MWCNTs) of quality-controlled specifications were used to prepare a germination medium together with different concentration of  $\text{Fe}^{2+}$  in agarose solution. To assess the effect of MWCNTs on seed germination, nutrient uptake and growth of corn (*Zea mays* L.), physical parameters were measured and the essential nutrient uptake rate quantified using nuclear microscopy technique of Particle Induced X-ray Emission, (PIXE). The application of CNTs of concentration between 10-20 mg/L significantly increased seed germination rate, Fresh and dry biomass of shoots and roots. The trace elemental nutrient uptake by the roots was quantitatively measured and reported as well.

**Keywords:** Carbon nanoparticles (CNPs), Carbon nanotubes (CNTs), corn, nutrient uptake, PIXE.

### **1. INTRODUCTION**

In recent years, we have seen an increased use of nanotechnology especially carbon nanotubes (CNTs) in agriculture due to their favorable biocompatibility, good conductivity, large scale production and eco-friendly synthesis methods [1]. The impact of the using CNTs includes

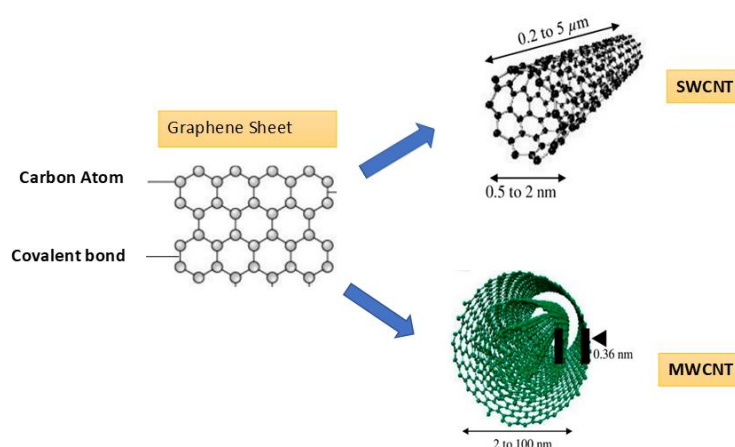
stimulating and enhancing seed germination and rate, elongation of root and shoot structures, increased vegetative biomass and photosynthesis hence improved plant growth, enhancing stress tolerance, regulating nutrient uptake, diagnosing plant diseases, and reduction of agrochemical input [2,3,4, 5, 6]. CNTs used as additives and active components make up 40% of all contributions of nanotechnology in agriculture [7]. Carbon nanotubes (CNTs) have proven to be the dominant and most versatile of the carbon nanotubes (CNTs) since the inception of their use in agricultural production and have shown significant positive result on improving crop yield necessary to meet the high food demand for both human and livestock [8, 9,10]

The multifaceted dynamics associated with CNT-plant interactions constitutes various effects on the physiological [11], biochemical [12], and molecular levels [13]. The resulting benefit to plant includes improved photosynthesis, nutrient uptake efficiency, and increased antioxidant activity, which together leads to better plant growth and stress resilience. In addition, there is also the potential phytotoxic implications associated with the use of CNTs for plant growth [14]. Two important features that play a central role in shaping plant health, growth and stress tolerant includes microbial interactions within the rhizosphere and plant microbiome [15, 16]. CNTs have been found to have much influence on these intricate microbial networks, with potential implications for plant-microbe symbiosis and soil health [17, 18]. Similarly, CNTs has been found to have a strong efficacy against plant pathogens including fungi and bacteria [19,20,21], a property that is so essential in improving crop yields. Due to their high surface area and reactivity, CNTs provide an effective way of delivering agrochemical compounds to plants efficiently. This property has revolutionized the precision and targeted delivery of fertilizers, pesticides, and other essential agricultural inputs [22] hence improving yields especially in the face of the climate change that threatens to affect weather phenomena that adversely affect agricultural production and output all over the world, needed to feed the ever-increasing human population. CNTs can enter plants through various pathways, including root uptake and foliar application. Once inside the plant, CNTs may be transported through the vascular system, affecting nutrient and water transport.

Additionally, their environmental persistence poses challenges, CNTs do not degrade easily, meaning they can accumulate in soil and water, potentially leading to long-term contamination. Another concern is the impact on soil chemistry and nutrient availability, as CNTs can interact with organic matter, altering the way nutrients and pollutants behave in the soil. This paper aims to provide a detailed exploration of the multifaceted dynamics of CNTs in modulating plant processes. It delves into the positive aspects of CNT-plant interactions, elucidating the mechanisms behind growth enhancement, stress tolerance, and nutrient dynamics. It reports the results of the quantitative PIXE analysis of the germinated corn roots to establish nutrient uptake levels.

## 2. CATEGORIES OF CARBON NANOTUBE (CNTs)

Carbon Nano-based materials were first discovered in 1985 as a 60-carbon atom fullerene [23]. By 1991, the first carbon nanotubes were manufactured [24]. CNTs are categorized into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) based on the layers on their respective structures. MWCNTs have a 2 to 100 nm diameter made of multiple layer of high purity graphene with a thermal conductivity in the range of 3000 W/m.K. On the other hand, SWCNTs have a 0.4 -2 nm diameter made of a single layer of low purity graphene with a high thermal conductivity in the range of 6000 W/m.K [25].



**Figure 1: The typical dimensions of single width carbon nanotube (SWCNT) and multi-width carbon nanotube (MWCNT).**

## 3. EFFECT OF CARBON NANOTUBES (CNTs) ON SEED GERMINATION AND SEEDLING GROWTH

Both SWCNTs and MWCNTs possess chemically inert and hydrophobic sidewalls with carboxyl groups on the tube walls, which significantly improve biocompatibility in terms of adsorption capacity, covalent bonding, and electrostatic contact. This property allows them to penetrate the thick seed coat and activate the water uptake process which might be responsible for rapid germination and early growth [26]. Research shows that oxidized MWCNTs facilitated the water-absorbing potential of the seeds for rapid regeneration. Biologically, aquaporins facilitate the water uptake inside the cells, however their efficiency is highly affected by factors like pH; concentrations of the heavy metal ions; osmotic pressure; and water channel expression genes such as plasma membrane intrinsic protein (PIP), small basic intrinsic protein (SIP), and so on [27]. The potential for aquaporins to also reduce the flow of different ions through membranes and control the electrochemical potential of the membrane could be the reason for the rapid regeneration of seeds in the presence of oxidized MWCNTs [27]. CNTs unlike many other nanoparticles, has the

ability to effectively penetrate through the hard coatings of most seeds and interact with the embryo and other organs, thereby promoting seed germination and seedling growth. This has led to recent increase in the use of CNTs in physical and mechanical method in seed priming to break seed dormancy [28]. Various studies have explored the effect of CNTs on plant germination of different seeds including tomato, rice, cucumber, onion, radish, corn, soybean, switchgrass, and broccoli [28-34.].

#### **4. EXPERIMENTAL PROCEDURE**

##### **4.1 Preparation of Growth Medium**

The first experiment (Set 1) was meant to determine the effect of various concentrations of MWCNT on the germination and growth of maize seeds. The seeds were germinated and grown in Bacteriological agar (BA) gel medium spiked with different concentrations of MWCNT. The BA powder was weighed and mixed with de-ionized water to create a 15 g/L concentration before being autoclaved at 120°C temperature for 20 minutes. The right masses of MWCNT were weighed and mixed with the BA solution and stirred mechanically for 10 minutes. The concentrations of the resulting growth medium shown in table 1 were then placed in sterilized petri dishes and then maize seeds cleaned in 70% ethanol solution and rinsed in de-ionized water planted in the set substrate gels. The petri dishes were then placed in a climate control chamber with temperature range between 23°C -25°C and relative humidity of 70% and allowed to germinate and grow for 8 days.

**Table 1: Concentrations of the MWCNT prepared in bacteriological agar gel.**

Sample	MWCNT Conc. (mg/L)
1	0
2	5
3	10
4	20
5	40
6	60

The second experiment (Set 2) was meant to check the absorption of nutrients when the seeds are grown in different concentrations of  $\text{Fe}^{2+}$  with or without 20 mg/L of MWCNT. To prepare the germinating and growth medium, agarose powder was weighed and mixed in deionized water at a concentration of 8 g/L. The mixture was heated with constant magnetic stirring at a temperature of 95°C for 3 hours until the agarose was completely dissolved. The agarose solution was cooled to room temperature and then mixed with different concentration of MWCNT and/ or with  $\text{Fe}^{2+}$

introduced as a solution of  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  to produce the germinating and growth medium, as shown in table 2. The agarose solutions containing the different substrates were poured in different sterile petri dishes for seed growth. The corn seeds were cleaned in ethanol solution of 70% concentration before being dried and planted in respective medium each containing 20-22 seeds. The growth for all seedlings was carried out in the dark for 8 days at the room temperature of  $23^\circ\text{C}$  and humidity of 70%. The seeds were grown in the dark to prevent the photo-oxidation of  $\text{Fe}^{2+}$  [35].

**Table 2: Geminating media of MWCNTs concentrations in bacteriological agar with some containing  $\text{Fe}^{2+}$  of different concentrations.**

Sample set	MWCNTs concertation (mg/L)
S1	Agarose
S2	Agarose +MWCNT (20 mg/L)
S3	Agarose + Fe (II) $1 \times 10^{-3} \text{ M}$
S4	Agarose + Fe (II) $1 \times 10^{-3} \text{ M}$ + MWCNT (20 mg/L)
S5	Agarose + Fe (II) $3 \times 10^{-3} \text{ M}$
S6	Agarose + Fe (II) $3 \times 10^{-3} \text{ M}$ + MWCNT (20 mg/L)

## 4.2 Morphological measurements

After eight days, the germinated seeds in experimental set 1 were counted. Samples were harvested from each set and the seedlings were gently rinsed with deionized water then gently dried using paper towel before excising the roots and shoot for fresh weight (FW) measurements. The roots and shoots were then dried in an oven at  $60^\circ\text{C}$  for 3 days before weighing them to determine the dry weight (DW).

## 4.3 Sample preparation for Nuclear Microscopy to measure nutrient uptake

The root radical of the seedling from each set 2 were excised about 5mm long at the base of the seed and inserted in a plastic tube containing polyethylene tissue-freezing medium. This was quickly cryo-frozen in a container of isopentane (2-Methylbutane) cooled with liquid nitrogen, which provides superior cryogenic condition without Leiden frost phenomenon. The samples were then put in a deep freezer at  $-80^\circ\text{C}$  and stored for 3 hours. The frozen samples were cryo-sectioned with a thickness of  $60 \mu\text{m}$ . The sections were freeze-dried for about 2 hours and then carefully mounted on aluminum sample holders ready for PIXE irradiation.

## 4.4 PIXE Analysis

The Particle Induced X-ray Emission (PIXE) elemental micro-imaging and analysis was done using a 2-MeV focused ( $5 \mu\text{m}$  spot size) proton beam with a beam current of 50 pA. Simultaneous PIXE and proton backscattering spectrometry (PBS) were performed using a Canberra GUL0110

HPGe X-ray detector with a resolution of 154 eV FWHM at 5.9 keV and a Canberra PIPS detector, respectively. The spectrometry system is calibrated for standard-free quantitative analysis [36]. From the PIXE data collected, quantitative elemental image mapping was done using GeoPIXE software package [37] that also provides the tools to extract elemental concentrations. Matrix correction is based on the information on the organic matrix components extracted from PBS data using SIMNRA [38]. For each sample, several sections of the roots ( $n = 7 - 9$ ) were analyzed and the elemental concentrations measured in parts per million (ppm). The average concentration was then determined with a 95% confidence interval.

#### 4.5 Results

Figure 1 (a-d) shows the various indices studied when the concentration of MWCNT was varied. In fig 1 (a) the % germination of the *Zea mays* seeds in various concentration of MWCNT are shown. Each medium contained about 20-22 seeds. The Germination percentage was calculated as:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{number of total seeds}} \times 100 \quad (1)$$

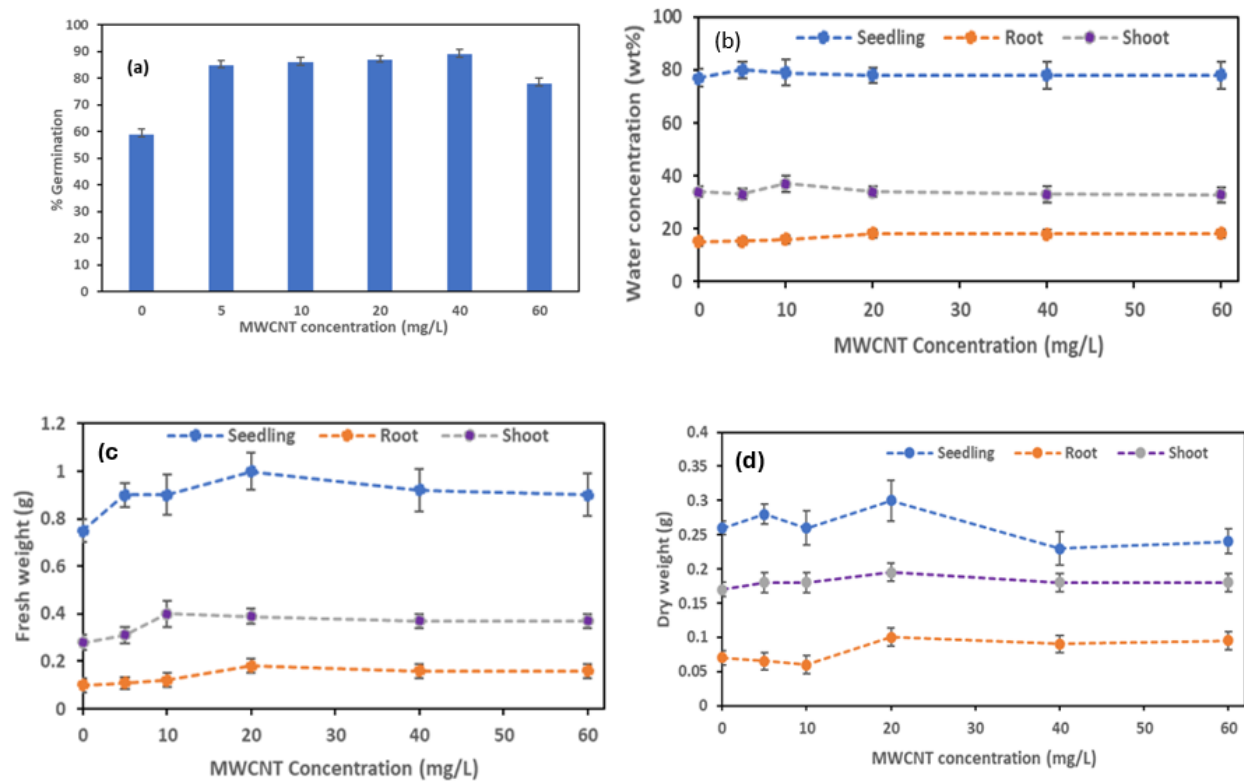
The germination percentage was higher in all the medium containing MWCNT compared to the control. It peaked at a medium of 20mg/L concentration of MWCNT which was about 30% higher than the control and then dropped slightly for higher concentrations of MWCNT. This observation was consistence with other reported studies [8]. The morphological results are illustrated in the figures 1(b-d) shows a significant effect of MWCNTs concentration and the water concentration of the seedlings, fresh weight of the seedlings, and dry weight of the seedlings. The results include 95%-tile confidence interval error bars. There was a limit noted that the growth was relatively higher for lower concentration of MWCNTs (10-20 mg/L) and reduced when the concentration reached 40 mg/L.

The percentage water content was calculated as:

$$\text{Water content (\%)} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Fresh Weight}} \times 100 \quad (2)$$

The MWCNT caused an increase of the water content of the shoot especially for low concentrations of MWCNT. The trace for the whole seedling follows the combined response of the shoot and the root (Fig 1 b).





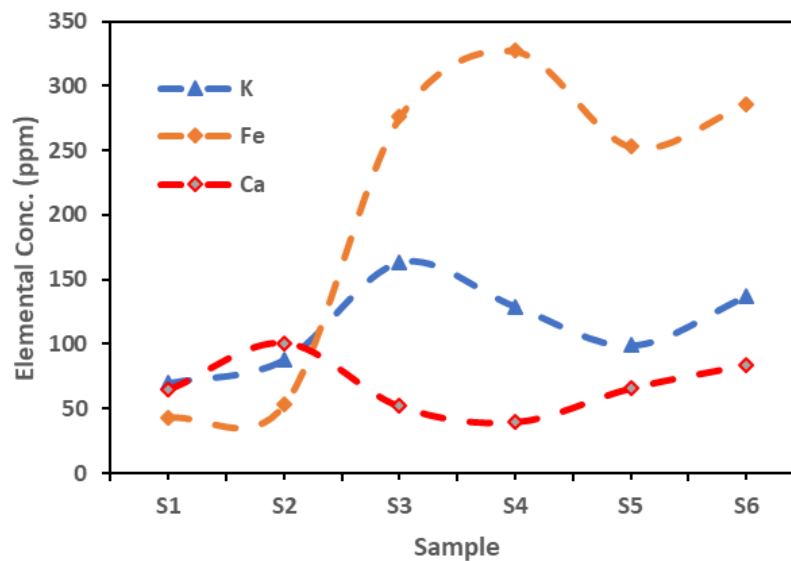
**Fig. 2: Effect of multi-walled carbon nanotubes (MWCNT) on the 8-day germination and growth of *Zea mays* (maize) in the BA medium of different concentrations (a) %germination, (b) % water content of the whole seedling, the root and shoot versus the MWCNT concentrations, (c) fresh weights (FW) and (d) Dry weights (DW) of the different morphological parts versus MWCNT concentrations. The root and shoot DWs have been multiplied by a factor of 10 to accommodate them in the same graph.**

Since the fresh weight is simply due to tissue water content, Fig 1c of fresh weight followed the same trends as those of freshwater content in Fig 1b. The dry weight (Fig 1d) is not much different however there is a slight sudden jump at concentration of 20 mg/L of MWCNT especially for the root and then levels lower for higher concentrations of MWCNT.

The PIXE elemental analysis of the roots yielded elemental concentration especially of the three primary elements Fe (that was added at different concentration in the growth medium with or without 20% MWCNT), Ca and K (Fig 3). The result shows that the iron uptake significantly increased in the medium where  $\text{Fe}^{2+}$  was added and even increased further when the MWCNT was part of the medium compared to the control. This is true for the different concentrations of  $\text{Fe}^{2+}$  used. The trend of Fe and Ca is interesting to note here as they appear to be mirror image of each other. Since only Fe was added to the growth medium and not Ca, which may be present only



in cell walls of the seedlings, the variation of Ca could be due to the interaction of Fe and Ca in the media and plant cells with the mediation of the MWCNT.



**Fig. 3: Elemental concentration (Fe, K, and Ca) uptake of seedling samples corresponding to table 2 obtained by PIXE analysis.**

## 5. DISCUSSION

MWCNT enhanced both seed germination, water and nutrient uptake by the seedlings. The results show that the germination rate increased by about 30% when MWCNT was added to the Agar medium for germination. The water and oxygen molecules enter the seed through the region of black layer of the seed coat where the radicle emerges [39]. Since MWCNT can penetrate seed coats, they would get into the seed through this point hence enhancing their effect of improving uptake and absorption of water by the seedlings. The introduction of low concentration of MWCNT enabled a high degree of porosity which greatly facilitate the uptake of water and nutrients. This explains the high-water content for low concentrations of MWCNT reported in Fig 1 b - c which peaked at about 20 mg/L. At high concentrations above 40 mg/L of MWCNT, the nanotubes aggregate more which resulted in in reduced functionality thus affecting the delivery of water molecules to the part of the seed in contact [40]. Another factor that may have played a role in reduced water uptake at higher concentration of MWCNT is the oxidative damage resulting from the penetration of the plasma membrane of the seedling cells [40,41].

In the second experiment, the elemental concentration especially of Fe that was added to the growth medium with or without MWCNT shows that the uptake was significantly increased with the presence of MWCNT (Fig 3). As expected, the medium with a higher Fe concentration shown a higher ppm of Fe in the PIXE of the seedling root that that with a lower concentration of Fe.

Similarly, the presence of MWCNT showed an increase in the uptake of Fe in the corresponding media. The increased flow of water into the root especially in the presence of MWCNT brings with it the increased uptake of nutrients presents in the growth media. The case of Ca was observed to interestingly behave in opposite direction to that of Fe. Ca is present in the cell walls of the seedling in the form of heteropoly-saccharide, pectin as calcium pectate [42, 43]. The presence of MWCNT alone does not affect the uptake of Ca but when Fe is added, either alone or with MWCNT, a reduction of Ca in the seedling occurs diametrically in opposite manner to the Fe which suggests that Ca is cat-ionically exchanged by  $\text{Fe}^{2+}$  in the cell walls of the seedlings as seen in Fig 3.

Despite the broad potential of MWCNTs in agriculture, their widespread adoption faces significant challenges. Research has shown that certain CNTs exhibit toxicity at physiological, cellular and genetic levels in artificial plant culture, but long-term studies under real-world conditions remain scarce [44]. Evaluating CNTs toxicity in soil is particularly complex due to factors such as low solubility, interactions with organic matter, and high background carbon levels, all of which hinder detection and mechanistic understanding. Additionally, little is known about how CNTs affect plant nutrition, genetic stability and transgenerational traits. Perhaps most critically, the transfer of CNTs from soil to plants and subsequently through the food chain remains unexplored [45]. Given these uncertainties, the results of this study show a promising effect of CNTs on productivity that needs further study.

## 6. CONCLUSION

This work has showed that the presence of MWCNT affects the germination of *Zea mays* L. seeds and subsequently improves the water and nutrient uptake by the seedlings for a certain level of concentration. The peak concentration for water uptake and nutrient uptake was found to be 20 mg/L of MWCNT. At higher concentrations of MWCNT, the uptake was diminished. These findings suggest that at an optimum concentration, MWCNT can be used in the manufacture of fertilizers to enhance not just seed germination but also yield output since it enhances the uptake of essential nutrients by crops. This can revolutionize agricultural production to provide food for the growing global population.

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