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Impacts of multi-walled carbon-nanotubes on the growth of pearl millet

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ABSTRACT

Nanotubes (NTs) penetrate plant cells and trigger plant growth. Recent research has shown that carbon NTs (CNTs) play an important factor in mitigating the oxidative stress and strengthening plant growth. In the present study, the effect of pearl millet seed priming with CNTs was evaluated based on seed germination, photosynthetic pigmentation, proline concentration, and Indole acetic acid (IAA) content of *Pennisetum glaucum* (pearl millet) during the seedling stage was investigated in this work. Different concentrations of NT's were used, including 30, 60, 90, 120, and 150 parts per million (ppm). In seed germination, 90 ppm multi-walled carbon nanotubes (MWCNTs) resulted in 80% germination. The substantial enhancements in the seedlings shoot and root length, fresh weight, and dry weight were seen at 90 ppm. With increasing MWCNTs treatment, the proline concentration continually increased. MWCNTs 90 ppm treatment decreased the quantity of malondialdehyde. At 90 ppm, the total chlorophyll content in seedling leaves was 27.74 mg/g fresh weight, which was greater than the other treatment levels. When compared to the control, the IAA concentration in the seedlings increased to 37 µg/g at 150 ppm, up from 21.5 µg/g. In conclusion, the results demonstrated that MWCNTs can promote plant growth and plant growth-promoting features to a certain extent. It may trigger special metabolic activities. As a consequence, employing CNTs as nano-fertilizers in agriculture to increase plant development may be preferable along with chemical fertilizers. However, a more in-depth examination of the mechanism of action of CNTs through seed priming is suggested, possibly using omics sciences.

1. INTRODUCTION

Numerous industries, including those in pharmacology, farming, food technology, and environmental protection, use nano-particles [1]. Due to their unique physical-chemical features, Nanoparticles (NPs) are gaining more attention in agricultural applications to augment plant development and yield. Nanomaterials easily enter different plant tissues and cells due to their small size, allowing for targeted delivery and precise release [2]. Despite these benefits, more studies have shown the inhibitory effects of nanomaterials on plants, including cellular membrane disruption, the release of heavy metal ions, the generation of reactive oxygen species (ROS), and alterations to DNA and proteins [3]. Different kinds of man-made nano-particles have been examined for their valued effects on the progress, productivity, and quality of important crops as nano-pesticides, nano-sensors, and nano-fertilizers [4]. To boost seedling growth and stress tolerance, which, in turn, increases plant productivity and food's nutritional value, NTs are also being used as a coating agent, an exercise known as "nano-priming," for seeds [5-7]. These nano-priming advantages are related to enhancements in seed germination, as well as changes in several biochemical and physiological and signaling pathways in seeds, like upholding ROS homeostasis [6,8-11]. Inquiries of nano-priming effects on seed germination have made increased use of NPs worldwide, such as zinc oxide and zinc, silver, iron, and titanium oxide nano-particle [12-16]. Furthermore, under drought stress conditions, nano-priming with Multi-walled carbon nanotubes (MWCNTs) improved the percentage of seed sprouting for *Dodonaea viscose* and *Alnus subcordata* seeds [17,18].

CNTs, for example, have been shown in studies to penetrate plant roots and then translocate to the vascular bundles and aerial part through the xylem through the transpiration process [19]. They can also penetrate the cell wall and membrane through pores or channels formed by the apoplastic route and endocytosis, allowing them to act as carriers of substances or chemicals within cells [20].

Pennisetum glaucum (L.) R.Br. (Pearl millet), commonly referred to as bajra, is a grain crop that is grown generally in tropical, dry regions of Asia and Africa. It is a large genus of the Poaceae family, with about 140 species that thrive in a variety of environments worldwide [21]. It is regarded as one of the most important farming crops, with a 6th rank worldwide [22]. The most basic food source for families in the world's undeveloped nations and among the unfortunate people is pearl millet. Among millets and grains, it is also one of the crops that can endure drought the best. More than 90 million people eat pearl millet, which is grown on 27 million hectares around the world [23]. It can be full-grown in drastic conditions; meanwhile, it is well adapted to

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production systems with low soil fertility, high temperatures, and low rainfall (200–600 mm) [24]. Millet grains contain more micronutrients such as iron and zinc than maize, rice, wheat, and sorghum, as well as more fiber (1.2 g/100 g), a small amount of starch, and 8–19% protein [25]. To make bread, pasture, flour, and couscous, pearl millet is utilized [26]. It is majorly cultivated to produce silage, green chop, pasture, hay, and stand over fodder that is directly eaten as cattle fodder [21,27].

Pearl millet, a climate-resilient crop with a high nutritional value, can be used to improve nutritional quality and combat malnutrition. It is almost free of major diseases and insect attacks and could be grown with a good yield [28].

Fullerenes, graphene, and nanotubes (NTs) are some of the carbon nano-materials among other nano-particles [29]. According to reports, CNTs promote seed germination [30-32]. Contrary effects on seed germination result from the fact that the effect of CNTs depends on conc., the solubility of applied nano-material, and size [30]. Moreover, it has been shown that CNTs can lessen the adverse effect of different abiotic stressors together with salinity revealing their efficiency to augment productivity and quality under unfavorable conditions [31].

Martínez-Ballesta *et al.*, 2016, demonstrated that broccoli plants (*Brassica oleracea*) treated with MWCNTs significantly increased their growth (32.7%), and a positive effect was also observed under saline stress conditions [33]. MWCNTs improved water absorption in seeds through aquaporins and cation exchange in the cell wall matrix, increasing nutrient concentrations such as Ca and Fe. These minerals can help with germination and plant growth and development, which can have an impact on performance [34]. Furthermore, the interaction of CNTs with proteins and polysaccharides was discovered to cause a cascade of signaling that resulted in the accumulation of compounds that led to cell wall thickening and subsequent growth [35].

It is especially important to look for new nanotechnology-based alternatives that allow for improvements in agricultural crop operation and are simple to implement. As a result, it is critical to investigate the impact that carbon nanomaterial can have on production systems in order to improve them. In the present study, seedling growth, proline content, lipid peroxidation, chlorophyll content, indole acetic acid (IAA) content, and seed germination level of *P. glaucum* seedlings treated with diverse concentrations of CNTs were studied. The use of MWCNTs for the growth of pearl millet can be better understood by the results obtained in this study.

2. MATERIALS AND METHODS

2.1. Plant Material

Seeds of *P. glaucum* (L.) R.Br. were procured from the Rajasthan Agricultural Research Institute (RARI) Durgapura, Jaipur, Rajasthan with a variety of Rajasthan hybrid (RHB) 173.

2.2. Dispersion of Nanomaterial and Seed Treatment

MWCNT Type 10 Carbon NTs (CNTs) (-COOH Functionalized) has been procured directly from the company named sisco research limited (SRL Pvt. Ltd), India. The Specifications for MWCNTs are approximately 95% pure, 10–20 nm in diameter, and 10–30 µm long and suspended using ultrasonication as defined earlier in Park *et al.*, 2014 [32]. Without combining any surfactants, 200 mL of autoclaved distilled water was mixed with 200 mg of nanomaterials before being subjected to ultrasonic vibration at 150 W and 40 kHz for 30 min. in a sonicator. Before use, a stock of MWCNTs was diluted with many concentrations of autoclaved distilled water.

The pearl millet seeds were carefully cleaned with 20% [v/v] Extran® (Merck, India) for 3 to 4 min. followed by washing with distilled water 3 times. The seeds were surface disinfected with a Laminar air flow hood fitted with UV light using a 0.1% $HgCl_2$ [Merck, India] solution for at least 3 min, and then washed 3 times with autoclaved distilled water. Further, different concentrations of multi-walled CNTs (MWCNTs), including 30 ppm (MW30), 60 ppm (MW60), 90 ppm (MW90), 120 ppm (MW120), and 150 ppm (MW150) were prepared from the stock solution for the experiment. Seeds were imbibed in the prepared nano-suspension for 24 h at 100 rpm in a shaking incubator. The experiment was done in triplicate and the seed incubated in pure de-ionized water was used as a control, denoted by C_0.

2.3. Seed Germination and Seedling Development

The blotting paper method was used to identify seed viability tests. Treated pearl millet seeds were placed (10 seeds per Petri plate) in glass Petri plates having germination beds saturated with distilled water. To prepare the germination bed, an autoclaved absorbent cotton sheet of 3–5 mm in thickness was placed on the Petri plate, which was then covered with autoclaved filter paper cut into the same shape, and 5 ml of autoclaved distilled water was added, following the instructions of the International Seed Testing Association [36]. This whole exercise was done under laminar airflow to avoid the unwanted growth of microorganisms on the germination bed as well as germinating seeds, which can alter the results significantly. The Petri plates were kept in a growth compartment with a temperature control of 26 ± 1° C, a controlled dark/light cycle of 16/8 h, and the illumination of 24 mol/m^2/s for 15 days. After that, measurements of morphological factors such as shoot and root length, fresh and dry weight, and seed germination were made.

2.4. Proline Content

The Bates *et al.*, 1973, method was used to assess proline. The 0.5 g expunged seedling was crushed in 5000 µL of 3% aqueous sulfosalicylic acid using mortar and pestle after being refrigerated in a deep freezer at −20°C [37]. The even mixture was then centrifuged for 10 min at 13,000 rpm at a temp. of 4°C. A reaction mixture was formed by mixing 2000 µL of supernatant with 2000 µL each of acid ninhydrin and glacial acetic acid. At 100° C, this reaction mixture was heated for 1 h. By keeping it in the ice bath for 5 min. after this treatment, the reaction was completed. After that, 4000 µL of toluene was added, and a UV-visible absorbance was taken at 520 nm with a spectrophotometer (UV-1800, Shimadzu). 1 mg/mL of proline was used as the standard in glacial acetic acid. By using a standard curve, the unknown concentration of the proline was estimated as µmole/g F.wt.

2.5. Lipid Peroxidation

Calculating the gathered malondialdehyde (MDA) by the procedure described by Heath and Packer., 1968 followed for the study of lipid peroxidation [38]. After homogenizing 0.2 g of pearl millet seedlings in 2000 µL of 5% TCA, the sample was centrifuged for 15 min. at 10,000 rpm. Following that, 1 mL of 0.5% 2-Thiobarbituric acids was added to 1 mL of supernatant (prepared in 20% TCA). This solution was heated in a boiling water bath for 30 min. After that, it was rapidly cooled on ice to stop the reaction and then centrifuged for 10 min. at 10,000 rpm. By taking the absorbance at 600 nm, the absorbance at 532 nm was attuned for non-specific turbidity. An extinction coefficient of 155 mM-1 cm^{-1} was used to determine the quantity of MDA. The values were given in terms of µM MDA/gm F. wt.

2.6. Total Chlorophyll Content

To determine the total chlorophyll content, the Arnon method., 1949 was used [39]. 0.5 g of fresh leaves of seedlings were collected and after that, chilled in a deep freezer (−20°C). By homogenizing the leaves in 80% acetone and centrifuging them for 10 min at 10,000 rpm, the pigments were extracted. The supernatant was collected for pigment estimation. The pigment analysis was performed by spectrophotometer immediately after the supernatant was separated. At 663 and 646 nanometers, absorbance was measured. As a blank, acetone at 80 % was used. To estimate the total chlorophyll conc., Lichtenthaler and Wellburn's [40] equations method was used: Chlorophyll a = $2.81A_{646}-12.21A_{663}$, Chlorophyll b = $5.03A_{663}-20.13A_{646}$, Total chlorophyll content = Chlorophyll a + Chlorophyll b, where, A_{646} and A_{663} are the absorbance at 646 nm and 663 nm wavelengths, respectively. The results were expressed as mg per gm F.wt.

2.7. IAA Content

The conc. of IAA was assessed using the Gordon and Weber technique [40]. After being chilled in a deep freezer, 1 g of seedlings was crushed in 10 mL of PO_4 buffer (0.05M) of pH 7.0. This homogenate was centrifuged for 15 min. at 10,000 rpm and 4°C. Then, after centrifuging once more at 5000 rpm for 10 min, 30% cold acetone was added to 70% of the supernatant to a final concentration of 100%. After mixing 1000 µL of supernatant with 2000 µL of Salkowaski reagent, the mixture's optical density (OD) at 530 nm was measured after 25 min. To determine the unidentified IAA concentrations, the OD value was plotted on the standard curve. IAA (1 mg/mL) was used to make the standard curve. The IAA content was specified in terms of µg/g.

3. RESULTS AND DISCUSSION

3.1. Seed Germination

The crop production depends on fast and uniform seed sprouting [41]. When pearl millet seed was treated with MWCNTs at MW30-MW150 concentrations, higher germination was determined at MW90, that is, 80% germination. Further germination of seeds declined at maximum MW120 and MW150 CNTs levels, compared to control seeds treated with sterilized distilled water. Germination increased by raising MWCNTs concentration to MW90. Consequently, it was observed that MWCNTs work as a promoter at a certain level after they can generate stress conditions for seed germination. According to Lin and Xing., 2007, seed germination rates of *Brassica napus, Raphanus sativus, Lolium perenne, Zea mays, Lactuca sativa, and Crocus sativus* improved by MWCNTs treatment [42]. The improvement in germination rates of pearl millet seeds by AgNPs was also reported by Parveen and Rao., 2015 [43]. Carbon nanomaterial-treated has the potential to uptake more water than untreated seeds that are responsible for increasing germination rates [44]. However, in the study by Park and Ahn., 2016, the germination of carrot seed treated with MWCNTs showed a 25% reduction concerning control [41]. This can occur because of the blocking of seed coat pores with micron-sized sheets of graphene that lead to cause less water permeability and a reduction in germination [44]. According to Thuesombat *et al.*, 2014, sprouting and germination rate of rice are found to be affected by the level of dose of nanoparticles (NPs) [45]. Thus, the results of this study suggested that improvement in seed germination by MWCNTs treatment is exhibited in a dose-depended manner.

3.2. Seedling Growth

Plants can quickly respond to NTs since roots are the chief tissues through which NMs enter plants [41]. The root length of pearl millet was increased up to (15.3 cm) at MW90 treatment of MWCNTs. At MW120 and MW150 length of pearl millet roots drastically decreased to 6.1 cm and 4 cm, respectively, in comparison with the control, which have a 13 cm root length. Likewise, among all concentrations of MWCNTs treatment, MW90 led to the highest shoot length at 2.7 cm, followed by MW60 with 2.5 cm and MW30 with 1.7 cm as compared to the shoot length of untreated seeds (1.6 cm) in Figure 1.

Above the MW90, the length of the shoot was found to be increased as specified in Figure 1. Likewise, Shekhawat *et al*., 2021, noted a little rise in the shoot and root length of *Vigna radiata* with 5.67% and 16.65%, respectively, at 100 µM level of CNTs, compared to control, but at higher concentrations, both lengths declined [46]. At all provided MWCNTs levels, length of shoot-root and seed vigor index of *Dodonaea viscosa* under drought stress conditions were reported with the highest values by Yousefi *et al*., 2017, in comparison with control [18].

Rahimi *et al*., 2016, also revealed positive results of the shoot and root length, d.wt., and seed vigour index of *A. subcordata* seedlings under drought conditions at 0–100 mg/L content of MWCNTs [17]. According to Li *et al*., 2016, the length of the root and shoot was increased up to 0.4 mg/mL of fluorescent C dots before being reduced at higher treatment levels [47]. In a study by Nair *et al*., 2012, rice seeds treated with 50 g/mL CNTs had the lengthiest roots and shoots compared to the control group [44]. However, Canas *et al*., 2008, reported that CNTs treatment enhanced the root length of *Allium cepa* and *Crocus sativus* but reduced the elongation of lettuce and tomato roots [48]. It was observed due to the differential response of nanoparticles on plant species or genotype. In addition, the impact of MWCNTs treatment on the fresh weight of pearl millet seedlings was higher with 0.09 g at MW90. A slightly increment with 0.06 g was observed at MW30 compared to the control (0.05 g). Drastically reduction in fresh weight was recorded comparatively at MW120 and MW150 treatment with 0.01 gm and 0.001 gm, respectively, Table 1.

Similarly, in Figure 2, the maximum dry weight of MWCNTs treated *P. glaucum* was observed at MW90 with 0.0096 g and thereafter declined up to 0.0003 g at MW150. The average weight of dry seedlings was recognized with 0.008 g and 0.0089 g, at MW30 and MW60, respectively.

Shekhawat *et al*., 2021, reported 1.20 and 1.14 times higher fresh and dry weight of *V. radiata* at 100 µl MWCNTs content than the control [46]. The fresh weight of *Brassica juncea* seedlings was

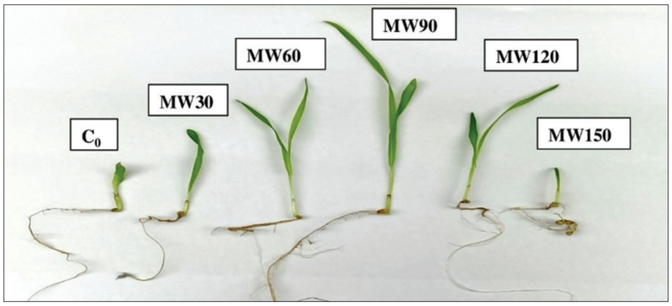

Figure 1: Effect of Multi-walled carbon nanotubes on shoot length and root length development of *Pennisetum glaucum* at different concentrations at 15 days of the seedling stage.

augmented at 50 mg/l AgNPs [49]. Applications of CNTs, according to Liang *et al*., 2013, enhance the concentration of potassium and nitrogen in plant organs, which is responsible to plant growth improvement [50]. A reduction in plant growth at higher CNTs levels can be because of CNTs accumulation on the roots that control the permeability of plant minerals [51].

3.3. Proline Content

Common physical responses in plant cells uncovered to variable environmental conditions comprise the gathering of proline [46]. The improved concentration of proline in pearl millet treated with variable levels of MWCNTs is described in Table 2.

The proline content was significantly augmented by the gradually raised carbon- NTs concentration. As in Figure 3, the maximum level of MWCNTs (MW150) showed a 0.84 µmole/gm F.wt. concentration of proline, which is the highest as compared to other treatments. Control showed 0.32µmole/gm F.wt. proline content. When exposed to 100 µM CNTs, Shekhawat *et al*., 2021 found that the proline levels in *V. radiata* leaves and roots were 1.48 and 6.29 times, those in the control group [46]. The enhancement of proline concentration with increasing CNTs treatment indicates water stress conditions due to variations in cell permeability [52,53]. As scavengers of ROS, plants often endorse a variety of total antioxidant activity, such as thiols, carotenoids, and proline, as well as enzymatic antioxidant activity, like catalase, superoxide dismutase and peroxidase [54-56].

3.4. Lipid Peroxidation

MDA, a byproduct of LPO, serves as an oxidative stress indicator. Consequently, under stress conditions, the MDA level is augmented in the plant [57]. With an exact dosage of MWCNTs up to MW90, the enhancement of MDA content was seen in the recent learning. As in Figure 3, the gradual reduction in MDA concentration was determined at MW30, MW60, and MW90 with 0.26, 0.21, and 0.15 µM MDA/g F.wt. as compared to control (0.29 µM MDA/g F.wt.). A progressive increment in MDA level was recorded at MW120 and MW150, that is, 0.45 and 0.59 µM MDA/g F.wt. as specified in Table 2. The concentration of MDA in both the leaves and the roots of *V. radiate* was reduced in Shekhawat *et al*., 2021, study up to 100 Molar of CNTs and further at 150 Molar [46]. With leaf and root sections, MDA was 1.09 and 1.06 times higher. Due to the small size of carbon nanoparticles, which have better diffusion capacity in plant cells and enhance nutrient availability, LPO can be reduced to a certain amount of CNTs. Nutrient absorption augments the tolerance ability of plants under stress conditions; therefore, the MDA level is reduced [58]. But at higher concentrations of CNTs, MDA content increases due to the stimulation of CNTs-mediated oxidative stress [59].

Table 1: Morphological parameters of *Pennisetum glaucum* 15 days seedlings treated with variable levels of multi walled carbon-nanotubes.

Sample	Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)
C0	1.6 ± 0.12^{b}	13 ± 0.09^{d}	0.05 ± 0.01^{b}	0.007 ± 0.001^{c}
MW30	1.7 ± 0.16^{b}	13.4 ± 0.18^{c}	0.06 ± 0.01^{b}	0.008 ± 0.0004^{bc}
MW60	2.5 ± 0.21^{a}	14.1 ± 0.02^{b}	0.07 ± 0.02^{ab}	0.0089 ± 0.0002^{ab}
MW90	2.7 ± 0.10^{a}	15.3 ± 0.21^{a}	0.09 ± 0.01^{a}	0.0096 ± 0.0001^{ab}
MW120	2 ± 0.24^{b}	6.1 ± 0.14^{e}	0.01 ± 0.01^{c}	0.006 ± 0.0006^{d}
MW150	0.5 ± 0.10^{c}	4 ± 0.29^{f}	0.001 ± 0.003^{c}	0.0003 ± 0.0002^{e}

*All the triplicate values are present in Mean±SD; F.wt; Fresh Weight. Means with different superscripts within same parameters based on different concentrations are significantly different ($P < 0.05$).

3.5. Total Chlorophyll Content

Total chlorophyll conc. has a vital role in the determination of plant development and commonly it is a sign of detrimental effect on the plants [51]. According to El-Badri *et al*., 2021, nano-priming reduces biosynthesis pigments and ROS by alleviation of O^{2-} and H_2O_2 accumulation and chlorophyll degradation along with enhancement of anti-oxidant to increase the defensive mechanism in the plant [16]. The impact of CNTs on the chlorophyll level of pearl millet leaf was evaluated in this study. As compared to the control (14.88 mg/g F.wt.), the maximum concentration of chlorophyll, that is, 27.74 mg/g F.wt., was recognized at MW90. At MW150, chlorophyll level declined significantly up to 21.24 mg/g F.wt. comparatively as mentioned Table 2. Consequently, it was suggested that MWCNTs facilitate the synthesis of chlorophyll in pearl millet leaves at a particular concentration, and higher levels of MWCNTs lead to cause a negative impact on chlorophyll content. Likewise, Shekhawat *et al*., 2021, lead a study in which a steady rise in total chlorophyll concentration was observed up to 100 µM of CNTs treatment, followed by a reduction recognized at higher 150 and 200 µM concentrations of carbon-nanoparticles [46]. The application of MWCNTs (50 mL) in carrot leaf with 25–30% enhancement in chlorophyll level was recorded by Park and Ahn, 2016 [41]. In the research, it has been also reported a 14.8% increment in chlorophyll biosynthesis in mung bean sprouts by carbon dots. This is most likely because CNTs improve the activity of the photosystem (Rubisco activity and chlorophyll concentration) by increasing the rate of e-transfer. But at higher CNTs levels, reduction in chlorophyll synthesis occurs due to the direct association of engineered nanoparticles with the chloroplast and oxidative stress takes place in the cell's plastid [51].

3.6. IAA Content

For plant development and growth, IAA is an essential plant hormone [60]. Many auxin-responsive genes are controlled at the

Table 2: Bio-chemical analysis of *Pennisetum glaucum* 15 days old seedlings treated with variable levels of carbon nanotubes.

Sample	Proline content (µmole/g F.wt.)	LPO assay (µM MDA/g F.wt.)	Total chlorophyll content (mg/g F.wt.)	IAA content (µg/gm)
C0	0.32 ± 0.01^{f}	0.29 ± 0.02^{c}	14.88 ± 0.27^{f}	21.5 ± 0.37^{f}
MW30	0.46 ± 0.005^{e}	0.26 ± 0.01^{d}	20.8 ± 0.21^{e}	25.12 ± 0.10^{e}
MW60	0.59 ± 0.01^{d}	0.21 ± 0.02^{e}	26.48 ± 0.05^{b}	28.12 ± 0.03^{d}
MW90	0.67 ± 0.02^{c}	0.15 ± 0.005^{f}	27.74 ± 0.15^{a}	33.37 ± 0.02^{c}
MW120	0.72 ± 0.005^{b}	0.45 ± 0.01^{b}	23.023 ± 0.08^{c}	34.75 ± 0.082^{b}
MW150	0.84 ± 0.02^{a}	0.59 ± 0.005^{a}	21.24 ± 0.01^{d}	37 ± 0.05^{a}

*All the triplicate values are present in Mean±SD; F.wt; Fresh Weight. Means with different superscripts within same parameters based on different concentrations are significantly different ($P < 0.05$).

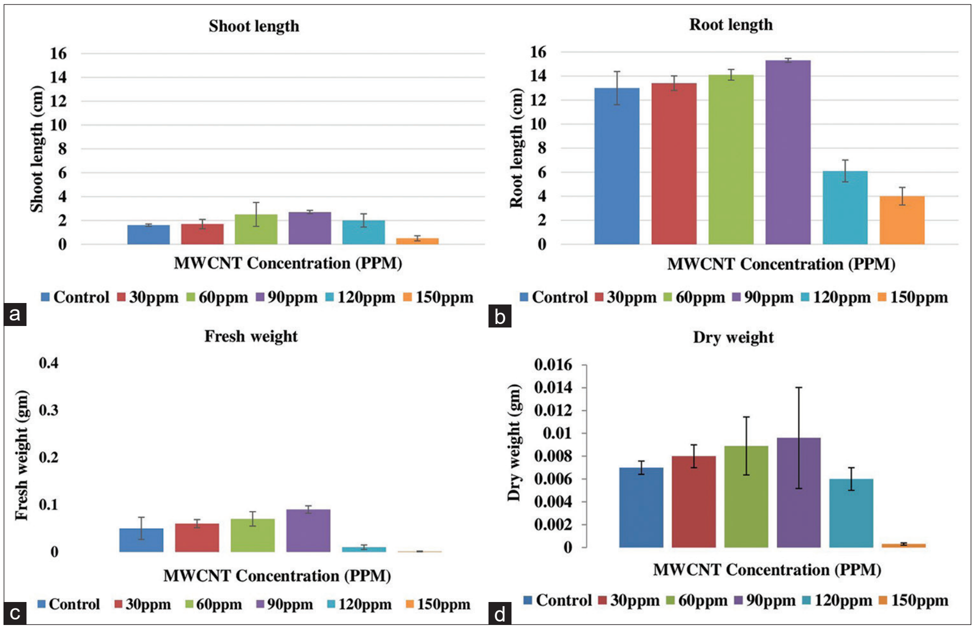

Figure 2: Morphological parameters of *Pennisetum glaucum* 15 days seedlings treated with variable levels of multi walled carbon-nanitubes (MWCNTs).

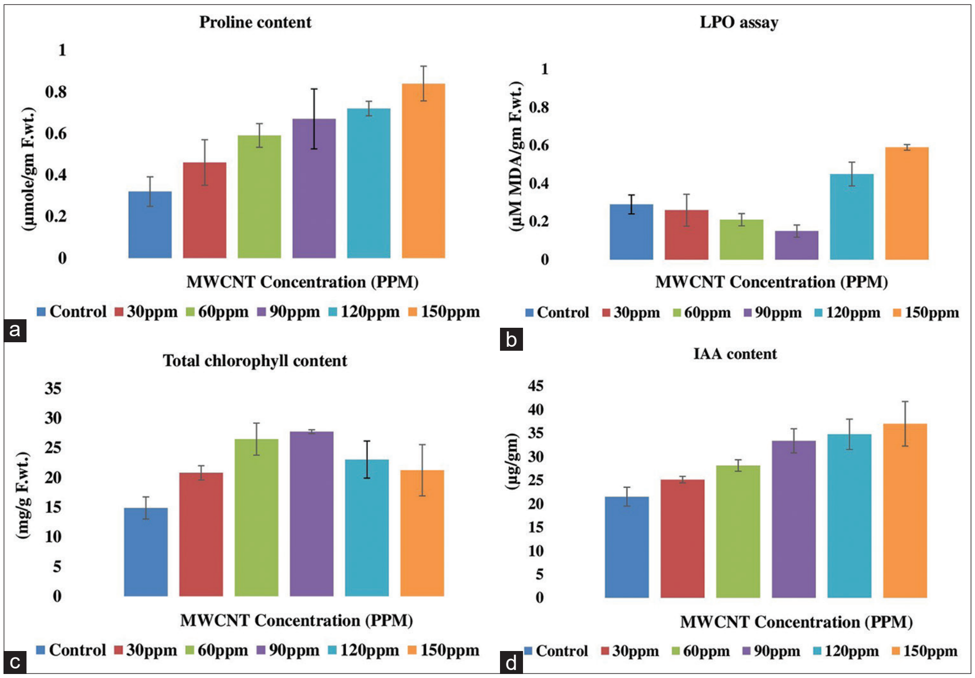

Figure 3: Bio-chemical analysis of *Pennisetum glaucum* 15 days old seedlings treated with variable levels of multi walled carbon-nanotubes (MWCNTs).

transcriptional level in plants with the help of auxin through auxin/indole-3-acetic acid-Auxin Response Factor (ARF) auxin-signaling modules [61]. Therefore, proper IAA production, signaling, and transport are vital for rooting [62]. As shown in Table 2, when the exposure level of MWCNTs was amplified from MW30 (30ppm) to MW150 (150ppm) in comparison to control, the amount of IAA in pearl millet seedlings progressively improved. The control showed 21.5µg/gm IAA that was raised to 37µg/gm at MW150 higher quantity of MWCNTs. Li *et al.* [60] reported 39% and 56% higher endogenous IAA concentrations in shoots and root parts of wheat treated with chitosan nanoparticles. Cheng *et al*., 2016, detected an increment in the abundance of ARF and IAA in *B. napus* by 50 mg/l of graphene oxide [63]. Sun *et al*., 2020 reported enhancement of auxin contents, stem cell niche activity, signaling of root apex, and potential of meristematic cell division of *Arabidopsis* by treatment with nano priming application of SWCNTs [64]. Thus, the result of this study indicated that MWCNTs treatment can increase IAA content in pearl millet at a particular concentration.

4. CONCLUSION

The growth and biochemical parameters of pearl millet seedlings improved by the use of MWCNTs. This study shows that MWCNTs affect plant development differentially. At a specific concentration of MWCNTs, 90 ppm (MW90), the height of the shoot and root as well as the fresh weight and dry weight of pearl millet seedlings were augmented. Above the MW90, the length and biomass both declined. Likewise, a significant improvement in LPO, proline, chlorophyll, and IAA content was also identified at MW90. However, at higher concentrations of MWCNTs, proline, and IAA concentrations were increased; it could be possible due to, regulating the stress condition. In the present work, it was observed that a certain MWCNTs level (MW90) favors the growth of pearl millet seedlings.

Seed priming with MWCNTs elicited favorable responses, which may improve the development of the pearl millet crop. These findings suggested that treating pearl millet seeds with MWCNTs could be a good way to induce biostimulation while also demonstrating an easy method of application. Overall, the impact of CNTs on the development of pearl millet seedlings was strongly dependent on application rates.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

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REFERENCES

1. Tripathi DK, Shweta, Singh S, Singh S, Pandey R, Singh VP, *et al*. An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity. Plant Physiol Bioch 2017a;110:2-12.
2. Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS. Nanoparticulate material delivery to plants. Plant Sci 2010;179:154-63.
3. Van Aken B. Gene expression changes in plants and microorganisms exposed to nanomaterials. Curr Opin Biotechnol 2015;33:206-19.
4. Rizwan M, Ali S, Qayyum MF, Ok YS, Adrees M, Ibrahim M, *et al*. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. J Hazard Mater 2017;322:2-16.
5. Ye Y, Cota-Ruiz K, Hernandez-Viezcas JA, Valdes C, Medina-Velo IA, Turley RS, *et al*. Manganese nanoparticles control salinity-modulated molecular responses in *Capsicum annuum* L. through priming: A sustainable approach for agriculture. ACS Sustain Chem Eng 2020;8:1427-36.
6. Pereira AE, Oliveira HC, Fraceto LF, Santaella C. Nanotechnology potential in seed priming for sustainable agriculture. Nanomaterials 2021;11:267.
7. Shah T, Latif S, Saeed F, Ali I, Ullah S, Alsahli AA, *et al*. Seed priming with Titanium Dioxide nanoparticles enhances seed vigor, leaf water status, and antioxidant enzyme activities in Maize (*Zea Mays* L.) under salinity stress. J King Saud Univ Sci 2021;33:101207.
8. Tripathi DK, Singh S, Singh VP, Prasad SM, Dubey NK, Chauhan DK. Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (*Triticum aestivum*) seedlings. Plant Physiol. Biochem 2017b;110:70-81.
9. Latef AA, Srivastava AK, El-Sadek MS, Kordrostami M, Tran LS. Titanium dioxide nanoparticles improve growth and enhance tolerance of broad bean plants under saline soil conditions. Land Degrad Dev 2018;29:1065-73.
10. Faizan M, Faraz A, Yusuf M, Khan ST, Hayat S. Zinc oxide nanoparticle mediated changes in photosynthetic efficiency and antioxidant system of tomato plants. Photosynthetica 2018;56:678-86.
11. Khalaki MA, Moameri M, Lajayer BA, Astatkie T. Influence of nano-priming on seed germination and plant growth of forage and medicinal plants. Plant Growth Regul 2021;93:13-28.
12. Latef AA, Alhmad MF, Abdelfattah KE. The possible roles of priming with ZnO nanoparticles in mitigation of salinity stress in lupine (*Lupinus termis*) plants. J Plant Growth Regul 2017;36:60-70.
13. Castiglione MR, Giorgetti L, Geri C, Cremonini R. The effects of nano-TiO2 on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. J Nanopart Res 2011;13:2443-9.
14. Panyuta O, Belava V, Fomaidi S, Kalinichenko O, Volkogon M, Taran N. The effect of pre-sowing seed treatment with metal nanoparticles on the formation of the defensive reaction of wheat seedlings infected with the eyespot causal agent. Nanoscale Res Lett 2016;11:92.
15. Mahakham W, Sarmah AK, Maensiri S, Theerakulpisut P. Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. Sci Rep 2017;7:8263.
16. El-Badri AM, Batool M, Mohamed IA, Khatab A, Sherif A, Wang ZK, *et al*. Modulation of salinity impact on early seedling stage via nano-priming application of zinc oxide on rapeseed (*Brassica napus*, L.). Plant Physiol Biochem 2021;166:376-92.
17. Rahimi D, Kartoolinejad D, Nourmohammadi K, Naghdi R. Increasing drought resistance of *Alnus subcordata* CA Mey. Seeds using a nano priming technique with multi-walled carbon nanotubes. J For Sci 2016;62:269-78.
18. Yousefi S, Kartoolinejad D, Naghdi R. Effects of priming with multi-walled carbon nanotubes on seed physiological characteristics of hopbush (*Dodonaea viscosa* L.) under drought stress. Int J Environ Stud 2017;74:528-39.
19. Jordan JT, Singh KP, Cañas-Carrell JE. Carbon-based nanomaterials

elicit changes in physiology, gene expression, and epigenetics in exposed plants: A review. Curr Opin Environ Sci Health 2018;6:29-35.

20. Majeed N, Panigrahi KC, Sukla LB, John R, Panigrahy M. Application of carbon nanomaterials in plant biotechnology. Mater Today Proc 2020;30:340-5.
21. Zhou S, Wang C, Frazier TP, Yan H, Chen P, Chen Z, *et al*. The first illumina-based de novo transcriptome analysis and molecular marker development in Napier Grass (*Pennisetum purpureum*). Mol Breeding 2018;38:95.
22. Khan I, Raza MA, Awan SA, Khalid MH, Raja NI, Min S, *et al*. *In vitro* effect of metallic silver nanoparticles (Agnps): A novel approach toward the feasible production of biomass and natural antioxidants in pearl millet (*Pennisetum glaucum* L.). Appl Ecol Environ Res 2019;17:12877-92.
23. Varshney RK, Shi C, Thudi M, Mariac C, Wallace J, Qi P, *et al*. Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. Nat Biotechnol 2017;35:969-76.
24. Gupta V, Singh AP, Gupta N. Importance of pearl millet and its health benefits. Just Agriculture e-Magazine 2022;2:2582-8223.
25. Tako AA, Kotiadis K. PartiSim: A multi-methodology framework to support facilitated simulation modelling in healthcare. Eur J Oper Res 2015;244:555-64.
26. Badi S, Hoseney R. Use of *Sorghum* and pearl millet flours in cookies. Cereal Chem 1976;53:733-8.
27. Thiombiano L, Meshack M. Scaling up Conservation Agriculture in Africa: Strategies and Approaches. Ethiopia, Kirkos Sub-City: The FAO Sub-regional Office for Eastern Africa; 2009.
28. Satyavathi CT, Ambawat S, Khandelwal V, Srivastava RK. Pearl Millet: A climate-resilient nutricereal for mitigating hidden hunger and provide nutritional security. Front Plant Sci 2021;12:659938.
29. Zaytseva O, Neumann G. Carbon nanomaterials: Production, impact on plant development, agricultural and environmental applications. Chem Biol Technol 2016;3:17.
30. Husen A, Siddiqi KS. Carbon and fullerene nanomaterials in plant system. J Nanobiotechnol 2014;12:16.
31. Khan MN, Mobin M, Abbas ZK, AlMutairi KA, Siddiqui ZH. Role of nanomaterials in plants under challenging environments. Plant Physiol Biochem 2017;110:194-209.
32. Park H, Ko E, Ahn Y. Small heat shock proteins can confer tolerance to nanomaterial-induced toxicity. HortScience 2014;49:1116-21.
33. Martínez-Ballesta MC, Zapata L, Chalbi N, Carvajal M. Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. J Nanobiotechnology 2016;14:42.
34. Villagarcia H, Dervishi E, de Silva K, Biris AS, Khodakovskaya MV. Surface chemistry of carbon nanotubes impacts the growth and expression of water channel protein in tomato plants. Small 2012;8:2328-34.
35. Talebi SM. Nanoparticle-induced morphological responses of roots and shoots of plants. In: Tripathi DK, Ahmad P, Sharma S, Chauhan DK, Dubey NK, editors. Nanomaterials in Plants, Algae, and Microorganisms. Ch. 6. United States: Academic Press; 2018. p. 119-41.
36. International Seed Testing Association. International Rules for Seed Testing. Seed Science and Technology. Vol. 4. Switzerland: International Seed Testing Association; 1976. p. 52-70.
37. Bates L, Waldren R, Teare I. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:205-7.
38. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 1968;125:189-98.
39. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 1949;24:1-15.
40. Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. Plant Physiol 1951;26:192-5.
41. Park S, Ahn YJ. Multi-walled carbon nanotubes and silver nanoparticles differentially affect seed germination, chlorophyll content, and hydrogen peroxide accumulation in carrot (*Daucus carota* L.). Biocatal Agric Biotechnol 2016;8:257-62.
42. Lin D, Xing B. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environ Pollut 2007;150:243-50.
43. Parveen A, Rao S. Effect of nanosilver on seed germination and seedling growth in *Pennisetum glaucum*. J Clust Sci 2015;26:693-701.
44. Nair R, Mohamed MS, Gao W, Maekawa T, Yoshida Y, Ajayan PM, *et al*. Effect of carbon nanomaterials on the germination and growth of rice plants. J Nanosci Nanotechnol 2012;12:2212-20.
45. Thuesombat P, Hannongbuab S, Akasitb S, Chadchawan S. Effect of silver nanoparticles on rice (*Oryza sativa* L. Cv. KDML 105) seed germination and seedling growth. Ecotox Environ Saf 2014;104:302-9.
46. Shekhawat GS, Mahawar L, Rajput P, Rajput VD, Minkina T, Singh RK. Role of engineered carbon nanoparticles (CNPs) in promoting growth and metabolism of *Vigna radiata* (L.) Wilczek: Insights into the biochemical and physiological responses. Plants (Basel) 2021;10:1317.
47. Li Y, Zheng H, Zhang Z, Liu W, Su S, Chen Y, *et al*. Phytotoxicity, uptake, and translocation of fluorescent carbon dots in mung bean plants. ACS Appl Mater Interfaces 2016;8:19939-45.
48. Canas JE, Long MQ, Nations S, Vadan R, Dai L, Luo MX, *et al*. Effects of functionalized and nonfunctionalized single-walled carbon nanotubes on root elongation of select crop species. Environ Toxicol Chem 2008;27:1922-31.
49. Sharma P, Bhatt D, Zaidi MG, Saradhi PP, Khanna PK, Arora S. Silver nanoparticle-mediated enhancement in growth and antioxidant status of *Brassica juncea*. Appl Biochem Biotechnol 2012;167:2225-33.
50. Liang TB, Yin QS, Zhang YL, Wang BL, Guo WM, Wang JW, *et al*. Effects of carbon nano-particles application on the growth, physiological characteristics and nutrient accumulation in tobacco plants. J Food Agric Environ 2013;11:954-8.
51. Ghoto K, Simon M, Shen ZJ, Gao GF, Li PF, Li H, *et al*. Physiological and root exudation response of maize seedlings to TiO2 and SiO2 nanoparticles exposure. BioNanoScience 2020;10:473-85.
52. Tripathi BN, Singh V, Ezaki B, Sharma V, Gaur JP. Mechanism of Cu-and Cd-induced proline hyperaccumulation in *Triticum aestivum* (Wheat). J Plant Growth Regul 2013;32:799-808.
53. Mahawar L, Khator K, Shekhawat GS. Role of proline in mitigating NaCl induced oxidative stress in *Eruca sativa* miller: An important oil yielding crop of Indian Thar Desert. Vegetos Int J Plant Res 2018;31:55-63.
54. Mahawar L, Kumar R, Shekhawat GS. Evaluation of heme oxygenase 1 (HO 1) in Cd and Ni induced cytotoxicity and crosstalk with ROS quenching enzymes in two to four leaf stage seedlings of *Vigna radiata*. Protoplasma 2017;255:527-45.
55. Mahawar L, Shekhawat GS. EsHO 1 Mediated mitigation of NaCl induced oxidative stress and correlation between ROS, antioxidants and HO 1 in seedlings of *Eruca sativa*: Underutilized oil yielding crop of arid region. Physiol Mol Biol Plants 2019;25:895-904.
56. Mahawar L, Popek R, Shekhawat GS, Alyemeni MN, Ahmad P. Exogenous hemin improves Cd2+ tolerance and remediation potential in *Vigna radiata* by intensifying the HO-1 mediated antioxidant defence system. Sci Rep 2021;11:2811.
57. Al-Huqail A, El-Dakak RM, Sanad MN, Badr RH, Ibrahim MM, Soliman D, *et al*. Effects of climate temperature and water stress on plant growth and accumulation of antioxidant compounds in sweet Basil (*Ocimum basilicum* L.) leafy vegetable. Scientifica (Cairo) 2020;2020:3808909.
58. Verma SK, Das AK, Gantait S, Kumar V, Gurel E. Applications of carbon nanomaterials in the plant system: A perspective. Sci Total Environ 2019;667:485-99.
59. Shaw A, Hossain Z. Impact of Nano-CuO stress on rice

(*Oryza sativa* L.) seedlings. Chemosphere 2013;93:906-15.

60. Li R, He J, Xie H, Wang W, Bose SK, Sun Y, *et al*. Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). Int J Biol Macromol 2019;126:91-100.
61. Ivanchenko MG, Napsucialy-Mendivil S, Dubrovsky JG. Auxin-induced inhibition of lateral root initiation contributes to root system shaping in *Arabidopsis thaliana*. Plant J 2010;64:740-52.
62. Peret B, De Rybel B, Casimiro I, Benkova E, Swarup R, Laplaze L, *et al*. *Arabidopsis* lateral root development: An emerging story. Trends Plant Sci 2009;14:399-408.
63. Cheng F, Liu YF, Lu GY, Zhang XK, Xie LL, Yuan CF, *et al*. Graphene oxide modulates root growth of *Brassica napus* L. and regulates ABA and IAA concentration. J Plant Physiol 2016;193:57-63.
64. Sun L, Wang R, Ju Q, Xu J. Physiological, metabolic, and transcriptomic analyses reveal the responses of *Arabidopsis* seedlings to carbon nanohorns. Environ Sci Technol 2020;54:4409-20.

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