ISSN: 2347-4688, Vol. 12, No.(3) 2024, pg. 1090-1105

Current Agriculture Research Journal

www.agriculturejournal.org

Synthesis, Characterization, and Impact of Different Carbon-Based Nanomaterials on Gram (*Cicer arietinum*) Plant Growth and Soil Properties

KALPANA AWASTHI[1], KRITIKA GUPTA[2] and RAJANI SRIVASTAVA[2*]

[1]Department of Physics, K.N. Govt. P. G. College, Gyanpur, Bhadohi, India.
[2]Environmental Science (Env. Tech.), RGSC, Institute of Environment & Sustainable Development, Banaras Hindu University, India.

Abstract

The ecology and general public health are badly impacted by the prolonged usage of chemical fertilizers. Applying carbon-based nanomaterials is one of the best options available for accelerating plant growth while reducing harm to the environment. The current study aims to assess the effects of graphene oxides (GO), functionalized carbon nanotubes (FCNTs), and carbon nanotubes (CNTs) on plant growth and soil nutrient content. To observe the impact on gram plant growth and soil parameters, we synthesized and applied GO, FCNTs, and CNTs at a rate of 100µg/mL (120 g per kg soil) in the corresponding pots. After 90 days of seed sowing, GO-treated crops showed a 41% increase in crop height compared to the control (no nanomaterials), but this increase was 33% and 40% in CNTs and FCNTs-treated crops, respectively. When compared to the control, the GO-treated plants shown a twofold increase in root length, in contrast, the FCNTs and CNTs-treated plants showed increases of 60% and 25%, respectively. The highest increases in plant biomass, soil organic matter, total nitrogen, microbial biomass, and enzymatic activity were observed in plants treated with GO. A 52% increase in SDA was seen in the GO-treated soil as compared to the control; in the FCNTs and CNTs-treated soils, this increase was 32% and 19%, respectively. An organic material with a carbon base is a carbon-based nanomaterial, which has the ability to control the soil microenvironment and activate soil enzyme activity. The results verified that incorporating carbon-based nanomaterials, particularly GO, into the soil might enhance the growth of gram plants and the sustainability of the soil.

Article History
Received: 03 September 2024
Accepted: 18 October 2024

Keywords
Carbon Nanotubes;
Functionalized Carbon Nanotubes;
Graphene Oxide;
Nanomaterials;
Plant Growth;
Sustainability.

CONTACT Rajani Srivastava ✉ rajani.srivastava25@gmail.com Environmental Science (Env. Tech.), RGSC, Institute of Environment & Sustainable Development, Banaras Hindu University, India.

Doi: http://dx.doi.org/10.12944/CARJ.12.3.05

Introduction

Owing to rapid population growth and climate change worldwide, there is a greater need for efficient food cultivation and food security in the finite land resources of this planet. Modern chemical-based agricultural practices have marginally increased food production, however, evidence indicates that these practices develop genetic resistance in crops, contaminate the soil, and adversely affect productivity and human health.[1-3] Under such conditions, greater food production with sustainable resource usage within planetary boundaries[4] may not be sufficient to fulfil the needs of the various UN SDGs, like SDGs 1, 2, 3, 12, and 13. Many previous works have also evolved strategies to meet out the objectives of SDGs[5,6] and in continuation to that, one more revolutionary method was developed that minimizes environmental harm while optimizing plant growth acceleration through the use of synthetic nanoparticles. Rapid advancements in nanotechnology are essential for tackling issues facing the agricultural sector, such as crop yield, seedling growth, deficiencies in soil nutrients, and water stress conditions.[7-9] Because of their large surface area, high stability, high adsorption capacity, and active sites on their surface, nanoparticles are used more efficiently than bulk particles. In agriculture, nanomaterials facilitate the controlled delivery of biomolecules, nutrients, and pesticides into the plant and increase crop yield. Nanosensors monitor plant health and soil quality.[10-12] Metals, metal oxides, polymers, and carbon nanoparticles are examples of nanomaterials. Among these, carbon-based nanomaterials, such as fullerene, carbon nanotubes (CNTs), graphene, and graphene oxides (GO), have drawn attention because of their exceptional physicochemical qualities, which include their small size, large surface area, high mechanical strength, and distinctive electrical, thermal, and chemical properties.[13,14] Graphene is a two-dimensional crystal with only one layer of carbon atoms. It can be wrapped into a cylinder to form a single-walled CNT[15] or Multi-walled CNTs (MWCNTs) using multiple sheets of graphene.[16] Due to their exclusive chemical and physical properties, these carbon-based nanomaterials have potential in various fields, including electronic devices, energy storage, catalysts, biosensors, drug delivery, and environmental remediation, and are gaining attention in the agriculture field as well.[17-19] The effect of CNTs on germination of seeds and plant development has been reported by many research groups.[18,20-24] One of the drawbacks related to the use of as-grown CNTs in agriculture is their poor water solubility. Chemical modifications (i.e. functionalization) have improved the CNTs solubility in water and reduced the aggregation of CNTs.[25] Another nanocarbon material that has recently garnered attention is graphene and graphene oxide (GO, derivative of graphene) used in plant growth. GO are effective fertilizer and increases the effectiveness of nutrient use.[26-28] Recent studies have explored the beneficial effect of GO on plant development at different stages, like seed germination, flowering, and root and shoot growth.[28-31] Conversely, several studies have shown the adverse effect of high concentrations of GO on plants.[31-34] Hematological parameters, protein and nucleic acid content, and a few oxidative stress physiology markers in *A. testudineus* were all observed to be affected by GO doses.[35]

Because CNTs are made entirely of carbon, they have high stability, low toxicity, and environmental friendliness.[14,19] However, research on the impact of CNTs, functionalized CNTs (FCNTs), and GO on overall plant morphological growth and soil physicochemical and microbial properties is still scarce and primarily conducted on laboratory scales. The majority of agricultural soils in the Indian Vindhyan tropical region are red loam red laterite in texture with low drainage. Vindhyan soil is rainfed and is therefore invariably low in potash, poor in nitrogen and phosphorus, and moderate in potash. The soil of the study site was sandy loam in texture with low drainage having 59% sand, 19% silt, and 22% clay.[36] Additionally, having low carbon (0.37%), nitrogen (153.32 kg/ha), and medium in available P (14.15 kg/ha) and potassium (218 kg/ha) and poor in sulphur (12.14 kg/ha). It was reported in previous studies that soil moisture and water holding capacity were very low, varying from 4-10% and 24-25% respectively. Whereas, soil organic carbon and total nitrogen vary from 0.4 - 0.5% and 0.03 - 0.04% respectively, in different seasons in dry tropical Vindhyan soil.[37-39]

In the present study, it was hypothesized that carbon-based nanomaterials (CNTs, FCNTs and GO) may have the capacity to promote plant growth and improve soil quality, and microbial biomass especially

GO-treated soil in the dry tropical/subtropical regions where most soils inherit low organic carbon content. This study aimed to compare the effects of applying CNTs, FCNTs, and GO on three different aspects of soil qualities: (1) physicochemical and microbiological properties of the soil; (2) growth and nutrient availability of gram (*Cicer arietinum*) plants; and (3) enzymatic activities. The results are expected to improve our understanding of CNTs carbon nanomaterials application potential in crop production and soil quality improvement. Also, comparative assessment helps us in choosing the best material for agricultural purposes with minimum environmental contamination. Gram seeds were selected as the test crop for this study as it is the most significant Indian pulse crop. Gram seeds are also known as "chickpea" or Bengal gram. This ancient crop was first cultivated by Neolithic people and is now grown worldwide. It ranks third in terms of grain legume production, after sweet pea (*Pisum sativum* L.) and dry bean (*Phaseolus vulgaris* L.).

Materials and Methods

The present experiment was carried out at the south part of the Mirzapur, Vindhyan plateau, which is the vicinity of the Rajiv Gandhi South Campus (RGSC) (25"10'N, 82"37" E, 146 m asl), Banaras Hindu University, India. The study site is in India, Uttar Pradesh, approximately 650 km from Delhi and Kolkata both, nearly 89 km away from Allahabad, and 57 KM from Varanasi.

Chemicals and Reagents

Graphite powder (<50μm) was purchased from Sigma-Aldrich (purity ~99%), India. Sulphuric acid (H_2SO_4), nitric acid (HNO_3), hydrochloric acid (HCl), and benzene (C_6H_6) were procured as analytical grades from Sigma-Aldrich. Ferrocene ($C_{10}H_{10}Fe$) was purchased from Alfa Aesar with a purity of 99.5%. Potassium chlorate ($KClO_3$) (< 99%) was purchased from Merck. The chemicals were of analytical grade.

Crop and Soil

Gram (*Cicer arietinum*) was the crop chosen for the experiment. It is a crop that grows best in regions with moderate annual rainfall, 60 to 90 cm. The best-suited soil is well-drained deep loam, or silty clay loam with a pH ranging from 6 to 8. C. arietinum makes a substantial contribution to higher soil fertility through its capacity to fix atmospheric nitrogen. The soil utilized in this pot experiment was a local soil made up of red laterite-textured Vindhyan rocks with a sandy loam composition. It had low drainage. The experimental soil was collected from an agricultural field of RGSC, Banaras Hindu University, Mirzapur. The soil is slightly acidic in reaction (pH 6.5 -6.8), poor in nitrogen and phosphorus, and moderate in potash. The soil has a low amount of organic carbon below 0.5%.[22] Approximately 20 kg of soil was excavated from the upper layer of the crust. From different sites, soil was collected and mixed to represent a single composite soil. For the initial physical, chemical and biological properties analysis of the soil, a few samples of soil were taken and set aside. The soil was sieved with a 5 mm sieve in the laboratory to homogenize the soil and add farmyard manure in a ratio of 50:50 to ensure initial growth. This is the common practice of this dry tropical Vindhyan region. Other workers have also used manures or fertilizers for acceleration of growth.[40] The prepared soil was spread for drying to remove soil moisture and then filled into the pots. The size of pots used in the experiment was 5 cm in diameter at the top of the earthen pot and the height was 10 cm. Approximately 250 g of soil was filled in one pot.

CNTs, FCNTs, and GO preparation and Synthesis

The CNTs were synthesized through chemical vapor deposition (CVD) assisted spray pyrolysis of a ferrocene and benzene precursor solution at 850° under an argon ambient.[41] The benzene solution (25 mg/ml) was used to dissolve catalyst ferrocene and sprayed into a preheated (850°C) quartz tube for 15 minutes using argon gas (100 standard cubic centimeters per minute). The tube (quartz) was mounted inside a tube furnace (30 cm long, diameter~ 2.5 cm). The black deposition was collected from the quartz tube and used for the synthesis of FCNTs. The FCNTs carboxylic group were synthesized by the chemical oxidation of CNTs through hard oxidizing agents concentrated (conc.) H_2SO_4 and conc. HNO_3 as described earlier.[22] The GO was prepared using graphite oxide's thermal exfoliation.[42] In a typical experiment, graphite powder (1 g) was treated with conc. H_2SO_4 (18 ml), HNO_3 (9 ml), and a strong oxidizing agent ($KClO_3$) (11g) were added slowly into the reaction solution at room temperature. The mixture solution was stirred for 5 days at room temperature. After the reaction, the graphite oxide sample was washed with distilled water several times. After that, to remove sulphate and other ion impurities, it was washed with HCl (10%) solution and dried at 80 ºC in oven. The graphite oxide powder (~ 200 mg) was kept in a quartz tube (dia.: ~2.5mm and length ~130 cm) and flushed with argon gas for 15 min. Then this tube was inserted into a furnace preheated to 1050 ºC for 30 seconds. The complete experimental procedure for the synthesis of CNTs FCNTs and GO is represented in Figure 1.

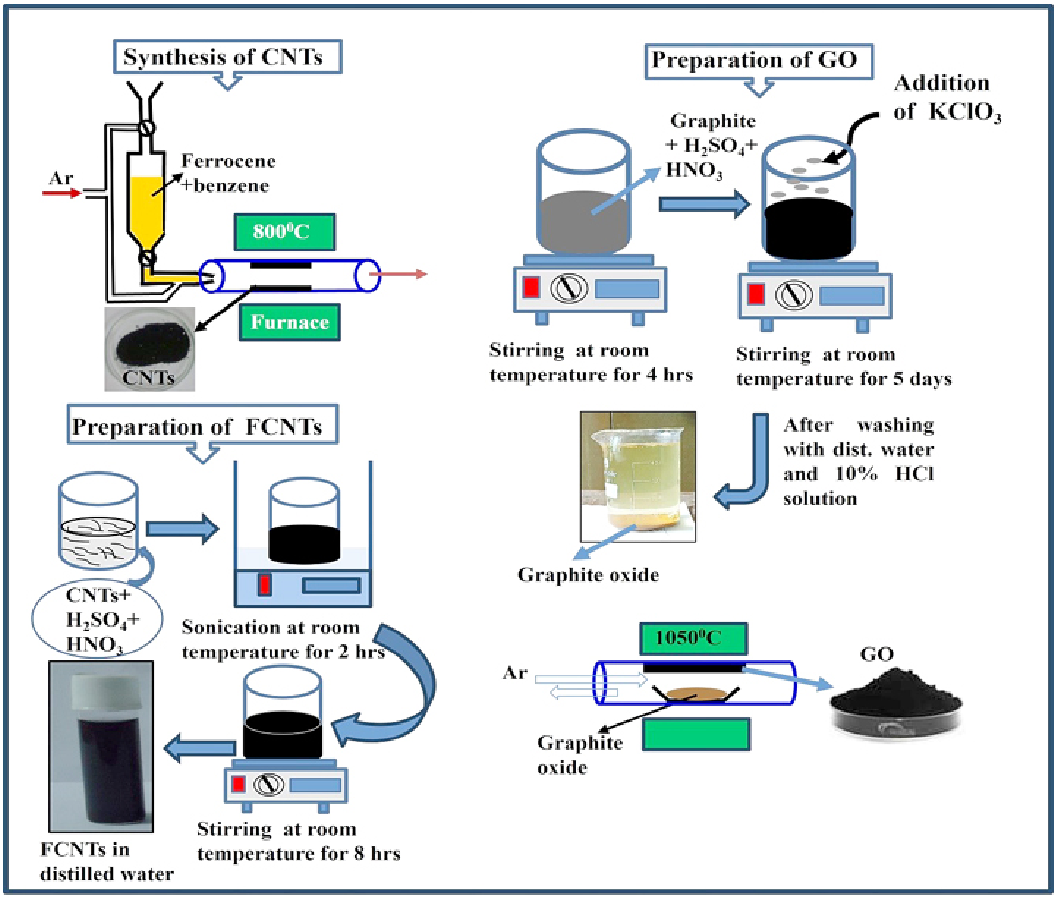

Fig. 1: Detail process of synthesis of carbon nanomaterials

Characterization of the Prepared Carbon Nanomaterials

The microstructure characterization of the as-prepared carbon nanomaterials i.e. CNTs, FCNTs and GO was carried out by scanning electron microscopy (SEM) (FEI: Quanta 200) and transmission electron microscopy (Technai 20 G^2) (TEM). Fourier transform infrared (FTIR) [Perkin-Elmer (Spectrum 100, USA)] spectroscopy was used for the examination of the presence of functional groups on FCNTs and GO. Samples for SEM investigation were mounted onto the sample holder with silver glue. For TEM characterization, a small amount of CNTs/FCNTs/GO was dispersed in a mixture of water and a few drops of ethanol solution using an ultrasonic bath for 20 minutes. After being immersed into the dispersed CNTs/FCNTs/GO solution, a copper TEM grid was dried. The prepared sample was finely powdered, combined in potassium bromide powder, and then formed into pellets for FTIR analysis. The synthesis of CNTs and GO and the characterization of all these were performed at the Nanoscience Laboratory, Department of Physics, Institute of Science, Banaras Hindu University, Varanasi. The functionalization process of CNTs and the preparation of graphite oxide were carried out at K. N. Govt. P.G. College, Gyanpur.

Seedling Preparation and Transplantation of Crop

Initially, seeds were surface sterilized with 10% sodium hypochlorite and then thoroughly washed twice with distilled water. Seeds were then soaked in distilled water for 10 h. Subsequently, viable seeds were taken from the soaked water and separately dipped in different carbon nanomaterial solutions for the entire night at room temperature as described by Rahman and others.[43] There is mounting evidence that seed treatments provided by nanotechnology increase germination rate,[44] speed up germination, and boost resilience. Seeds were then transplanted into 40 different pots (10 replicates for each treatment and one set for control), each of which was labelled. All seeds were sowed approximately two inches deep in the prepared soil in each treatment pot and the control pots (without nanomaterials). Initially, five seeds were put in each pot, but after thinning, only three remained. Before the seeds were transplanted, a soil sample was collected to evaluate the preliminary study of the physical, chemical and microbiological properties of the soil. The different carbon nanomaterials GO, CNTs, and FCNTs, were applied at the rate of 100µg/mL (50 ml water per pot given three times a week for up to 2 weeks, after that only normal water was applied). Overall, in all two weeks, about 30 mg per pot (120 g per kg soil) nanomaterials were applied in respective pots. It was reported that 50 g per kg was the optimal application rate for GO and other nanomaterials.[40-41,45] High-concentration application of nanomaterials may harm plant growth. Plant growth parameters like plant height, fruit number, root length, and above- and below-ground biomass were monitored. The heights of plants were determined using a ruler at 15, 30, 45, 60, 75, and 90 days afterward seed sowing, and the number of flowers counted in each pot at 40, 60, and 90 days after seed sowing. For measurements of root length, and above-ground and below-ground biomass of plants, three earthen pots (containing 9 plants, 3 per pot) were destroyed at 40, 60, and 90 days after seed sowing in such a way that roots and other parts of the crop were not damaged. Then, with the help of running tap water wash the soil and measure the length of the root. After that, separate the aboveground part of the plant by cutting the belowground root just below the aboveground part of the plant. Then, keep both types of plant samples in the oven at 70 ºC for 48 hours and take the dry weight. This was reported as the above-and below-ground biomass of plants in respective nanomaterial-treated pots.

Soil Analysis

After completion of the experiment, the final physical and chemical properties of soil and biological properties were analyzed in all potted nanomaterial-treated soil (from 20 samples each) and compared with the control. All physical-chemical and biological analyses were performed in the Environmental science soil analysis lab, RGSC. Different soil analyses were performed by standard methods. Bulk density (dry weight per unit volume of soil) was measured by inserting metallic tubes (of known internal volume) into the soil and oven-drying the enclosed soil core. For the analysis of soil moisture, the soil was excavated from the respective pots and determined by oven drying at 105ºC. A Systronics digital pH and EC meter (335) was used in the laboratory to measure the properties of electrical conductivity (EC) and pH of the soil. To measure the soil's WHC, the brass cup method was used.[46] Soil was collected from various pots treated with nanomaterials and subjected to chemical analysis. The dichromate oxidation and titration method[47] was used to quantify soil organic carbon, whereas, the Kjeldahl method[48] was used to estimate total nitrogen. The microbial biomass carbon and microbial biomass nitrogen content of the soil was measured using the fumigation-extraction method.[49] Soil dehydrogenase activity (SDA) was analyzed by triphenyl tetrazolium chloride (TTC) as a substrate.[50] Five grams of moist soil were mixed with 0.3-0.4 g/100 ml of TTC solution, and the mixture was incubated for 24 hours at 30°C. Following incubation, 40 ml of acetone was added, and the absorbance at 546 nm was measured using a spectrophotometer. Dehydrogenase activity was reported as µg TTC g^{-1} h^{-1}. For comparison, a blank was used in each experiment.

Statistical Analysis

Excel 2019 was used to collate the experimental data. The SPSS package (IBM SPSS Statistics 26, New York, USA) was used for statistical analysis of the data. Values are expressed as mean ± standard error. Mean values were compared by using the LSD, least significant difference range test procedure at the 5% level of significance. SPSS 26.0 statistical software was used to conduct One-way ANOVA and Duncan post-hoc test to compare means of all physical and chemical parameters with MBC and MBN under three nanomaterial treated pots i.e. CNTs, FCNTs, and GO compared with control pots during one crop cycle at 0.05 level of significance ($p < 0.05$).

Results and Discussion

Microstructural Characterization of CNTs, FCNTs, and GO

The morphology of the as-prepared carbon nanomaterials (CNTs, FCNTs, and GO) was investi-gated, and observed by SEM and TEM. The SEM image shows the formation of CNTs and nanotubes that are agglomerated due to van der Waals interaction. The surface of the CNTs is smooth, and the diameter of the CNTs varies between 25 and 55 nm. The CNTs were chemically treated with H_2SO_4 and HNO_3 acids, which are responsible for the presence of the -COOH group on the surface of the CNTs.[22] Due to chemical treatment, roughness is observed on the surface of the CNT.

The SEM analysis exhibits stacked layered GO with a fluffy morphology in the microstructure of the prepared GO. The GO sheets have a wave-like structure. The TEM image of GO sheets also shows the transparent wrinkled paper-like structure. The wrinkle structures in graphene sheets confirm the presence of oxygen functionalities.[51]

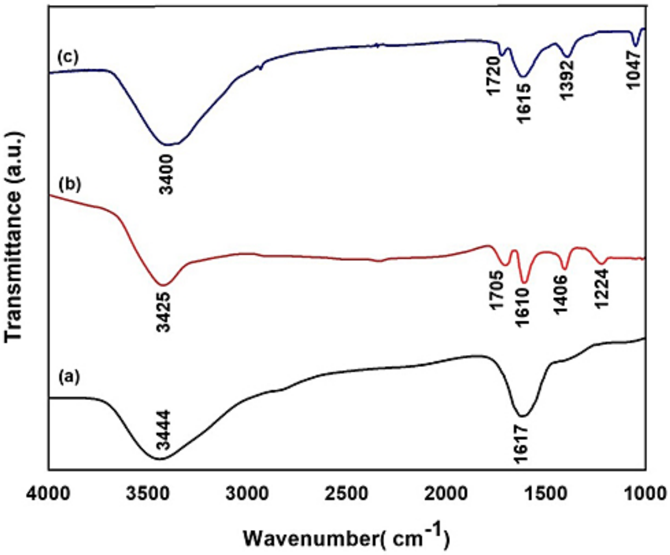

Fig. 2: FTIR spectrum of CNTs (a), FCNTs (b) and GO (c) used in the present experiment.

FTIR analysis of CNTs, FCNTs, and GO

FTIR is a suitable technique for identifying the functional groups presence in the sample. This technique shows the absorption of radiation (infrared) by the sample versus wavelength. The infrared absorption bands/ peaks correspond to the molecular components and structures. The FTIR spectra of carbon nanomaterials are shown in Figure 2. Figure 2 (a) represents the FTIR spectra of the as-synthesized CNTs. The observed band at 3444 cm^{-1} corresponds to the -OH group on the exterior of CNTs and it is due to the existence of ambient atmospheric moisture. The peak at 1617 cm^{-1} is associated with the stretching C=C vibration of the CNTs. When CNTs are functionalized with carboxylic groups, the presence of carboxyl (-COOH) moieties can be observed in the infrared spectra of FCNTs as indicated in Figure 2 (b). The peak at 1705 cm^{-1} is indicative of the stretching C=O vibration of the carboxyl group (COOH).[20] The peaks at 1406 and 1224 cm^{-1} are related to stretching O-H and C-O vibrations of the carboxylic group, respectively.

These peaks did not appear in the CNT spectra, thus confirming that this functionalization process introduced an oxygen-containing functional group, i.e. COOH onto the surface of the CNTs. GO is derived from graphite through oxidation processes, which introduce oxygen-containing functional groups such as carboxyl, hydroxyl, and epoxy onto the graphene structure. In the GO FTIR spectra [Figure 2 (c)], a broad peak at 3400 cm-1 is attributed to the stretching vibrations of the hydroxyl (O-H) group. The peak at 1720 cm^{-1} is associated with the stretching vibration of the carbonyl (C=O) groups, indicating the presence of carboxyl (COOH) functionalities. The peak at 1615 cm^{-1} is due to the C=C stretching vibrations of the graphene skeleton. The peaks that appear at 1392 cm^{-1} and 1047 cm^{-1} are related to C-OH and C-O bonds, respectively, which reveal the presence of hydroxyl and epoxy groups on the GO.

Application of Nanomaterials and Gram Plant Growth

By contrasting the effects of treating gram crops such as CNTs, FCNTs, and GO with control (non-nanomaterial) crops, the findings were examined. During the studies, morphological traits of the crop (*C. arietinum*) were observed at intervals of 15 days (for plant height), and 20-30 days (for flower numbers, root length, and above-and below-ground biomass). The application of carbon nanomaterials (CNTs, FCNTs and GO) showed differences in plant height [Figure 3 (A)], number of flowers [Figure 3 (B)], root length [Figure 3 (C)] and plant biomass [Figure 3 (D)]. In the case of plant height, maximum plant height is achieved 90 days after seed sowing in all nanomaterial-treated crops. Compared with the control, there was an increase in plant height in all nanomaterial-treated crops. Among treatments, no difference in crop height was noticed after 15 days of sowing. Differences were marked after 30 days of seed sowing, and maximum height was achieved by the GO-treated crop after 90 days. After 90 days, GO-treated crops showed a 41% increase in crop height compared to the control; in contrast, CNTs- and FCNTs-treated crops showed increases of 33% and 40%, respectively [Figure 3 (A)].

Forty days following crop sowing, there was no discernible variation in the amount of flowers between crops treated with nanomaterials [Figure 3(B)]. Following 60 and 90 days, the amount of flowers produced by the CNT-treated plants was comparable to the control (2 flowers at 60 days in both and 4 and 5 flowers at 90 days in respective pots). In contrast, the FCNTs and- GO treated pots (7 and 8 at 60 and 90 days, respectively) displayed more flowers per plant than the control (9 and 10 at 60 and 90 days, respectively).

Differences in root length were recorded at 40, 60, and 90 days following crop sowing in all nanomaterial-treated plants [Figure 3(C)]. Maximum root growth was measured at 60 days after seed sowing in all treatments. Among various treatments, extreme root length was noticed in GO-treated crops followed by FCNTs and then in CNTs-treated crops. In comparison to the control, the GO-treated plants displayed a twofold increase in root length; in the FCNTs and CNTs-treated plants, this increase was 60% and 25%, respectively. In all treatments, the biomass of above- and below-ground plants increased dramatically between 40 and 60 days following seed sowing [Figure 3(D)]. Thereafter, no significant increase was noted in all treatments up to crop maturity (90 days). Among the treatments, GO-treated crops showed greater accumulation of above-ground and below-ground plant biomass. GO-treated crops showed a 103% increase, FCNTs showed an 82% increase, and CNT showed a 23% increase compared to the control in above-ground plant biomass. In the case of below-ground plants biomass increases were 93%, 67%, and 45% in GO, FCNTs, and CNTs-treated plants, respectively [Figure 3(D)].

The effects of nanomaterials on different plants have been extensively studied, and both promotion and inhibition of growth have been recorded (in germination of seeds, growth in root and shoot, and flowering). We exposed gram seeds and soil to CNTs, FCNTs, and GO to assess their effect on different growth phases. The results showed that exposure to GO increased plant height, flower count, root dry weights, and shoot dry weights whereas CNT and FCNTs-treated plant and soil had a limited effect on seedling growth. Carbon nanomaterials in low doses have progressive effects on gram plant growth.[18,29, 52] Zhao with his co-workers reported that carbon nanomaterials initiate physiological processes in plants at low application doses.[53] They asserted that the amendment applied at the ideal rate of 200 mg carbon nanomaterials kg^{-1} resulted in a significant boost in maize development, as seen by improved plant height, biomass yield, nutrient absorption, and nutrient use efficiency. The mechanism behind better growth in nanomaterials applied plants is the effective uptake and transport of water and nutrients by aquaporins.[54] It is assumed that nanoparticles laden nutrients onto the surface of the rhizosphere and transported them through the epidermis, cortex and finally to the xylem. When the loading of carbon nanomaterials was modest, they improved the adsorption of nutrients and water to the plant roots without aggregating on the surface of the roots. Using various nanoparticles greatly raised the amount of chlorophyll b and the number of flowers per plant when compared to the control.[14] It was shown that carbon nanomaterial application on crops significantly increased plant height (21%) and root and shoot dry biomass (27% and 57%, respectively). It was also reported that the incorporation of graphene nanoparticles in salinity-stressed soil increased the flower count up to 58%.[55]

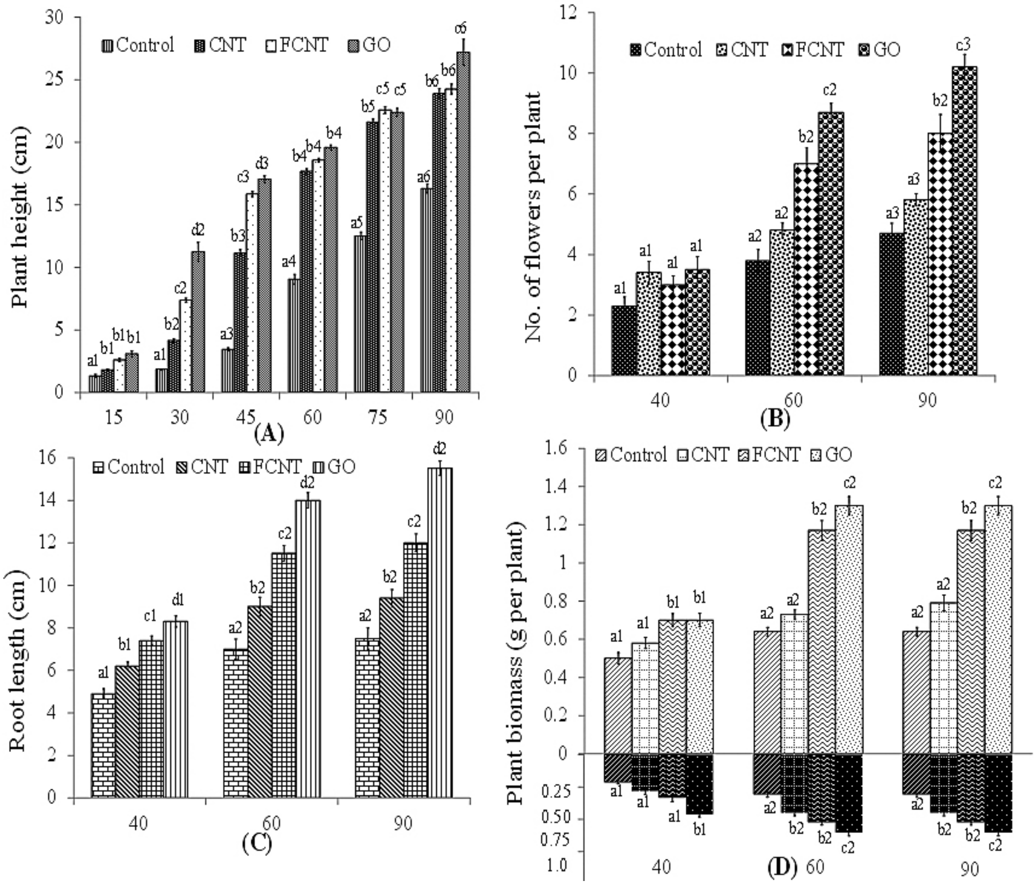

Fig. 3: Effect of carbonous nanomaterials (carbon nanotubes, CNTs; functionalized carbon nanotubes, FCNTs; and graphene oxides, GO) on plant growth. (A) Plant height (mean ±SE); (B) Number of flowers per plant (mean ±SE); (C) Root length (mean ±SE), and; (D) Plant biomass per plant (above ground and below ground plant biomass, (mean ±SE). Gram plant height was measured at 15, 30, 45, 60 75, and 90 days intervals. Whereas, the number of flowers, root length, and plant biomass were analyzed at 40, 60, and 90-day intervals. Lowercase alphabets represent the significant differences among different types of nanomaterials used and numbers represent differences among days in respective treatments. Duncan test, N = 10, $p < 0.05$ in all (A), (B) (C), and (D) figures.

The current study found that there were substantial differences in the impacts of different nanomaterials on the growth of gram plants concerning shoot and root biomass, number of flowers, and plant height.

Water molecules are drawn to the oxygen-containing functional groups of GO and then carried to the soil by the hydrophobic sp^2 domains.[56] According to our previous study, nanomaterials are more effective in enhancing crop growth and among nanomaterials, GO is more effective in increasing plant height, flower number, and root and shoot biomass. In one study, GO in the soil in different concentrations for the growth of mung beans and proved that a suitable amount of GO application had a good influence on plant growth.[57] Nanoparticles positively impacted plant development.[58]

The GO derivative is an effective fertilizer because it has oxygen-containing groups (such as carboxyl, epoxy, hydroxyl, and carbonyl) on its surface and edges.[25] The oxygen functionalities of GO attract the water molecules and transport them to the soil via the hydrophobic sp^2 domains. Thus, the GO improves the ability to store and transport water into the soil and accelerates the growth of the gram root system. It also lowers the rate of release and increases the effectiveness of nutrient use.[30-31] Recent studies have explored the beneficial effect of GO on crop growth at different stages, like seed germination, flowering, and root and shoot growth.[30-31]

Moreover, the size of GO nanoparticles is lesser compared to cell walls, they can more readily penetrate them and function as intelligent treatment delivery systems to control plant growth. The GO nanoparticles affect both physiological and genetic processes in plants and act as plant growth regulators.[59] In contrast to CNTs and FCNTs, GO improves the soil's ability to hold and move water, hastening the development of plant root systems. This variance suggests that the characteristics of nanomaterials their size, shape, and carbon content have a significant impact on the outcomes.

The concentration of GO did not gather on the gram plant root surface in the current investigation and adsorbed water and nutrients, thus providing proper simulation to the roots. Our previous study showed that the height, leaves, and fruit count of tomato plants grown in soil treated with FCNTs were higher than those grown in soil treated with CNTs and control. In this study, gram plant growth was improved with the application of GO compared with FCNTs. The oxygen functionalities in GO are more than those in FCNTs; thus plant growth increased with GO loading. Other studies have revealed that excessive formation of reactive oxygen species causes oxidative stress and cell death[,60-61] which is a disadvantage of nanomaterials.[62] Therefore, for the use of nanomaterial in plant growth, it would be important to optimize the appropriate concentrations of nanomaterials to prevent cell death for good cell viability.

Application of Nanomaterials on Soil Properties

Applying different materials, like CNTs, FCNTs, and GO, showed differential impacts on soil physicochemical properties and biological and enzymatic activities (Table 1). There were no differences in the soil bulk density among the GO, CNTs and FCNTs-applied pots and the control. However, GO-treated soil showed a lower value. Compared with the control, GO-treated soil showed maximum soil moisture and water-holding capacity, after that in FCNTs and then CNTs-treated soil. Due to the application of nanomaterials, soil moisture increases four times and water holding capacity increases one and a half times in GO-treated soil. Whereas, FCNTs and CNTs showed 128% and 42% increases in soil moisture and 22% and 11% water holding capacity in their respective soil. Additionally, compared to FCNTs and CNTs, GO-treated soil had a better electrical conductivity. The pH of the soil treated with CNTs and FCNTs did not differ; on the other hand, the pH of the soil treated with GO was a little acidic. All soils treated with nanomaterials showed significantly greater amounts of total nitrogen and carbon. (Table 1). The addition of GO to soil modifies its water content and, hygroscopic and adsorptive properties and, lessens the effects of drought stress.[31,63] This effect was also evident in this study in GO-treated soil. GO amendment also acts as a carrier and increases the uptake of mineral micronutrients by plants via controlled release.[64-65]

GO-treated soil had a considerably ($P<0.05$) higher amount of organic carbon (126% compared to control), followed by soil treated with FCNTs and CNTs (62% and 33%, respectively, compared to control). Total nitrogen increased more than twofold in GO-treated soil compared with control, whereas, this amount was 116% and 65% in FCNTs- and CNTs-treated soil. Carbons to nitrogen (C/N) ratios among treatments vary from 8.6 to 12, with the lowest value in GO-treated soil. By addressing inadequate soil nutrient conditions, the unique and practical nanomaterial graphene reduces chemical fertilizer contamination and enhances plant nutrient uptake and increases soil absorption of nutrient components. GO nanocarbon has a mixed effect on soil characteristics, exhibiting both positive and negative consequences. GO alone and with different concentrations of nano- sulphur was tested in one research.[66] When GO was applied alone, soil respiration was shown to improve the most, and this effect persisted even when high nano-S was added. They partially proved that GO enhanced nano-S oxidation and increased phosphatase activity. Their investigation showed the impacts of graphene application on soil physicochemical parameters, maize development, and nutritional content, Increased aboveground fresh weight, dry weight, plant height, and stalk thickness were also reported in the study.[40] Their research verified that by boosting soil fertility and optimizing the soil environment, applying graphene to the topsoil may increase maize plant biomass. Our investigations show that soil treated with FCNTs has higher levels of carbon and nitrogen than soil treated with CNTs.[7,22] The microbial biomass C and N also showed variation after the application of different nanomaterials in soil. Microbial biomass C and N were both more than three times higher in GO-treated soil and more than two times higher in FCNTs-treated soil compared with the control. However, these increases were 70% and 42% N in CNT-treated soil for C and N, respectively. Microbial biomass is an excellent indicator of fertility in the soil and represents the fraction of organic matter in the soil.[67] It is generally used to characterize microbial status in soil and is sensitive to different treatments. GO in soil may damage microorganisms by removing phospholipids from cell walls and penetrating them[68] as well as by reducing the abundance of several functional microbial groups connected to respiration and nutrient transformation activities.[69] It was reported that the most affected soil characteristics like basal and substrate-induced respiration, significantly increased in the soil enriched with GO compared with all other variants.[67] Apart from several studies that referred to the adverse effect of GO on the soil microbiome,[70-72] the positive effect on microbial growth and activity is assumed to be due to the reported improved delivery of macro- and micronutrients via adsorption.[27,73]

Table 1: Changes in physicochemical and microbial characteristics of soil after application of carbon nanotubes (CNTs); functionalized carbon nanotubes (FCNTs); and graphene oxides (GO) (mean ± SE, N = 15). Least Significant Difference (LSD, $p < 0.05$) compares the mean among different types of soil under different treatments.

Properties of soil	Control	CNTs	FCNTs	GO	LSD
Bulk density (g/cm³)	1.51 ±0.006	1.48 ±0.007	1.46 ±0.007	1.23 ±0.006	.020
Soil moisture (%)	5.42 ±0.168	7.74 ±0.36	12.1 ±0.780	22.8 ±0.42	1.45
Water holding capacity (%)	22 ±0.706	22.4 ±0.68	26.8 ±1.24	31.8 ±0.66	2.57
EC (dsm^{-1})	0.17 ±0.006	0.20 ±0.007	0.21 ±0.005	0.34 ±0.012	0.03
pH	6.96 ±0.075	6.8 ±0.001	6.8 ±0.001	6.7 ±0.040	0.13
Organic carbon (%)	0.45 ±0.014	0.60 ±0.010	0.73 ±0.022	1.02 ±0.044	0.077
Total nitrogen (%)	0.037 ±0.001	0.061 ±0.005	0.08 ±0.004	0.12 ±0.003	0.01
C/N ratio	12.1 ±0.37	10.1 ±.71	.9.3 ±0.57	8.6 ±0.25	1.52
Microbial biomass C (μgg^{-1})	83.2 ±0.968	97.0 ±0.005	124.2 ±6.78	256.8 ±3.56	12.6
Microbial biomass N (μgg^{-1})	8.38 ±0.298	10.2 ±0.582	14.6 ±0.58	21.1 ±0.64	1.63
Soil Dehydrogenase Activity (μgg^{-1}h^{-1})	13.4 ±0.59	15.9 ±0.28	17.7 ±0.29	20.3 ±0.45	1.28

Soil enzymatic activities like soil dehydrogenase activity (SDA) are linked to the physicochemical and biological properties of soil and respond rapidly to changes in treatments and environmental conditions, as estimated in the present study. Our study showed higher SDA in GO-treated soil than in FCNTs and

CNTs. A 52% increase in SDA was seen in the GO-treated soil as compared to the control, in the FCNTs- and CNTs-treated soils, the increase was 32% and 19%, respectively. An organic material with an activating influence on the enzyme activity of soil and the ability to control the soil microenvironment is called a carbon-based nanomaterial. According to reports, it can control the soil microenvironment and has a certain activating influence on the activity of soil enzymes.[74-75] Dehydrogenase is a soil enzyme that represents the entire spectrum of oxidative activity of the microflora present in soil. Because of its sensitivity and quick response, it is frequently used in research.[76] The occurrence of low enzymatic activities in the control and CNTs was mainly due to low soil moisture. However, high moisture and reduced soil density provide suitable conditions for SDA in FCNTs- and GO-treated soil. Because many enzymatic activities, such as SDA, are connected to the hydrological process, which entails the hydrolytic transformation of enzymes, they have been extensively used for the estimation of changes in soil quality when moisture is available (as in the present case GO-treated soil). An enzyme called dehydrogenase is present in every living microorganism. These enzymes can be used to measure soil microbe's metabolic conditions.[77] Its activity depends on similar factors that influence microorganism's abundance and activity, as reflected in the present study. According to our findings, applying GO and other carbon nanomaterials at a rate of 120 g per kg is the best way to boost soil productivity, control enzymatic activity, and promote gram plant growth in dry tropical Vindhyan soil. GO performed the best out of all the nanomaterials used. Even though GO acts as a possible novel tool for accelerating agricultural and plant growth, there are still many issues, such as cytotoxicity and implications for animal and human health. Thus, more investigation is needed to ascertain the effects of nanomaterials on plant and agricultural growth, both directly and indirectly. We propose that using nanomaterials in appropriate concentrations would be a useful tactic to evaluate the impact on crop development and soil fertility.

Conclusion

The addition of carbon-based nanomaterials could significantly enhance the growth of gram plants, as evidenced by increased plant height, number of flowers, and aboveground and belowground biomass. Concerning soil nutrients and plant growth, GO outperformed the other nanomaterials in terms of efficiency, followed by FCNTs and CNTs. The application of the same amount of GO to the soil resulted in higher soil moisture and water-holding capacity, soil nutrients, and improved the amount of soil organic matter and total nitrogen, which improved soil microbial biomass and soil enzymatic (SDA) activity. These are reflected in terms of maximum plant growth (increased plant height, greater flower production) and dry matter accumulation (more root length and higher plant above-ground and below-ground biomass) in GO-treated soil. Our findings provide valuable guidance for controlling the application of graphene oxide and other carbon-based nanomaterials in the dry tropical Vindhyan soils of India. The current study only showed improvements in microbial biomass and growth parameters following the application of nanomaterials; more in-depth research on the mechanism and microbial profile analysis is needed.

Acknowledgment

We would like to thank the Head and Course Co-ordinator of Environmental Science (Environmental Technology), RGSC, Institute of Environment and Sustainable Development, and the late Prof. O.N. Srivastava, Department of Physics, Banaras Hindu University for providing the field and laboratory facilities. KA thanks the higher education department of Uttar Pradesh for providing financial support under the research development scheme.

Funding sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

All data underlying this study are available as part of the article and no additional source data are required.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Author Contributions

- **Dr. Kalpana Awasthi:** Synthesis and characterization of nanomaterials were done by Dr. Kalpana Awasthi. She participated in designing the study and also participated substantially in data interpretation. She was the co-supervisor of this study.
- **Kritika Gupta:** participated in determining the design, performing experiments, analyzing the data, creating Figures and Tables and writing the first draft of the manuscript.
- **Rajani Srivastava:** Dr. Rajani conceived the study and was the principal person in designing the study, interpreting of data, helping in making Figures and Tables, writing this manuscript and supervising this work.

All authors participated in contributing to the text and the content of the manuscript, including revision and edits. All three authors approved the content of the manuscript and agreed to be held accountable for the study.

References

1. Ramankutty N, Mehrabi Z, Waha K, Jarvis L, Kremen C, Herrero M and Rieseberg LH. Trends in Global Agricultural Land Use: Implications for environmental health and food security. *Annu Rev Plant Biol.* 2018; 69:789-815. doi:10.1146/annurev-arplant-042817-040256
2. Singh A, Dubey PK, Chaurasiya R, Mathur N, Kumar G, Bharati S and Abhilash PC. Indian spinach: An underutilized perennial leafy vegetable for nutritional security in developing world. *Energy Ecol Env* 2018; 3:195-205. https://doi.org/10.1007/s40974-018-0091-1
3. Srivastava R. Effect of global warming on agricultural systems. *Amer Eurasian J Agricul.* Env. Sci. 2013; 13: 677-682. https://doi.org/10.5829/idosi.aejaes.2013.13.05.1951
4. Ferretto A, Matthews R, Brooker R and Smith P. Planetary boundaries and the doughnut frameworks: A review of their local operability. *Anthropocene* 2022; 39:100347. https://doi.org/10.1016/j.ancene.2022.100347
5. Srivastava R. 2022. Strategies to achieve sustainable development Goals (SDGs): A road map for global development (Editor: Srivastava R.)- https://doi.org/10.52305/YNDL2610 - NOVA Science Publishers, USA, 388p.
6. Abd-Elmaboud M E, Saqr A M, El-Rawy M, Al-Arifi N and Ezzeldin R. Evaluation of groundwater potential using ANN-based mountain gazelle optimization: A framework to achieve SDGs in East El Oweinat. *Egypt. J Hydrology*: Regional Studies 2024; 52: 101703. https://doi.org/10.1016/j.ejrh.2024.101703
7. Srivastava R, Awasthi K and Tripathi D. Nanotechnology towards sustainable agriculture. *Int J Sci Res Phys Appl Sci* 2018; 6:155-158.https://doi.org/10.26438/ijsrpas/v6i6.155158
8. Srivastava R. Emerging trends of nanotechnology in environment and sustainability: A review-based approach. *Aust J Env Manag* 2019; 26:191-192. https://doi.org/10.1080/14486563.2019.1584490
9. Sembada AA and Lenggoro IW. Transport of nanoparticles into plants and their detection methods. *Nanomaterials* 2023; 14:131. https://doi.org/10.3390/nano14020131
10. Giraldo JP, Landry MP, Faltermeier SM, McNicholas TP, Iverson NM, Boghossian AA, Reuel NF, Hilmer AJ, Sen F, Brew JA and Strano MS. Plant nanobionics approach to augment photosynthesis and biochemical sensing [published correction appears in *Nat Mater.* 2014 May;13(5):530]. *Nat Mater.* 2014;13(4):400-408. doi:10.1038/nmat3890
11. Kah M, Tufenkji N and White JC. Nano-enabled strategies to enhance crop nutrition and protection. Nat Nanotech 2019; 14:532-540. https://doi.org/10.1038/s41565-019-0439-5
12. Mittal D, Kaur G, Singh P, Yadav K and Ali SA. Nanoparticle-based sustainable agriculture and food science: Recent advances and future outlook. *Front Nanotech* 2020; 2: 579954. https://doi.org/10.3389/fnano.2020.579954
13. Yadav TP and Awasthi K. Carbon nanomaterials: Fullerene to graphene. *Transact Indian* Nat Acad Engg 2022; 7:715-737. https://doi.org/10.1007/s41403-022-

00348-w

14. Ahmadi SZ, Zahedi B, Ghorbanpour M and Mumivand H. Comparative morpho-physiological and biochemical responses of *Capsicum annuum* L. plants to multi-walled carbon nanotubes, fullerene C60 and graphene nanoplatelets exposure under water deficit stress. *BMC Plant Biol.* 2024; 24(1):116. Published 2024 Feb 17. doi:10.1186/s12870-024-04798-y
15. Geim AK and Novoselov KS. The rise of graphene. *Nat Mater* 2007; 6: 183-191. https://doi.org/10.1038/nmat1849
16. Iijima S. Helical microtubules of graphitic carbon. *Nature* 1991; 354: 56-58. https://doi.org/10.1038/354056a0
17. Mukherjee A, Majumdar S, Servin AD Pagano L, Dhankher OP and White JC. Carbon nanomaterials in agriculture: A critical review. *Front Plant Sci* 2016; 7:172. https://doi.org/10.3389/fpls.2016.00172
18. Safdar M, Kim W, Park S Gwon Y, KimY-O and Kim J. Engineering plants with carbon nanotubes: a sustainable agriculture approach. *J. Nanobiotech* 2022; 20:275. https://doi.org/10.1186/s12951-022-01483-w
19. Chen Z, Zhao J, Cao J ZhaoY, Huang J, Zheng Z, Li W, Jiang S, Qiao J, Xing B and Zhang J. Opportunities for graphene, single-walled and multi-walled carbon nanotube applications in agriculture: A review. *Crop Design* 2022; 1: 100006. https://doi.org/10.1016/j.cropd.2022.100006
20. Villagarcia H, Dervishi E, de Silva K, Biris AS and Khodakovskaya MV. Surface chemistry of carbon nanotubes impacts the growth and expression of water channel protein in tomato plants. *Small.* 2012; 8(15): 2328-2334. doi:10.1002/smll.201102661
21. Tiwari DK, Schubert ND, Cendejas LMV Villegas, J, Montoya LC and García SEB. Interfacing carbon nanotubes (CNT) with plants: enhancement of growth, water and ionic nutrient uptake in maize (*Zea mays*) and implications for nano agriculture. *Appl Nanosci* 2014; 40: 577-591. https://doi.org/10.1007/s13204-013-0236-7
22. Awasthi K, Tripathi DK and Srivastava R. Functionalization of multi-walled carbon nanotubes and its impact on growth of tomato plant (*Solanum lycopersicum* L.). *J Expt Biol Agr Sci* 2020; 8:469-478. http://dx.doi.org/10.18006/2020.8(4).469.478
23. Kharisov BI, Kharissova OV, Gutierrez HL and Mendez UO. Recent advances on the soluble carbon nanotubes. *Ind Eng Chem Res.* 2009; 48:572-590. https://doi.org/10.1021/ie800694f
24. Srivastava A and Rao DP. Enhancement of seed germination and plant growth of wheat, maize, peanut and garlic using multiwalled carbon nanotubes. *Eur Chem Bulletin* 2014; 3(5): 502-504. https://doi.org/10.17628/ECB.2014.3.502
25. Liu J, Chen S, Liu Y and Zhao B. Progress in preparation, characterization, surface functional modification of graphene oxide: A review. *J Saudi Chem Soc* 2022; 26:101560. https://doi.org/10.1016/j.jscs.2022.101560
26. Andelkovic IB, Kabiri S, Tavakkoli E, Kirby JK, McLaughlin MJ and Losic D. Graphene oxide-Fe(III) composite containing phosphate-A novel slow release fertilizer for improved agriculture management. *J Clean Prod* 2018; 185:97-104. https://doi.org/10.1016/j.jclepro.2018.03.050
27. Kabiri S, Degryse F, Tran DNH da Silva RC, McLaughlin MJ and Losic D. Graphene oxide: A new carrier for slow release of plant micronutrients ACS. *Appl Mater Inter* 2017; 9: 43325-43335. https://doi.org/10.1021/acsami.7b07890
28. Guo X, Zhao J, Wang R Zhang H, Xing B, Naeem M, Yao T, Li Rm, Xu R, Zhang Z and Wu J. Effects of graphene oxide on tomato growth in different stages. *Plant Physiol Biochem* 2021; 162: 447- 455. https://doi.org/10.1016/j.plaphy.2021. 03.013
29. Zhang X, Cao H, Zhao J, Wang H, Xing B, Chen Z, Li X and Zhang J . Graphene oxide exhibited positive effects on the growth of Aloe vera L. *Physiol Mol Biol Plants.* 2021; 27(4):815-824. doi:10.1007/s12298-021-00979-3
30. Yang Y, Zhang R, Zhang X, Chen Z, Wang H and Li PCH. Effects of graphene oxide on plant growth: A review. *Plants* 2022; 11: 2826. https://doi.org/10.3390/plants11212826
31. Zhao S, Wang W, Chen X, Gao Y, Wu X, Ding M and Duo L. Graphene oxide affected

root growth, anatomy, and nutrient uptake in alfalfa. *Ecot Env Safety* 2023; 250: 114483. https://doi.org/10.1016/j.ecoenv.2022.114483

32. Zhang P, Guo Z, Luo W, Monikh FA, Xie C, Jones EV, Lynch I and Zhang Z. Graphene Oxide-Induced pH Alteration, Iron Overload, and Subsequent Oxidative Damage in Rice (Oryza sativa L.): A New Mechanism of Nanomaterial Phytotoxicity. *Environ Sci Technol.* 2020; 54(6): 3181-3190. doi:10.1021/acs.est.9b05794
33. Song J, Cao K, Duan C, Luo N and Cui X. Effects of graphene on *Larix olgensis* seedlings and soil properties of haplic cambisols in Northeast China. Forests 2020; 11: 258. https://doi.org/10.3390/f11030258
34. Pikula K, Johari SA, Oliveira RS and Golokhvast K. The comparative toxic impact assessment of carbon nanotubes, fullerene, graphene, and graphene oxide on marine microalgae *Porphyridium purpureum.* Toxics 2023; 11: 49. https://doi.org/10.3390/toxics11060491.
35. Paital B, Guru D, Mohapatra P, Panda B, Parida N, Rath S, Kumar V, Saxena PS and Srivastava A. Ecotoxic impact assessment of graphene oxide on lipid peroxidation at mitochondrial level and redox modulation in fresh water fish Anabas testudineus. Chemosphere 2019; 224: 796-804. https://doi.org/10.1016/j.chemosphere.2019.02.156
36. Vishwakarma SP, Singh JK, Sharma P, Singh AK and Kumar NM. Sulphur and boron application for enhanced yield of blackgram (*Vigna mungo* L.) under custard apple (*Annona squamosa* L.) based agroforestry system in Vindhyan region. *Eco. Env*. & Cons. 2024 (sp.issue); S409-S417. http://doi.org/10.53550/EEC.2024.v30i04s.068
37. Srivastava R, Mohapatra M and Latare A. Impact of land use changes on soil quality and species diversity in the Vindhyan dry tropical region of India. *J Trop Ecol.* 2020; 36: 72-79. https://doi.org/10.1017/S0266467419000385
38. Srivastava R and Bharti V. Importance of Dalbergia sissoo for restoration of degraded land: A case study of Indian dry tropical Vindhyan regions. *Ecol Eng,* 2023. 194: 1-10. https://doi.org/10.1016/j.ecoleng.2023.107021 Engineering 194: 107021.
39. Singh A and Srivastava R. Impact of Plant growth promoting Rhizobacteria on restoring soil and crop attributes. *Indian J. Agric. Sci*.2022. https://doi.org/10.18805/IJARe.A-5962
40. Wang S, Liu Y, Wang X, Xiang H, Kong D, Wei N, Guo W and Sun H. Effects of concentration-dependent graphene on maize seedling development and soil nutrients. *Scientific Reports* 2023; 13: 1-13. https://doi.org/10.1038/s41598-023-29725-3
41. Srivastava A, Srivastava O N, Talapatra S, Vajtai R and Ajayan P M. Carbon nanotube filters. Nature Mater.2004;3: 610-614.
42. Awasthi K, Kumar R, Raghubanshi H Awasthi S, Pandey R, Singh D. Yadav TP and Awasthi K. Synthesis of nano-carbon (Nanotubes, nanofibres, graphene) materials. *Bull Mat Sci* 2011; 34:607- 614.
43. Rahman M S, Chakraborty A, Kibria A and Hossain M J. Effects of silver nanoparticles on seed germination and growth performance of pea (*Pisum sativum*). Plant Nano Biology, 2023; 5, 100042. https://doi.org/10.1016/j.plana.2023.100042
44. Sharma D, Afzal S and Singh NK. Nanopriming with phytosynthesized zinc oxide nanoparticles for promoting germination and starch metabolism in rice seeds. *J. Biotech.*, 2021; 336, 64-75. https://doi.org/10.1016/j.jbiotec.2021.06.014
45. Nepal J, Xin X, Landry G, Ahmad W, Pereira J, Santra S, Wright AL, Ogram A, Stofella P J and He Z. Carbon nanomaterials are a superior soil amendment for sandy soils than biochar based on impacts on lettuce growth, physiology and soil biochemical quality. *NanoImpact,* 2023; 31, 100480. https://doi.org/10.1016/j.impact.2023.100480
46. Piper C. Soil and Plant Analysis. 1966; Hans Publishers, Bombay
47. Kalembasa SJ and Jenkinson DS. A comparative study of titrimetric and gravimetric methods for the determination of organic carbon in soil. *J Sci Food Agric* 1973; 24: 1085-1090. https://doi.org/10.1002/jsfa.2740240910
48. Jackson ML. Soil Chemical Analysis. 1958; Prentice-Hall Inc, Englewood Cliffs, NJ
49. Vance ED, Brookes PC and Jenkinson DS. An extraction method for measuring soil

microbial biomass C. *Soil Biol Biochem* 1987; 19: 703-707. https://doi.org/10.1016/0038-0717(87)90052-6

50. Thalmann Zur Methodik der bestimmung der dehydrogenaseaktivit at im bodenmittels triphenyltetrazoliumchlorid (TTC). *Forsch* 1968; 21: 249-258.
51. Gan L, Li B, Chen Y Yu B and Chen Z. Green synthesis of reduced graphene oxide using bagasse and its application in dye removal: A waste-to-resource supply chain. *Chemosphere* 2019; 219: 148-154. https://doi.org/10.1016/j.chemosphere.2018.11.181
52. Begum P, Ikhtiari R and Fugetsu B. Graphene phytotoxicity in the seedling stage of cabbage, tomato, red spinach, and lettuce. *Carbon* 2011; 49: 3907-3919. https://doi.org/10.1016/j.carbon.2011.05.029
53. Zhao F, Xin X, Cao Y, Su D, Ji P, Zhu Z and He Z. Use of carbon nanoparticles to improve soil fertility, crop growth and nutrient uptake by corn (*Zea mays* L.). *Nanomaterials* 2021; 11: 2717. https://doi.org/10.3390/nano11102717
54. Ali S, Mehmood A and Khan N. Uptake, Translocation, and Consequences of Nanomaterials on Plant Growth and Stress Adaptation. *J Nanomater* 2021; 6677616. https://doi.org/10.1155/2021/6677616
55. Khodakovskaya MV, Kim BS, Kim JN, Alimohammadi M, Dervishi E, Mustafa T and Cernigla CE. Carbon nanotubes as plant growth regulators: Effects on tomato growth, reproductive system, and soil microbial community. Small 2013; 9:115-123. https://doi.org/10.1002/smll.201201225
56. He Y J, Hu RR, Zhong YJ, Zhao X, Chen Q and Zhu H. Graphene oxide as a water transporter promoting germination of plants in soil. *Nano Res* 2018; 11: 1928-1937. https://doi.org/10.1007/s12274-017-1810-1
57. Mirza FS, Aftab Z-e-H, Ali MD, Aftab A, Anjum T, Rafiq H and Li G. Green synthesis and application of GO nanoparticles to augment growth parameters and yield in mungbean (*Vigna radiata* L.). *Front Plant Sci* 2022; 13:1040037. https://doi.org/10.3389/fpls.2022.1040037
58. Mahmoud NE and Abdelhameed RM. Superiority of modified graphene oxide for enhancing the growth, yield, and antioxidant potential of pearl millet (*Pennisetum glaucum* L.) under salt stress. Plant Stress 2021; 2: 100025. https://doi.org/10.1016/j.stress.2021.100025
59. Chakravarty D, Erande MB and Late D. Graphene quantum dots as enhanced plant growth regulators: effects on coriander and garlic plants. *J Sci Food Agric* 2015; 95:2772-2778. https://doi.org/10.1002/jsfa.7106.
60. Apel K and Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Ann. Rev. Plant Biol.* 2004; 55: 373-399. https://doi.org/10.1146/annurev.arplant.55.031903.141701
61. Wen F, Xing D and Zhang LR. Hydrogen peroxide is involved in high blue light-induced chloroplast avoidance movements in arabidopsis. *J Exp Bot* 2008; 59: 2891-2901
62. Nel A, Xia T, Madler L and Li N. Toxic potential of materials at the nanolevel. Science 2006; 311: 622-627. https://doi.org/ 10.1126/science.1114397
63. Zhao D, Fang, Tang Y and Tao J. Graphene oxide as an effective soil water retention agent can confer drought stress tolerance to Paeonia ostii without toxicity environmental. *Sci Tech* 2020; 54: 8269-8279. https://doi.org/10.1021/acs.est.0c02040
64. Carneiro JSD, Leite DAD, de Castro GM, Franca JR, Botelho L, Soares JR, de Oliveira JE and Melo LCA. Biochar-graphene oxide composite is efficient to adsorb and deliver copper and zinc in tropical soil. *J. Clean. Prod* 2022; 360: 13. https://doi.org/10.1016/j.jclepro.2022.132170
65. Mohammadi AS, Ramezanzadeh AH, Dolatabadi N, Khalaki MA, Moameri M, Lajayer BA and van Hullebusch ED. Impact on nutritional status of plants treated with nanoparticles. In: Rajput VD, Minkina T, Sushkova S, Mandzhieva S S, Rensing C (eds) *Toxicity of nanoparticles in plants*, Massachusetts: Academic Press, Cambridge, 2022; pp 333-358.
66. Hammerschmiedt T, Holatko J, Zelinka R, Kintl A, Skarpa P, Bytesnikova Z, Richtera L, Mustafa A, Malicek O and Brtnicky M. The combined effect of graphene oxide and elemental nano-sulfur on soil biological properties and lettuce plant biomass. *Front Plant Sci* 2023; 14: 1057133. https://doi.org/10.3389/fpls.2023.1057133

67. Logah V, Safo EY, Quansah C and Danso I. Soil microbial biomass carbon, nitrogen and phosphorus dynamics under different amendments and cropping systems in the semi-deciduous forest zone of Ghana. West African *J Appl Ecol* 2010; 17:121-133.
68. Tu Y, Lv M, Xiu P, Huynh T, Zhang M, Castelli M, Liu Z, Huang Q, Fan C, Fang H and Zhou R. Destructive extraction of phospholipids from Escherichia coli membranes by graphene nanosheets. *Nat Nanotechnol* 2013; 8: 594-601. https://doi.org/ 69.10.1038/nnano.2013.125
69. Du J, Zhou Q, Wu J, Li G, Li G and Wu Y. Soil bacterial communities respond differently to graphene oxide and reduced graphene oxide after 90 days of exposure. *Soil Ecol Lett* 2020; 2: 176-179. https://doi.org/ 10.1007/s42832-020-0043-5
70. Gurunathan S, Han JW, Dayem AA, Eppakayala V and Kim JH. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in *Pseudomonas aeruginosa. Int J Nanomedicine* 2012; 7: 5901-5914. https://doi.org/10.2147/IJN.S37397
71. Du J, Hu X and Zhou Q. Graphene oxide regulates the bacterial community and exhibits property changes in soil. *RSC Adv* 2015; 5: 27009-27017. https://doi.org/10.1039/ c5ra01045d
72. Xie J, Ming Z, Li H, Yang H , Yu B ,Wu R, Liu X , Bai Y and Yang ST. Toxicity of graphene oxide to white rot fungus phanerochaete chrysosporium. *Chemosphere* 2016; 151: 324-331. https://doi.org/ 10.1016/j.chemosphere. 2016.02.097
73. Navarro DA, Kah M, Losic D, Kookana RS and McLaughlin MJ. Mineralisation and release of 14C-graphene oxide (GO) in soils. *Chemosphere* 2020; 238: 124558. https://doi.org/10.1016/j.chemosphere. 2019.124558
74. Jaisi DP, Saleh NB, Blake RE and Elimelech M. Transport of single-walled carbon nanotubes in porous media: Filtration mechanisms and reversibility. *Environ Sci Technol* 2008; 42: 8317-8323. https://doi.org/ 10.1021/es801641v
75. Lin J, Ma K, Chen H Chen Z and Xing B. Influence of different types of nanomaterials on soil enzyme activity: A global meta-analysis. *Nano Today* 2022; 42: 101345. https://doi.org/10.1016/ j.nantod.2021.101345
76. Burns RG and Dick RP. Enzymes in the environment: Activity, ecology, and applications (1st ed.). CRC Press. Boca Raton. 2002; 614. https://doi.org/10.1201/9780203904039
77. Jarvan M, Edesi L, Adamson A and Vosa T. Soil microbial communities and dehydrogenase activity depending on farming systems. *Plant Soil Env* 2014; 60:459-463. https://doi.org/ 10.17221/410/2014-PSE