MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Reclamation of sandy soil using multiwalled carbon nanotubes alone or loaded with soil conditioners for cultivation of kidney bean plants

Mohammed N. A. Hasaneen, Heba M. M. Abdel-Aziz and Sara H. Helal

Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

Received:9/10/2019
Accepted: 18/11/2019

Abstract: A pot experiment was conducted in which kidney bean seeds were planted in sandy soil either alone or amended with different concentrations of urea, peat moss and multi-walled carbon nanotubes. In general, soil conditioners amended to sandy soil induced significant increases in all growth and reproductive parameters of kidney bean throughout the four successive growth and developmental stages under study. In addition, soil conditioners induced pronounced significant increases in photosynthetic pigments at vegetative growth stage. Furthermore, the addition of CNTs alone or in combination with urea and/or peat moss to sandy soil induced significant increases in all enzymatic and non-enzymatic antioxidants determined in kidney bean plants.

In conclusion, the disparity of changes in photosynthetic pigments, enzymatic and non-enzymatic antioxidants determined in kidney bean plants at vegetative stage, and the associated changes in all growth and developmental parameters, appeared to be a function of metabolic changes as influenced by different concentrations of CNTs and/or soil conditioners used. Furthermore, the present results give a clear indication that CNTs (foliar spray) + urea + peat moss was superior than CNTs (soil incorporation) + urea + peat moss, and could be recommended as the most effective soil conditioner for best reclaiming sandy soil. These new and novel results may also be a new nanotechnology strategy that can be further applicable for reclaiming sandy soil to be ready for cultivation of different crops.

keywords: Antixoidants, growth, carbon nanotubes, photosynthetic pigments, sandy soil, soil conditioners, kidney bean **.**

1.Introduction

Sandy soils widely exist in arid and semi-arid regions such as the east and west desert areas of Egypt. Increasing productive lands is now considered one of the major targets of the agricultural policy. However, the productivity of sandy soils is mostly limited by several agronomic obstacles. Sandy soils usually have poor properties including low specific surface area, low water retention, low organic matter content, low fertility and high infiltration rates. Such poor physical properties cause insufficient water use, especially in arid and semi-arid regions. These adverse factors can be solved by several means such as different natural conditioners [1, 2].

A major influence on the reclamation of sandy desert soils not only results from the amount of water applied, carbon and nitrogen in the soil, but also from the water and nutrient distribution in the root zone and the prevention of water movement in the soil profile, either by seepage or evaporation [3, 4, 5]. Another major influence on immobilization of water in the root zone by evaporation is due to the temperature gradient at the soil surface. To reduce evaporation, the top soil should be rendered hydrophobic [6]. A treatment over a depth of few centimeters with bitumen emulsion gives good results [7].

Organic materials such as peat moss tend to be hydrophobic and may be difficult to rewet if allowed to become too dry. [5, 8] found that peat-based media become more efficient at absorbing applied water as the moisture content of the medium increased before irrigation. The state of decomposition of the peat moss may also affect the ability to rewet after drying. Peats with a greater state of degradation also have a greater amount of humic acid. Humic acid plays an important role in cation exchange capacity of peat moss based root media [5].

Nanotechnology has potential to bring revolution in the field of arid afforestation and agriculture. It facilitates enhanced and elegant properties of molecules that could be developed in material if used at nanoscale. They have intrinsic properties of different kinds, so the majority of the atoms are in different environment showing different physical, chemical, mechanical, optical properties that can be exploited for different uses. These nano-structured molecules can change the concept of fertilizers and irrigation methods. Thus, arid area agriculture and desert rehabilitation could be techno-economically feasible [9, 10, 11, 12].

Foliar application of nanofertilizer also increases fertilizer use efficiency. Zeolite-based synthetic substrate, termed zeoponics (nanograde fertilizer), consists of NH_4^+ and K^+. These ions occur in free form that can easily be exchanged. It contains clinoptilolite and a synthetic apatite whose water solution provides essential nutrients through mineral dissolution and ion exchange to the whole plant. This fertilizer should remain in the form of colloidal suspension which readily spreads into plant tissues due to its nanostructured form. The nanostructured fertilizer easily crosses plasma membrane after the spray through the xylem tissues which appear on the surface of the leaf. A nanostructure fertilizer is expected to be translocated into the plant. This enhances the vegetative growth during the early stage, so the plant survives even under drought condition [13]. The free NH_4^+ ion could then be easily absorbed by the plant [13].

Enhancement of many physiological parameters related to plant growth and development were reported that included photosynthetic activity and nitrogen metabolism by metal based nanomaterials in a few crops including soybean [14], spinach [15, 16, 17], and peanut [18] and by multiwalled carbon nanotubes in tomato [19].

Different effects of CNTs either alone or loaded with NPK fertilizers on plant growth could be attributed to the type of plant, physical and chemical features of CNTs, the concentration used, exposure duration to nanotubes and the culture conditions [20]. CNTs and CS- nanoparticles had the ability to increase the growth parameters of foliary treated French bean plants compared with control [21]. This could be explained on the basis that CNTs and CS- nanoparticles either alone or loaded with NPK increased water absorption by the plant root and enhanced cell division by improving the expression of specific genes.

Increased peroxidase activity was found to be associated with oxidative stress caused by nanomaterials [20]. Accumulation of MWCNTs at the root surface would increase their penetration to the cell walls of epidermal cells. This was assumed to represent a mechanical injury resulted in rising the level of peroxidase activity that was increased in alliance with nanofertilizers concentration stimulation the growth of roots and stems of plants [22]. Fe_3O_4 nanoparticles significantly increased the levels of SOD and CAT in the root tissue of *Cucurbita mixta* [23]. It was found that at 50 µg/L concentration of nano-ZnO, a positive response of the plant was recorded but at higher concentration phytotoxic activity of nanoparticles was reported. Consequently, the level of superoxide radical and hydrogen peroxide, total phenolics, SOD and POX activities were increased [24].

Thus, the objective of this study was designed to investigate the effects of classical soil conditioners (urea and peat moss) as well as multiwalled carbon nanotubes either alone or loaded with urea and/or peat moss for reclaiming sandy soil and concomitant physiological effects on growth, development and certain metabolic changes in kidney bean plants.

MATERIALS AND METHODS

1- Experimental design

The experiment was a factorial design with nine treatments, each at optimum rate of urea, peat moss and CNTs (20 µg for foliar spray and 0.5 g mixed with the soil). These treatments were applied either singly or in combination, with kidney bean plants (*Phaseolus vulgaris* L., Cv. Giza 6). Each treatment and control was replicated five times.

2- Soil and pot installation

Sandy top soil, 0-30 cm depth arable layer, was collected from the northern area of Klapsho region, Dakahlia Governorate, Mansoura, Egypt. All pots (30×28×26 cm) contained equal amounts of sandy soil (8 kg). Analysis of such sandy soil was as follow:

Physical characteristics					Chemical characteristics (%)								
Soil texture (%)			% W.H.C	% Porosity	pH	EC ($mS.cm^{-1}$)	O.C	Total N	Na^+	K^+	Ca^{++}	Mg^{++}	P
Sand	Silt	Clay											
90.00	8.70	1.30	22.00	30.80	8.35	0.35	0.90	0.37	0.06	0.04	1.22	0.28	0.001

W.H.C; water holding capacity, **EC**; electrical conductivity, **O.C**; organic carbon

The normal water holding capacity of the soil used was 22 % and all pots were irrigated with tap water every three days to maintain the soil at the field capacity throughout the entire period of experiment. Superphosphate was applied with irrigation water as 0.5 g/pot weekly [25].

3- Time course experiment

A uniformly-sized lot of kidney bean (*Phaseolus vulgaris* L., Cv. Giza 6) seeds were selected. The seeds were surface sterilized by soaking in 10^{-3} M $HgCl_2$ solution for 3 minutes, then washed with sterile water. The seeds were then sown in sandy soil in pots (50 pots; 30×28×26 cm). All pots contained equal *amounts of sandy soil (8 kg).*

Fifty pots were classified into 10 groups each of 5 pots, one group was left without treatment to serve as a control and the other 9 groups were treated with different soil conditioners according to the following scheme:

Group	Treatment
1	Control (sandy soil alone)
For foliar treatment	
2	Urea (7.09 mM)
3	CNTs (20 µg/L)
4	CNTs (20 µg/L) + Urea (7.09 mM)
For soil incorporation treatment	
5	Peat moss (2 kg)
6	CNTs (0.5 g)
7	CNTs (0.5 g) + Urea (7.09 mM)
8	CNTs (0.5 g) + Peat moss (2 kg)
9	CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)
For foliar treatment+ Soil incorporation treatment	
10	CNTs (20 µg/L) + Urea (7.09 mM) (F) + Peat moss (2 kg)

Samples were taken after 20, 32, 50 and 80 days from the date of sowing representing four successive growth and developmental stages (seedling, vegetative, flowering and fruiting respectively) to determine growth and developmental parameters the concentration of photosynthetic pigments, and enzymatic and non-enzymatic antioxidants in kidney bean plants.

ANALYTICAL METHODS

1. Determination of photosynthetic pigments

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method as developed by [26].

2. Determination of antioxidant system

2.1. Determination of antioxidant compounds

2.1.1. Determination of hydrogen peroxide

The content of H_2O_2 was determined according to [27].

2.1.2. Determination of total phenols

The content of total phenolic compounds was determined according to the method described by [28].

2.1.3. Determination of reduced glutathione (GSH)

According to the method of [29].

2.1.4. Determination of ascorbic acid (AsA)

The AsA content was estimated using [30] method.

2.2. Determination of activity of antioxidant enzymes

Enzyme extraction.

Enzyme extracts were prepared by grinding 200 mg of fresh kidney bean leaves with 5 cm^3 chilled phosphate buffer. For APX and SOD, the extraction medium was 0.1 M phosphate buffer at pH 7.8 and for CAT, GR, POX and PPO, 0.1 M phosphate buffer at pH 6.8 was used. The homogenate was filtered through cheesecloth and the filtrate was centrifuged in a refrigerated centrifuge at 10,000 rpm for 20 min. The supernatant served as enzyme extract. All operations were carried out at 4 °C [31].

2.2.1. Determination of superoxide dismutase activity (SOD, EC 1.15.1.1).

In the present study, SOD activity was determined according to the method of [32]. As applied recently by [31].

2.2.2. Determination of catalase activity (CAT, EC 1.11.1.6).

Catalse activity was measured at 240 nm over 1 minute by monitoring the removal of H_2O_2 [33].

2.2.3. Determination of ascorbate peroxidase activity (APX, EC 1.11.1.11).

Ascorbate peroxidase activity was assayed by measuring the decrease in absorbance at 290 nm due to ascorbate oxidation; as adopted and described by [34].

2.2.4. Determination of glutathione reductase activity (GR, EC 1.8.1.7).

GR activity was assayed as the decrease in absorbance at 340 nm due to the reduction of GSSG in the presence of NADPH which is oxidized to $NADP^+$ as described by [35].

2.2.5. Determination of peroxidase activity (POX, EC 1.11.1.7).

POX activity was assayed by the method of [36].

2.2.6. Determination of polyphenol oxidase activity (PPO, EC 1.10.3.1).

PPO activity was assayed as the increase in absorbance at 420 nm due to the formation of purpurogallin [36].

RESULTS AND DISCUSSION

Changes in growth and developmental parameters

The results reported herein (Tables 1, 2, 3, and 4) show that the addition of urea, peat moss and/or CNTs (foliar spray or soil incorporation) either alone or in combination, to sandy soil induced marked significant increases in all growth and developmental parameters determined. The following sequence was displayed with respect to growth and developmental parameters determined: CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S) > CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg) > CNTs (0.5 g) + Peat moss (2 kg) > Peat moss (2 kg) > CNTs (20 µg) > CNTs (20 µg) + Urea (7.09 mM) > CNTs (0.5 g) + Urea (7.09 mM) > CNTs (0.5 g) > Urea (7.09 mM) > Control.

In this connection, [25] reported that, soil conditioners amended to sandy soil induced significant increases in all growth parameters of cotton throughout all stages of growth. Application of two conditioners (bitumen and ureaformaldhyde polymer) to sandy soil planted with groundnuts gave significant increases in nodulation, dry matter yield and nitrogen accumulation [37]. It has been also reported [19] that the use of nanomaterials for improving plant growth, generally related to the concentrations of nanoparticles applied to various plant organs or tissues.

Furthermore, [20] showed that the application of nano-composite CS-PMAA and nano-engineered CNTs either alone or in combination with NPK, at different concentrations, to French bean plants induced marked significant variable increases in case of foliar application and significant decreases with both seed priming and soil incorporation techniques regarding all growth parameters determined (root and shoot length, fresh and dry weight, water content and leaf area) at growth and developmental stages. The magnitude of increased growth parameters was most pronounced with low concentration of nanofertilizers (CS 10% and CNTs 20 µg/L).

The increased growth and developmental parameters of kidney bean plants grown in sandy soil amended with urea, peat moss and CNTs either alone or in combination at seedling, vegetative, flowering and fruiting stages, as a result of application of soil conditioners (See tables 1, 2, 3, and 4) could be tentatively attributed to variable effects of the conditioner used on: (a) Structure stability of the sand and (b) effects on water conservation in a sandy soil [38].

In conformity of the present results, [4, 5, 11, 20, 25, 39, 40, 41, 42, 43] stated that the effects of different soil conditioners on soil physical and chemical properties and consequently on growth and yield of different plant species were as follows: (a) decreased bulk density, (b) increased voids, (c) increased stability of the sand, (d) improved available nutrient status, (e) decreased evaporation and (f) increased fertility and hence increased growth and yield [25, 38, 43, 44, 45].

Table 1: Effect of different concentrations of urea, peat moss and carbon nanotubes (CNTs) either alone or in combination as soil conditioners on growth and development of 20-day-old kidney bean plants (seedling stage) grown in sandy soil. *Mean values listed are significantly different from control at $p \leq 0.05$.

Parameter Treatment	ShootLength (cm plant^{-1})	% change	Shootfresh weight (g palnt^{-1})	% change	Shoot Dryweight (g palnt^{-1})	% change	ShootWatercontent (g plant^{-1})	% change	Leafarea (cm^{2}plant^{-1})	% change
Control	15.20		2.60		0.41		2.19		15.84	
Soil incorporation treatment										
Peat moss (2 kg)	17.05*	12.17	4.90*	88.46	0.58*	41.46	4.32*	97.26	18.60*	17.42
CNTs (0.5 g)	15.50*	1.97	3.10*	19.23	0.47	14.63	2.63*	20.09	16.07*	1.45
CNTs (0.5 g) + Urea (7.09 mM)	15.95*	4.93	3.80*	46.15	0.51*	24.39	3.29*	50.23	17.20*	8.59
CNTs (0.5 g) + Peat moss (2 kg)	17.03*	12.04	5.76*	121.54	0.62*	51.22	5.14*	134.70	20.60*	30.05
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	17.40*	14.47	5.95*	128.85	0.68*	65.85	5.27*	140.64	27.60*	74.24

Table 1 (cont.):

Parameter Treatment	Rootlength (cm plant^{-1})	% change	Rootfresh weight(g palnt^{-1})	% change	Root dryweight (g palnt^{-1})	% change	Root watercontent (g plant^{-1})	% change
Control	5.05		0.25		0.03		0.22	
Soil incorporation treatment								
Peat moss (2 kg)	5.40*	6.93	0.72*	188.00	0.08	166.67	0.64*	190.90
CNTs (0.5 g)	5.20*	2.97	0.40*	60.00	0.04	33.33	0.36*	63.64
CNTs (0.5 g) + Urea (7.09 mM)	5.35*	5.94	0.51*	104.00	0.06	100.00	0.45*	104.55
CNTs (0.5 g) + Peat moss (2 kg)	5.63*	11.49	0.86*	244.00	0.15*	400.00	0.71*	222.73
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	6.20*	22.77	0.94*	276.00	0.26*	766.67	0.68*	209.09

Table 2: Effect of different concentrations of urea, peat moss and carbon nanotubes (CNTs) either alone or in combination as soil conditioners on growth and development of 32-day-old kidney bean plants (vegetative stage) grown in sandy soil. *Mean values listed are significantly different from control at $p \leq 0.05$.

Parameter Treatment	Shootlength (cm plant^{-1})	% change	Shootfresh weight(g palnt^{-1})	% change	Shootdry weight (g palnt^{-1})	% change	Shootwater content (g plant^{-1})	% change	Leafarea(cm^2 plant^{-1})	% change
Control	25.20		3.10		0.64		2.46		18.91	
Foliar treatment										
Urea (7.09 mM)	25.90*	2.78	3.90*	25.81	0.65	1.56	3.25*	32.11	19.29	2.01*
CNTs (20 µg)	33.90*	34.52	5.00*	61.29	0.70	9.38	4.30*	74.80	23.62	24.91*
CNTs (20 µg) + Urea (7.09 mM)	33.60*	33.33	5.10*	64.52	0.69	7.81	4.41*	79.27	22.58	19.41*
Soil incorporation treatment										
Peat moss (2 kg)	35.80*	42.06	6.20*	100.00	0.72	12.50	5.48*	122.76	29.33	55.10*
CNTs (0.5 g)	27.60*	9.52	4.10*	32.26	0.66	3.13	3.44*	39.83	21.21	12.16*
CNTs (0.5 g) + Urea (7.09 mM)	28.00*	11.11	4.40*	41.94	0.67	4.69	3.73*	51.63	22.08	16.76*
CNTs (0.5 g) + Peat moss (2 kg)	38.10*	51.19	6.80*	119.35	0.76*	18.75	6.04*	145.53	30.00	58.65*
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	40.40*	60.32	7.20*	132.26	0.80*	25.00	6.40*	160.16	31.58	67.00*
Foliar treatment + Soil incorporation treatment										
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	41.00*	62.70	7.40*	138.71	0.84*	31.25	6.56*	166.67	32.43	71.50*

Table 2 (cont.):

Parameter Treatment	Rootlength (cm plant^{-1})	%change	Rootfresh weight(gplant)	% change	Rootdry weight(gplant^{1})	% change	Rootwatercontent(g plant^{-1})	% change
Control	6.00		0.43		0.09		0.34	
Foliar treatment								
Urea (7.09 mM)	6.30*	5.00	0.49	13.95	0.10	11.11	0.39	14.71
CNTs (20 µg)	7.30*	21.67	0.79*	83.72	0.12	33.33	0.67*	97.06
CNTs (20 µg) + Urea (7.09 mM)	7.25*	20.83	0.78*	81.40	0.12	33.33	0.66*	94.12
Soil incorporation treatment								
Peat moss (2 kg)	7.80*	30.00	0.81*	88.37	0.14	55.56	0.67*	97.06
CNTs (0.5 g)	6.50*	8.33	0.51	18.60	0.10	11.11	041	20.59
CNTs (0.5 g) + Urea (7.09 mM)	7.00*	16.67	0.70*	62.79	0.11	22.22	0.59*	73.53
CNTs (0.5 g) + Peat moss (2 kg)	8.00*	33.33	0.82*	90.70	0.14	55.56	0.68*	100.00
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	8.50*	41.67	0.90*	109.30	0.17	88.89	0.73*	114.71
Foliar treatment + Soil incorporation treatment								
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	8.90*	48.33	0.92*	113.95	0.20*	122.22	0.72*	111.76

Parameter Treatment	Shootlength(cmplant^{-1}	%chane	Shootfresh weight (gpalnt^{1})	%chane	Shoot dryweigt (g palnt^{-1})	% change	Shootwater content (gplant^{1})	% change	Leafarea (cm^{2}plant)	% change	No. of flowers	% change
Control	30.85		6.60		0.96		5.64		20.25		11.00	
Foliar treatment												
Urea (7.09 mM)	31.30*	1.46	7.83*	18.64	1.07*	11.46	6.76*	19.86	20.90*	3.21	11.00	0.00
CNTs (20 µg)	36.60*	18.64	14.87*	125.30	1.63*	69.79	13.24*	134.75	24.50*	20.99	18.00*	63.64
CNTs (20 µg) + Urea (7.09 mM)	36.35*	17.83	13.71*	107.73	1.54*	60.42	12.17*	115.78	23.30*	15.06	18.00*	63.64
Soil incorporation treatment												
Peat moss (2 kg)	36.80*	19.29	15.92*	141.21	1.80*	87.50	14.12*	150.35	26.65*	31.60	21.00*	90.91
CNTs (0.5 g)	33.30*	7.94	10.64*	61.21	1.30*	35.42	9.34*	65.60	21.80*	7.65	15.00*	36.36
CNTs (0.5 g) + Urea (7.09 mM)	35.76*	15.92	12.77*	93.48	1.43*	48.96	11.34*	101.06	23.00*	13.58	16.00*	45.45
CNTs (0.5 g) + Peat moss (2 kg)	37.50*	21.56	17.64*	167.27	2.17*	126.04	15.47*	174.29	26.83*	32.49	23.00*	109.09
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	42.50*	37.76	18.70*	183.33	2.40*	150.00	16.30*	189.01	31.65*	56.30	23.00*	109.09
Foliar treatment + Soil incorporation treatment												
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	43.60*	41.33	22.26*	237.27	2.71*	182.29	19.55*	246.63	32.50*	60.49	23.00*	109.09

Table 3: Effect of different concentrations of urea, peat moss and carbon nanotubes (CNTs) either alone or in combination as soil conditioners on growth and development of 50-day-old kidney bean plants (flowering stage) grown in sandy soil. *Mean values listed are significantly different from control at p ≤ 0.05.

Table 3 (cont.):

Parameter Treatment	Root length(cm plant^{-1})	% change	Rootfresh weight(gplant^{-1})	% change	Rootdry weight(gplant^{1})	% change	Rootwater content(g plant^{-1})	% change
Control	9.00		0.51		0.10		0.41	
Foliar treatment								
Urea (7.09 mM)	9.60*	6.67	0.57	11.76	0.11	10.00	0.46*	12.20
CNTs (20 µg)	12.85*	42.78	1.98*	288.24	0.18	80.00	1.80*	339.02
CNTs (20 µg) + Urea (7.09 mM)	12.80*	42.22	1.61*	215.69	0.17	70.00	1.44*	251.22
Soil incorporation treatment								
Peat moss (2 kg)	13.25*	47.22	2.20*	331.37	0.19*	90.00	2.01*	390.24
CNTs (0.5 g)	11.10*	23.33	0.72	41.18	0.13	30.00	0.59*	43.90
CNTs (0.5 g) + Urea (7.09 mM)	11.75*	30.56	1.02	100.00	0.16	60.00	0.86*	109.76
CNTs (0.5 g) + Peat moss (2 kg)	13.90*	54.44	2.86*	460.78	0.20*	20.00	2.66*	548.78
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	16.20*	80.00	3.50*	586.27	1.20*	1100.00	2.30*	460.98
Foliar treatment + Soil incorporation treatment								
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	18.10*	101.11	3.90*	664.71	1.76*	1660.00	2.16*	426.83

Table 4: Effect of different concentrations of urea, peat moss and carbon nanotubes (CNTs) either alone or in combination as soil conditioners on growth and development of 80-day-old kidney bean plants (fruiting stage) grown in sandy soil. *Mean values listed are significantly different from control at $p \leq 0.05$.

Parameter Treatment	Shoot length (cm plant^{-1})	% change	Shootfresh weight (g palnt^{-1})	% change	Shoot dryweight (g palnt^{-1})	% change	Shootwater content (gplant^{-1})	% change	Leafarea (cm^2 plant^{-1})	% change
Control	31.20		7.22		1.17		6.05		21.70	
Foliar treatment										
Urea (7.09 mM)	32.20*	3.21	8.46*	17.17	1.30*	11.11	7.16*	18.35	22.00*	1.38
CNTs (20 µg)	35.50*	13.78	15.12*	109.42	2.54*	117.09	12.58*	107.93	25.20*	16.13
CNTs (20 µg) + Urea (7.09 mM)	34.90*	11.86	14.33*	98.48	2.21*	88.89	12.12*	100.33	24.20*	11.52
Soil incorporation treatment										
Peat moss (2 kg)	41.70*	33.65	20.01*	177.15	3.64*	211.11	16.37*	170.58	26.10*	20.28
CNTs (0.5 g)	33.50*	7.37	10.43*	44.46	1.82*	55.56	8.61*	42.31	22.90*	5.53
CNTs (0.5 g) + Urea (7.09 mM)	34.50*	10.58	13.66*	89.20	1.90*	62.39	11.76*	94.38	23.70*	9.22
CNTs (0.5 g) + Peat moss (2 kg)	42.10*	34.94	23.92*	231.30	3.99*	241.03	19.93*	229.42	27.30*	25.81
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	43.50*	39.42	25.29*	250.28	4.05*	246.15	21.24*	251.07	28.10*	29.49
Foliar treatment + Soil incorporation treatment										
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	44.30*	41.99	26.88*	272.30	4.19*	258.12	22.69*	275.04	28.90*	33.18

Table 4 (cont.):

Parameter Treatment	Rootlength (cm plant^{-1})	% change	Rootfresh weight(g palnt^{-1})	% change	Rootdry weight(g palnt^{-1})	% change	Rootwater content(g plant^{-1})	% change
Control	11.10		0.60		0.12		0.48	
Foliar treatment								
Urea (7.09 mM)	12.50*	12.61	0.65	8.33	0.15	25.00	0.50	4.17
CNTs (20 µg)	14.70*	32.43	1.24*	106.67	0.20	66.67	1.04*	116.67
CNTs (20 µg) + Urea (7.09 mM)	13.90*	25.23	1.10*	83.33	0.19	58.33	0.91*	89.58
Soil incorporation treatment								
Peat moss (2 kg)	16.00*	44.14	1.51*	151.67	1.05*	775.00	0.46	-4.17
CNTs (0.5 g)	12.80*	15.32	0.83*	38.33	0.17	41.67	0.66*	37.50
CNTs (0.5 g) + Urea (7.09 mM)	13.70*	23.42	0.94*	56.67	0.17	41.67	0.77*	69.42
CNTs (0.5 g) + Peat moss (2 kg)	16.90*	52.25	1.87*	211.67	1.18*	883.33	0.69*	43.75
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	17.80*	60.36	2.00*	233.33	1.26*	950.00	0.74*	54.17
Foliar treatment + Soil incorporation treatment								
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	18.80*	69.37	2.44*	306.67	1.35*	1025.00	1.09*	127.08

Table 4 (cont.):

Parameter Treatment	Podlength (cm pod^{-1})	% change	PodWidth (cm pod^{-1})	% change	No. of pods plant	% change	Wt. of pod (g pod^{-1})	%change	No. of branches / plant	% change	No.ofseeds/ pod	% change	Wt. of seeds (g pod^{-1})	% change
Control	3.00		0.40		4.00		0.17		5.00		0.00		0.00	
Foliar treatment														
Urea (7.09 mM)	4.20*	40.00	0.40	0.00	4.00	0.00	0.22	29.41	5.00	0.00	0.00	0.00	0.00	0.00
CNTs (20 µg)	9.90*	230.00	0.80*	100.00	6.00*	50.00	1.70*	900.00	7.00*	40.00	3.00*	300.00	0.30*	30.00
CNTs (20 µg) + Urea (7.09 mM)	9.50*	216.67	0.80*	100.00	6.00*	50.00	0.96*	464.71	7.00*	40.00	3.00*	300.00	0.28*	28.00
Soil incorporation treatment														
Peat moss (2 kg)	9.98*	232.67	1.00*	150.00	6.00*	50.00	2.32*	1264.71	7.00*	40.00	3.00*	300.00	0.36*	36.00
CNTs (0.5 g)	7.40*	146.67	0.60*	50.00	4.00	0.00	0.76*	347.06	7.00*	40.00	3.00*	300.00	0.05	5.00
CNTs (0.5 g) + Urea (7.09 mM)	7.50*	150.00	0.70*	75.00	5.00*	25.00	0.78*	358.82	7.00*	40.00	3.00*	300.00	0.10*	10.00
CNTs (0.5 g) + Peat moss (2 kg)	10.20*	240.00	1.10*	175.00	6.00*	50.00	2.74*	1511.76	7.00*	40.00	3.00*	300.00	0.41*	41.00
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	10.60*	253.33	1.30*	225.00	6.00*	50.00	3.66*	2052.94	7.00*	40.00	3.00*	300.00	0.57*	57.00
Foliar treatment + Soil incorporation treatment														
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	11.00*	266.67	1.50*	275.00	7.00*	75.00	4.00*	2252.94	7.00*	40.00	3.00*	300.00	0.62*	62.00

Table 5: Effect of different concentrations of urea, peat moss and carbon nanotubes (CNTs) either alone or in combination as soil conditioners on chlorophyll a (Chl a), Chlorophyll b (Chl b) & and their ratio (Chl a/b), carotenoids (Cars) and total; total photosynthetic pigments (mg/ 100 fresh weight) of 32-day-old kidney bean plants (vegetative stage) grown in sandy soil. In each case, the % change from the control is also shown. Each value is a mean of five replicates. *The values listed are significantly different from control at $p \leq 0.05$

Parameter Treatment	Chl a	% change	Chl b	% change	Chls a+b	% change	Chl a/b ratio	% change	Cars	% change	Total pigments	% change
Control	71.53		24.22		95.75		2.95		25.04		120.79	71.53
Foliar treatment												
Urea (7.09 mM)	75.62*	5.72	29.64*	22.38	105.26*	9.93	2.55*	-13.56	26.30*	5.03	131.56*	8.92
CNTs (20 µg)	106.13*	48.37	37.34*	54.17	143.47*	49.84	2.84*	-3.73	33.68*	34.50	177.15*	46.66
CNTs (20 µg) + Urea (7.09 mM)	92.74*	29.65	33.66*	38.98	126.40*	32.01	2.76*	-6.44	31.47*	25.68	157.87*	30.70
Soil incorporation treatment												
Peat moss (2 kg)	116.07*	62.27	45.62*	88.36	161.69*	68.87	2.54*	-13.90	34.64*	38.34	196.33*	62.54
CNTs (0.5 g)	86.30*	20.65	28.54*	17.84	114.84*	19.94	3.02	2.37	26.71*	6.67	141.55*	17.19
CNTs (0.5 g) + Urea (7.09 mM)	87.51*	22.34	30.79*	27.13	118.30*	23.55	2.84*	-3.73	27.10*	8.23	145.40*	20.37
CNTs (0.5 g) + Peat moss (2 kg)	131.35*	83.63	49.93*	106.15	181.28*	89.33	2.63*	-10.85	46.13*	84.23	227.41*	88.27
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	135.80*	89.85	58.71*	142.40	194.51*	103.14	2.31*	-21.69	51.00*	103.67	245.51*	103.25
Foliar treatment + Soil incorporation treatment												
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	136.22*	90.44	67.07*	176.92	203.29*	112.31	2.03*	-31.19	46.58*	86.02	249.87*	106.86

Table 6: Effect of different concentrations of urea, peat moss and carbon nanotubes (CNTs) either alone or in combination as soil conditioners on the non-enzymatic antioxidants ascorbic acid (ASA; mmol AsA/ 100 g fresh weight), reduced glutathione (GSH; mmol GSH/ 100 g fresh weight), hydrogen peroxide (H_2O_2; mmol H_2O_2/100 g fresh weight), total phenolic contents (TPC; mg catechol/ 100 g dry weight) of 32-day-old kidney bean plants (vegetative stage) grown in sandy soil. *Mean values listed are significantly different from control at $p \leq 0.05$.

Parameter Treatment	AsA	% change	GSH	% change	H_2O_2	% change	TPC	% change
Control	487.50		177.60		1.66		288.02	
Foliar treatment								
Urea (7.09 mM)	510.71*	4.76	183.15*	3.13	1.93*	16.27	296.57*	2.97
CNTs (20 µg)	757.14*	55.31	209.79*	18.13	2.34*	40.96	340.00*	18.05
CNTs (20 µg) + Urea (7.09 mM)	641.07*	31.50	203.13*	14.38	2.29*	37.95	334.07*	15.99
Soil incorporation treatment								
Peat moss (2 kg)	775.00*	58.97	222.56*	25.32	2.41*	45.18	349.86*	21.47
CNTs (0.5 g)	519.64*	6.59	187.59*	5.63	2.07*	24.70	313.68*	8.91
CNTs (0.5 g) + Urea (7.09 mM)	526.78*	8.06	197.03*	10.94	2.13*	28.31	323.55*	12.34
CNTs (0.5 g) + Peat moss (2 kg)	800.00*	64.10	255.30*	43.75	2.73*	64.46	361.71*	25.59
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	803.57*	64.83	264.18*	48.75	2.89*	74.10	365.60*	26.94
Foliar treatment + Soil incorporation treatment								
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	823.21*	68.86	280.00*	57.66	2.97*	78.91	386.05*	34.04

Table 7: Effect of different concentrations of urea, peat moss and carbon nanotubes (CNTs) either alone or in combination as soil conditioners on the activities of antioxidant enzymes superoxide dismutase (SOD; U/ g fresh weight), catalase (CAT; U/ g fresh weight), ascorbate peroxidase (APX; U/ g fresh weight), glutathione reductase (GR; U/ g fresh weight), peroxidase (POX; U/ g fresh weight), polyphenol oxidase (PPO; U/ g fresh weight) of 32-day-old kidney bean plants (vegetative stage) grown in sandy soil. *Mean values listed are significantly different from control at $p \leq 0.05$.

Parameter Treatment	SOD	%change	CAT	% change	APX	%change	GR	% change	POX	% change	PPO	% change
Control	193.97		145.07		1.61		1.33		23.16		21.00	
Foliar treatment												
Urea (7.09 mM)	219.83*	13.33	181.16*	24.88	2.25*	39.75	1.87*	40.60	24.90*	7.51	23.10*	10.00
CNTs (20 µg)	271.55*	40.00	217.26*	49.76	4.50*	179.50	3.01*	126.32	41.58*	79.53	31.26*	48.86
CNTs (20 µg) + Urea (7.09 mM)	245.69*	26.66	191.99*	32.34	2.89*	79.50	2.89*	117.29	38.32*	65.46	30.06*	43.14
Soil incorporation treatment												
Peat moss (2 kg)	284.48*	46.66	202.49*	39.58	5.36*	232.92	3.44*	158.65	42.36*	82.90	31.50*	50.00
CNTs (0.5 g)	232.76*	20.00	173.29*	19.45	2.68*	66.46	2.29*	72.18	26.40*	13.99	23.88*	13.71
CNTs (0.5 g) + Urea (7.09 mM)	232.76*	20.00	174.60*	20.36	3.54*	119.88	2.35*	76.69	27.60*	19.17	24.90*	18.57
CNTs (0.5 g) + Peat moss (2 kg)	297.41*	53.33	221.85*	52.93	5.36*	232.92	3.80*	185.71	45.12*	94.82	33.42*	59.14
CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg)	297.41*	53.33	228.74*	57.68	6.21*	285.71	3.98*	199.25	45.60*	96.89	32.22*	53.34
Foliar treatment + Soil incorporation treatment												
CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S)	310.34*	60.00	237.28*	63.56	8.36*	419.25	4.28*	221.80	47.28*	104.15	29.88*	42.29

Changes in photosynthetic pigments

In relation to the control kidney bean plant, the addition of urea, peat moss and CNTs either alone or in combination to sandy soil by foliar application and soil incorporation, induced significant increases in the total amount and in the relative composition of photosynthetic pigment fractions (chl a, chl b and carotenoids) throughout the vegetative stage of growth (Table 5).

The ratio between chl a and chl b, in response to different treatments, showed variable changes in relation to control kidney bean plants grown in sandy soil alone (Table 5). The following sequence of treatments CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S) > CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg) > CNTs (0.5 g) + Peat moss (2 kg) > Peat moss (2 kg) > CNTs (20 µg) > CNTs (20 µg) + Urea (7.09 mM) > CNTs (0.5 g) + Urea (7.09 mM) > CNTs (0.5 g) > Urea (7.09 mM) > Control was displayed with respect to chls a+b and total pigment contents of kidney bean plants. In this respect, the mentioned increases in photosynthetic pigment fractions and their total contents were generally in proportion to the corresponding increased growth rate (See table 5). In conclusion, the observed enhancements in photosynthetic pigment content of kidney bean leaves, with respect to amendment of sandy soil with different soil conditioners maight be attributed to (a) increased photosynthetic electron transport or (b) stimulation of pigment biosynthesis [5, 20, 25, 43, 46], and (c) increased carotenoid biosynthesis [11, 12, 25, 47].

Changes in enzymatic and non-enzymatic antioxidants

The results obtained (Tables 6 and 7) indicated that urea, peat moss and CNTs either alone or in combination (urea; 7.09 mM, peat moss; 2 kg and CNTs; 20 µg for foliar spray) and 0.5 g CNTs mixed with soil as soil incorporation act as stimulators for all enzymatic and non-enzymatic antioxidants in kidney bean plants at vegetative stage of growth. The following sequence of treatments CNTs (20 µg) + Urea (7.09 mM) (F) + Peat moss (2 kg) (S) > CNTs (0.5 g) + Urea (7.09 mM) + Peat moss (2 Kg) > CNTs (0.5 g) + Peat moss (2 kg) > Peat moss (2 kg) > CNTs (20 µg) > CNTs (20 µg) + Urea (7.09 mM) > CNTs (0.5 g) + Urea (7.09 mM) > CNTs (0.5 g) > Urea (7.09 mM) > Control was displayed with respect to enzymatic and non-enzymatic antioxidants.

The stimulation of growth of kidney bean plants in consequence to the addition of soil conditioners (urea, peat moss and CNTs, either alone or in combination), may presumably be attributed to the increase in both antioxidants system; enzymatic and non-enzymatic (See tables 6 and 7) through the stimulation in their biosynthesis [10, 11, 12, 20, 43].

Different treatments (foliar application, seed priming and soil incorporation) of French bean plants with chitosan nanoparticles and carbon nanotubes either alone or loaded with NPK, at increasing concentrations, induced varied significant increases in antioxidants (either compounds or enzymes) with all treatments, as compared to control [48]. Tea leaves treated with chitosan nanoparticles showed significant increase in accumulation of POX, PPO, SOD, CAT and phenol content. In chitosan nanoparticles treated leaves, both enzyme activity and mRNA expression levels of CAT and SOD were found to be significantly higher than control plants. Hence, the increased expression of SOD and CAT might protect the plants from the oxidative stress [49].

The present results are in agreement with those of [50], where antioxidant enzymes in rice cells were significantly induced as a result of treatment with 20 mg/L of MWCNTs. Such increment in enzyme activity indicated that the cells were exposed to an oxidative stress that might lead to a reduction in cell proliferation or cell death through the apoptotic pathway or necrosis.

Chickpea plants exhibited significantly increased APX, CAT and POX activities in relation to control when plant seeds were treated with silver chitosan nanoparticles [51]. High CAT and POX level indicates less ROS formation and less toxicity to the plants [52]. A tentative explanation the present work was that exposure of kidney bean plant cells to MWCNTs stress, nanotube could interact with the cell wall through the adhesion forces (such as hydrogen bonding between components polysaccharides or proteins of cell walls) or through physical wrapping that allows the MWCNTs to penetrate into the space between the residues of the proteins and polysaccharides. This interaction between MWCNTs and cell wall components might change the dimensional structure of the signal molecules such as polysaccharides or proteins, and such change in structure would then lead to ROS induction [50

References

1 A. M. Al-Omran, A. M. Falatah, A. S. Sheta, and A. R. Al-Harbi (2004), "Clay deposits for water management of sandy soils," Arid Land Research and Management, vol. **18,** pp. 171-184,.

2 M. T. Wafaa, E. M. Aly, and T. A. Eid, (2016). "Effect of irrigation regime and natural soil conditioner on crop productivity in sandy soil," *Egypt. J. Soil. Sci*, vol. **56**, pp. 327-350,

3 G. A. Constable, and I. J. Rochester, (1988) "Nitrogen application to cotton on clay soil: Timing and soil testing," *Agronomy Journal,* vol. **80**, pp. 498-502,.

4 M. D. Boodt, J. Pannier, and H. Verplanke (1990,) "A major aspect in reclaiming sand desert soils. Minimal water management and its distribution in the root-zone," *Egyptian Journal of Soil Science*, vol. **30**, pp. 107-117,.

5 A. S. Ali, (1997) "Physiological studies on cotton plants grown in reclaimed sandy soil," M. Sc. Thesis, Mansoura Univ., Mansoura, Egypt,.

6 J. Boll, R. B. G. Van-Ryijn, K. W. Weiler, J. A. Ewen, J. Daliparthy, S. J. Herbert, and T. S. Steenhuis, (1996) "Using ground-penetrating radar to detect layers in a sandy field soil," Geoderma, vol. **70**, pp. 117-132,.

7 R. Stern, A. J. Van Der Merwe, M. C. Laker, and I. Shainberg, (1992) "Effect of soil surface treatments on runoff and wheat yields under irrigation," *Agronomy Journal*, vol. **84**, pp. 114-119,.

8 W. R. Agro, and J. A. Biernbaum, (1994) "Irrigation requirements, root-medium pH, and nutrient concentrations of *Easter lilies* grown in five peat-based media with and without an evaporation barrier," *Journal of the American Society for Horticultural Science*, vol. **119**, pp. 1151-1156,.

9 H. M. M. Abdel-Aziz, M. N. A. Hasaneen and A. M. Omer, (2016) "Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil," *Spanish Journal of Agricultural* Research, vol. **14**, pp. 0902,.

10 M. N. A. Hasaneen, H. M. M. Abdel-Aziz, and A. M. Omer, (2016) "Effect of foliar application of engineered nanomaterials: carbon nanotubes NPK and chitosan nanoparticles NPK fertilizer on the growth of French bean plant," Biochemistry and Biotechnology Research, vol. **4**, pp. 68-76,.

11 M. N. A. Hasaneen, H. M. M. Abdel-Aziz, and A. M. Omer, (2017)

"Characterization of carbon nanotubes loaded with nitrogen, phosphorus and potassium fertilizers," American. *J. Nano Res. and Appl.*, vol. **5**, pp. 12-18,.

12 H. M. M. Abdel-Aziz, M. N. A. Hasaneen and A. M. Omer, (2018) "Foliar application of nano chitosan NPK fertilizer improves the yield of wheat plants grown on two different soils," Egypt. *J. Exp. Biol. (Bot.),* vol. **14**, pp. 63-72,.

13 H. Mochizuki, P. K. Gautam, S. Sinha, and S. Kumar, (2009) "Increasing fertilizer and pesticide use efficiency by nanotechnology in desert afforestation, arid agriculture," *Journal of Arid Land Studies*, vol. **19**, pp. 129-132,.

14 C. M. Lu, C. Y. Zhang, J. Q. Wen, G. R. Wu, and M. X. Tao, (2002) "Research of the effect of nanometer materials on germination and growth enhancement of Glycine max and its mechanism," Soybean Science, vol. **21**, pp. 168-171,.

15 F. Hong, F. Yang, C. Liu, Q. Gao, Z. Wan, F. Gu, C. Wu, Z. Ma, J. Zhou, and P. Yang, (2005a) "Influences of nano-TiO_2 on the chloroplast aging of spinach under light," Biological trace element research, vol. **104**, pp. 249-260,.

16 F. Hong, J. Zhou, C. Liu, F. Yang, C. Wu, L. Zheng, and P. Yang, (2005b) "Effect of nano-TiO_2 on photochemical reaction of chloroplasts of spinach," Biological trace element research, vol. **105**, pp. 269-279,.

17 M. Linglan, L. Chao, Q. Chunxiang, Y. Sitao, L. Jie, G. Fengqing, and H. Fashui, (2008) "Rubisco activase mRNA expression in spinach: modulation by nanoanatase treatment," Biological trace element research, vol. **122**, pp. 168-178,.

18 X. M. Liu, F. D. Zhang, S. Q. Zhang, X. S. He, R. Fang, Z. Feng, and Y. Wang, (2005) "Effects of nano-ferric oxide on the growth and nutrients absorption of peanut," Plant Nutr. Fert. Sci, vol. **11**, pp. 14-18,.

19 M. Khodakovskaya, E. Dervishi, M. Mahmood, X. Yang, Z. Li, W. Fumiya, and A. S. Biris, (2009) "Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth," ACS nano, vol. **3**, pp. 3221-3227,.

20 H. M. M. Abdel-Aziz, M. N. A. Hasaneen and A. M. Omer, (2019) "Impact of engineered nanomaterials either alone or loaded with NPK on growth and productivity of French bean plants: Seed priming vs foliar application," *South African Journal of Botany*, vol. **125**, pp. 102-108,

21 M. Khodakovskaya, K. De Silva, E. Dervishi, A. S. Biris, and H. Villagarcia, (2012) "Carbon nanotubes induce growth enhancement of tobacco cells," ACS nano, vol. **6**, pp. 2128-2135,.

22 E. A. Smirnova, A. A. Gusev, O. N. Zaytseva, A. G. Tkachev, E. V. Kuznetsova, E. M. Lazareva, G. E. Onishchenko, A. V. Feofanov, and M. P. Kirpichnikov, (2012) "Uptake and accumulation of multiwalled carbon nanotubes change the morphometric and biochemical characteristics of Onobrychis arenaria seedlings," Frontiers of Chemical Science and Engineering, vol. **6**, pp. 132-138,.

23 H. Wang, X. Kou, Z. Pei, J. Q. Xiao, X. Shan, and B. Xing, (2011) "Physiological effects of magnetite (Fe_3O_4) nanoparticles on perennial ryegrass (Lolium perenne) and pumpkin (Cucurbita mixta) plants," Nanotoxicology, vol. **5**, pp. 30-42,.

24 S. S. Sanjay, A. C. Pandey, M. Singh, and S. M. Prasad, (2015) "Effects of functionalized ZnO nanoparticles on the phytohormones: growth and development of Solanum melongena L.(Brinjal) plant," World *Journal of Pharmaceutical* Research, vol. **5**, pp. 1990-2009,.

25 H. M. El-Saht, S. A. Abo-Hamed, M. N. A. Hasaneen, and A. S. Aly, (2001) "Physiological effects on cotton grown on sandy soil amended with different soil conditioners," *Egyptian Journal of Agronomy*, vol. **23**, pp. 15-45,.

26 H. Metzner, H. Rau, and H. Senger, (1965) "Untersuchungen Zur Synchronisier barkeep ein Zelner pigment. Mango I Mutanten Von Chlorella," Planta, vol. **65**, pp. 186-194,.

27 V. Alexieva, I. Sergiev, S. Mapelli, and E. Karanov, (2001) "The effect of drought

and ultraviolet radiation on growth and stress markers in pea and wheat," Plant, Cell and Environment, vol.**24**, pp. 1337-1344,.

28 E. A. Ainsworth, and K. M. Gillespie, (2007) "Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent," Nature protocols, vol. **2**, pp. 875-877,.

29 E. Beutler, O. Duron, and B. M. Kelly, (1963) "Improved method for the determination of blood glutathione," *J. lab. clin. Med.*, vol. **61**, pp. 882-888,.

30 S. T. Omaye, J. D. Turnbull, and H. E. Sauberlich (1979)., "Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids," Methods in Enzymology, vol. **62**, pp. 3-11,

31 S. Agarwal, and R. Shaheen, (2007). "Stimulation of antioxidant system and lipid peroxidation by abiotic stresses in leaves of Momordica charantia," Brazilian *Journal of Plant Physiology,* vol. **19**, pp. 149-161,

32 M. Nishikimi, N. A. Roa, and K. Yogi, (1972) "Measurement of superoxide dismutase," Biochem. Biophys. Res. Commun., vol. **46**, pp. 849-854,.

33 R. A. Gomes-Junior, P. L. Gratão, S. A. Gaziola, P. Mazzafera, P. J. Lea, and R. A. Azevedo, (2007) "Selenium-induced oxidative stress in coffee cell suspension cultures," Functional Plant Biology, vol. **34**, pp. 449-456,.

34 Y. Nakano, and K. Asada, (1981) "Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts," Plant and cell physiology, vol. **22**, pp. 867-880,.

35 D. M. Goldberg, and R. J. Spooner, (1983) "Methods of enzymatic analysis," Bergmeyer H.V., vol. **3**, pp. 258-265,.

36 P. Devi, "Principles and methods in plant molecular biology, biochemistry and genetics," 1st edition, Agrobios, India.

37 S. M. S. El-Din, and H. Moawad, (1988) "Effect of soil conditioners on some biological properties of sandy soil and the diversity of microbes involved in their biodegradation," *Egypt. Jour. Of Microbiology*, vol. **23**, pp. 99,.

38 H. M. El-Saht, S. A. Abo-Hamed, and A. S. Ali, (2000) "The effect of different soil conditioners on the growth and metabolism of cotton plants," *Egypt. J. Agron.*, vol. **22**, pp. 125-148,.

39 J. R. Gregory, (1984) "Urea-sulphuric acid shows promise as fertilizer and in soil and water treatment," Sulphur in Agriculture, vol. **8**, pp. 15,.

40 D. Pal, and S. B. Varade, (1985) "Transpiration and energy status of water in corn as influenced by aerial environment, soil water potential and texture," *Journal of Agronomy and Crop Science*, vol. **154**, pp. 129,.

41 H. Fluehler, W. Durner, and M. Flury, (1996) "Lateral solute mixing processes: A key for understanding field-scale transport of water and solutes," Geoderma, vol. **70**, pp. 165-183,.

42 A. W. J. Heijs, J. R. Goen, and W. D. Louis, (1996) "Three-dimensional visualization of preferential flow patterns in two soils," Geoderma, vol. **70**, pp. 101-116,.

43 A. S. Ali, (2005) "Studies on growth and productivity of cotton plants grown on sandy soil enriched with soil conditioners," Ph.D. Thesis, Mansoura Univ., Mansoura, Egypt,

44 B. C. Biswass, D. S. Yadav, and S. Maheshwari, (1985) "Nutrient management in salt affected and acid sandy soils," Fertilizer-news, vol. **30**, pp. 28,.

45 C. A. M. De-Klein, and R. S. P. Van-Logtestin, (1994) "Denitrification in the top soil of managed grasslands in the Netherlands in relation to soil type and fertilizer level," Plant and Soil, vol. **163**, pp. 33-44,.

46 A. J. Young, J. Britton, and D. Musker, (1989) "A rapid method for the analysis of the mode of action of bleaching herbicides," Pesticide Biochemistry and Physiology, vol. **35**, pp. 244-250,.

47 N. I. Krinsky, (1966) "The role of carotenoid pigments as protective agents against photosensitized oxidations in chloroplasts," Biochemistry of Chloroplasts, vol. **1**, pp. 423-430,.

48 A. M. Omar, (2017) “Comparative studies on the effect of chitosan nanoparticles and carbon nanotubes loaded with NPK on the growth and productivity of French bean,” Ph.D. Thesis, Mansoura Univ., Mansoura, Egypt,.

49 S. Chandra, N. Chakraborty, A. Dasgupta, J. Sarkar, K. Panda, and K. Acharya, (2015) “Chitosan nanoparticles: a positive modulator of innate immune responses in plants,” Scientific Reports, vol. **5**, pp. 15195,.

50 X. Tan, C. Lin, and B. Fugetsu (2009), “Studies on toxicity of multi-walled carbon nanotubes on suspension rice cells,” Carbon, vol. **45**, pp. 3479-3487,.

51 S. Anusuya, and K. N. Banu, (2016) “Silver-chitosan nanoparticles induced biochemical variations of chickpea (*Cicer arietinum* L.),” Biocatalysis and Agricultural Biotechnology, vol. **8**, pp. 39-44,.

52 C. Krishnaraj, E. G. Jagan, R. Ramachandran, S. M. Abirami, N. Mohan, and P. T. Kalaichelvan, (2012) “Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism,” Process Biochemistry, vol.**47**, pp. 651-658,.