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Impact of foliar application of carbon nanotube and benzyladenine on broccoli growth and head yield

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Abstract

The experiment were performed on the broccoli (*Brassica oleracea* var. *Italica*) at an a private farm in, Tema, city, Sohag, Egypt located at N 26° 52' 12.1908", E 31° 24' during the two successive winter seasons of 2018 and 2019 respectively, to study the effect of foliar applications with carbon nanotube (CNTs), benzyladenine (BA) and the combined effect of them on vegetative growth, head yield and head quality of broccoli in a clay loam soil. CNTs at 3 g/L, 5 g/L and 7 g/L concentrations and 50, 100 and 150 ppm of BA and the interaction of them, was sprayed on broccoli seedlings after two weeks of transplanted. A random complete block design (RCBD) with three replications was used. The data showed that positively effect of CNTs at 7 g/L and 100 ppm of BA. Also, CNTs at 5 g/L plus BA at 100 ppm scored the maximum values of broccoli growth characters and flower head yield.

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Keywords: carbon nanotube, benzyladenine, broccoli, *Brassica oleracea*.

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1. Introduction

Sprouting broccoli (*Brassica oleracea* L. var. *italica*) belongs to the family Brassicaceae. Broccoli is grown in very limited scattered areas in Egypt, while it is widely cultivated in many European and American countries. Broccoli has a great importance, enormous nutritional and medicinal values attributed to being vitamins, minerals, number of anti-oxidants which decrease the formation of cancer (Doklega and Abd El-Hady, 2017) of the case-controlled studies, 56% demonstrate a strong association between increased broccoli consumption and the protection against cancer (Verhoeven *et al*., 1996). This protective effect has largely been attributed to the complement of phytochemicals, in broccoli which include the vitamins C and E, the flavonols quercetin and kaempferol, the carotenoids β-carotene, lutein and the glucosinolates (Podsędek, 2007). For these reasons, the cultivation of broccoli started to spread lately where Egypt is ranking the fifteenth in the world production with a total production of 121127 tones valuing, while china is the top world producer of broccoli (FAO, 2019). Despite the growing importance of cultivating broccoli under Egyptian conditions, very little studies have been carried out on enhancing the growth and production of such crop under local agricultural conditions. For this reason, growers are playing safe by adding extra amount of fertilizers in order to avoid any possible loss in production. However, the new trend in modern agricultural production requires efficient, sustainable and environmentally sound fertilizer management practices in order to save environment and human health. Plant hormones, such as cytokinins, play a key role in the stimulation of cell division, nucleic acid metabolism, and root-shoot interactions, particularly under stress (Hare *et al*., 1997; Ha *et al*., 2012). Cytokinins are one of the five major types of plant hormones that play an important role in the cell cycle and affect the growth and development of plants. In addition to promoting cell division and plant growth and development, cytokinins also impede plant senescence by inhibiting the decomposition of chlorophyll, nucleic acids, proteins, and other substances in plants and redistributing necessary amino acids, hormones, inorganic salts, and other compounds to other parts of the plant (Yun *et al*., 2020). Three most commonly detected and most physiologically active cytokinins include zeatin, dihydrozeatin and iso-pentyladenine (IPA). Kinetin and benyladenine (BA) are highly active. Benzyladenine riboside has been extracted from petunia (Salisbury and Ross, 1990). Cytokinins (CKs) are plant hormones known to be key regulators of various aspects of plant growth and development, including cell division, leaf senescence, apical dominance, lateral root formation, stress tolerance, and nutritional signaling (Argueso *et al*., 2009; Sakakibara, 2006; Werner *et al*., 2003). Exogenous application of synthetic CKs, such as 6-benzylaminopurine (BA) and N-(2-chloro-pyridin-4-yl)-N’-phenyl-urea (CPPU), can induce fruit set and development in fruit crops such as grape, kiwifruit, melon, watermelon, apple, and pear (Flaishman *et al*., 2001; Hayata *et* *al*., 1995, 2000; Kim *et al*., 2006; Stern *et al*., 2003; Zabadal and Bukovac, 2006). Furthermore, endogenous levels of CKs have been linked with fruit growth (Gillaspy *et al*., 1993; Srivastava and Handa, 2005). Therefore, CKs may play important roles in fruit development. The mechanism of action by cytokinins varies in different tissues hence the variability of cytokinin effects. Fosket (1977) reported that cytokinins promote cell division by increasing the transition of cells from G2 to mitosis, (G2 refers to the period of cell growth after DNA replication that preceeds mitosis). Cytokinins increase the transition of cells from G2 to mitosis by increasing the rate of protein synthesis. Some of these proteins could be enzymes needed for mitosis. Stimulating formation of mRNAs that code for protein could also increase protein synthesis. Cytokinins effect seems to be specifically on translation. The ribosomes in treated cells are frequently grouped in large protein synthesizing polysomes rather than in small polysomes or as free mono-ribosomes characteristic of slowly dividing cells. Experimental evidence has also shown that cytokinins promote faster incorporation of radioactive amino acids into proteins and inhibition of the physiological response by inhibitors of protein synthesis. Cytokinins effect may also be as a result of their effect on the cell's osmotic potential (Bewli and Witham, 1976; Huff and Ross, 1975). In plants, endogenous CK content is known to be spatially and temporally regulated by a fine balance between synthesis and catabolism (Hirose *et al*., 2008). In many plant species, the initial step of CK biosynthesis is catalysed by adenosine phosphate-isopentenyltransferase (IPT), producing isopentenyladenine (iP) nucleotides as CK precursors (Kakimoto, 2001; Takei *et al*., 2001; Sakamoto *et al*., 2006). In *Arabidopsis* (*Arabidopsis thaliana*), the iP-nucleotides are converted into *trans*-zeatin (tZ) nucleotides by the cytochrome P450 monooxygenases, CYP735A1 and CYP735A2 (Takei *et al*., 2004). To become biologically active, CK nucleotides produced by IPTs and CYP735As must be converted to the free-base form. A CK-activating enzyme (LOG), which directly converts CK nucleotides to the active nucleobases, was recently identified in rice and *Arabidopsis* (Kurakawa *et al*., 2007; Kuroha *et al*., 2009). Inactivation of CKs occurs by degradation or conjugation, and cytokinin oxidase/dehydrogenase (CKX) catalyses the irreversible degradation of CKs in many plant species. CKX is a flavin adenine dinucleotide-containing oxido-reductase that selectively cleaves unsaturated N^6 side chains from tZ, iP, and their corresponding ribosides and it is primarily responsible for metabolic CK inactivation (Jones and Schreiber, 1997; Mok and Mok, 2001; Werner *et al*., 2006). When radish cotyledons are grown in weak light, cytokinins increase the internal production of reducing sugars (mainly glucose, sucrose and fructose) and these sugars act osmotically to cause water uptake that drives growth (Bewli and Witham, 1976; Huff and Ross, 1975). Siddiqui *et al*. (2011) estimated that treatments with 10 and 15 ppm 6benzylaminopurine can be used to extend shelf life of fresh-cut broccoli florets during storage at 6±1°C at commercial level. Another study conducted by Siddiqui *et al*. (2015b) reported that 6benzylaminopurine (BAP) with 200 ppm and 300 ppm can be used to increase quality and enhanced antioxidant activity of cauliflower upto 12 days at 25oC. In his study, fresh cauliflower curds were treated with 6benzylaminopurine at three concentrations (100, 200, or 300 ppm w/v) and its effects on lipid peroxidation, membrane integrity, bioactive molecules, antioxidant activity, soluble sugar, etc. were observed during storage at ambient conditions. BAP profoundly delayed lipid peroxidation and loss of membrane integrity of the tissue, which was associated with the ageing and senescence processes. A positive effect of BAP on maintaining higher bioactive molecules (ascorbic acid and total phenols), antioxidant activity, and soluble sugar was also observed at 200 and 300 ppm, which was decreased in control curds. Therefore, 200 ppm BAP is recommended for practical application considering the cost of 6-benzylaminopurine. Dandan *et al*. (2018) observed that 6-BAP significantly inhibited decay incidence of harvested litchi, associated with a direct inhibition on Peronophythora litchii, the major pathogenic fungi and also reduced H2O2 accumulation and lipid peroxidation, which may account for browning inhibition to an extent and higher contents of anthocyanin and total phenolic compounds. Furthermore, higher activities of SOD, CAT and APX and DPPH radical scavenging capacity in BAP-treated fruit possibly benefited reducing ROS accumulation and lipid peroxidation. Overall, application of BAP showed great potential to control decay and browning and extend shelf life of harvested litchi. The field of nanotechnology has gained increased attention and has quickly intensified over the years due to its unique properties and diverse applications in different fields of science and technology. Different kinds of nanomaterials and their applications have been introduced; however, among them, carbon-based nanomaterials, including graphite, diamond, fullerene, graphene, and carbon nanotubes (CNTs), have been extensively studied. CNTs are widely used in plant sciences (Phytonanotechnology), which alter conventional plant production systems, detoxify chemicals (pesticides and fertilizers), increase disease resistance, and act as plant growth regulators (Anuradha *et al*., 2020). New promising applications, like nano fertilizers, could handle cultivation in poor desert conditions. Nanofertilizers are nanomaterials which contain nano-particles having unique physicochemical properties, *i.e*., large surface area, high reactivity, compatible pore size and particle morphology (Giraldo *et al*., 2014). They provide one or more nutrients to plants for improving their growth and yields. They have high efficiency and consequently reduce the undesirable environmental effects that result from the massive usage of conventional fertilizers (Lahiani *et al*., 2013). Nanofertilizers such as nano-phosphorus-fertilizer, nano-calcium carbonate, iron, magnesium, manganese, zinc, molybdenum oxides (Siddiqui *et al*., 2015b) were investigated on plants and many of them showed positive responses according to the concentration used. Otherwise, Carbon nanotubes (CNTs) could be used as a nutrient carrier for macro and micro elements that may reduce their higher concentrations which are usually used. Carbon nanotubes applications in agriculture showed very promising results (Liu and Lal, 2015). It takes an important role due to its competitive mechanical, electrical, thermal and chemical properties (Siddiqui *et al*., 2015a). Single and multi-walled CNTs special properties could be benefit for researches in the field of agriculture and food. Recently, some researches had shown that carbon nanotubes treatment encouraged growth, branching and other aspects of plant growth parameters. Multi-walled CNTs might act as regulators for seed germination and growth or could organize the marker genes to enhance cell culture growth by increasing cell divisions, cell wall formation and water transport. It was found that CNTs can penetrate coat of tomato seeds and induce germination and growth (Khodakovskaya *et al*., 2009; 2012). In addition, the engineered CNTs could induce germination of seed, growth and development of plants (Siddiqui and Al-Whaibi, 2014). However, in some researches, multi-walled CNTs did not found to show a positive effect on seed germination in many plants (Husen and Siddiqi, 2014; Lin and Xing, 2007). Some other studies faced the potential toxicity of multi-walled CNTs in plant cells (Lin *et al*., 2009; Stampoulis *et al*., 2009; Tan *et al*., 2009; Tan and Fugetsu, 2007). This could be due to the higher concentrations used or the sensitivity of some plants or some of their growth stages in those investigations (Taha *et al*., 2016). Recently, the *in vitro* effect of CNTs on date palm cultures was studied (Taha *et al*., 2016). It was indicated that CNTs increased callus fresh weight while decreased the number of embryos compared with the control. However, germinated embryos number increased and a significant enhancement of shoot length and leaf number in elongation stage was observed. Moreover, root number, root length, plantlet length and hairy roots were enhanced. It was found that CNTs could organize nutrients absorption in the plant. They increased nitrogen, phosphorus, potassium and calcium while decreased sodium percent. Consequently, it increased total chlorophyll a and b. More knowledge about nanofertilizers in agriculture and the relationships between physico-chemical characteristics of nano materials and biological interactions are necessity, but also more care about the risk with handling nano particles application in this important field is needed (Taha, 2016). Scientific research is increasingly needed to study the effect of nanofertilizers on plant and their effect on human and animals' health. CNTs have been used in the agriculture, more specifically, for the growth of the plants. However, results revealed mixed effects of CNTs on plants. It improves the biomass of the plant; however, it creates acute cytotoxicity and genetic alteration in many plants (Mukherjee *et al*. 2016). So, the goal of the present study was to illustrate the influence of CNTs, BA and the combined effect on vegetative growth and head yield as safe tool increase broccoli productivity.

2. Materials and methods

2.1 Experimental site

The experiment were performed on the broccoli (*Brassica oleracea* var. *iItalica*) at an a private farm in, Tema, City, Sohag, Egypt located at N 26° 52' 12.1908", E 31° 24' during the two successive winter seasons of 2018 and 2019 respectively, to study the effect of foliar applications with carbon nanomaterials and benzyladenine on vegetative growth, head yield and head quality of broccoli in a clay loam soil as presented in Table (1).

Table (1): The physical and chemical properties of the samples taken from experimental soil during the two cultivated seasons.

Characteristic	Value	Characteristic	Value
O.M. (%)	0.0062	Mg^{+2} (%)	0.036
$CaCO_3$ (%)	1.62	Na^{+} (%)	6.5
Sand (%)	55.2	K^{+} (%)	0.035
Silt (%)	20.8	Available (ppm)	---
Clay (%)	24	NH_4 (%)	48.0
Texture class	Sand clay loam	N (%)	0.032
pH	7.4	P (%)	0.0054
EC (dS/m)	2.4	Zn (%)	2.5
Cl	0.355	Ca^{+2} (%)	0.03

2.2 Experimental materials

Carbon nanotube as a form of carbon nanomaterial was obtained from Scientific and Technological Applications Unit, Al-Azhar University (Assuit Branch), Assuit, Egypt. Also, benzyladenine were obtained from Al-Gomhoria Company, Assuit, Egypt. The commercial Waltham 29 cv. of broccoli (importer Mecca TRADE Co. as PETO SEED product, USA) was used. The seedlings were initially grown in a greenhouse and fertilized with 19: 19: 19 for N: P: K soluble fertilizers. Seedlings (about 35 days old) were transplanted in rows (80 cm apart with an intra-row spacing of 60 cm). During the plant-growing period, furrow irrigation was used. Insecticide was applied to avoid crop damage by cabbage worms and aphids. Weeds were kept under control manually.

2.3 Experimental design

A random complete block design (RCBD) with three replications was used. The seedlings were initially grown in a greenhouse and fertilized with 19: 19: 19 for N: P: K soluble fertilizers. Seedlings (about 35 days old) were transplanted in rows (80 cm apart with an intra-row spacing of 60 cm). Each plot size consisted of six rows with each row being 3.5 m long. During the plant-growing period, furrow irrigation was used. Insecticide was applied to avoid crop damage by cabbage worms and aphids. Weeds were kept under control manually.

2.4 Experimental treatments

The experimental treatments are explained and described in the following paragraph and Table (2). Carbon nanotube treatments: Freshly prepared Carbon nanotube (CNTs) were dissolved in distilled water at the rate of (T_1) 3, (T_2) 5 and (T_3) 7 CNTs g L^{-1}. Benzyladenine treatments: Stock solution of Benzyladenine was prepared as follow: added 1000 mg of benzyladenine to a 1000 ml volumetric flask to obtain a final concentration of 1000 ppm, then added 2-5 ml of 1 N NaOH to dissolve the powder with stirring to completely dissolved, then added 50, 100 or 150 ml of the stock solution to 1 liter of distilled water to obtain a final concentration of (T4) 50, (T5) 100 and (T6) 150 ppm.

Table (2): Carbon nanotube (CNTs) and benzyladenine (BA) treatments.

Treatment code	Treatment	Carbon nanotube (CNTs) g/L	Benzyladenine (BA) (ppm)
T_0	T_0	0.0	0.0
T_1	T_1	3	0.0
T_2	T_2	5	0.0
T_3	T_3	7	0.0
T_4	T_4	0.0	50
T_5	T_5	0.0	100
T_6	T_6	0.0	150
T_7	($T_1 \times T_4$)	3	50
T_8	($T_1 \times T_5$)	3	100
T_9	($T_1 \times T_6$)	3	150
T_{10}	($T_2 \times T_4$)	5	50
T_{11}	($T_2 \times T_5$)	5	100
T_{12}	($T_2 \times T_6$)	5	150
T_{13}	($T_3 \times T_4$)	7	50
T_{14}	($T_3 \times T_5$)	7	100
T_{15}	($T_3 \times T_6$)	7	150

Mixture treatments: The mixture treatments were T7 (T1×T4), T8 (T1×T5), T9 (T1×T6), T10 (T2×T4), T11 (T2×T5), T12 (T2×T6), T13 (T3×T4), T14 (T3×T5) and T15 (T3×T6). The control plants were sprayed with distilled water (T0). Carbon nanotube (CNTs), benzyladenine (BA), mixture (CNTs+ BA) and control treatments were applied as foliar spray after seven days from transplanting broccoli seedlings. The spraying was conducted once a week until the appearance of floral primordia.

2.5 Agricultural operations

At soil preparation time, the full dose of triple superphosphate (P_2O_5) 200 Kg

/feddan (feddan = 4200 m² = 0.420 hectares = 1.037 acres) and 20 m3 of farmyard manure (FYM) per feddan, were added. Nitrogen fertilizer in the form of ammonium sulfate (20.6%) and potassium in the form of potassium sulphate (48% K_2O) was applied at the rate of 150 and 100 Kg/feddan respectively, of which one-third was applied at one week after transplanting of broccoli seedlings as basal dose, one-third at the early vegetative phase (30 days after transplanting) and the last one-third applied at 30 days from the previous one. All the other agricultural practices used for commercial broccoli production were carried out in this experiment (Hassan, 1991).

2.6 Data collected

Harvesting took place in December in the two years. The inner rows were used for sampling and harvest. Entire plants were harvested at ground level from each plot when the terminal buds were swollen but not opened. Plant height (PH cm) was determined by measuring from ground level to head bottom. Number of leaves per plant (NL/P) and leave area (LA cm) was measured. The plants were then cut 20 cm below the top of head, which was trimmed to obtain a marketable product. Head weight (HW g), head diameter was measured across the widest part of head (HD cm), as well as Heads yield as ton per feddan (HY ton /feddan).

2.7 Statistical analysis

The analysis of variance of the data was carried out on the mean values of the tested treatments according to the procedures described by Gomez and Gomez (1984). The least significant differences (LSD) at 5% levels were used for testing the significance of the differences among the mean values of the tested treatments for each character.

3. Results

The following chapter shows the effect of applying CNTs, BA and interactions effect on vegetative growth, head traits and head yield of broccoli crop. Data of plant height in cm are presented in Table (3). Results revealed that, non-significantly effect of CNTs and BA concentration treatments on broccoli plant height. Broccoli plant height was increased with increasing of CNTs concentrations. Meanwhile, 100 ppm of BA scored the highest values of plant height compared the control treatment and the other BA concentrations in both successive seasons of 2018 and 2019. Significant interactions were detected between CNTs and BA concentration treatments. Broccoli plants at CNTs 5 g/L + BA 100 ppm (T_{11}) treatment recorded the longest plants than the other treatments followed by CNTs 7 g/L + BA 100 ppm (T_{14}) in both seasons, respectively.

Table (3): Effect of CNTs and BA foliar application treatments on broccoli plant height (PH cm) of 2018 and 2019 cultivated seasons.

Treatment	2018	2019
T_0 (Control)	68.03	66.13
T_1 (CNTs 3g/L)	67.80	65.87
T_2 (CNTs 5g/L)	69.27	66.87
T_3 (CNTs 7g/L)	72.47	69.87
LSD 0.05	NS	NS
T_4 (BA 50 ppm)	68.13	65.53
T_5 (BA 100 ppm)	72.80	69.48
T_6 (BA 150 ppm)	68.80	68.07
LSD 0.05	NS	NS
T_7 (CNTs 3g/L) + (BA 50 ppm)	68.80	66.87
T_8 (CNTs 3g/L) + (BA 100 ppm)	71.40	71.33
T_9 (CNTs 3g/L) + (BA 150 ppm)	70.40	72.40
T_{10} (CNTs 5g/L) + (BA 50 ppm)	72.80	72.53
T_{11} (CNTs 5g/L) + (BA 100 ppm)	85.60	86.80
T_{12} (CNTs 5g/L) + (BA 150 ppm)	72.73	75.20
T_{13} (CNTs 7g/L) + (BA 50 ppm)	83.53	84.07
T_{14} (CNTs 7g/L) + (BA 100 ppm)	84.33	86.33
T_{15} (CNTs 7g/L) + (BA 150 ppm)	83.33	84.73
LSD 0.05	5.61	6.65

Meanwhile, CNTs 3 g/L + BA 50 ppm treatment (T_7) scored the shortest plants in both seasons, respectively. Non-significant affect was obtained from the data which, presented in Table (4) for the leaves number per plant as affected by CNTs and BA concentration treatments in both cultivated seasons of 2018 and 2019. A slight increase of leaves number/plant was detected with increasing of CNTs concentrations. Also, BA treatment at the middle concentration (100 ppm) (T_5) scored the same slight increment in both seasons of 2018 and 2019. The interaction effect showed that, highly effect of CNTs and BA concentration treatments on leaves number /plant. CNTs 5 g/L + BA 100 ppm (T_{11}) and CNTs 7 g/L + BA 100 ppm (T_{14}) scored the maximum number of leaves per plant in both seasons of 2018 and 2019, respectively, with non-significant between them. It is revealed from the Table (5) that the maximum broccoli leave area in cm was recorded with CNTs at 7 g/L (T_3) in both cultivated seasons. Also, BA at 100 ppm (T_5) scored the maximized leave area than the lowest and highest BA concentrations. Significant increase in leave area were found by spraying CNTs 5 g/L + BA 100 ppm (T_{11}) followed by CNTs 7 g/L + BA 100 ppm (T_{14}), in the tow cultivated seasons of 2018 and 2019 respectively. On the other hand, CNTs 3 g/L + BA 150 ppm (T_9) scored the lowest values of leave area in cm in both seasons.

Table (4): Effect of CNTs and BA foliar application treatments on broccoli number of leaves per plant (NL/P) of 2018 and 2019 cultivated seasons.

Treatment	2018	2019
T_0 (Control)	20.95	21.07
T_1 (CNTs 3g/L)	23.40	22.27
T_2 (CNTs 5g/L)	23.20	22.40
T_3 (CNTs 7g/L)	23.80	22.80
LSD 0.05	NS	NS
T_4 (BA 50 ppm)	21.60	21.67
T_5 (BA 100 ppm)	22.80	21.87
T_6 (BA 150 ppm)	22.13	20.22
LSD 0.05	NS	NS
T_7 (CNTs 3g/L) + (BA 50 ppm)	22.07	22.33
T_8 (CNTs 3g/L) + (BA 100 ppm)	22.07	22.13
T_9 (CNTs 3g/L) + (BA 150 ppm)	21.80	21.37
T_{10} (CNTs 5g/L) + (BA 50 ppm)	22.13	22.93
T_{11} (CNTs 5g/L) + (BA 100 ppm)	24.86	25.27
T_{12} (CNTs 5g/L) + (BA 150 ppm)	24.07	24.00
T_{13} (CNTs 7g/L) + (BA 50 ppm)	23.40	23.67
T_{14} (CNTs 7g/L) + (BA 100 ppm)	24.47	25.27
T_{15} (CNTs 7g/L) + (BA 150 ppm)	23.93	23.40
LSD 0.05	1.05	1.90

Table (5): Effect of CNTs and BA foliar application treatments on broccoli leave area (LA cm) of 2018 and 2019 cultivated seasons.

Treatment	2018	2019
T_0 (Control)	526.14	583.35
T_1 (CNTs 3g/L)	592.13	532.85
T_2 (CNTs 5g/L)	615.83	571.23
T_3 (CNTs 7g/L)	621.91	622.33
LSD 0.05	61.94	56.91
T_4 (BA 50 ppm)	623.17	565.50
T_5 (BA 100 ppm)	726.70	663.15
T_6 (BA 150 ppm)	645.44	540.53
LSD 0.05	61.94	56.91
T_7 (CNTs 3g/L) + (BA 50 ppm)	639.40	624.17
T_8 (CNTs 3g/L) + (BA 100 ppm)	659.91	663.89
T_9 (CNTs 3g/L) + (BA 150 ppm)	600.15	577.70
T_{10} (CNTs 5g/L) + (BA 50 ppm)	685.83	666.24
T_{11} (CNTs 5g/L) + (BA 100 ppm)	813.93	871.69
T_{12} (CNTs 5g/L) + (BA 150 ppm)	720.64	793.03
T_{13} (CNTs 7g/L) + (BA 50 ppm)	797.06	749.67
T_{14} (CNTs 7g/L) + (BA 100 ppm)	798.67	800.65
T_{15} (CNTs 7g/L) + (BA 150 ppm)	713.17	771.56
LSD 0.05	69.47	80.68

There was significant difference between the broccoli head weight in the study (Table 6). Application of CNTs at 3 g/L (T_1), 5 g/L (T_2) and 7 g/L (T_3) treatments led to increasing the head weigh gradually. Meanwhile, maximum values of head weigh were observed with BA at 100 ppm (T_5) than the low and high BA concentrations (50 and 150 ppm) T_4 and T_6 respectively, in both seasons of 2018 and 2019. The combined effect of CNTs and BA treatments showed extreme positive values of (CNTs 5 g/L) + (BA 100 ppm) (T_{11}) on head weight than the other combination treatments in both seasons respectively.

Table (6): Effect of CNTs and BA foliar application treatments on broccoli head weight (HW g) of 2018 and 2019 cultivated seasons.

Treatment	2018	2019
T_0 (Control)	325.79	277.42
T_1 (CNTs 3g/L)	395.50	382.73
T_2 (CNTs 5g/L)	400.13	405.40
T_3 (CNTs 7g/L)	416.00	424.20
LSD 0.05	64.48	71.97
T_4 (BA 50 ppm)	304.33	326.80
T_5 (BA 100 ppm)	477.87	366.40
T_6 (BA 150 ppm)	357.75	332.17
LSD 0.05	64.48	71.97
T_7 (CNTs 3g/L) + (BA 50 ppm)	478.20	456.80
T_8 (CNTs 3g/L) + (BA 100 ppm)	591.60	590.27
T_9 (CNTs 3g/L) + (BA 150 ppm)	568.60	532.00
T_{10} (CNTs 5g/L) + (BA 50 ppm)	615.47	556.40
T_{11} (CNTs 5g/L) + (BA 100 ppm)	845.20	872.87
T_{12} (CNTs 5g/L) + (BA 150 ppm)	582.20	562.60
T_{13} (CNTs 7g/L) + (BA 50 ppm)	571.33	584.60
T_{14} (CNTs 7g/L) + (BA 100 ppm)	696.00	696.80
T_{15} (CNTs 7g/L) + (BA 150 ppm)	678.40	644.20
LSD 0.05	91.45	102.04

On the other hand, CNTs 3 g/L + BA 50 ppm (T_7) scored the lowest means of head weight in both seasons. Data presented in Table (7) exhibit the effect of sixteen proportions of CNTs and BA treatments on head diameter of broccoli (cm). Application of CNTs with increasing dos from 3 g/L to 7 g/L led to increasing broccoli head diameter. But, application of BA at 100 ppm only scored the widest diameter of broccoli than the other concentrations of BA in both seasons. The results showed that there are significant differences among the treatments by interaction of CNTs application with BA in head diameter. CNTs 5g/L + BA 100 ppm (T_{11}) and CNTs 7g/L + BA 100 ppm (T_{14}) treatments recorded the widest diameter of broccoli heads than the other interaction treatments in both cultivated seasons of 2018 and 2019.

Table (7): Effect of CNTs and BA foliar application treatments on broccoli head diameter (HD cm) of 2018 and 2019 cultivated seasons.

Treatment	2018	2019
T_0 (Control)	20.40	19.65
T_1 (CNTs 3g/L)	23.33	22.03
T_2 (CNTs 5g/L)	23.33	22.40
T_3 (CNTs 7g/L)	23.80	23.80
LSD 0.05	1.12	1.34
T_4 (BA 50 ppm)	20.33	20.60
T_5 (BA 100 ppm)	23.53	21.80
T_6 (BA 150 ppm)	22.13	20.15
LSD 0.05	1.12	1.34
T_7 (CNTs 3g/L) + (BA 50 ppm)	24.40	23.57
T_8 (CNTs 3g/L) + (BA 100 ppm)	26.73	26.00
T_9 (CNTs 3g/L) + (BA 150 ppm)	25.50	24.00
T_{10} (CNTs 5g/L) + (BA 50 ppm)	26.60	26.40
T_{11} (CNTs 5g/L) + (BA 100 ppm)	31.67	32.40
T_{12} (CNTs 5g/L) + (BA 150 ppm)	25.40	25.37
T_{13} (CNTs 7g/L) + (BA 50 ppm)	26.73	26.00
T_{14} (CNTs 7g/L) + (BA 100 ppm)	28.73	28.60
T_{15} (CNTs 7g/L) + (BA 150 ppm)	27.87	27.33
LSD 0.05	1.58	1.91

It was very clear that there is a positive relationship between the applied concentration of CNTs, BA and interactions effect and the response of the plant in terms of head yield (ton/feddan) (Table 8). The recorded differences between treatments were significant compared to control treatment. All applied concentrations of CNTs, BA and interactions give positive and significant effect on head yield compared to control. In CNTs treatments the highest yield was observed with the maximum dos at 7 g/L (T_3) in both seasons. While, the middle concentration of BA (T_5) recorded the highest yield of broccoli head weight as ton/feddan in both seasons. Concerning the interaction effects between different proportion of CNTs and BA, the highest values of head yield per feddan were obtained from CNTs 5g/L + BA 100 ppm (T_{11}) followed by CNTs 7g/L + BA 100 ppm (T_{14}) treatment. In the same context CNTs 3g/L + BA 50 ppm treatment (T_7) scored the lowest head yield as ton per feddan in both seasons of 2018 and 2019. On the contrary, the control treatment T_0 was significantly the lowest compared to all treatments in both cultivated seasons respectively.

Table (8): Effect of CNTs and BA foliar application treatments on broccoli heads yield (HY ton/feddan) of 2018 and 2019 cultivated seasons.

Treatment	2018	2019
T_0 (Control)	4.57	3.88
T_1 (CNTs 3g/L)	5.54	5.36
T_2 (CNTs 5g/L)	5.60	5.68
T_3 (CNTs 7g/L)	6.89	6.23
LSD 0.05	0.76	0.81
T_4 (BA 50 ppm)	4.26	4.57
T_5 (BA 100 ppm)	6.69	6.13
T_6 (BA 150 ppm)	4.84	4.65
LSD 0.05	0.76	0.81
T_7 (CNTs 3g/L) + (BA 50 ppm)	6.69	6.38
T_8 (CNTs 3g/L) + (BA 100 ppm)	8.28	8.26
T_9 (CNTs 3g/L) + (BA 150 ppm)	8.03	7.44
T_{10} (CNTs 5g/L) + (BA 50 ppm)	8.62	7.75
T_{11} (CNTs 5g/L) + (BA 100 ppm)	11.83	12.21
T_{12} (CNTs 5g/L) + (BA 150 ppm)	8.15	7.88
T_{13} (CNTs 7g/L) + (BA 50 ppm)	7.91	8.18
T_{14} (CNTs 7g/L) + (BA 100 ppm)	9.72	9.55
T_{15} (CNTs 7g/L) + (BA 150 ppm)	9.51	9.15
LSD 0.05	1.11	1.14

4. Discussion

The present study aimed to reduce chemical fertilizers for sweet potato plants without reducing tuberous roots Development in agricultural management is a must in order to increase production efficiency and grower income. Growers are usually playing safe by increasing the amount of added fertilizers so that decrement in crop production may not occur. Unquestionably, insufficiency of agricultural development will guide to environmental aggravation problems, particularly in low potential areas, where expansion and increased production are crucial to help renovate the fragile natural resources base. For this reason, this study is focusing on the effect of using plant hormones, such as cytokinins and new promising applications, like nano fertilizers. In recent years, the use of nanotechnology in different areas has increased. Proof of this includes the large number of studies on the application of nanomaterials, such as metal nano-particles (NPs) and carbon nano-materials (CNMs), including single-walled carbon nanotubes, multi-walled carbon nanotubes (MWCNTs), graphene, and fullerenes (Jeevanandam, 2018). In addition, Plant hormones, such as cytokinins, play a key role in the stimulation of cell division, nucleic acid metabolism, and root-shoot interactions, particularly under stress (Banowetz and Ammar, 1999; Ha *et al*., 2012; Hare *et al*., 1997; Liu *et al*., 2020). Thus, literately both CNTs and BA have protective agents on broccoli plants. In this study, all plant growth parameters were positively and significantly affected. This may be due to the role of BA and/or CNTs of crop enhancement of broccoli plants. CNTs can enter the plant either by foliar route or through the root. In the foliar route, they can enter through the stomata or through the cuticle, although the entrance is greater by the cuticle due to the large area it has in the leaves compared to the stomata (Avellan, 2019). After their entry, nanomaterials (NMs) reach the tissues of the epidermis and the mesophyll where they can interact with these tissues and their structures, and later they can be translocated to the whole plant through the vascular bundles (Hubbard *et al*., 2020). In addition, CNTs act as elicitors in the regulation of plant growth (Patel *et al*., 2017), since they activate the biosynthesis of indole acetic acid and abscisic acid (Cheng *et al*., 2016). They also promote the expression of marker genes of cell division (CycB) and elongation of the cell wall (NtLRX1) that directly influence growth, hence productivity (Khodakovskaya *et al*., 2012). Martínez-Ballesta *et al*. (2016) showed that broccoli plants (*Brassica oleracea* var. *italica*) treated with MWCNTs significantly increased their growth under saline stress conditions. Younes *et al*. (2019) denoted that the activation of photosynthetic activities by graphene nanosheet (GNS) may give reason for increased growth, productivity and crop yield of pepper and eggplants. The presence of GNS inside chloroplast may act as carbon source that facilitate carbon fixation, thus induced sugar metabolism so fructose, sucrose as well as starch enhanced progressively in leaves of GNS treated plants. With regard to BA, cytokinins are substances derived from an adenine nitrogenous base. Their physiological effects on plants are related to elongation and differentiation, capacity of division, formation and activity of apical meristems, mobilization of nutrients, break of apical dominance, germination and break of seed and dormancy, induction of parthenocarpy in fruits, flowering induction and delayed senescence (Davies, 2004; Rivero *et al*., 2007). This indicated that the concentrations and combinations used in the present work of CNTs and/or BA were decisive in the biochemical and yield traits. Lin *et al*. (2009) mentioned that CNTs had physicochemical properties as molecular transporters in the cell walls of plants, which stimulated the growth of crops and promoted the metabolism of crop growth. With regard to BA, cytokinins are substances derived from an adenine nitrogenous base. Their physiological effects on plants are related to elongation and differentiation, capacity of division, formation and activity of apical meristems, mobilization of nutrients, break of apical dominance, germination and break of seed and dormancy, induction of parthenocarpy in fruits, flowering induction and delayed senescence (Davies, 2004; Rivero *et al*., 2007). This indicated that the concentrations and combinations used in the present work of CNTs and/or BA were decisive in the biochemical and yield traits. The obtained results showed that the broccoli plants under CNTs, BA and more specifically their combination had better than non-sprayed plants (Talebi, 2018). Also, Younes *et al*. (2019) reported similar enhancements of eggplant and pepper growth and yield characters under carbon nanoparticles known as graphene nanosheets. López *et al*. (2020) reported also similar induction of growth and fruit yield under the use of carbon nanotubes of tomato plants.

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