International Journal of New Technology and Research (IJNTR)
ISSN:2454-4116, Volume-4, Issue-4, April 2018 Pages 125-128

Toxicity and Influence of Multi-Wall Carbon Nanotubes on the Reproductive Ability to Strawberry

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***Abstract*—Multi-wall carbon nanotubes (MWCNTs) are widely used in various fields due to their superior properties. Due to its wide-ranging research and application, the biosafety of MWCNTs should be carefully considered. In the study, we reported that the toxicity of MWCNTs and the influence on the reproductive ability to strawberry. The growth status and reproductive ability of strawberry were monitored after 60 days of soil culture in the presence of MWCNTs. MWCNTs showed adverse effects on the photosynthesis of strawberry leaves and the reproductive capacity of strawberry has been suppressed. Our results collectively suggested that the biosafety of MWCNTs should be highly concerned.**

***Index Terms*—Multi-Wall Carbon Nanotubes; biosafety; photosynthesis; reproductive ability.**

I. Introduction

Carbon nanomaterials are composed of carbon elements and presented in many forms [1]. In recent years, the research of carbon nanotechnology has been quite diversified. A variety of nano-carbon forms have emerged in an endless stream. Carbon nanomaterials can be classified into three categories according to their dimensions: zero-dimensional (including fullerenes [2], fullerols, carbon quantum dots [3, 4], etc.), one-dimensional (including carbon nanotubes, carbon fiber tubes [5], etc.), and two-dimensional (graphene and its derivatives). Carbon nanotubes (CNTs), also known as buckytubes, belong to fullerene and are one of the hottest materials for carbon materials [6]. In most instances, it is a seamless nano-tube structure formed by single-layer or multi-layer hexagonally arranged carbon atoms sheet curled around the same central axis by a certain helix angle [7, 8]. These carbon atoms sheets also have defects including five-membered ring and seven-membered rings. The distance between the layers is fixed, about 0.34 nm, and the diameter is generally 2-20 nm. According to the number of carbon element layers can be roughly divided into: single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). Its unique structure is predestinated to have many superior properties and wide applications [9].

In recent years, with the continuous in-depth study of carbon nanotubes and its derivatives, its wide application prospects are continuously exhibited. Firstly, due to the fact that most carbon atoms in carbon nanotubes adopt SP2 hybridization, CNTs materials have high modulus and high strength. The tensile strength of CNTs reaches 50 to 200 GPa, which is 100 times that of steel and its density is only 1/6 that of steel. It can produce materials with the highest specific strength [10]. Predictively, CNTs have great application prospects in superconducting and capacitor fields [11, 12]. Carbon nanotubes have a high thermal conductivity, and as long as the carbon nanotubes are doped in the composite material, the thermal conductivity of the composite material may be greatly improved [13]. Biomolecules such as amino acids and proteins can react with the walls of carbon nanotubes and enter the carbon tube lumen. Therefore, in biological applications, carbon nanotubes can be used as sustained release agents for drugs [14]. However, before many applications are produced, we must seriously consider its biological safety [15].

CNTs have been found to be toxic to different organisms, in particular animal and plant models [16-18]. The translocation and fate of MWCNTs was reported in 2007 by Dend et al.[19] The biodistribution and translocation of MWCNTs were diagnosed in mice at 1, 7 and 14 d post-exposure. MWCNTs mostly accumulated in liver and retained for long time by intravenous injection. Not only that, in 2008 Yang et al.[20] reported that the long-term accumulation of SWCNTs in the main organs was evidenced by using Raman and TEM in mice. In the same year, Yang et al. found that TEM image confirming the presence of PEG-SWNTs in the liver of mice by intravenous injection [21]. There are also studies on phytotoxicity that have also been found to be biologically toxic [22]. As Lin et al. reported that the agglomerates of MWCNTs are biological toxicity to the Arabidopsis T87 suspension cells [23]. MWCNTs can induce DNA fragmentation and apoptosis in Allium root cells in 2011 by Ghosh et al [24]. Begum et al . explored that the potential toxicity and influence of 0-1000 mg/L the multi-walled CNTs on red spinach at 15 days [25]. They found that the leaves accumulated MWCNTs through Raman spectroscopy. The results of the study demonstrate this phenomenon- plants and plant cells showed high tendencies to accumulate CNTs. This will make the plants as an important link in the pathway by which CNTs enter the food chain and biological cycles.

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In this study, we evaluated the growth toxicity and reproductive ability of MWCNTs to Strawberry that were planted in soil culture systems. MWCNTs was supplemented into sand, vermiculite, and Hoagland nutrient solution for plant exposure. The leaf number, photosynthetic parameters and fruit number were investigated to compare the toxicity of strawberry in the culture system containing MWCNTs. The ecological environmental hazard assessment of MWCNTs materials was discussed in combination with the potential growth toxicity and reproductive capacity.

II. EXPERIMENTAL

A. Materals

Multi-Wall Carbon Nanotubes (main range of diameter 10-30 nm; length >2μm; purity >97%) was obtained from Shenzhen Nanotech Port Co. Ltd. China. Vermiculite (diameters of 1-3 mm) was bought from North Mining Processing Co., China. Sandy soil was obtained from the local outskirts of Chengdu in Sichuan province in China. Strawberry seedlings (plant height 15-30 cm) and cultivated pots (400 mL) were purchased from lvshang Agriculture Planting Base in Guangxi Province Co., China. Hoagland nutrient solution was used for the plant cultivation. The constitution of 10-times modified Hoagland solution was $Ca(NO_3)_2 \cdot 4H_2O$ (9450 mg/L), $KNO_3 \cdot 4H_2O$ (5060 mg/L), NH_4NO_3 (800 mg/L), KH_2PO_4 (1360 mg/L) and $MgSO_4$ (4930 mg/L), supplemented with micronutrients $FeSO_4 \cdot 7H_2O$ (139 mg/L), EDTA·2Na (186.5 mg/L), KI (8.3 mg/L), H_3BO_3 (62 mg/L), $MnSO_4$ (223 mg/L), $ZnSO_4 \cdot 7H_2O$ (86 mg/L), $Na_2MoO_4 \cdot 2H_2O$ (2.5 mg/L), $CuSO_4 \cdot 5H_2O$ (0.25 mg/L), and $CoCl_2 \cdot 6H_2O$ (0.25 mg/L). The reagents were analytical grade, and were used without purification. When used, it needs to be diluted.

B. Material Characterization

MWCNTs was tested morphology by TEM (JEM-200CX, JEOL, Japan), SEM (JSM-6610A, JEOL, Japan). It was detected characteristic Raman signal using Raman spectroscopy (inVia, Renishaw, UK).

C. Cultivation of Strawberry

The cornerstone of culture was prepared with local sandy soil and vermiculite at a ratio of 1:2 by volume. three seedlings of strawberry were cultivated in 400 mL pots containing 0-5 mg/mL MWCNTs (3 replicate pots for each concentration). Detailed mixing formulas was shown in Tab. 1, and the real picture was presented in Fig. 1. Strawberry plants were cultivated in a greenhouse where the temperature was maintained at 25 °C (day-12h), 20 °C (night-12h). Other culture conditions are 24 000 lx-light intensity and 80%-humidity. Each pot is added with 200ml of culture solution for the first time. Then, it needs to replenish the suitable amount of plant nutrient solution every day. Hoagland nutrient solution was added daily in amounts of 10 mL (1-15 d), 20 mL (16-30 d), 30 mL (31-35 d), and 40 mL (41-60 d).

Tab. 1. The detailed mixing formulas of planting cornerstone.

MWCNTs cocentration (mg/mL)	MWCNTs (g)	Sandy soil (mL)	Vermiculite (mL)	Total volume (mL)
0	0	400	800	1200
0.01	0.012	400	800	1200
1	1.2	400	800	1200
5	6	400	800	1200

Fig.1. Planting strawberries exposed to MWCNTs at concentration of 0-5 mg/mL in soil culture after 1 day.

D. Toxicity Evaluations

After harvesting, the Strawberry leaf number and fruit number were recorded ,and the leaf chlorophyll levels were recorded on a chlorophyll meter (SPAD-502plus, Konica Minolta Co., Japan) at 5 independent sites. For the photosynthesis measurements (including the net photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration), each seedling was measured at 3 independent sites by a portable photoactivator (Yaxin-1102, Beijing YaXin Liyi Technology Co., China).

E. Statistical Analysis

All datas are presented as the mean of 3 individual observations with the standard deviation (mean ± SD). The single factor analysis of variance (ANOVA) with LSD test was used to analyze the significance level at $P < 0.05$.

III. RESULTS AND DISCUSSION

A. Characterization of MWCNTs

MWCNTs was obtained as a black dispersion upon sonication that showed microscopic characteristics under TEM (Fig. 2a) and SEM (Fig. 2b). As the picture shows the elongated filaments, TEM and SEM photograph of MWCNTs revealed of the main range of diameter 10-30 nm and length >2μm. MWCNTs was checked with Raman at 532 nm. The typical sp^2 carbon atoms were reflected by the Raman G band signal at 1598 cm^{-1}(Fig. 2c). The five- and seven-membered carbon atom-rings structure defects were indicated by the strong D band signal at 1344 cm^{-1}. The strong 2D bands suggested the stacking of MWCNTs is composed of polycyclic arranged carbon atoms forming a multi-layer coaxial tube at 2677 cm^{-1}. The characterization data collectively suggested that the MWCNTs sample was of high purity and suitable for the later experiment.

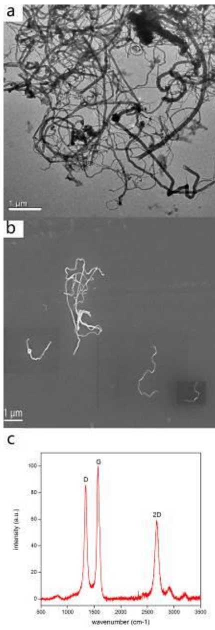

Fig.2. Characterization data for MWCNTs. (a) TEM image; (b) SEM image; (c) Raman spectrum.

B. Toxicity of MWCNTs to Strawberry

Growth inhibition

Effects of leaf number and chlorophyll content. We conducted a series of tests of the potential effects of the MWCNTs on the seedling growth of strawberry. We performed same experiment without MWCNTs as control. Little or no effect of the leaf number was noted in our experiment in Fig. 3a. Except for significant decrease at 1 mg/mL, there was no significant difference in other groups of data. Strawberry chlorophyll content does not change at low concentrations (Fig. 3b). However, It has a significant decrease with increasing concentration (MWCNTs concentration exceeds 1 mg/mL, P<0.05). It was found that it had an adverse effect on the chlorophyll content of plant leaves. Based on the results, we concluded that the MWCNTs into the soil medium maybe have triggered the changes of photosynthesis parameters in the strawberry leaf, with the intensity of symptoms (Decreased chlorophyll content) correlating to MWCNTs concentration.

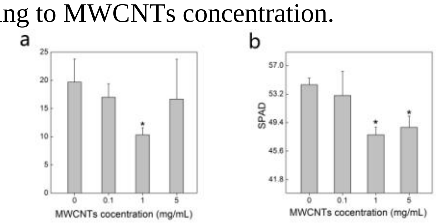

Fig.3. After harvesting the leaf number (a) and chlorophyll level (b) of strawberry exposed to MWCNTs in soil culture (n =3). * $p < 0.05$ compared to the control group.

Effects of photosynthetic parameters. The following experiment is focused on the results obtained with photosynthetic parameters. Compared to the untreated seeding, Net photosynthetic rate has such a trend - first increase then decrease, as shown in Fig. 4a. It has a significant decrease at 5 mg/mL (P<0.05). However, there was a significant increase in transpiration rate as shown in Fig. 4b (P<0.05). Intercellular CO2 concentration appears similar to the phenomenon in Fig. 4d.This phenomenon may be caused by the penetration of MWCNTs into plants. There have also been similar reports in other literature. CNTs are able to penetrate tomato seed coat and root by using TEM and Raman [22]. Enrichment of MWCNTs in red spinach leaves revealed significant material characteristic peaks detected by Raman [25]. No significant difference in stomatal conductance (Fig. 4c). Next, we further explore its potential impact on the reproductive capacity of strawberries.

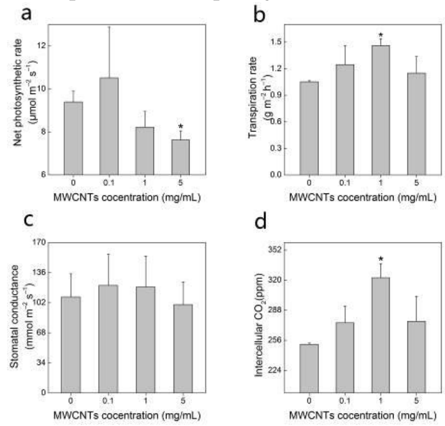

Fig.4. After harvesting the net photosynthetic rate (a), transpiration rate (b), stomatal conductance (c), and intercellular CO2 concentration (d) of strawberry exposed to MWCNTs in soil culture (n =3). * $p < 0.05$ compared to the control group

C. Reproductive Ability Influence of MWCNTs to Strawberry

Soil exposure for 60 days to MWCNTs at different concentrations (0, 0.1, 1, 5 mg/mL) resulted in toxicity symptoms and retardation of plant reproductive ability with increasing CNTs concentration compared to control plants. We found that the number of fruits in strawberries is decreasing, with the increase of MWCNTs concentration as in Fig. 5. At the highest concentration, the strawberry lost its ability to reproduce (no fruit was produced). As shown in the real map (Fig. 6), the shape of the stems and leaves of strawberries did not change much, but the number of fruits was significantly less. The result predicts that MWCNTs have strong biological toxicity. This will directly lead to the collapse of the ecosystem due to the loss of plant reproductive capacity.

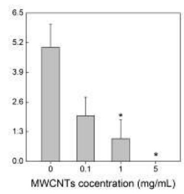

Fig.5. After harvesting the fruit number of strawberry exposed to MWCNTs in soil culture (n =3). * $p < 0.05$ compared to the control group.

Fig.6. Planting strawberries exposed to MWCNTs in soil culture after 60 day (a-0 mg/mL; b-0.1 mg/mL; c-1 mg/mL; d-5 mg/mL).

IV. CONCLUSIONU

In summary, MWCNTs has serious phenomenon that the Potential Toxicity and Reproductive Ability Influence of Multi-Wall Carbon Nanotubes in Strawberry. Although MWCNTs do not affect the appearance of strawberries (such as the number of leaves), it affect the physiological effects of the leaves and even affect the reproductive physiology. It is very likely to cause severe ecological disasters due to the destruction of the plant's reproductive capacity.

ACKNOWLEDGMENT

Xin Guan thanks the Undergraduate Innovation and Entrepreneurship Training Program in Southwest Minzu University (No. 201710656021).

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