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MULTIWALLED CARBON NANOTUBES ENHANCING NITROGEN UPTAKE AND USE EFFICIENCY OF UREA FERTLIZER BY PADDY

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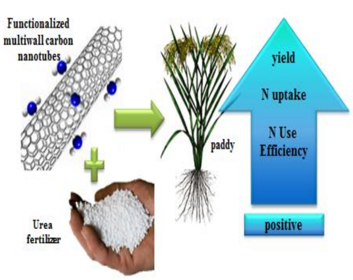
Graphical abstract



Abstract

Efficient use of urea fertilizer (UF) as important nitrogen (N) source in the world's rice production has been a concern for the economic sustainability of cropping systems. The use of carbon-based materials to enhance UF efficiency still facing a great challenge. Hence, N Nano-carrier is developed based on functionalized multiwall carbon nanotubes (f-MWCNTs) grafted with UF to produce urea-multiwall carbon nanotubes (UF-MWCNTs) for enhancing the nitrogen uptake (NU) and use efficiency (NUE). The grafted N was found efficiently absorbed and utilized by rice, and overcome the N propensity for loss from soil-plant systems when UF-MWCNTs are applied. The UF-MWCNTs shown tremendous NUE up to 96% and NU at 1180mg/pot. The chemical changes were monitored by Raman spectroscopy. Hence, UF-MWCNTs provides a promising strategy in enhancing plant nutrition for rice.

Keywords: Urea, functionalized MWCNTs, nitrogen uptake, nitrogen use efficiency

1.0 INTRODUCTION

The larger demand of N by the agricultural sector was emphasised by the International Fertilizer Industry Association's (IFA) in the Fertilizer Outlook for the year of 2013-2017 that reported the global N demand was projected to grow into 107.5 million metric tons of N. In order to increase the NUE and NU was tremendously difficult as plants normally take up N in the form of nitrate or ammonium ions [1]. It was reported that between 50 and 70% of the nitrogenous fertilizer is lost through leaching and gas emission of ammonia and nitrogen oxides to the atmosphere [2]. These may contribute to unfavourable environmental impact and higher operational cost to farmers. Uncontrolled applications of UF create an adverse effect to aquatic life due to eutrophication which had caused an excessive algae growth. Therefore, high NUE and NU of fertilizer N are important characteristics to ensure enough and lower cost N source for efficient paddy growth.

Recent development in carbon nanomaterials including MWCNTs as smart delivery system for efficient plant growth was widely explored due to their interface with plant organelles which imparting new and enhanced function of this part. Earlier, research on smart delivery systems in agriculture was reported on the delivery of pesticides encapsulated in carbon NMs for UV-shielding [3] and assisted delivery of genetic material for crop improvement [4]. The potential applications of nanomaterials in the agricultural sector have been highlighted in the previous research conducted [5]. In fact, nanomaterials had been proven are capable to increase the agriculture yield by optimizing the nutrient management of the plants [6]. Nanomaterials delivery system is targeting the plant to take up nutrients efficiently and enhance the germination rate of plants by improving the intake of water as well as oxygen [7]. Hence, leaching and losses of nutrients to unintended targets like soil are reduced.

Owing to their special physicochemical properties, MWCNTs having a transport properties [8] and unique ability as molecular delivery through plant cells walled [6], which stimulates crop growth, improves the soil environment and promotes crop growth metabolism [7]. These scenarios made MWCNTs very promising for recent progress in the agricultural research activities and utilization. Research on the impact of carbon-based nanomaterials combines with fertilizer on plants was reported [8]. They had found that the presence of nanomaterials has increased the yield and quality of winter wheat crop, which indicating a significant saving effect on the utilization of nitrogenous type fertilizer. A study of the effects of carbon nanomaterials fertilizer on late rice [9] in the double rice season area at the south of China, revealed that the use of carbon NMs fertilizer would increase the number of glume flowers per year, fertility and the rice yield. Concurrently, the carbon nanomaterials fertilizer was observed to slow down the fertilizer release rate, hence reducing the amount of the amount of fertilizer utilization which improving the NUE. These positive results agreed well with the role of carbon nanomaterials in fertilizer application for the enhancement of plant growth and yield. Hence, this study was encouraged to further exploring the potential of functionalized MWCNTs to be integrated with urea fertilizer (UF) for the sake of agricultural field

2.0 METHODOLOGY

A chemical vapour deposition grown MWCNTs were purchased from a commercial source (stock: 214 Cahaya Tech (M) Private Ltd.) were characterized as follows; purity of 95%, outside diameter of 10-20 nm, inside diameter of 5-10 nm and length ranging from 0.5 µm to 1.0 µm. Analytical chemical grade (Merck) of nitric acid (HNO_3) (69%) were used as received. Rice plants (code MR 219) were collected from farmers at Tanah Merah, Kelantan, Malaysia.

Typically, 1.50 mg of as-received MWCNTs was added to a 500-ml round bottom flask containing 450 ml of nitric acid for functionalization process. After being refluxed at 21h, the suspension was naturally cooled down to room temperature. The black solution known as f-MWCNTs was vacuum-filtered using a 3µm particle retention filter paper and washed with deionized water to remove excess HNO_3 until it neutral. The f-MWCNTs were dried in a vacuum oven at 105 °C for at least 2 hrs. F-MWCNTs were sonicated, then stirred together with urea for 6 hrs at 150 rpm to produce UF-MWCNTs. The samples were dried in oven at 70°C for at least 5 hours.

The MR219 rice seeds were rinsed thoroughly, soaked 24 hrs in water and germinated in plastic containers. Seedlings were transferred into pots containing rice field soil of 0.2% N-content. After sowing, the UF-MWCNTs fertilizer was applied three times (at 14, 35 and 55 days) with a rate of 120 kg ha-1. Ordinary fertilisers such as phosphorus (DAP) and potassium (muriate of potash) were applied once at a rate of 50 $kgha^{-1}$.

Raman spectroscopy was carried out with excitation laser source 532 nm, UniRAM-3500, micro Raman Mapping chamber**.** NUE analysis was carried out in isotopic-aided fertilizer experiments, where UF-MWCNTs fertilizer labelled with ^{15}N urea isotope (5% atom, Isotec) is used and the amount of N fertilizer that a plant has taken up is determined. The plant samples for ^{15}N were analyzed by using emission spectrometer detector (NO17), FAN (Fisher Analysen Instrumen), Germany. In this way N fertilizer uptake and use by paddy can be studied. Basically, 2% atom ^{15}N urea was enough to be detected by the emission spectrometer. Fertilizer N utilization by crops and retention in the soil were calculated using ^{15}N Direct technique calculation as showed in equations (1)-(4) [10]:

$$\%Ndff = \frac{atom\%^{15}Nexcess_{(plant)}}{atom\%^{15}Nexcess_{(fertilizer)}} \times 100 \quad (1)$$

$$N\ uptake\ by\ plant = Dry\ weight_{plant} \times \frac{\%N}{100} \quad (2)$$

$$Fertilizer\ N\ uptake\ by\ plant = N\ uptake\ by\ plant \times \frac{\%Ndff_{plant}}{100} \quad (3)$$

$$Fertilizer\ N\ utilization\ by\ plant = \frac{Fertilizer\ N\ uptake\ by\ plant}{N\ applied} \times 100 \quad (4)$$

Where, *Ndff* is fraction of N in the plant derived from the ^{15}N labelled fertilizer.

3.0 RESULTS AND DISCUSSION

3.1 Raman Spectroscopy

Figure 1 depicts Raman analysis of covalently f-MWCNTs in comparison to pristine MWCNTs. Referring to Figure 1, covalent functionalization of f-MWCNTs was observed does not generally result in obvious changes in D-band to G-band intensity ratios (I_D/I_G). Three characteristic peaks concerned for MWCNTs were their D-band at 1333.33cm^{-1}, G-band at 1572cm^{-1} and second order harmonic G'-band at 2677.54cm^{-1}[11]. Clearly, from Figure 1, the tangential G-band derived from the graphite-like in-plane mode [12] was still retain after functionalization at 21 hrs reflux time, reveal that the graphitic carbon structure with sp2 bonds of f-MWCNTs was preserved.

A very intense D band and a significant broadening effect showed by the Raman spectra of MWCNTs is found similar to the spectra of chars and carbon blacks[13]. Besides, the lower peaks of D-band and G'-band than G-band observed in Figure 1 reflecting f-MWCNTs samples with high purification.

Similar observation was also reported elsewhere [14]–[16].

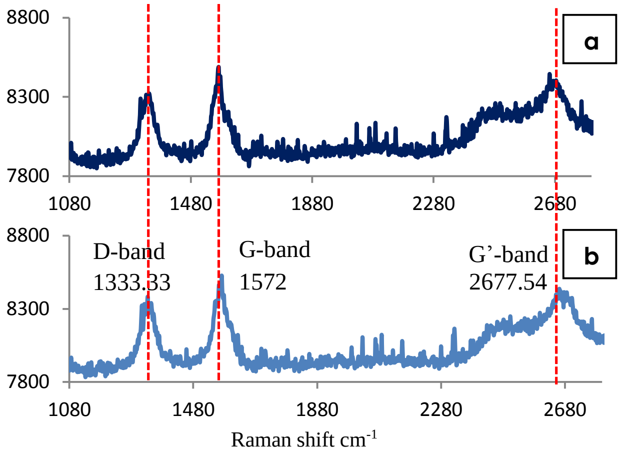


Figure 1 Raman spectra of (a) f-MWCNTs in comparison to (b) MWCNTs

Further comparison on the Raman spectra of UF-MWCNTs and UF was depicted in Figure 2. The most pronounced Raman feature in the spectrum subjected to highly crystalline structure of urea [17] is a strong sharp and narrow peak around ~1000 cm^{-1} for both UF-MWCNTs and UF samples. The urea bands centered at around ~1000 and ~1170cm^{-1} were correspond to N-C-N stretching and NH_2 rocking, respectively [18]-[19] were maintain after grafting with 21 hrs f-MWCNTs revealing the successful ofgrafting process to produce high performance UF-MWCNTs fertilizer.

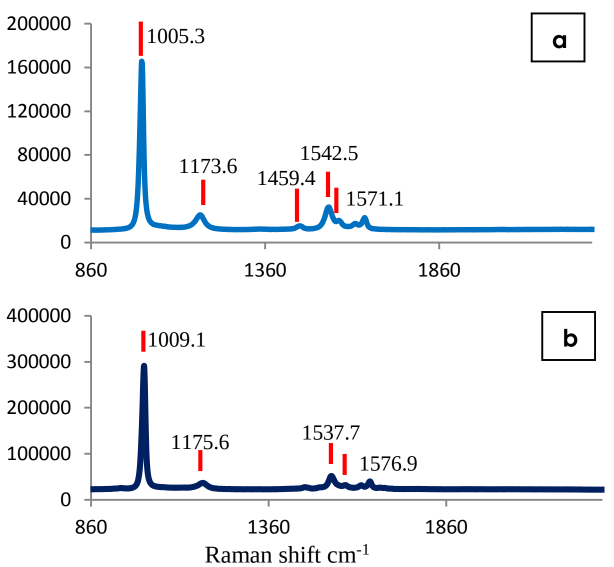


Figure 2 Raman spectra of (a) UF-MWCNTs in comparison to (b) UF

Significantly, the characteristic absorption peak for f-MWCNTs which is D-band at 1333.33cm^{-1} was strongly attenuated with new peak observed at 1459cm-1 for UF-MWCNTs after urea was grafted to f-MWCNTs. Furthermore, the G-band at 1572cm^{-1} for f-MWCNTs was split into two peaks at 1542.5 and 1571.1 cm-1 for UF-MWCNTs sample. This observation might be strongly correlated to grafting process of N from UF to f-MWCNTs that take place at opened end tubes and significantly changes their graphitic structure.

3.2 Evaluation of N fertilizer uptake (NU)

The current result regarding evaluation of NU for UF-MWCNTs as compared to conventional UF (control) was shown in Figure 3. Generally, as the early stages of growth from 5 to 9th weeks, attributed to vegetative and reproductive stage (tillers and panicles production), plants pursue high demand for nutrients especially N, hence result in remarkable increase in NU by paddies for both samples. The differences become significance start from 9th weeks and become apparent at 11th weeks of paddy growth. During the 11th weeks, the remarkable increase in NU for UF-MWCNTs treatment was 200% (1363.6 mg/pot) higher as compared to control. Here, paddies were at the ripening stage [20], and the maximum NU from soil occurred because after that paddies start to utilize N that is already available in plant tissues. Obviously, the UF-MWCNTs treatment promotes NU from UF efficiently and consequently and avoids N losses. The tremendous high NU might be explained by penetration capability of f-MWCNTs into the plant cells and promote NU as claimed by Khodakovskaya *et al.* (2009) for MWCNTs that create new pores and encourages nutrients uptake.

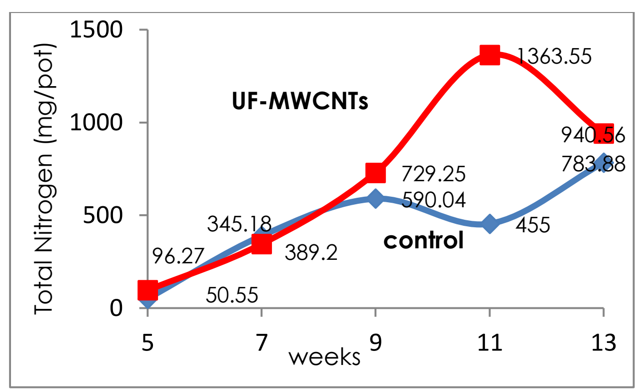


Figure 3 Trend of N fertilizer uptake by paddy straw for UF-MWCNTs compared to control

The lower NU from conventional UF paddies might be related to losses of N through leaching to the groundwater and via ammonia (NH_3) volatilization to the environment. Once, the UF applied to soil, NO_3^- and ammonium efflux from plant roots can be occurred and will result in a reduction of NU. As external NO_3^- and ammonium concentrations are increased, particularly into the range of concentrations that are typical of agricultural soils, elevated rates of NO_3^- and ammonium efflux result [22]. In sum, these several factors conspire to limit rates of plant NU [22]. Hence, f-MWCNTs increase the NU of UF by paddies through efficient N absorption from soil, and subsequently reduce NO_3^- and ammonium efflux from plant roots.

The scenario occurred at 13th week of paddies growth is more complicated. Contradict with UF-MWNTs, control shows increment in NU at 13th weeks. Only N-starved plant roots will absorb with both high capacity and high affinity. This might be correlated with reduction of NU at 11th weeks that urged paddies under UF treatment increase NU at 13th weeks to ensure enough N supplied for paddies growth. Thus, paddies under control have to continue for NU even at 13th weeks of paddy growth, while paddies under UF-MWCNTs treatment already have supplied enough N in their plants tissues for filling and maturation period. Thus, their energy can be utilized efficiently for growth and yield production as compared to control due to outstanding roles of f-MWCNTs for the NU and distribution in the plants.

3.3 Evaluation of NUE

Improving nitrogen use efficiency (NUE) of crop plants is thus of key importance for productive agriculture. The trend of NUE by paddies straw for UF-MWCNTs treatment as compared to conventional UF was illustrated in Figure 4. In general, the NUE followed similar trend with NU which consistently increased from vegetative stage at 5th weeks up to maximum tillering stage at 11th week before decrease at 13th weeks for filling and maturity stage. This trend reveal the close relationship between NUE and NU by paddies in respond to N fertilizer which reported also elsewhere [23] . The differences become significance start from 9th weeks and become obvious at 11th weeks of paddy growth. Specifically, during 9th week, UF-MWCNTs obviously show high NUE by paddy straw with 62% as compared to control with 43%. Then, by the time NUE reached the maximum at 11th weeks, the NUE trend for UF-MWCNTs treatment continue to increase and displays high NUE achievement with 88.7%, while UF WITH 50% NUE only. Finally, towards the end of growing period, the NUE by paddies was decrease for UF-MWCNTs paddies samples and slightly increase for UF.

The good performance of UF-MWCNTs might be explained also by the grafted N which can be absorbed and utilized by paddies efficiently to overcome the N propensity for loss from soil-plant systems usually less than 50% worldwide for UF which associated with leaching to the groundwater and via ammonia (NH_3) volatilization to the environment. Actually, NUE in plants is complex and much depends on N availability from fertilizer in the soil and on how plants utilized N throughout their life span. This includes NU, redistribution within the cell and balance between storage and current use at the cellular and whole plant level [23].

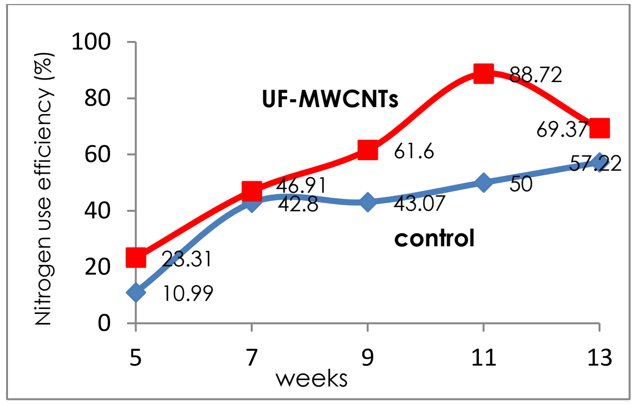


Figure 4 Trend of NUE by paddy straw for UF-MWCNTs compared to control

Hence, the good performance of UF-MWCNTs might also be strongly correlated to high N storage and balanced usage at the cellular level for UF-MWCNTs treatment. Under practical condition, NUE can be considered as the amount of N taken up from the soil by plants within a certain period of time compared with the amount of N applied [1]. Therefore, high N storage will give high NUE and NU. This observation supported earlier arguments regarding advantages of f-MWCNTs as N carrier into the plants due to f-MWCNTs structure which make them mobile to penetrate the plant cell walls and result in high NU and NUE. Obviously, the UF-MWCNTs treatment promotes NUE efficiently and consequently will save the environment and lowers the operational cost for N fertilizer. Hence, this new urea grafted f-MWCNTs (UF-MWCNTs) approach provides a promising strategy in enhancing NUE for better plant nutrition for paddies.

4.0 CONCLUSION

In conclusion the functionalization of MWCNTs by strong acid treatment resulted in the formation of opened ends tubes and still retaining their hollow sidewalls structure, as analyzed via Raman spectroscopy analysis. This condition encouraged excellent biocompatibility between f-MWCNTs and UF. The 0.50 wt.% amount of f-MWCNTs which have reflux in nitric acid for 21 hrs for functionalization process developed UF-MWCNTs with great performances up to 96% NUE and 1180 mg/pot for NU. This new urea grafted f-MWCNTs (UF-MWCNTs) approach delivers a promising improvement in NU and NUE for efficient plant nutrition of rice.

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