Effect of multi-walled carbon nanotubes and cytokinin 6-Benzylaminopurine on the growth and nutraceutical content of *Coriandrum sativum* microgreens

Project Work Report

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Abstract

Microgreens are compact food sources, in the sense that there is a high amount of nutrients per unit mass as compared to other plants. In addition, most microgreens take a significantly shorter period of time to grow fully. This study sought to determine whether carbon nanotubes and cytokinin both together and separately, can increase growth rates and nutraceutical content. Results reveal that 6-Benzylaminopurine (6-BAP) could best increase growth rates and overall nutraceutical content. On the other hand, carbon nanotubes inhibited growth rates. For all experimental set-ups, antioxidant content was increased significantly, but Vitamin C content was lower, compared to the control. These results also revealed that 6-BAP used alone is the best option for increasing growth rates and overall nutraceutical content. Our results also revealed that 6-BAP and carbon nanotubes are incompatible with each other.

1. Introduction

The United Nations Food and Agriculture Organization (FAO) estimates that about 815 million people of the 7.6 billion people in the world, or 10.7%, suffered from chronic undernourishment in 2016 (FAO, 2016). Food shortage is a severe and real issue. In recent years, a new "superfood" has emerged: microgreens. Microgreens contain nutrients many times that of the fully-grown plants (Warner, 2012). An example is coriander microgreens, *Coriandrum Sativum*. Coriander is a widely used fragrant, vegetable in many forms of Asian cuisine, and often praised for its ability to provide a range of health benefits to the consumer. Coriander has the innate ability to hinder the formation of low-density lipoproteins, which are unhealthy cholesterol (Kansal, Sharma, & Lodi, 2011). Coriander is able to inhibit the biosynthesis of cholesterol and triglycerides, and can therefore be used as a prevention for hyperlipidemia, the clogging up of our blood arteries with cholesterol, culminating in high blood pressure. Furthermore, coriander has also been shown to have high levels of antioxidants such as linalool. According to Rathore, Saxena and Singh(2013), when coriander powder was fed to rats, there was a significant decrease in the induced free radical level in the rats' kidneys, thereby acting as a prevention for different forms of cancer, prominently in the liver. This can be beneficial to the international community, as cancer is one of the top death causes in the world, as in the US alone, cancer took the lives of an estimated 700,000 people (Miller & Jemal, 2017). Thus, coriander could be a potential panacea to prevent cancer from developing in people. Moreover, research studies (Rathore, Saxena, & Singh, 2013) have also found that the consumption of coriander can play a pivotal role in the treatment of diabetes, one of the top causes for death in Singapore.

In an attempt to solve food shortage, a new field of research has emerged recently: use of carbon nanotubes in agriculture. Carbon nanotubes are carbon allotropes that are macromolecules. Carbon nanotubes are used in the production of carbon fiber. In recent years, the application of carbon nanotubes has fascinated scientists due to its useful physical properties. Thus far, carbon nanotubes have mostly been used for electronic and electrochemical purposes as well as for mechanical reinforcements in high performance composites (Ajayan & Zhou, n.d.). More recently, research on carbon nanotubes has extended to agriculture. There have been investigations that suggest that carbon nanotubes are able to increase germination rate, length of shoot and root, and vegetative biomass of seedlings (Ballesta, 2016). Carbon nanotubes were also found to be able to penetrate the coats of tomato seeds, increasing germination rates and stimulating growth in young seedlings (Khodakovskaya, et al, 2009). Carbon nanotubes can increase aquaporin protein count and aquaporin transduction in Tobacco calluses (Khodakovskaya et. al, 2012). This increased water uptake of the plants can lead to faster growth rates. Despite such promising features, it was also discovered that an excessive amount of carbon nanotubes may also take a toll on the germinating seeds. It was observed at high concentrations, the maize seedlings grown in agar had their growth and water uptake drastically reduced (Tiwari, et al, 2013). It is thus important to conduct further research on the effects of carbon nanotubes on plant growth. Multi-walled carbon nanotubes (MWCNT) were chosen to be studied in this project instead of single walled carbon nanotubes (SWCNT) as MWCNT are more rigid and have a higher surface tension than SWCNTs.

Cytokinin has also been used in agriculture to enhance plant growth (Massolo et al., 2014). Cytokinins are a type of plant hormone that promotes tissue growth and flower budding. Research has shown that cytokinins have had positive effects on plant growth and plant nutrient absorption. 6-BAP is a synthetic cytokinin that can increase plant growth rate in a variety of plants. This has been demonstrated specifically in *Brassica oleracea* var. *italica* with the use of 6-BAP (Massolo et al., 2014). 6-BAP was found to be able to reduce the amount of chlorophyll degrading genes, allowing the plant to photosynthesise without the reduction of chlorophyll. In another research, tissue treated for 24 hours with 6-BAP incorporated up to twice as much ^{14}C-leucine as untreated tissue(Tavares et al., 1970). This shows that 6-BAP has a positive effect on the uptake of proteins and hence nutrients. It is intriguing to consider the interactions between MWCNT and 6-BAP. Their combined effects on plant growth may work synergistically to give rise to even greater acceleration of plant growth. To our knowledge, this has yet to be investigated in *Coriandrum sativum*.

2. Objectives and hypothesis

The objective of this study is to determine whether cytokinins and carbon nanotubes have positive effects on the growth of coriander microgreens. In our experiment, we plan to investigate the effect of MWCNT, 6-BAP, both individually and together, on the rate of seed germination, plant growth and nutraceutical content of *Coriandrum sativum.* We aim to gain insights on the concentration of carbon nanotubes that would produce optimal results on the plant. Findings may inform future work in the advancement of nano agriculture as the use of carbon nanotubes in agriculture is still a fairly new concept. Success in further developments may give rise to a widespread use of carbon nanotubes in agriculture to enhance the overall nutritional content of plants per unit mass and increase the efficiency of agriculture, helping to mitigate the problem of food shortages in the future. As mentioned, previous research has found that MWCNT and 6-BAP, individually, increase plant growth in various plants. We hypothesise the same result for *Coriandrum sativum.* We also hypothesise that the use of both MWCNT and 6-BAP will result in even greater rates of plant growth, compared to using them individually. In addition, we also expect the antioxidant and vitamins C and E contents to be higher in microgreens grown in the presence of MWCNT and 6-BAP. As a result of expected higher rate of growth of microgreens in higher carbon nanotube concentrations, we can also expect photosynthetic activity of these microgreens to be higher.

3. Materials and methods

3.1 Materials

The multi-walled carbon nanotubes were purchased from US Research Materials (Stock#: US4309PVP) and cytokinin, 6-Benzylaminopurine, was purchased from Sigma Aldrich (CAS number 1214-39-7).

3.2 Methods

3.2.1 Preparation of growth media

Murashige and Skoog (MS) media solution (half-strength) was prepared by adding 4.34 g of MS media powder to 2 L of deionised water. A stock suspension of multi-walled carbon nanotubes (MWCNT) with concentration 50 mg/L was prepared by adding 25 mg of the powder to 500 mLof MS solution. To disperse the particles, the suspension was sonicated at 40W for 3 minutes. 6-Benzylaminopurine (6-BAP) stock solution (552 mg/L) was prepared by adding 138 mg of 6-BAP powder to 250 mL of 1M sodium hydroxide. The control set-up was prepared by adding 50mL of MS solution into a GA-7 vessel. The set-up containing MWCNT only was prepared by adding 40 mL of MS solution and 10mL of MWCNT suspension to a GA-7 vessel. The set-up containing 6-BAP only was prepared by adding 0.25 mL of 6-BAP stock solution and 49.75 mL of MS solution to a GA-7 vessel. The set-up containing both MWCNT and 6-BAP was prepared by adding 39.75 mL of MS solution, 10 mL of MWCNT suspension, and 0.25 mL of 6-BAP stock solution to a GA-7 vessel. The final concentration of MWCNT was 10 mg/L, and 2.17 mg/L for 6-BAP. Each set-up was replicated 4 more times, giving a total of 5 replicates per set-up and a total of 20 GA-7 vessels. The pH of the media were tested using a pH probe. The pH was adjusted to pH 6.5 with 1M sodium hydroxide and 1M hydrochloric acid. The GA-7 vessels were then autoclaved for 3 hours at 121° C and then left to cool and solidify.

3.2.2 Cultivation of microgreens

Coriandrum sativum seeds were soaked in deionised water for 3 hours, before they were sterilised with 5% bleach solution and rinsed with deionised water. 50 seeds were spread evenly on MS agar in a GA-7 vessel. The seeds were left to germinate in the dark at 22° C in a plant cultivator. Upon germination, they were allowed to grow in the cultivator, exposed to 12 hours of

LED light. Carbon dioxide sachets were also placed in the cultivators to provide carbon dioxide. The microgreens were left to grow for 26 days from the day where they were planted.

3.2.3 Collection of results

3.2.3.1 Determination of germination rate, photosynthetic activity and total growth.

Before the microgreens were harvested, germination rate (% seeds germinated) was calculated. Photosynthetic activity was measured with a SPAD 502 Chlorophyll Meter, 3 different leaves from different plants in the same vessel were taken for measurement for every vessel. The microgreens were then uprooted from the plant media. The height of the microgreens were measured from the start of the stem to the apex of the leaf of the tallest shoots.

3.2.3.2 Preparation of nutraceutical test solutions

The microgreens in each vessel were divided into 2 equal portions. The first portion was ground with a mortar and pestle in deionised water. The ratio of the mass of microgreens used/g to the volume of water used/mL was 1:9. The second portion was ground with a mortar and pestle in homogenate solution provided by the Vitamin test kits. Similarly, the ratio used was 1:9. The plant material from the first portion was centrifuged at 4800 rpm to obtain the supernatant. The supernatant was used as the test solution for the determination of the total antioxidant and vitamin content.

3.2.3.3 Determination of total antioxidant content

The control solution was prepared by adding 0.50 mL of DPPH to 0.95 mL of methanol and 0.05 mL of deionised water in a 1.5 mL cuvette. The solution was left in the dark for 10 minutes, before the absorbance of the solution was measured at 517 nm, with a solution of 1.45 mL methanol and 0.05 mL deionised water as the blank. The same was repeated for the test solution, but replacing deionised water with the test solution, i.e. the supernatant obtained beforehand. The radical scavenging activity (antioxidant content) of the microgreen extracts (%) was then calculated using the following formula: ((Control absorbance - Test absorbance) / Control absorbance) x 100%

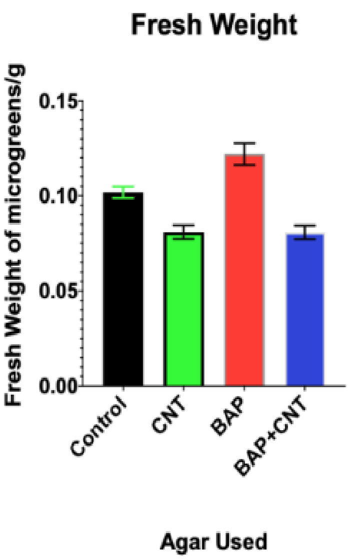
3.2.3.4 Determination of Vitamin Content

The Vitamin C content in the microgreen extracts are calculated by the following equation:

VC content (μg/ mL) = A_{sample}-A_{blank}/ $A_{standard}$-A_{blank} x 6 μg/ mL x 4, where A_{sample} , 7 A_{blank} , $A_{standard}$, stand for the absorbance of the sample, blanJk and standard solutions respectively, 6 μg/ mL is the concentration of vitamin C in the standard solution and 4 is the dilution factor that results from the steps listed in the test kit. The sample solution was prepared according to the test kits.

4. Results and discussion

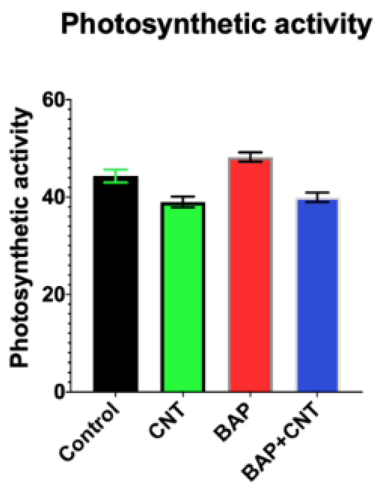
4.1 Germination rate, average height and yield of microgreens



Mann Whitney U Test

Vessel	P-Value
CNT	0.0287
BAP	0.0232
CNT+BAP	0.0678

Fig 1.1

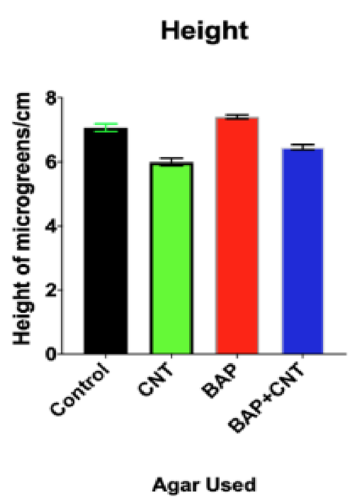


Mann Whitney U Test

Vessel	P-Value
CNT	0.0127
BAP	0.0107
CNT+BAP	0.0208

Fig 1.3

Figures 1.1 and 1.4 indicate that the set-ups with only 6-BAP had the greatest weight and height of microgreens compared to the control set-up. However, the set-ups containing MWCNT only and MWCNT + 6-BAP produced similar fresh weights and heights, but less compared to the control set-up. This is contrary to previous research that has shown that MWCNT increases plant growth rate in other plants. (Khodakovskaya



Mann Whitney U Test

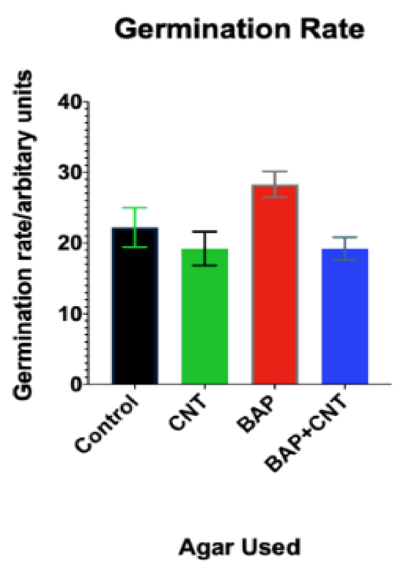
Vessel	P-Value
CNT	0.0450
BAP	0.0397
CNT+BAP	0.0438

Fig 1.4

et. al, 2012). The Mann-Whitney U test conducted revealed that the results obtained for the MWCNT only set-up and 6-BAP only set-up were statistically significant, where $p < 0.05$ for both fresh weight and height. However, results for the 6-BAP and MWCNT set-up were statistically insignificant, where $p > 0.05$. The results for the 6-BAP set-up confirm existing research that cytokinin increases plant growth rate (Massolo et al., 2014). The set-ups with MWCNT, however, showed to give lower growth rates than the control set-up. Further research is required to investigate the reasons. Fig 1.2 shows that germination rate for the set-up with 6-BAP only was the highest, with CNT and 6-BAP + CNT set-ups having lower germination rates than the control. This is contrary to previous research that carbon nanotubes increase germination rate. (Khodakovskaya et. al, 2012). One possible reason for the lower germination and growth rate with MWCNT is that the concentration used was incompatible with coriander microgreens, leading to phytotoxicity due to the accumulation of reactive oxygen species, which eventually leads to cell death (Tan et al., 2009). Further experimentation with even lower concentrations of MWCNT can be conducted to confirm this hypothesis.

4.2 Photosynthetic activity of microgreens

Fig 1.3 shows results for photosynthetic activity in a similar pattern to the abovementioned results. Plants in 6-BAP set-ups performed the best, while those in set-ups with MWCNT with and without 6-BAP had lower photosynthetic activity compared to the control. Data for all set-ups are significant, with $p < 0.05$ through the Mann-Whitney U Test. Reasons for the trend in results are similar to germination and growth rate.



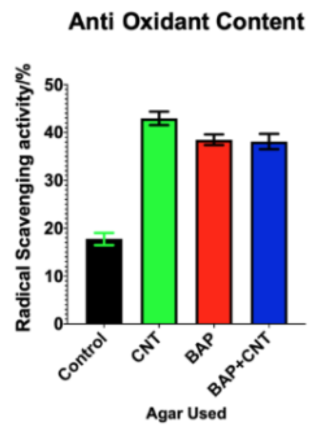
Mann Whitney U Test

Vessel	P-Value
CNT	0.0209
BAP	0.0340
CNT+BAP	0.0222

Fig 1.2

4.3 Antioxidant content (radical scavenging activity) of microgreens

Results for antioxidant content show a change in trend of results. Fig 1.5 shows that the MWCNT, 6-BAP, and MWCNT set-ups had significantly greater antioxidant content compared to the control, with $p < 0.05$ for all set-ups. Results for 6-BAP set-up confirm previous research that cytokinins increases radical scavenging activity of plants (Miller, 1992). However, research has yet to be conducted on the effect of MWCNT on the radical scavenging activity and hence antioxidant content of plants. Hence, the observation that the MWCNT set-up had the highest antioxidant content provides further information on the effect of carbon nanotubes on plant growth, as it has not been discovered to be able to increase antioxidant content of plants, according to the available research. Further research is also required to confirm why plants in the 6-BAP + MWCNT set-up did not perform as well as those in set-ups that contained these additives separately. Our findings show that it MWCNT or 6-BAP can be added to plant growth medium if an increase in the antioxidant content of crops is desired.



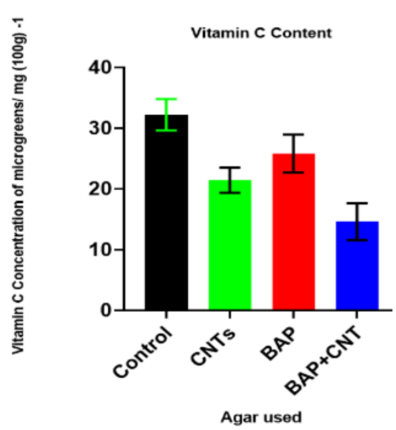
Mann Whitney U Test

Vessel	P-Value
CNT	0.0039
BAP	0.0107
CNT+BAP	0.0074

Fig 1.5

4.4 Vitamin C content of microgreens

Results for Vitamin C content follow a similar trend in results across the set-ups. Fig 1.6 shows that the plants in the control set performed better than those in the experimental set-ups. 6-BAP had the second highest Vitamin C content, followed by the MWCNT set-up, then the 6-BAP + MWCNT set-up. These results run contrary to the results for the antioxidant content of the microgreens and imply that the treatment may have increased the production of antioxidants other than Vitamin C. Research on the effect of MWCNTs or cytokinin on Vitamin C content of microgreens have yet to be conducted. These



Mann Whitney U Test

Vessel	P-Value
CNT	0.0079
BAP	0.0159
CNT+BAP	0.0079

Fig 1.6

findings suggest that neither MWCNT nor 6-BAP increase the Vitamin C content in coriander microgreens.

5.1 Conclusion

Our results show that cytokinin 6-BAP has a positive effect on germination rate, growth rate, photosynthetic activity and antioxidant content. This confirms previous findings. Our results also show that MWCNT alone, and 6-BAP with MWCNT, have a negative impact on growth rate and Vitamin C content. However, they do increase antioxidant content significantly. The fact that growth rate is decreased when MWCNT is used does not make MWCNT irrelevant in culturing coriander microgreens, since antioxidant content is still higher when they are used. Our findings also show that it is not beneficial to use both MWCNT and cytokinin in increasing either growth rate or nutraceutical content of coriander, since plants grown in the set-ups containing MWCNT and 6-BAP performed worse when compared to those that were grown in set-ups that contained the additives separately. 6-BAP and MWCNT, used either together or separately, decreased Vitamin C content. Our results show that 6-BAP used alone is the best option in increasing growth rates and nutraceutical content. Contrary to our hypothesis, the interactions between 6-BAP and MWCNT do not further increase growth rates and nutraceutical content.

5.2 Future work

Possible future work includes the investigation of the effect of MWCNT and 6-BAP on the Vitamin E content of microgreens, as well as the antibacterial properties of microgreens. In addition, our finding that MWCNT and 6-BAP do not have a positive effect on Vitamin C opens up possible paths for research to find another growth enhancer which increases Vitamin C content. Such growth enhancers, when combined with MWCNT or 6-BAP may increase growth rates, antioxidant content, and Vitamin C content all at once, possibly leading to the development of a future “superfood” with even higher nutraceutical content. The same experiments can also be conducted on microgreens other than coriander to source for more viable options, such as ones that grow more quickly. Lastly, the reasons as to why 6-BAP and MWCNT do not work together could be investigated to understand how MWCNT interacts with other growth enhancers.

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