EFFECT OF PURE AND N-DOPED CARBON QUANTUM DOTS ON THE PHOTOSYNTHESIS AND GROWTH OF *BRASSICA JUNCEA (L.) CZERN.*

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By

YAMUNA A/P CHOWMASUNDARAM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

EFFECT OF PURE AND N-DOPED CARBON QUANTUM DOTS ON THE PHOTOSYNTHESIS AND GROWTH OF *BRASSICA JUNCEA (L.) CZERN*.

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YAMUNA A/P CHOWMASUNDARAM

July 2023

Chairman : Professor Ir Ts. Suraya binti Abdul Rashid, PhD
Institute : Nanoscience and Nanotechnology

Nitrogen-doped quantum dot (N-CQD) is a novel nanomaterial that has attention in the agriculture field. In this work, the N-CQD was synthesized and characterized. The mechanism of photosynthesis is still not entirely understood, especially for plants growing indoors. The effects of pure and N- CQD on the plants that grow in indoor hydroponic systems are studied. The CQD at various concentrations (ranging from 0-400 ppm) applied via the foliar method toward the leaves of the green mustard plant *(Brassica Juncea).* A statistical analysis was performed on 54 plant samples (n=54). According to the findings, 150 ppm of both CQD types was determined to be the optimum concentration for promoting plant growth and photosynthesis parameters. The CQD-treated plant dramatically increased the number of leaves produced, leaves area, height, fresh weight, and dry weight compared to the control plant by 28.8%, 40.6%, 34.6%, 161%, and 255%, respectively. The N-CQD treated plant significantly enhanced the number of leaves production, leaves area, height, fresh weight, and dry weight by 79%, 187%, 71.5%, 383%, and 707%, respectively. Furthermore, the CQD treated plant increased transpiration rate, net assimilation, stomatal conductance, and iWUE by 11.9%, 55.7%, 30%, and 28%, respectively. Besides that, N-CQD significantly raised the plant transpiration rate by 28.1%, net assimilation by 114.6%, stomatal conductance by 49.1%, and iWUE by 57.5%. In this study, N-CQD improved the growth and photosynthesis rate of the green mustard plants compared with CQD. The effects of CQD on plants under various light spectrums, including full light spectrum and red/blue light spectrum, have also been studied. The result demonstrates that both CQD types effectively enhanced plant photosynthesis under full light spectrums rather than red/blue light spectrum. For instance, the 150 ppm treated pure CQD plants exposed to the full light spectrum had phiPSII higher than plants exposed to R/B light by 10.2%. Moreover, the 150 ppm treated doped CQD plants exposed to the full light spectrum had phiPSII higher than plants exposed to red/blue light spectrum by 11.2%. The study provides an explanation of how electron transfer works in

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the CQD/chloroplast complex. This study's findings emphasized the potential of CQD as an efficient method to enhance plant growth and photosynthesis for indoor plants. According to the research, CQD can applied in many plant species and growth environments as an artificial photosynthetic pigment. The innovative methodology created in this study to examine the impacts of CQD under various light spectrums can offer insightful information for maximizing the use of CQD in agriculture.

Keyword: Carbon quantum dots, growth, photosynthesis parameters, electron transfer mechanism, green mustard.

SDG: GOAL 2: Zero Hunger, GOAL 12: Responsible Consumption and Production, GOAL 13: Climate Action, GOAL 15: Life on Land

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KESAN KUANTUM KARBON TULEN DAN N-DIDOP PADA FOTOSINTESIS DAN PERTUMBUHAN *BRASSICA JUNCEA (L.) CZERN.*

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Kuantum nitrogen didop (N-CQD) adalah nanomaterial novel yang mempunyai perhatian dalam bidang pertanian. Dalam kajian ini, N-CQD telah disintesis dan dicirikan. Mekanisme fotosintesis masih belum difahami dengan sepenuhnya, terutamanya untuk tumbuhan dalaman. Kesan CQD dan N- CQD pada tumbuhan dalaman yang tumbuh dalam sistem hidroponik dikaji. Pelbagai kepekatan CQD di antara 0 hingga 400 ppm diaplikasi pada daun tanaman pokok sawi (*Brassica Juncea*) dengan mengunakkan cara semburan. Kajian analisis statistik dilakkukan ke atas 54 sampel tumbuhan (n=54). Menurut kajian, 150 ppm bagi kedua-dua jenis CQD ditentukan sebagai kepekatan optimum untuk menggalakkan pertumbuhan tanaman dan parameter fotosintesis. Sebagai perbandingan dengan pokok kawalan, pokok sawi yang dirawat dengan CQD meningkat secara dramatik dengan jumlah daun, luas daun, tinggi tumbuhan, berat segar, dan berat kering sebanyak 28.8%, 40.6%, 34.6%, 161%, dan 255%, masing-masing. Tumbuhan yang dirawat N-CQD meningkat jumlah daun, luas daun, tinggi tumbuhan, berat segar dan berat kering sebanyak 79%, 187%, 71.5%, 383% dan 707 % berbanding dengan tumbuhan kawalan, masing-masing. Sebagai perbandingan dengan kawalan, tumbuhan yang dirawat CQD meningkatkan kadar transpirasi, asimilasi, kekonduksian stomata, dan iWUE masing-masing sebanyak 11.9%, 55.7%, 30%, dan 28%. Selain itu, N-CQD meningkatkan secara signifikan kadar transpirasi tumbuhan sebanyak 28.1%, asimilasi sebanyak 114.6%, konduktansi stomata sebanyak 49.1%, dan iWUE sebanyak 57.5%. Dalam kajian ini, N-CQD meningkatkan pertumbuhan dan kadar fotosintesis tanaman pokok sawi secara berkesan berbanding dengan CQD. Kesan CQD pada tanaman di bawah pelbagai spektrum cahaya, termasuk spektrum cahaya putih dan spektrum cahaya merah/biru, juga telah dikaji. Hasilnya menunjukkan bahawa kedua-dua jenis CQD meningkat secara lebih berkesan pada fotosintesis tumbuhan di bawah spektrum cahaya putih berbanding dengan spektrum cahaya merah/biru. Sebagai contoh, 150 ppm tanaman CQD yang didedah kepada spektrum cahaya putih mempunyai phiPSII lebih tinggi daripada tanaman yang terdedah kepada spektrum cahaya

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merah/biru sebanyak 10.2%. Tambahan lagi, tanaman yang dirawat dengan 150 ppm N-CQD terdedah kepada spektrum cahaya putih mempunyai phiPSII lebih tinggi daripada tanaman yang didedah kepada spektrum cahaya merah/biru sebanyak 11.2%. Kajian ini memberikan penjelasan komprehensif mengenai bagaimana pemindahan elektron berfungsi di kompleks CQD/kloroplas. Potensi CQD sebagai kaedah yang cekap untuk meningkatkan pertumbuhan tanaman dan fotosintesis untuk tumbuhan dalaman diserlahkan oleh penemuan kajian ini. Menurut kajian, CQD dapat digunakan dalam berbagai spesies tanaman dan lingkungan pertumbuhan sebagai pigmen fotosintetik buatan. Metodologi inovatif yang dibuat dalam kajian ini untuk mengkaji kesan CQD di bawah pelbagai spektrum cahaya dapat memberi maklumat yang mendalam untuk memaksimumkan penggunaan CQD dalam pertanian.

Kata Kunci: Kuantum karbon, pertumbuhan, parameter fotosintesis, mekanisme pemindahan elektron, pokok sawi.

SDG: MATLAMAT 2: Sifar Kebuluran, MATLAMAT 12: Penggunaan dan Pengeluaran Bertanggungjawab, MATLAMAT 13: Tindakan Memerangi Perubahan Iklim, MATLAMAT 15: Kehidupan Darat

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LIST OF ABBREVIATIONS

A	Net Assimilation
ADP	Adenosine Diphosphate
Ag	Silver
ATP	Adenosine Triphosphate
$C_6H_{12}O_6$	Glucose
CNT	Carbon Nanotubes
CO_2	Carbon Dioxide
CQD	Carbon Quantum Dot
CVD	Chemical Vapour Deposition
DCPIP	2,6-Dichlorophenolindophenol
DNA	Deoxyribonucleic Acid
E	Transpiration Rate
EFB	Empty Fruit Bunch
ETC	Electron Transport Chain
ETRI	Electron Transfer Rate of PS I
ETRII	Electron Transfer Rate of PS II
FTIR	Fourier Transform Infrared
Fv/Fm	Quantum Efficiency
G3P	Glyceraldehyde 3-Phosphate
GQD	Graphene Quantum Dots

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GSFI	Global Security Food Index
gsw	Stomatal Conductance
H^+	Hydrogen ion
H_2O	Water
H_2O_2	Hydrogen Peroxide
HRP	Horseradish Peroxidase
HRTEM	High-Resolution Transmission Electron Microscopy
IPA	Isopropanol
iWUE	Intrinsic Water Use Efficiency
LED	Light-Emitting Diode
LICOR	Lambda Instruments Corporation.
Mg	Magnesium
MS	Murashige & Skoog
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide–Adenine Dinucleotide Phosphate
N-CQD	Nitrogen Doped Carbon Quantum Dot
NM	Nanomaterial
O_2	Oxygen
PAR	Photosynthetically Active Radiation
PGA	3C Molecules, 3-Phosphoglycerate
PhiPSII	Effective Quantum Yield

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PL	Photoluminescence
PPFD	Photosynthetic Photon Flux Density
PSI	Photosystem I
PSII	Photosystem II
R/B	Red/Blue Light
Rubisco	Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase
RuBP	Rubulose 1,5-Bisphosphate
TBA	Thiobarbituric Acid
TiO_2	Titanium Dioxide
UV-A	Ultraviolet A
UV-B	Ultraviolet B
ZnO	Zinc Oxide

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

According to the Global Security Food Index (GSFI), Malaysia is currently ranked 41st in food availability and sustainability. Nevertheless, six years ago, our nation was ranked 40th place (Hanif, 2023). The country's agriculture production is insufficient to meet demand and is unsustainable. This is mainly because of low productivity and quality of agricultural products (Kuen, 2022). Additionally, the news stated that approximately 45% of the nation's typical income comes from agriculture (Kuen, 2022). Comprehensive approaches that strengthen regional and national food systems should be planned to avoid a significant problem in the future. Engineered nanomaterials have the potential to be a solution to maintain the stability and availability of food in the nation. NM approach toward the agriculture field is the best decision and well-planned strategy due to the ability of NM to permeate into the layer of the plant and allow modification of the light-capturing organelle to fasten the growth of plants effectively (Banerjee et al., 2019; Pérez-de-Luque, 2017).

Carbon quantum dot (CQD) is the most promising nanomaterial because able to promote the photosynthesis rate of plants and also increase the crop yield. CQD is zero-dimensional (0D) which typically have a particle size of less than 10 nm (Sharma & Dave, 2020; Youfu Wang & Hu, 2014), are the most often studied carbon-based NM. CQD offers several outstanding characteristics that rank it among the most promising materials that have emerged in the agricultural sector in the previous century. CQD stands out from the competition due to its biocompatibility, low toxicity, antioxidant activity, UV protection, antibacterial activity, and sustainability (Guo et al., 2022; Kaur & Verma, 2022; Manzoor et al., 2023; Sharma & Dave, 2020; Tan et al., 2021; Woo et al., 2022) . These qualities improve their ability to be more resource-efficient and to be more environmentally friendly. Additionally, it means that CQD is a sustainable technique when used in agriculture, especially in terms of enhancing plant growth and photosynthesis rates to produce enough food for present and future generations.

1.2 Problem Statement

Many studies regarding the achievement of artificial photosynthesis via NM approaches are numerous nowadays. It has been demonstrated by numerous investigations that NM increases agricultural productivity. Planned strategies engineered nanomaterials, such as TiO_2, ZnO, Mg, Ag NM, and others, have

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been tested on plants and shown have beneficial effects (Ferdous & Nemmar, 2020; Ebesta et al., 2021; Segatto et al., 2020). Several NM-based fertilizers are not environmentally favorable because they were made from inorganic and harmful precursors (An et al., 2022). For example, metal and metal oxide NM have a number of drawbacks, one of which is their toxicity (Jamkhande et al., 2019; Mitra & De, 2016). Although these NM may show improved plant growth and yield, the majority of metal and metal oxide NM are hazardous to plants at high doses and are believed to pose a concern to the environment (Rastogi et al., 2017).

The toxicity of these metal and metal oxide NM is not only harmful to the environment but will also cause huge side effects to the consumers who ingest them for long periods (Alengebawy et al., 2021; Jamuna & Ravishankar, 2015). For example, it has been reported in 182 studies that zinc oxide NM has adverse effects on the cardiovascular, neurological system, alimentary canal, reproductive system, and respiratory systems of the human body system (Keerthana & Kumar, 2020). Several chemical approaches to produce metal oxide NM have also been reported to include harmful substances such as H_2S, toxic material, and metallic precursors (Raghavendra, 2017; Sabir et al., 2014). Other than that, the cost of the precursor is very expensive and has limitations in scaling up (Seabra & Durán, 2015), which would not make it a sustainable approach for future agriculture. Carbon quantum dots (CQD) is one of the promising novel nanomaterials which able to fulfill all the requirements, whereby, it is environmentally friendly (Desmond et al., 2021). Among all of these engineered NM, CQD has emerged as a rising star because of its numerous advantages such as chemical inertness, photoluminescence properties, excellent solubility, non-toxicity, high biocompatibility, and relatively inexpensive cost (Lim et al., 2015; Peng et al., 2012; Xi Wang et al., 2019; Zhao et al., 2018).

A lot of work has been carried out on the effects of utilizing the CQD towards various types of plants. For example, CQD effects on the growth of Rome lettuce, mung bean, rice plant, wheat and others (Li et al., 2018; Li et al., 2016; Swift et al., 2020; Wang et al., 2018a; Zheng et al., 2017). The complex process of photosynthesis, which takes place in plants, involves the absorption of light energy, its conversion to chemical energy, and the production of organic compounds (Lopez & Barclay, 2017). Despite intensive study, the mechanism of photosynthesis is still not entirely understood, especially for plants growing indoors. The green mustard plants are frequently grown in hydroponic systems, this study will investigate the effects of CQD on the physical characteristics and photosynthesis parameters of these plants. In this study, comparison between pure and doped CQD were studied. Therefore, this will contribute to the knowledge of the difference in the effects of both CQD types on the growth and photosynthesis parameters of green mustard plants.

Nitrogen is a crucial component for plant growth (Wahocho et al., 2016), and it has been demonstrated that nitrogen doped carbon quantum dots (N-CQD) or doped CQD can improve crop growth by serving as a source of nitrogen (Li et al., 2021). They may also encourage the synthesis of plant hormones, which may

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aid in subsequent growth. The doped CQD has grab more attention in the agriculture research field recently, however limited research has been done so far. According to a recent study, N-CQD increased the growth and yield of tomato and maize plants (Chen et al., 2021; Wang et al., 2021). Furthermore, impacts of N-CQD on photosynthetic parameters, including reduced 2,6-Dichlorophenolindophenol (DCPIP) is activity, ferricyanide, enzyme expression, total chlorophyll a and chlorophyll b of rice plant have also been reported (Li et al., 2021). There are limited studies reported on the photosynthesis enhancement of the light response of CQD treated plants under different light spectrums. In this study, full light and red/blue light spectrums are investigated

The light response of plants is a crucial factor in determining their growth and development. A promising substance for improving plant development and stress tolerance is CQD. Yet, the majority of CQD research has concentrated on the effect of plants under full light spectrum conditions, paying little attention towards the varied light spectra affect CQD behavior in plants. By examining the effects of pure and doped CQD under both full light spectrum (400 - 700 nm) and red/blue light spectrum (400 - 500 nm and 620 -750 nm), the current work intended to address this knowledge gap. This study aimed to offer new insights into the mechanisms underlying CQD plant interactions and their potential applications in agriculture by evaluating the light response of CQD under various lighting conditions. These results imply that the light spectrum has a significant impact on the effectiveness of CQD on plants. This research recognizes the significance of investigating the CQD plant interactions that are impacted by various light spectrums.

Incorporating CQD into plants can change their photoluminescence characteristic which can reveal information about the photosynthetic processes. This study examines the CQD effect on the green mustard plants' photoluminescence traits to learn more about how effectively photosynthesis occurs in indoor hydroponic systems. The effects of CQD on the plants' light absorption, energy conversion, and organic compound production can be determined by observing changes in photoluminescence qualities like fluorescence. These studies will hopefully help for better understand the processes that indoor plants use for photosynthesis. This information can be used to enhance hydroponic systems and increase the effectiveness of growing plants indoors. Further investigation is required to completely comprehend the processes underlying these interactions and to maximize the application of CQD for boosting plant growth and stress tolerance in agriculture.

1.3 Research Objectives

a) To synthesize and characterize the CQD.
b) To evaluate the effects of CQD on plant growth and photosynthesis parameters under full light spectrum.
c) To explore the effects of light response on CQD treated plants under two different light spectrums.

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The first objective is to determine the characterizations of CQD. Nitrogen doped CQD (N-CQD) is a new type of CQD prepared from empty fruit bunch (EFB). The characterizations of doped CQD was studied. Second objective of the study is to evaluate the effects of CQD on plant growth and photosynthesis parameters. Pure CQD (CQD) was previously synthesized using a similar precursor and techniques. The plants were treated with both pure and doped CQD to analyze their effects on plant growth and photosynthesis parameters. Last objective is to explore the effects of light response on the CQD plants under two different spectrums. The two types of CQD were tested to determine their effects on light response under two different spectrums.

1.4 Scope of Work and Limitations

The first objective is to synthesize and characterize the CQD. In the prior publication, the characterization of pure CQD made from the EFB was reported (Jamaludin et al., 2020). To prepare the doped CQD (N-CQD), a similar preparation technique and an EFB precursor were used. The EFB natural-based biochar served as the primary precursor for the production of N-CQD, along with urea. Using high-resolution transmission electron microscopy (HRTEM), the morphology of the prepared N-CQD was investigated. Fourier transform infrared (FTIR) spectroscopy was used to conduct structural analyses of N-CQD. N-CQD was produced and diluted into a few concentrations, ranging from 0 ppm to 400 ppm. Then, using photoluminescence (PL) spectroscopy, the optical analyses of the prepared N-CQD at various concentrations were studied. In the first objective, the results of two CQD types were compared and discussed in depth.

The second objective of the studies is to evaluate the effects of CQD on plant growth and photosynthesis parameters under full light spectrum. Green mustard plants were treated with both pure and doped CQD. The green mustard plants received a foliar treatment of CQD on their leaves. The CQD concentration is a factor that impacts the plants. As a result, the green mustard plants were exposed to five different concentrations: control (zero parts per million), 50, 100, 150, 200, and 400 parts per million. The plant physiological characteristics like a number of leaves produced, area of leave, height, fresh and dry weight of green mustard plants were measured. The photosynthesis parameters such as net assimilation, transpiration rate, stomatal conductance, and water use efficiency were determined for each treated plant. The LICOR fluorometer photosynthesis machine was used to obtain these data. With the aid of the Rubisco Kit, the Rubisco activity of a few carefully chosen concentrations that have a major impact on the plant was investigated. PL spectroscopy was used to examine the PL characteristics of the isolated chloroplast from the treated plants. Comparisons were made between the physiological traits and photosynthetic parameters of the two types of CQD. The optimal concentration of one CQD type that effectively improves the physical characteristic and photosynthetic parameters is chosen at the end of the second objective.

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The third objective is to evaluate the impacts of light response on CQD plants under two different light spectrums. Different types of spectrums of light which include full light and red/blue light spectrums were chosen. The full light spectrum has 400 -750 nm wavelength. Red light has a wavelength of 620 - 750 nm while blue light has a 400 - 500 nm wavelength. The photometer was used to gauge each light's brightness. The green mustard plants were treated foliar applications of a few fixed doses of pure and doped CQD. The greatest quantum efficiency (Fv/Fm), effective quantum yield (PhiPSII), and light response curve were the parameters that collected for this third objective. The results from the various light wavelengths were then compared. The results of the pure and doped CQD were compared after that. Some limitations in this study were the space for the plantation of plant were not enough due to the availabilities of the limited number of holes in the one hydroponics system. The maximum number of holes that can be used to carried out the experiment is limited which make it harder to carry the large number of samples at one time. A greater sample size is necessary to produce a reliable statistical result. Therefore, the experiments were run a few times using the same hydroponics system due to space restrictions.

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