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GROWTH, LEAF GAS EXCHANGE AND BIOCHEMICAL CHANGES OF OIL PALM (Elaeis guineensis Jacq.) SEEDLINGS AS AFFECTED BY IRON OXIDE NANOPARTICLES

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By

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GROWTH, LEAF GAS EXCHANGE AND BIOCHEMICAL CHANGES OF OIL PALM (*Elaeis guineensis* Jacq.) SEEDLINGS AS AFFECTED BY IRON OXIDE NANOPARTICLES

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Iron oxide nanoparticles (Fe_3O_4 NPs) has a great potential to boost up plant growth. However, depending on the size, concentration and the plant type used, controversial results have been obtained. Therefore, their fate in the plant body should be investigated to lower the negative impacts and raising the beneficial impacts of the Fe_3O_4 NPs utilization on oil palm, major crop cultivated in Malaysia. Thus, the objectives of this study are 1) to investigate the impact of different Fe_3O_4 NPs concentrations i.e. (0, 800, 1600 and 2400 mg/L) on the growth, leaf gas exchange and biochemical changes of 3-months old oil palm seedlings and; 2) to study the physiological adaptation (growth, leaf gas exchange and biochemical properties) and uptake of Fe_3O_4 NPs by the 3-months old oil palm seedlings when exposed to low Fe_3O_4 NPs concentrations, i.e. (0, 200, 400 and 600 mg/L). Both experiments were arranged in a randomized complete block design (RCBD) and replicated three times. The first experiment revealed that the oil palm seedlings unable to tolerate even the lowest concentration of Fe_3O_4 NPs (800 mg/L). The plant growth was not significantly affected by Fe_3O_4 NPs, but it significantly ($p \leq 0.05$) reduced the SPAD chlorophyll value and the leaf total stomata density as compared to the control. Besides, the net photosynthesis is significantly reduced due to damage of photosynthetic apparatus of Fe_3O_4 NPs-stressed seedlings, in comparison to control. Moreover, the production of malondialdehyde (MDA) is positively correlated with the total volume of phenolics and total flavonoids. This observation indicates that an increase in MDA might be responsible for the up-regulation of the secondary metabolites production under high Fe_3O_4 NPs concentration application. Meanwhile, in the second experiment, the long-term exposure of low Fe_3O_4 NPs concentration application significantly reduced the plant height, total biomass, basal diameter, leaf number, total leaf area, and relative growth rate. Leaf gas exchange and chlorophyll fluorescence characteristics of treated seedlings decreased under high Fe_3O_4 NPs application, compared to the control. Electrolyte leakage (EL) and leaf respiration rate were gradually increased as the Fe_3O_4 NPs application elevated up to 600 mg/L. Proline, total phenolics, and iron content were significantly increased with Fe_3O_4 NPs application. The increasing

magnetic signal of electron spin resonance (ESR) spectra confirmed the existence of Fe_3O_4 NPs in root cells of the treatment plants. In conclusion, the uptake of Fe_3O_4 NPs at 200 to 2400 mg/L concentration dramatically reduced the oil palm seedlings growth as well as photosynthesis efficiency and production of secondary metabolites were elevated as physiological adaptation responses of oil palm seedlings to withstand the impact of Fe_3O_4 NPs application.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PERTUMBUHAN, PERTUKARAN GAS DAUN DAN PERUBAHAN BIOKIMIA ANAK POKOK KELAPA SAWIT (*Elaeis guineensis* Jacq.) YANG TERJEJAS AKIBAT NANOPARTIKEL BESI OKSIDA

Oleh

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Nanopartikel besi oksida (Fe_3O_4) mempunyai kebolehan untuk meningkatkan pertumbuhan pokok. Walaubagaimanapun, bergantung pada saiz, kepekatan dan jenis pokok, keputusan kontroversi telah ditemui. Oleh itu, kehadiran Fe_3O_4 dalam system pokok perlu dikaji untuk mengurangkan kesan negative dan meningkatkan kesan berguna bagi tujuan penggunaan pada pokok kelapa wait. Dengan itu, objektif eksperimen pertama adalah untuk menyiasat impak konsentrasi Fe_3O_4 yang berlainan (0, 800, 1600, 2400 mg/L) terhadap pertumbuhan, pertukaran gas daun dan perubahan biokimia anak pokok kelapa sawit berumur 3 bulan apabila dideahkan pada kepekatan yang tinggi dan; 2) untuk mengkaji adaptasi fisiologi (pertumbuhan, pertukaran gas daun dan biokimia) dan pengambilan Fe_3O_4 oleh anak pokok kelapa sawit berumur 3 bulan apabila terdedah kepada kepekatan Fe_3O_4 yang rendah iaitu (0, 200, 400 dan 600 mg/L). Kedua-dua eksperimen disusun dalam reka bentuk blok lengkap rawak (RCBD) dan direplikasi tiga kali. Eksperimen pertama menunjukkan bahawa anak kelapa sawit tidak dapat bertahan walaupun dengan kepekatan terendah Fe_3O_4 (800 mg/L). Pertumbuhan anak kelapa sawit tidak terjejas dengan ketara oleh Fe_3O_4, tetapi pengurangan nilai klorofil SPAD dan kepadatan keseluruhan stomata daun adalah ketara ($p \leq 0.05$). Selain itu, kadar fotosintesis menurun dengan ketara disebabkan gangguan pada peralatan fotosintesis pada pokok yang dirawat dengan Fe_3O_4. Tambahan pula, pengeluaran malondialdehid (MDA) telah membentuk korelasi positif dengan jumlah fenolik dan jumlah flavonoid. Ini menunjukkan bahawa peningkatan MDA mungkin bertanggungjawab terhadap pengawalseliaan pengeluaran metabolit sekunder di bawah kepekatan Fe_3O_4 yang tinggi. Sementara itu, dalam eksperimen kedua, pendedahan jangka panjang konsentrasi rendah Fe_3O_4 telah mengurangkan ketinggian anak pokok, jumlah biojisim, ukur lilit pangkal, jumlah daun, luas daun, dan kadar pertumbuhan relatif dengan ketara. Pertukaran gas daun dan ciri-ciri pendarfluor klorofil anak pokok yang dirawat telah menurun di bawah konsentrasi Fe_3O_4 yang tinggi, berbanding dengan kawalan. Kadar kebocoran elektrolit (EL) dan kadar pernafasan daun didapati telah menaik secara beransur-ansur dipertingkatkan dengan peningkatan kepekatan Fe_3O_4 sehingga 600 mg/L. Peningkatan pengeluaran proline, jumlah fenol dan kandungan besi

diperhatikan di bawah kepekatan Fe_3O_4 yang tinggi. Peningkatan isyarat magnetik spektrum oleh resonans spin elektron (ESR) mengesahkan kewujudan Fe_3O_4 dalam sel tisu akar anak pokok yang terawat. Kesimpulannya, pengambilan Fe_3O_4 dari konsentrasi 200 hingga 2400 mg/L telah menurunkan kadar pertumbuhan anak pokok sawit, kecekapan pertukaran gas daun dan peningkatan metabolit sekunder sebagai adaptasi fisiologi anak pokok sawit untuk bertahan dari kesan penggunaan Fe_3O_4.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS AND SYMBOLS

%	percent
‘	minute
*	significant at 0.05 probability level
**	significant at 0.01 probability level
***	significant at 0.001 probability level
µmol/m^2/s	micro mole per meter square per second
µmol/mol	micro mole carbon dioxide per mole air
^oC	degree-celcius
A	net photosynthesis
ANOVA	analysis of variance
B	boron
cm^2	centimeter square
C_i	intercellular carbon dioxide concentration
CO_2	carbon dioxide
Df	degree of freedom
D X P	*dura* cross by *pisifera*
DNMRT	duncan new multiple range test
E	transpiration rate
ENPs	engineered nanoparticles
ENM	engineered nanomaterials
ESR	electron spin resonance
F_m	maximal fluorescence
F_o	minimal fluorescence
F_v	variable fluorescence

Fe_3O_4	magnetic iron oxide
Fe^{2+}	ferrous ion
Fe^{3+}	ferric ion
F_v/F_m	maximum quantum efficiency of psII system
g	gram
g_s	stomata conductance
H_2O_2	hydrogen peroxide
IMP3	Third Industrial Master Plan
K	potassium
kg	kilogram
Mg	magnesium
mg/L	miligram per liter
$mmol/m^2/s$	millimole per meter square per second
mm	milimeter
mm/day	milimeter per day
MPOB	Malaysia Palm Oil Board
n	number of samples
N	nitrogen
nm	nanometer
NPs	nanoparticles
n.s	not-significant
OH	hydroxyl radical
p	probability
P	phosphorus
PEN	Project on Emerging Nanotechnologies

PSII	photosystem II
R	respiration rate
RCBD	randomized complete block design
RGR	relative growth rate
RH	relative humidity
ROS	reactive oxygen species
r.p.m	relations per minute
SPAD	soil plant analytical development
t	time
TE	Trace element
WUE	water use efficiency
WAT	weeks after start of treatment

CHAPTER 1

INTRODUCTION

Nanotechnology is an expanding industry that has practiced worldwide in all sectors of life including agriculture. A wide range of nanoparticles (NPs) impacts on plants has been proven, which exhibits that the positive and negative impacts depend on their size, concentration and the plant type used (Tombuloglu et al., 2019). It has been estimated that the production of NPs will rise to 58,000 tons by 2020 and iron oxide NPs will be the major contributor (Maynard et al., 2006).

Bulk iron oxides have been modified to nanosized particles (1-100nm size), which increasing supply of iron to plants. Thus, iron oxide NPs have a great potential in making their way in agriculture and other industries development due to their unique properties, such as high surface energy, increased surface area-to-volume ratio, and a number of catalytic properties (Sun et al., 2015). Based on the uniqueness of these characteristics, magnetite (Fe_3O_4), have gained more importance among the other iron oxide NPs (Wu et al., 2015).

The first research using Fe_3O_4 NPs in plants was made by Zhu et al. (2008), who showed a significant Fe_3O_4 NPs uptake, translocation and accumulation in various tissues of pumpkin (*Cucurbita maxima*) plants without toxic effects at concentration of 500 mg/L. Ghafariyan et al. (2013) showed that Fe_3O_4 NPs enter from soybean roots, translocated in the aerial part and increase the chlorophyll content and enzymatic efficiency of photosynthesis.

Differ from the beneficial uses of Fe_3O_4 NPs, researchers have identified contradicting findings when plants were treated with high concentration for long durations of exposure (Bombin et al., 2015; Shukla et al., 2003). The inhibitory effects impacted the seed germination, growth of seedling and metabolic process that are directly proportional to yield production (Bombin et al., 2015).

Along with positive and negative effects of Fe_3O_4 NPs on plant growth and development, the extensive use of Fe_3O_4 NPs in every aspect of life have raise concerns about its impacts on environmental issues. In example, Malaysia export huge amount of iron ore that mostly carrying 60% magnetite, which is one of the main raw materials to make steel. In Pengerang Johor, 146-hectare ex-bauxite mine land (formerly occupied by oxisols) have been planted with oil palm. Oxisols is soil of tropical region dominated by iron oxide. Over years, the oil palm trunk became smaller due to lack macronutrients and excess toxic metals present cause nutrient imbalance (Shamshuddin, 2016).

Hence, to obtain the maximum benefits from Fe_3O_4 NPs instead of adverse impacts, it is a crucial need for further research and more critical investigation about effects of Fe_3O_4 NPs on physiological, photosynthesis and biochemical in gaining a better understanding

about the plant health status after exposed to Fe_3O_4 NPs application. With that, the current study investigates the impact of Fe_3O_4 NPs to the growth and development of oil palm (*Elaeis guineensis* Jacq.), one of the widely cultivated and economically important crop in the world. The objectives of this study are 1) to investigate the impact of different Fe_3O_4 NPs concentrations i.e. (0, 800, 1600 and 2400 mg/L) on the growth, leaf gas exchange and biochemical changes of 3-months old oil palm seedlings and; 2) to study the physiological adaptation (growth, leaf gas exchange and biochemical changes) and uptake of Fe_3O_4 NPs by the 3-months old oil palm seedlings when exposed to low concentrations, i.e. (0, 200, 400 and 600 mg/L).

It is hypothesized that oil palm treated with high concentration of Fe_3O_4 NPs would initiate clogging effects and their potential adherence to the root surface causes adverse effects on growth due to decreasing leaf gas exchange characteristics. It is also hypothesized that secondary metabolites (total phenolics and total flavonoids) would be enhanced as adaptive response towards long exposure of low Fe_3O_4NPs concentration. The Fe_3O_4 NPs would be expected to show less aggregation and lead to higher uptake that has been shown with increased electron spin resonance signal.

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