

# **UNIVERSITI PUTRA MALAYSIA**

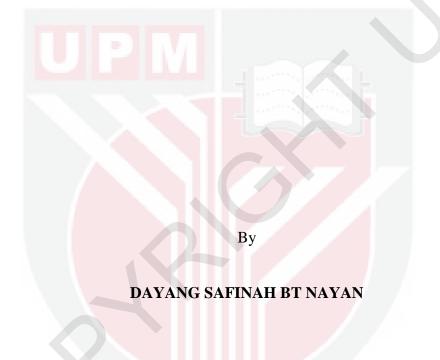
SELENIUM BIOFORTIFICATION OF GREEN SPINACH (Amaranthus spp.) AFFECTED BY SOIL TYPES, PHOSPHORUS FERTILIZATION AND SELENIUM SOURCES, AND APPLICATION TIMING

**DAYANG SAFINAH BT NAYAN** 

FP 2021 15



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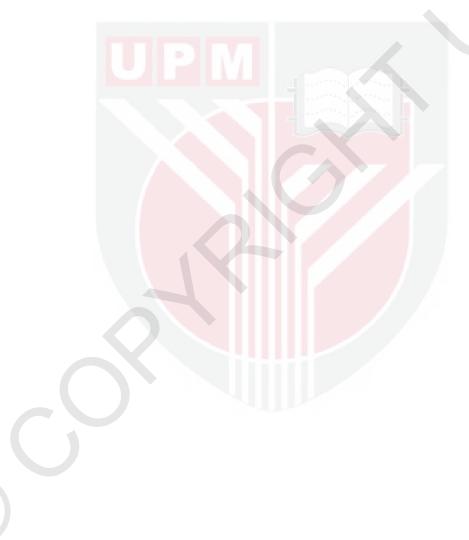
Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

July 2020

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

### SELENIUM BIOFORTIFICATION OF GREEN SPINACH (Amaranthus spp.) AFFECTED BY SOIL TYPES, PHOSPHORUS FERTILIZATION AND SELENIUM SOURCES, AND APPLICATION TIMING

By

### DAYANG SAFINAH BT NAYAN

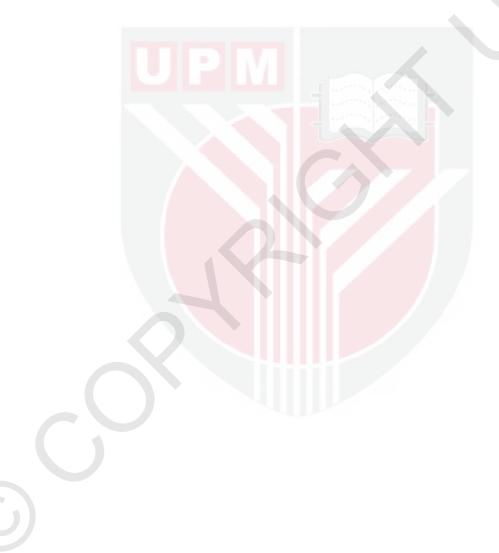
**July 2020** 

Chairman: Professor Che Fauziah bt Ishak, PhDFaculty: Agriculture

In selenium (Se) biofortification strategy of green spinach through fertilization, the mechanism or process that affect its availability in soils and uptake by plants is very important to understand. Therefore, in this study, selenite, Se (IV) and selenate, Se (VI) adsorption and desorption in soil type that are commonly grown with vegetables in Malaysia were evaluated. Adsorption-desorption study in soils of various textures was performed with Se concentration from 0-2 mg/L in the form of Se (IV) and Se (VI), respectively. Next, to determine the effect of various species of applied Se on dry matter, Se uptake, and antioxidant activity of green spinach, a glasshouse experiment was conducted in a factorial (2x5) completely randomized design, with two forms of Se (Se (IV) and Se (VI)) and five Se rates (0-60 g Se/ha as soil application in solution form). Then, a factorial (3x4) randomized complete block design experiment was conducted under a glasshouse condition to determine the effect of P on Se accumulation in green spinach. Green spinach was applied with Se (IV) at rates 0-180 g/ha as sodium selenite. Phosphorus fertilizer was applied at 0, 50, and 100% from the recommended amount of 23 kg/ha P<sub>2</sub>O<sub>5</sub>. And lastly, a field experiment was conducted in randomized complete block design to evaluate the effect of Se application timing on the dry matter, growth, and Se accumulation in plant. Based on adsorption-desorption study, selenite was adsorbed more (0.02 - 19.25 mg/kg) in all studied soil compared to selenate, (0.01 – 12.39 mg/kg). However, more Se (VI) was desorbed which is between 0 - 100%, as compared to Se (IV) which is only 0 - 66.7%. From glasshouse experiment, Se uptake by leaves was higher when plant was applied with Se (VI) which is 12.58  $\mu$ g/10 plants as compared to Se (IV), with only 9.01  $\mu$ g/10 plants. However, higher dry matter and antioxidant activity of green spinach were observed when Se (IV) was applied. In general, Se as Se (IV) has been shown to have more beneficial effect on plant yield, as well as the antioxidant activity in green spinach as compared to Se (VI) and conventionally grown green spinach (0 g/ha Se). Results from this study also show that the highest dry matter was obtained when Se



was applied at 120 g/ha with 100% of P fertilizer (106 g/10 plants). In leaves and stems, the highest uptake was observed when 120 g/ha Se with 100% of P fertilizer was applied with 23.07  $\mu$ g/10 plants and 7.40  $\mu$ g/10 plants, respectively. Applying P fertilizer as recommended could increase yield as well as the Se uptake by leaves and stems of green spinach. Selenium as Se (IV) at 120 g/ha applied to sandy clay soil was considered as the optimum rate as higher rates could reduce plant yield and decrease the Se uptake by green spinach. During the field experiment, plant yield was affected by the Se application timing. In general, single application of Se at 14 DAP gave the best result in terms of growth of the plant during the field experiment. Although the highest Se accumulation in leaves was observed when Se was applied at 7 DAP, the decrease in yield cause it to be regarded as inappropriate time of application.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

### BIOFORTIFIKASI SELENIUM PADA BAYAM HIJAU (Amaranthus spp.) YANG DIPENGARUHI OLEH JENIS TANAH, PEMBAJAAN FOSFORUS DAN SUMBER SELENIUM, SERTA WAKTU APLIKASI

Oleh

#### **DAYANG SAFINAH BT NAYAN**

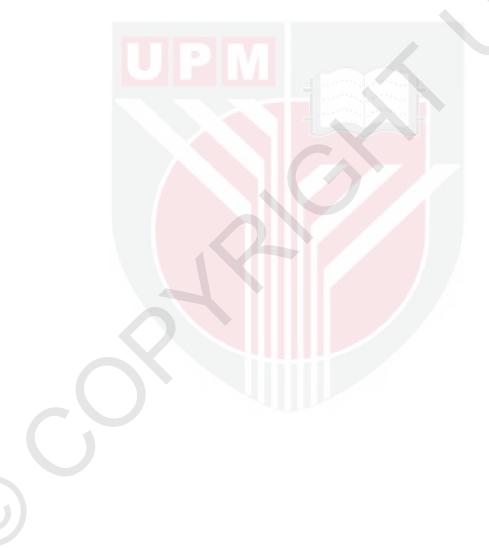
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Pengerusi : Profesor Che Fauziah Ishak, PhD Fakulti : Pertanian

Dalam strategi biofortifikasi selenium (Se) pada bayam hijau melalui pembajaan, mekanisme atau proses yang mempengaruhi ketersediaan Se di tanah dan ambilannya oleh tanaman adalah penting untuk difahami. Oleh itu, dalam kajian ini, adsorpsi dan desorpsi selenit, Se (IV) dan selenate, Se (VI) di jenis tanah yang lazimnya ditanam sayuran berdaun di Malaysia dinilai. Kajian adsorpsi-desorpsi di tanah pelbagai tekstur dilakukan pada kepekatan Se dari 0-2 mg/L masing-masing dalam bentuk Se (IV) dan Se (VI). Selepas itu, untuk menentukan kesan pelbagai spesies Se pada berat kering, ambilan Se, dan aktiviti antioksidan bayam hijau, eksperimen rumah kaca dilakukan dalam reka bentuk rawak faktorial (2x5) sepenuhnya, dengan dua bentuk Se (Se (IV) dan Se (VI)) dan lima kadar Se (0-60 g/ha Se sebagai aplikasi tanah dalam bentuk larutan). Kemudian, eksperimen reka bentuk blok lengkap secara faktorial (3x4) dilakukan di rumah kaca untuk menentukan kesan P terhadap ambilan Se pada bayam hijau. Bayam hijau diaplikasikan dengan Se (IV) pada kadar 0-180 g/ha Se sebagai natrium selenit. Baja fosforus digunakan pada kadar 0, 50, dan 100% dari jumlah yang disarankan iaitu 23 kg/ha P2O5. Akhir sekali, eksperimen lapangan dilakukan dalam reka bentuk blok lengkap secara rawak untuk menilai kesan masa aplikasi Se pada berat kering, pertumbuhan, dan ambilan Se oleh tanaman. Berdasarkan kajian adsorpsi-desorpsi, selenite dijerap lebih banyak (0.02-19.25 mg/kg) di semua tanah yang dikaji berbanding dengan selenate, (0.01–12.39 mg/kg). Namun, Se (VI) lebih banyak terdesorpsi iaitu antara 0-100%, dibandingkan dengan Se (IV) dengan hanya 0-66.7%. Dari kajian rumah kaca, pengambilan Se oleh daun lebih tinggi ketika tanaman diaplikasi dengan Se (VI) iaitu 12.58 µg/10 pokok dibandingkan dengan Se (IV), dengan hanya 9.01 µg/10 pokok. Walau bagaimanapun, berat kering dan aktiviti antioksidan bayam hijau yang lebih tinggi diperhatikan semasa Se (IV) digunakan. Secara umum, Se sebagai Se (IV) menunjukkan kesan yang lebih bermanfaat terhadap hasil tanaman, serta aktiviti antioksidan pada bayam hijau dibandingkan dengan Se (VI) dan bayam hijau yang ditanam secara konvensional (0



g/ha Se). Hasil dari kajian ini juga menunjukkan bahawa berat kering tertinggi diperoleh ketika Se diaplikasikan pada kadar 120 g/ha dengan 100% baja P (106 g/10 pokok). Pada daun dan batang, ambilan Se tertinggi diperhatikan ketika 120 g/ha Se dengan 100% baja P diaplikasi dengan masing-masing 23.07 µg/10 pokok dan 7.40 µg/10 pokok. Menggunakan baja P seperti yang disarankan dapat meningkatkan hasil serta ambilan Se oleh daun dan batang bayam hijau. Selenium sebagai Se (IV) pada 120 g/ha yang diaplikasi ke tanah liat berpasir dianggap sebagai kadar optimum kerana kadar yang lebih tinggi dapat mengurangkan hasil dan ambilan Se oleh bayam hijau. Senasa eksperimen lapangan, hasil tanaman dipengaruhi oleh masa aplikasi Se. Secara umum, aplikasi tunggal Se pada 14 DAP memberikan hasil terbaik dari segi pertumbuhan tanaman semasa eksperimen di lapangan. Walaupun ambilan Se tinggi pada daun ketika Se diaplikasi pada 7 DAP, pengurangan hasil menyebabkan ianya dianggap sebagai waktu aplikasi yang tidak sesuai.



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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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# LIST OF ABBREVIATIONS

WHO	World Health Organization
GPX	Glutathione Peroxidase
GSH-Px	Glutathione Peroxidase
SOD	Superoxide Dismutase
RDA	Recommended Daily Amount
NOAEL	Non-Observed Adverse Effect Level
LOAEL	Lowest Observed Adverse Effect Level
UL	Upper Tolerable Intake
FAO	Food and Agriculture Organization
RDA	Recommended Dietary Allowance
FA	Fulvic Acid
HA	Humic Acid
SeMet	Selenomethionine
SeCys2	Selenocystine
MeSeCys	Se-methylselenocysteine
TBARS	Thiobarbituric Acid Reactive Substances
OC	Organic Carbon
CEC	Cation Exchange Capacity
AAS	Atomic Absorption Spectrophotometer
GF-AAS	Graphite Furnace Atomic Absorption Spectrophotometer
EPA	United States Environmental Protection Agency
OM	Organic Matter
CRD	Completely Randomized Design

RCBD	Randomized Complete Block Design	
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- DPPH 2,2-Diphenyl-1-picryl-hydrazyl-hydrate
- HSD Honestly Significant Difference
- LSD Least Significant Difference
- ANOVA Analysis of Variance
- DOA Department of Agriculture
- NIST National Institute of Standard & Technology
- DAP Days After Planting
- LAI Leaf Area Index
- PAR Photosynthetically Active Radiation
- P Phosphorus
- TF Translocation Factor

### **CHAPTER 1**

#### **INTRODUCTION**

Selenium (Se) is important to humans as it contributed to many physiological processes in human health. According to Lenny et al. (2015), 30  $\mu$ g/day of Se is insufficient for humans, while intake more than 900  $\mu$ g/day of Se is considered harmful. Due to the narrow gap between essential and toxic intake of Se, World Health Organization (WHO) has established dietary requirements of Se and its upper safe intake level for adults at 55  $\mu$ g and 400  $\mu$ g/day, respectively (WHO, 2011). However, according to Haug et al. (2007), majority of the world is still facing suboptimal Se intake rather than toxicity, therefore, an increase in diseases that involve increasing levels of oxidative stress is expected.

The data on micronutrients especially for Se among populations in Malaysia are still lacking. A study by Ishak et al. (2005) on aborigines in Pahang and Perak shows that the mean of serum Se concentration for aborigines was significantly lower when compared to other healthy populations in Italy. In addition, a study done by Rejali et al. (2007) conducted in a hospital in Malaysia on sixty-two newly diagnosed breast cancer patients found that the mean Se concentration among the cases was significantly lower than that among the control. There was a significant relation between breast cancer and low Se serum level and an increased Se concentration contributes to a reduced risk of breast cancer. Hence, with all those issues and health problem associated with Se, biofortification of food crops is necessary for an enhancement of human nutrition and health status.

Biofortification has been defined as the process of increasing the concentration of essential nutrients in the edible parts of food crops through agronomic practices. Biofortification by mineral fertilizer application is frequently implemented in some countries by applying selenium-containing fertilizers and this is as a short-term solution for improving selenium content in plants (Rosell., 2016). In Malaysia, vegetables have been expressed as the lowest Se content in Malaysian food source as compared to seafoods, meats, eggs, rice and fruit (Ali et al., 1991). According to Dayang et al. (2012), Se concentration of the Malaysia soils ranges from 0.008 to 7 mg/kg with a mean concentration of 0.7 mg/kg. Meanwhile, Se concentrations in plants are also quite low (0.016 to 1.392 mg/kg) with mean concentration of 0.09 mg/kg.

Food crop such as vegetables can be low in Se because of low Se status in the soil, or because of the condition that might lower the availability of soil Se for plants uptake. As mentioned by Lenny et al. (2015), there are various factors that could affect Se accumulation by plants (e.g. soil pH, soil organic matter, Se species, competitive anions, etc). Therefore, it is important to determine which factors largely affect Se uptake by plants and to understand the mechanism responsible in order to achieve the more effective Se biofortification programme for vegetable.

Based on Petro et al. (2015), there are various vegetables that has been tested on the ability as Se accumulator plants, therefore, in this study, leafy vegetable that is frequently bought and consumed by various community in Malaysia, which is green spinach (*Amaranthus spp.*) was investigated for its ability to accumulate Se and to be explored for the possibilities to be used as a functional food. Moreover, biofortification of leafy vegetable with Se is a more economical practice since most of applied Se tends to accumulate in the edible parts on the plants (leaves & stems).

In this study, effect of Se species (e.g. selenite, selenate) and competitive anion (e.g. phosphorus) and application timing were emphasized in order to obtain the optimum Se biofortification programme for selected vegetable. Although many studies had observed higher uptake with selenate, Se (VI) application, study on the behaviour of both Se species in soil and its mobility specifically in soil that represent the vegetables cultivation areas in Malaysia is needed, since no such study has ever been done in Malaysia. Vegetable cultivation practices in Malaysia also apply high rates of macronutrients fertilizer. Interactions of Se with macronutrients can affect the effectiveness of agronomic biofortification (Valença et al., 2017). Hence, effect of the application of these macronutrients especially phosphorus on Se uptake by vegetables need to be studied. According to Ros (2016), Se application timing also was found to be the main factor that enhanced plant Se uptake. Although cultivation period for green spinach is short, appropriate Se application timing need to be identified to get an effective package for Se fertilization management.

## 1.1 Objectives

The general objective of this study was to develop an effective selenium biofortification package for green spinach. The specific objectives were as follow:

- i. To compare selenite, Se (IV) and selenate, Se (VI) adsorption/desorption capacity in soils of various texture,
- ii. To evaluate the effect of applied Se species (Se (IV) and Se (VI)) on dry matter and the uptake of Se by green spinach,
- iii. To determine the effect of phosphorus (P) fertilization on Se uptake by green spinach,
- iv. To determine optimum P & Se fertilization rates for high yield and Se uptake by green spinach, and
- v. To evaluate the effect of Se application timing on the dry matter, growth and Se accumulation in green spinach.

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### **BIODATA OF STUDENT**

Dayang Safinah Nayan is a research officer at Malaysia Agriculture Research & Development Institute (MARDI), Serdang, Selangor. In the institute, she was assigned to the Soil Science, Water and Fertilizer Research Center. After serving for five years, she was awarded with full-paid study leave to further her studies in Doctoral Degree at Universiti Putra Malaysia (UPM). She holds a degree in Bachelor of Science (Applied Chemistry) from Universiti Malaya and Master of Science (Land Resource Management) from Universiti Putra Malaysia. Her field of study is more focused on soil chemistry, nutrient management as well as plant nutrition. After graduation, she continues to serve in the same department to conduct research that can drive agricultural productivity and sustainability, especially in the field of fertilizer technology.



### LIST OF PUBLICATIONS

- Dayang, S.N., Farahzety, A.M., and Che Fauziah, I. 2016. Selenium Biofortification of Green Spinach Applied with Selenate and Selenite. Technical Report 2016, Crop & Soil Science Research Center MARDI
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