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

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

By

DAYANG SAFINAH BT NAYAN

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

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, PhD
Faculty : 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_2O_5. 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 µg/10 plants as compared to Se (IV), with only 9.01 µg/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 µg/10 plants and 7.40 µg/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

Julai 2020

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 P_2O_5. 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. Semasa 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.

ACKNOWLEDGEMENTS

First and foremost, I thank Allah S.W.T. for giving me bless and strength to complete this study. I also like to express the deepest appreciation to the chairman of my supervisory committee, Professor Doctor Che Fauziah Ishak for her support and guidance along the course of this study and during the preparation of this thesis.

I would also like to acknowledge my supervisory committee members, Dr. Arina Shairah Abdul Sukor, Dr. Martini Mohammad Yusof as well as Dr. Rozlalily Zainol for their useful comments, remarks and engagement throughout the course of this study.

I must express my sincere gratitude to my beloved parents, my husband, my son and my siblings for their prayers, love, sacrifice and support they have been given all this time. Last but not least, I would like to acknowledge all those who have helped me, which made this study a success.

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:

Che Fauziah Ishak, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chaiman)

Arina Shairah Abdul Sukor, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Martini Mohammad Yusof, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Rozlalily Zainol, PhD
Director
Industrial Crop Research Centre
Malaysia Agriculture Research and Development Institute
(Member)

____

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 January 2021

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: ________________________ Date: ____________________

Name and Matric No.: Dayang Safinah bt Nayan, GS46879

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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
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 µg/day of Se is insufficient for humans, while intake more than 900 µg/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 µg and 400 µg/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.

REFERENCES

Abat M., McLaughlin M.J., Kirby J.K., Stacey S.P., 2012. Adsorption and desorption of copper and zinc in tropical peat soils of Sarawak, Malaysia. Geoderma, 175-176: 58-63

Abdalla A.L., Regitano J.B., Tornisielo V.L., Marchese L., Pecanha M.R.S.R., Vitti D.M.S.S., Smith T., 2005. Biodegradation of polyethylene glycol (PEG) in three tropical soils using radio labelled PEG. Animal Feed Science and Technology, 122 (1-2): 187-193

Alfthan G., Eurola M., Ekholm P., Venäläinen E.R., Root T., Korkalainen K., Hartikainen H., Salminen P., Hietaniemi V., Aspila P., Aro A., 2015. Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. Journal of Trace Elements in Medicine and Biology, 31: 142–147

Ali D.A., Ismail G., Peterson P.J. 1991. Selenium in foods of animal and plant origin in Malaysia. Malaysian applied biology. ISSN: 0126-8643

Ali F. El M., Ray J. B. R., Christine N. P., Stormy D. L., Jennifer J. C., Sirine C. F., Elizabeth A. H. P. S., 2014. Analysis of selenium accumulation, speciation and tolerance of potential selenium hyper accumulator Symphyotrichum ericoides. Physiologia Plantarum, 152: 70–83

Ali M., Daniel L., Jeffers and Paul R. Henderlong., 2003. Interrelationship between leaf area, light interception and growth rate in a soybean-wheat system. Asian Journal of Plant Sciences, 2: 605-612

Alifar N., Zaharah A.R., Ishak C.F., Awang Y., Khayambashi B., 2014. Selenium enrichment of paddy rice grains in Malaysia. Asian Journal of Crop Science, 6 (4): 345-353

Avila F.W., Yang Y., Faquin V., Ramos S.J., Guilherme L.R., Thannhauser T.W., Li L., 2014. Impact of selenium supply on Se-methylselenocysteine and glucosinolate accumulation in selenium-biofortified Brassica sprouts. Food Chemistry, 165: 578-586

Black C.A and Klute A., (Ed)., 1965. Methods of soil analysis: Part 1 - Physical and mineralogical properties, including statistics of measurement and sampling, (Number 9.1 in the series, Agronomy). ASA, Madison Wisconsin USA

Black C.A., Evan D.D., White J.L., Ensminger L.E., and Clark F.E., (Ed)., 1965. Methods of soil analysis, Part 2 – Chemical and microbiological properties (Number 9 in the series, Agronomy). ASA, Madison Wisconsin USA

Blasco B., Rios J.J., Cervilla L.M., Sanchez-Rodrigez E., Ruiz J.M., Romero L., 2008. Iodine biofortification and antioxidant capacity of lettuce: potential benefits for cultivation and human health. Annals of Applied Biology, 152 (3): 289-299

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, 29:1199-1200

Bocchini M., D'Amato R., Ciancaleoni S., Fontanella M.C., 2018. Soil selenium (Se) biofortification changes the physiological, biochemical and epigenetic responses to water stress in *Zea mays* L. by inducing a higher drought tolerance. Frontiers in Plant Science, Volume 9: Article 389. DOI: 10.3389/fpls.2018.00389

Bray R.H. and Kurtz L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils. Soil Science, 59 (1): 39-46

Carter D.L, Robbins C.W and Brown M.J., 1972. Effects of phosphorus fertilization on the selenium concentration in alfalfa. Soil Science Society of America, Proceedings 36: 624–628

Cartes P., Gianfreda L., Mora M.L., 2005. Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. Plant and Soil, 276: 359–367

Carvalho S.M.P. and Vasconcelos M.W., 2013. Producing more with less: Strategies and novel technologies for plant-based food biofortification. Food Research International, 54: 961-971

Christophersen O.A., Lyons G., Haug A., Steinnes E., 2012. Chapter 16: Selenium in BJ Alloway (ed.) Heavy metals in soils: trace elements and metalloids in soils and their bioavailability. Third edition. Environmental Pollution 22. Dordrecht: Springer

Dayang S.N. and Che Fauziah I., 2013. Soil factors influencing heavy metal concentrations in medicinal plants. Pertanika Journal of Tropical Agricultural Science, 36 (2): 161-178

Dayang S.N., Che Fauziah I., Umi Kalsom Y., 2012. Influence of selenium on the antioxidant activity of Mas cotek (*Ficus deltoidea*) as affected by soil acidity and phosphorus (P) fertilization. Journal of Medicinal Plants Research, 6(45): 5662-5668

Denoroy P., 1996. The crop physiology of *Helianthus tuberosus L.*: A model-oriented view. Biomass and Bioenergy, 11 (1): 11-32

Department of Agriculture Malaysia, 2019. Booklet statistik tanaman (Sub-sektor tanaman makanan.

Diaz-Alarcon J.P., Navarro-Alarcon M., Garcia de la Serrana H.L., Asensio-Drima C., Lopez-Martinez M.C., 1996. Determination and chemical speciation of selenium in farmlands from Southeastern Spain: Relation to levels found in sugar cane. Journal of Agricultural and Food Chemistry, 44: 2423−2427

Dowdle P.R., Oremland R.S., 1998. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. Environmental Science and Technology, 32 (23): 3749-3755

Eich-Greatorex S., Krogstad T., Sogn T.A., 2010. Effect of phosphorus status of the soil on selenium availability. Journal of Plant Nutrition and Soil Science, 173: 337–344

Emanueli d.N.d.S., Aureli F., D'Amato M., Raggi A., Cadore S., Cubadda F., 2017. Selenium bioaccessibility and speciation in selenium-enriched lettuce: Investigation of the selenocompounds liberated after in vitro simulated human digestion using two-dimensional HPLC-ICP-MS. Journal of Agriculture and Food Chemistry, 65 (14): 3031-3038

FAMA, 2020. Retrieved from http://www.fama.gov.my/web/pub/harga-pasaran-terkini

Fan J., Wang R., Hu H., Huo G., Fu Q., Zhu J., 2015. Transformation and bioavailability of selenate and selenite added to a *Nicotiana tabacum L.* planting soil. Communications in Soil Science and Plant Analysis, 46 (11): 1362-1375

FAO/WHO., 2002. Selenium. in: Human vitamin and mineral requirements. Report of a Joint FAO/WHO Expert Consultation. FAO, Rome; pp 235-255

Ferrarese M., Sourestani M.M., Quattrini E., Schiavi M., Ferrante A., 2012. Biofortification of spinach plants applying selenium in the nutrient solution of floating system. Vegetable Crops Research Bulletin, 76: 127-136

Fordyce F., 2013. Selenium deficiency and toxicity in the environment. in: Selinus, O., (ed.) Essentials of medical geology. Elsevier, 373-415

Frias J., Gulewicz P., Villaluenga C.M., Pilarski R., 2009. Influence of germination with different selenium solutions on nutritional value and cytotoxicity of lupin seeds. Journal of Agricultural and Food Chemistry, 57 (4): 1319-1325

Gabos M.B., Goldberg S., Alleoni L.R.F., 2014. Modelling selenium (IV) and (VI) adsorption envelopes in selected tropical soils using the constant Capasitance Model. Environmental Toxicology and Chemistry, 33 (10): 2197- 2207

Garg M., Sharma N., Sharma S., Kapoor P., Kumar A., Chunduri V., Arora P., 2018. Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. Frontiers in Nutrition, 5:12

Garousi F., 2017. Toxicity of selenium, application of selenium in fertilizers, selenium treatment of seeds, and selenium in edible parts of plants. Acta Universitatis Sapientiae, Alimentaria, 10: 61-74

Goh K.H. and Lim T.T., 2004. Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption. Chemosphere, 55: 849–859

Goodson C.C. Parker D.R., Amrhein C., Zhang Y., 2003. Soil selenium uptake and root system development in plant taxa differing in Se-accumulating capability. New Phytologist, 159 (2): 391-401

Gribaldi G., Nurlaili N., Dewi N., Danial E., Sakalena F., Suwignyo R.A., 2017. Modified application of nitrogen fertilizer for increasing rice variety tolerance toward submergence stress. International Journal of Agronomy. Article ID 9734036, 6 pages. https://doi.org/10.1155/2017/9734036

Gupta M. and Gupta S. 2017. An overview of selenium uptake, metabolism and toxicity in plants. Frontiers in Plant Science, 7:2074. DOI: 10.3389/fpls.2016.02074

Hartikainen H., 2005. Biogeochemistry of selenium and its impact on food chain quality and human health. Journal of Trace Elements in Medicine & Biology, 18: 309-318

Hartikainen H., Xue T.L., Piironen V., 2000. Selenium as an antioxidant and pro oxidant in ryegrass. Plant and Soil, 225 (1-2): 193-200

Haug A., Graham R.D., Christophersen O.A., Lyons G.H., 2007. How to use world's scarce selenium resources efficiently to increase the selenium concentration in food. Microbial Ecology in Health and Disease, 19: 209-228.

He H., Pang J., Wu G.L., Lambers H., 2019. The application potential of coal fly ash for selenium biofortification. Advances in Agronomy, 157: 1-54

Hossain S.A.A.M., Wang L., Chen T., Li Z., 2017. Leaf area index assessment for tomato and cucumber growing period under different water treatments. Plant Soil Environment, 63 (10): 461-467

Irmak S., 2017. Effects of selenium application on plant growth and some quality parameters in peanut (*Arachis hypogaea*). Pakistan Journal of Biological Sciences, 20 (2): 92-99

ISO 11466. 1995. Soil quality: extraction of trace elements soluble in aqua regia. ISO, Geneva, Switzerland

Ji Y., Wang Y.T., 2017. Selenium reduction by batch cultures of *escherichia coli* strain EWB32213. Journal of Environmental Engineering, 143 (6): 1-7

Johnsson L., 1991. Selenium uptake by plants as a function of soil type, organic matter content and pH. Plant and Soil, 133: 57–64

Kapolna E., Fodor P., 2007. Bioavailability of selenium from selenium-enriched green onions (*Allium fistulosum*) and chives (*Allium schoenoprasum*) after 'in vitro' gastrointestinal digestion. International Journal of Food Science & Nutrition, 58 (4): 282-296

Kolachi N.F., Kazi T.G., Afridi H.I., Khan S., Wadhwa S.K., Shah A.Q., Shah F., Baig J.A., Sirajuddin., 2010. Determination of selenium content in aqueous extract of medicinal plants used as herbal supplement for cancer patients. Food and Chemical Toxicology, 48 (12): 3327-3332

Krystyna P., 2009. Selenium speciation in enriched vegetables. Food Chemistry, 114: 1183-1191

Kuan W.H., Lo S.L., Wang M.K., Lin C.F., 1998. Removal of Se (IV) and Se (VI) from water by aluminum-oxide-coated sand. Water Research, 32 (3): 915-923

Kuban P., Kuban P., Kuban V., 2004. Rapid speciation of Se (IV) and Se (VI) by flow injection-capillary electrophoresis system with contactless conductivity detection. Analytical and Bioanalytical Chemistry, 378 (2): 378-382

Kumar J., Gupta D.S., Kumar S., Gupta S., Singh N.P., 2016. Current knowledge on genetic biofortification in lentil. Journal of Agriculture and Food Chemistry, 64 (33): 6383-6396

Laitonjam W.S., Kongbrailatpam B.D., 2010. Studies on the chemical constituents and antioxidant activities of extracts from the roots of *Smilax lanceaefolia Roxb*. Natural Product Research, 24 (12): 1168-1178

Lavu R., Du Laing G., Van de Wiele T., Pratti V. L., Willekens K., Vandecasteele B., Tack F., 2012. Fertilizing soil with selenium fertilizers: Impact on concentration, speciation, and bioaccessibility of selenium in leek (*Allium ampeloprasum*). Journal of Agricultural and Food Chemistry, 60(44): 10930–10935

Lee S.E., Hwang H.J., Ha J.S., Jeong H.S., Kim J.H., 2003. Screening of medicinal plant extracts for antioxidant activity. Life Sciences, 73 (2): 167-179

Lenny H.E.W., Bas V., Gerrad D.J., Leila S.S., Elizabeth P.S. and Gary S.B., 2015. Selenium cycling across soil-plant-atmosphere interfaces: A critical review. Nutrients, 7: 4199-4239

Lessa J.H.L., Araujo A.M., Silva G.N.T., Guilherme L.R.G., Lopes G., 2016. Adsorption-desorption reactions of selenium (VI) in tropical cultivated and uncultivated soils under Cerrado biome. Chemosphere, 164: 271-277

Li H.F., McGrath S.P., Zhao F.J., 2008. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. New Phytologist, 178: 92–102

Li X., Wu Y., Li B., Yang Y., Yang Y., 2018. Selenium accumulation characteristics and biofortification potentiality in turnip (*Brassica rapa var. rapa*) supplied with selenite or selenate. Frontiers in Plant Science, 8:2207. DOI: 10.3389/fpls.2017.02207

Liu H., Shi Z., Li J., Zhao P., Qin S., Nie Z., 2018. The impact of phosphorus supply on selenium uptake during hydroponics experiment of winter wheat (*Triticum aestivum*) in China. Frontiers in Plant Science, 9:373. DOI: 10.3389/fpls.2018.00373

Liu Q., Wang D.J., Jiang X. J., Cao Z. H., 2004. Effects of the interactions between selenium and phosphorus on the growth and selenium accumulation in rice (*Oryza sativa*). Environmental Geochemistry and Health, 26: 325–330

Loganathan P. and Hedley M.J., 2006. Spatial and time dependent patterns of selenium (Se) release from selected Se fertiliser granules. Australian Journal of Soil Research, 44: 155-163

Lyons G.H., Gene Y., Soole K., Stangoulis J.C.R., Liu F., Graham R.D., 2009. Selenium increases seed production in *Brassica*. Plant and Soil, 318: 73-80

Lyons M.P., Papazyan T.T., Surai F.P., 2007. Selenium in food chain and animal nutrition: Lessons from nature. Asian-Australasian Journal of Animal Sciences, 20 (7): 1135-1155

Melanie W.R., Monika S., Susanne B., Dietmar S., Franziska S.H., Anna P.K., Daryl D.R., Kerry L.B.H., Marian J.M., 2017. Mechanisms of selenium enrichment and measurement in brassicaceous vegetables, and their application to human health. Frontiers in Plant Science, 8:1365. DOI: 10.3389/fpls.2017.01365

Metka S., and Walter G., 2005. Accumulation of selenium in natural plants and selenium supplemented vegetable and selenium speciation by HPLC-ICPMS. Chemical Speciation and Bioavailability, 17 (2): 63-73

Mikkelsen R.L., Page A.L., Bingham F.T. 1989. Factors affecting selenium accumulation by agricultural crops. In: Jacobs L.W. (ed), Selenium in Agriculture and Environment. SSSA Spec. Publ. 23. SSSA and ASA, Madison, WI. p. 65-94

Ministry of Health Malaysia, 2005. Selenium: Recommended nutrient intakes for Malaysia

Mora M.L., Duran P., Acuna A.J., Cartes P., Demanet R., Gianfreda L., 2015. Improving selenium status in plant nutrition and quality. Journal of Soil Science and Plant Nutrition, 15 (2): 486-503

Mora M.L., Pinilla L., Rosas A., Cartes, P., 2008. Selenium uptake and its influence on the antioxidative system of white clover as affected by lime and phosphorus fertilization. Plant and Soil, 303: 139-149

Moulick D., Santra S.C., Ghosh D., 2018. Effect of selenium induced seed priming on arsenic accumulation in rice plant and subsequent transmission in human food chain. Ecotoxicology and Environmental Safety, 152: 67-77

Murphy J., and Riley J.P., 1962. A modified single-solution method for the determination of phosphorus in natural waters: Analytica Chimica Acta, 27: 31-36.

Nawaz F., Ahmad R., Ashraf M., Waraich E., & Khan S., 2015. Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. Ecotoxicology and Environmental Safety, 113: 191–200

Ni Z., Liu Y., Qu M., 2014. Determination of 5 natural selenium species in selenium-enriched bamboo shoots using LC-ICP-MS. Food Science and Biotechnology, 23: 1049-1053

Nowak B.H., 2013. Comparative effects of selenite and selenate on growth and selenium accumulation in lettuce plants under hydroponic conditions. Plant Growth Regulation, 70:149–157

NutriPlus with Organic Selenium, 2014. Retrieved from http://layhong.com.my/website/nutriplus_selenium.html

Paul D., Dey S., 2014. Selenium content and uptake in crops as affected by soil selenium content and implications on human health. Communications in Soil Science and Plant Analysis, 45 (4): 429-436

Paulo F.B., Marislaine A.d.F., Yong Y., Hongmei L., Shree G., Jonathan J.H., Valdemar F., Luiz R.G.G., Threorodore W.R., Li. L., 2016. Selenium promotes sulfur accumulation and plant growth in wheat (*Triticum aestivum*). Physiologia Plantarum, 158 (1): 80-91

Paulo F.B., Valdemar F., Ramos S.J., Karina V.F.B., Fabricio W.A., Luiz R.G.G., 2013. Soil and foliar application of selenium in rice biofortification. Journal of Food Composition and Analysis, 31 (2): 238-244

Pedrero Z. and Madrid Y., 2009. Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. Analytical Chimica Acta, 634: 135-152

Petro E.M., Mkabwa L.K.M., Amra G., Paul A.F., Goran L., Mate E., Stephen S.N., 2015. Selenium accumulating leafy vegetables are a potential source of functional foods. International Journal of Food Science, Article ID 549676: 8 pages

Poldma P., Tonutare T., Viitak A., Luik A., Moor U., 2011. Effect of selenium treatment on mineral nutrition, bulb size, and antioxidant properties of garlic (*Allium sativum L*.). Journal of Agricultural and Food Chemistry, 59 (10): 5498-5503

Premarathna H.M.P.L., McLaughlin M.J., Kirby J.K., Hettiarachchi G.M., 2012. Influence of submerge and subsequent drainage on the partitioning and lability of added selenium fertilizers in a sulphur-containing Fluvisol. Europian Journal of Soil Science, 63: 514-522

Premarathna L., Mike J.M., Jason K.K., Ganga M.H., Samuel S., David J.C., 2012. Selenate-enriched urea granules are a highly effective fertilizer for selenium biofortification of paddy rice grain. Journal of Agricultural and Food Chemistry, 60: 6037-6044

Puccinelli M., Malorgio F., Pezzarossa B., 2017. Selenium enrichment of horticultural crops. Molecules, 22 (933): 1-21

Pyrzynska K., 2009. Selenium speciation in enriched vegetables. Food Chemistry, 114: 1183-1191

Qin H.B., Zhu J.M., Su H., 2012. Selenium fractions in organic matter from se-rich soils and weathered stone coal in selenosis areas of China. Chemosphere, 86: 626–633.

Qiuhui H., Xu J., Pang G., 2003. Effect of selenium on the yield and quality of green tea leaves harvested in early spring. Journal of Agricultural and Food Chemistry, 51(11): 3379-3381

Rahman M.M., Islam M.B., Biswas M., Alam A.H.M.K., 2015. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. BMC Research Notes, 8: 621. DOI: 10.1186/s13104-015-1618-6

Ramos S.J., Avila F.W., Boldrin P.F., Pereira F.J., Castro E.M., Faquin V., Reis A.R., Guilherme L.R.G., 2012. Response of *brachiaria* grass to selenium forms applied in a tropical soil. Plant, Soil and Environment, 58 (11): 521-527

Ramos S.J., Faquin V., Guilherme L.R.G., Castro E.M., Avila F.W., Carvalho G.S., Bastos C.E.A., Oliveira C., 2010. Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. Plant, Soil and Environment, 56 (12): 584-588

Rani N., Dhillon K.S., Dhillon S.K., 2005. Critical levels of selenium in different crops grown in an alkaline silty loam soil treated with selenite-Se. Plant and Soil, 277: 367-374

Rayman M.P., 2012. Selenium and human health. The Lancet 379 (9822): 1256-1268

Rios J.J., Rosales M.A., Blasco B., Cervilla L.M., Romero L., Ruiz J.M., 2008. Biofortification of Se and induction of the antioxidant capacity in lettuce plants. Scientia Horticulturae, 116: 248-255

Rodrigo S., Santamaria O., Lopex-Bellido F.J., Poblaciones M.J., 2013. Agronomic selenium biofortification of two-rowed barley under Mediterranean conditions. Plant, Soil and Environment, 59 (3): 115-120

Rodrigo S., Santamaria O., Poblaciones M.J., 2014. Selenium application timing: Influence in wheat grain and flour selenium accumulation under Mediterranean conditions. Journal of Agricultural Science, 6 (3): 23-30

Ros G.H., Rotterdam A.M.D., Bussink D.W., Bindraban P.S., 2016. Selenium fertilization strategies for bio-fortification of food: An agro-ecosystem approach. Plant and Soil, 404 (1/2): 99-112

Rosell, C.M., 2016. Fortification of grain-based foods. In Encyclopaedia of Food Grains (Second Edition). Academic Press, Oxford: 43-49.

Saffaryazdi A., Lahouti M., Ganjeali A., Bayat H., 2012. Impact of selenium supplementation on growth and selenium accumulation on spinach (*Spinacia oleracea L.*) plants. Notulae Scientia Biologicae, 4 (4): 95-100

Saha U., Fayiga A., Sonon L., 2017. Selenium in the soil plant environment: A review. International Journal of Applied Agricultural Sciences, 3 (1): 1-18

Salah K.B., Mahjoub M.A., Ammar S., Michel L., Millet-Clerc J., Chaumont J.P., Mighri Z., Aouni M., 2006. Antimicrobial and antioxidant activities of the methanolic extracts of three Salvia species from Tunisia. Natural Product Research, 20 (12): 1110-1120

Saygi K.O., Melek E., Tuzen M., Soylak M., 2007. Speciation of selenium (IV) and selenium (VI) in environmental samples by the combination of graphite furnace atomic absorption spectrometric determination and solid phase extraction on Diaion HP-2MG. Talanta, 71 (3): 1375-1381

Schiavon M., Dallacqua S., Mietto A., Pillon Smits E.A., Sambo P., Masi A., Malagoli M., 2013. Selenium fertilization alters the chemical composition and antioxidant constituents of tomato (*Solanum lycopersicon L.*). Journal of Agriculture and Food Chemistry, 61 (44): 10542-10554

Seppänen M.M., Kontturi J., Heras I.S., Madrid Y., Camara C., Hartikainen H., 2010. Agronomic biofortification of brassica with selenium - enrichment of *semet* and its identification in *brassica* seeds and meal. Plant and Soil, 337: 273–283

Sharma S., Bansal A., Dhillon S.K., Dhillon K.S., 2010. Comparative effects of selenate and selenite on growth and biochemical composition of rapeseed (*Brassica napus L.*). Plant and Soil, 329: 339-348

Singh M., Singh N., Bhandari D.K., 1980. Interaction of selenium and sulfur on the growth and chemical composition of raya. Soil Science (USA) 129 (4): 238-244

Strek M., Telesinski A., 2015. Assessment of selenium compounds use in limitation of petroleum impact on antioxidant capacity in sandy soil. Environmental Protection and Natural Resources, 26 No 3(65): 6-11

Sturm M., Marsic N.K., Zupanc V., Zeleznik B.B., Lojen S., Pintar M., 2010. Effect of different fertilisation and irrigation practices on yield, nitrogen uptake and fertiliser use efficiency of white cabbage (*Brassica oleracea var. capitata L.*). Scientia Horticulturae, 125: 103-109

Sun W., Pan W., Wang F., Xu N., 2015. Removal of Se (IV) and Se (VI) by MFe_2O_4 nanoparticles from aqueous solution. Chemical Engineering Journal, 273: 353-362

Supriantin S., Weng L., Coman R.N.J., 2015. Selenium speciation and extractability in Dutch agricultural soils. The Science of Total Environment, 532: 368-382

United States Environmental Protection Agency (EPA), 2008. Fate, transport and transformation test guidelines OPPTS 835.1230: Adsorption/Desorption (Batch Equilibrium). EPA 712-C-08-009

Valença A.W. d., Bake A., Brouwer I.D., Giller K.E., 2017. Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. Global Food Security, 12: 8–14.

Vaz Patto M.c., Amarowicz R., Aryee A.N.A., Boye J.I., Chung H.J., Martin-Cabrejas M.A., 2015. Achievements and challenges in improving the nutritional quality of food legumes. Critical Review in Plant Sciences, 34 (1-3): 105-143

Virginia F.C., Albert M.G., Roser R., Jose Fermin L.S., 2013. Selenium uptake by edible plants from enriched peat. Scientia Horticulturae, 164: 428-433

Wang H.F., Takematsu N., Ambe S., 2000. Effects of soil acidity on the uptake of trace elements in soybean and tomato plants. Applied Radiation and Isotopes, 52 (4): 803-811

Wen S., Zhu X., Wei Y., Wu S., 2013. Cloud point extraction-inductively coupled plasma mass spectrometry for separation/analysis of aqueous-exchangeable and unaqueous-exchangeable selenium in tea samples. Food Analytical Method, 6 (2): 506-511

White P. J., 2016. Selenium accumulation by plants. Annals of Botany, 117(2), 217–235. https://doi.org/10.1093/aob/mcv180

WHO, 2011. Selenium in drinking-water: Background document for development of who guidelines for drinking-water quality

Wu Z, Bañuelos GS, Lin Z-Q, Liu Y, Yuan L, Yin X and Li M (2015). Biofortification and phytoremediation of selenium in China. Frontiers in Plant Science, Volume 6, Article number 136

Xu S., Zheng M., Zhang X., Zhang J., Lee Y.I., 2012. Nano TiO_2-based preconcentration for the speciation analysis of inorganic selenium by using ion chromatography with conductivity detection. Microchemical Journal, 101: 70-74

Xue T., and Hartikainen H., 2000. Association of antioxidative enzymes with the synergistic effect of selenium and irradiation in enhancing plant growth. Agricultural and Food Science in Finland, 9: 177-186

Yong-Guan Z., Elizabeth A.H.P.S., Fang-Jie Z., Paul N.W. and Andrew A.M., 2009. Selenium in higher plants: understanding mechanisms for bio fortification and remediation. Trends in Plant Science, 14 (8): 436-442

Zahoor A., Honna T., Yamamoto S., Irshad M., Haytham E.S., Abou E.H.W.H., Faridullah., 2007. Wheat (*Triticum aestivum L.*) response to combined organic and inorganic phosphorus fertilizers application under saline conditions. Acta Agriculturae Scandinavica, Section B, Soil & Plant Science, 57 (3): 222-230

Zarcinas B.A., Che Fauziah I., McLaughlin M.J., Cozens G., 2004. Heavy metals in soils and crop in Southeast Asia. Environmental Geochemistry and Health, 26: 343-357

Zhang M., Tang S., Huang X., Zhang F., Pang Y., Huang Q., Yi Q., 2014. Selenium uptake, dynamic changes in selenium content and its influence on photosynthesis and chlorophyll fluorescence in rice (*Oryza sativa L*). Environmental and Experimental Botany, 107: 39-45

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

Dayang, S.N., Farahzety, A.M., Mohd Zulkhairi, A. and Che Fauziah, I. 2017. Effect of Selenium Biofortification on Selenium Concentration and Antioxidant Activity of Green Spinach. Abstract & Poster at 3rd International Conference on Food, Agriculture & Natural Resources (FANRES 2017), 21-23 Aug 2017, Universiti Putra Malaysia, Serdang, Selangor

Dayang Safinah N., Farahzety A.M., Rozlaily Z., Arina Shairah A.S., Martini M.Y., Che Fauziah I. 2018. Adsorption-desorption of Selenium in Soil. Poster & Proceedings article at Joint Symposium of 8th International Agriculture Congress 2018 & 6th International Symposium for Food & Agriculture 2018, 13-15 Nov 2018, Universiti Putra Malaysia, Serdang, Selangor

Dayang Safinah N., Farahzety A.M., Rozlaily Z., Arina Shairah A.S., Martini M.Y. & Che Fauziah I. 2019. Baja Selenium: Baja untuk Peningkatan Hasil & Nutrisi Sayuran Berdaun. Poster at Seminar TWG MARDI-DOA (TWG 3 Sayuran), 24 Jan 2019, Kementerian Pertanian Malaysia, Putrajaya

Dayang Safinah N., Farahzety A.M., Rozlaily Z., Arina Shairah A.S., Martini M.Y. and Che Fauziah I. 2019. Selenium fertilizer for leafy vegetables. Agromedia MARDI (in conjunction of SEAVEG 2019)

Dayang Safinah N., Farahzety A.M., Rozlaily Z., Arina Shairah A.S., Martini M.Y. and Che Fauziah I. 2019. Selenium uptake by green spinach as affected by phosphorus fertilization. Abstract at Southeast Asia Vegetable Symposium: Advancing Vegetable Technologies for High Productivity and Better Human Health (SEAVEG 2019), 9-11 July 2019, Hatten Hotel, Melaka

Dayang Safinah N., Farahzety A.M., Rozlaily Z., Arina Shairah A.S., Martini M.Y. and Che Fauziah I. 2019. Selenium Enriched Spinach: Agronomic Biofortification Strategies to Improve Human Nutrition. Abstract at 2nd International Food Research Conference (IFRC 2019), 27-29 August 2019, The Everly Hotel, Putrajaya

Dayang Safinah N., Rozlaily Z., Arina Shairah A.S., Martini M.Y., Che Fauziah I., 2020. Selenium biofortification of green spinach with optimum phosphorus fertilization and selenium application timing. Agrosystems, Geosciences & Environment, Volume 3, Issue1. https://doi.org/10.1002/agg2.20089

Dayang Safinah N., Rozlaily Z., Arina Shairah A.S., Martini M.Y. and Che Fauziah I. 2020. Selenium Adsorption Desorption Capacity and Influence on Selenium Uptake by Green Spinach. Agronomy Journal (submitted)