UNIVERSITI PUTRA MALAYSIA

GROWTH OF LETTUCE (Lactuca sativa L.) AFFECTED BY CADMIUM CONCENTRATIONS AND TOXICITY ALLEVIATION BY CALCIUM SUPPLEMENT

SANI AHMAD JIBRIL

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By

SANI AHMAD JIBRIL

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

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DEDICATION

To my beloved parents without whom I will not be where I am today, my loving wife and children for their patience, support and prayers

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

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Chairman : Associate Professor Siti Aishah bt Hassan, PhD
Faculty : Agriculture

Rapid urbanization and population growth contribute to shortage of fresh water globally. Farmers in many developing countries resort to the utilization of untreated urban wastewater in irrigation. This unconventional waters contains toxic elements from households and industries such as mercury, chromium, lead and cadmium (Cd) that can be toxic to both plants and animals. Lettuce (*Lactuca sativa* L.) is a good accumulator of these toxic elements, especially Cd in the leaves; and therefore was selected in this research to study the effect of Cd on morphological, physiological, biochemical and anatomical attributes of lettuce.

Seeds of five (5) lettuce varieties (Var. 163, Var. 168, Dankabo-D/Kabo, Italian lettuce-167 and Bombilasta-BBL) were germinated for varietal separation in a preliminary experiment. Two-weeks old seedlings were transplanted into a trough and supplied with the Cooper's nutrient formulation solution (Cooper, 1979) containing (mg L^{-1}): 236 N, 60 P, 300 K, 185 Ca, 50 Mg, 68 S, 12 Fe (EDTA), 2.0 Mn, 0.1 Zn, 0.1 Cu,0.3 B and 0.2 Mo, using a nutrient film technique system. The pH was maintained at 6 and electrical conductivity (E.C.) of 1.5 – 2.5 dS m^{-1}. Varieties BBL and Italian 167 were eventually selected and treated with $CdCl_2$ concentrations of 0, 0.5, 1, 2, and 4 mg L^{-1} to determine morphological, anatomical and physiological changes due to Cd toxicity. However, no visible toxicity sign was observed throughout the growing period on both varieties. Higher Cd concentrations of 0, 3, 6, 9 and 12 mg L^{-1} was used to re-examine toxicity effect on the two varieties. Significant negative effects were recorded in morphological, physiological, biochemical and nutrient element contents in the varieties. Despite the level of Cd used, variety BBL was taller and recorded higher leaf area, fresh and dry root weights than Italian variety 167.

There was higher increase in antioxidant activity and vitamin C with increase in Cd concentrations compared with the control plants; but it was higher in variety 167 than BBL variety. The varieties displayed similar responses to Cd toxicity symptoms of chlorosis and stunted growth from 6 mg L^{-1} to 12 mg L^{-1} Cd level compared with the untreated plants. Macronutrient elements in the roots and shoots of lettuce were significantly decreased compared with the control plants at the highest Cd concentration of 12 mg L^{-1}. Significant differences were found among the varieties of lettuce in the contents of micronutrients in the roots and shoots, however, variety 167 was found to absorb higher contents of Cd than variety BBL. Variety 167 had higher contents of Fe, Mn, Cu and Zn, implying synergistic effect of Cd and micronutrients in the roots and shoots of lettuce varieties.

Introduction of calcium supplement of 0, 6 and 9 mg L^{-1} to Cd polluted water significantly alleviated the negative effects imposed by Cd at 0, 6 and 9 mg L^{-1} on all the parameters of lettuce studied. Calcium improved morphological, phytochemical, anatomical and physiological attributes of lettuce. At the same time enhanced photosynthetic pigments efficiency, proline and flavonoids contents; increased mineral nutrient elements and yield and decrease in MDA. Addition of 6 mg L^{-1} and 9 mg L^{-1} of Ca into solution containing 6 mg L^{-1} Cd provided more significant results very close to the results of the control plants. This study indicated that application of Ca^{2+} had significant and antagonistic effect on Cd by improving plant growth and development. Italian 167 variety is not recommended for planting in Cd polluted environments as it absorbed more Cd than lettuce variety BBL. Calcium can therefore be recommended for utilization in heavy metal polluted environments, particularly Cd as a strategy to alleviate their harmful effects, to enhance plant metabolism and perform better in such polluted areas.

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

KESAN KEPEKATAN KADMIUM DAN PENGURANGAN KETOKSIKAN OLEH PENAMBAHAN KALSIUM TERHADAP PERTUMBUHAN SALAD (*Lactuca sativa* L.)

Oleh

SANI AHMAD JIBRIL

JulaI 2017

Pengerusi : Profesor Madya Siti Aishah bt Hassan, PhD
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Perbandaran yang pesat dan pertumbuhan penduduk menyumbang kepada kekurangan air tawar di peringkat global. Petani di negara membangun mengambil jalan keluar dengan menggunakan air sisa bandar yang tidak dirawat sebagai sumber pengairan. Pengairan tidak konvensional ini mengandungi bahan toksik dari isi rumah dan industri seperti Hg^{2+}, Cd^{2+}, Cr^{2+} dan Pb^{2+} yang boleh menjadi toksik kepada tumbuhan dan haiwan. Salad (*Lactuca sativa* L.) adalah pengumpul yang baik di dalam daun bagi unsur toksik, terutamanya Cd. Oleh itu, tanaman ini telah dipilih dalam kajian ini untuk mengkaji kesan Cd kepada ciri morfologi, fisiologi, biokimia dan anatomi.

Biji benih lima (5) jenis salad (Var. 163, Var. 168, Dankabo-D/Kabo, Itali salad-167 dan Bombilasta-BBL) telah dicambahkan dalam span dan diletakkan di bawah stuktur lindungan hujan untuk pemisahan varietal dalam eksperimen awal. Anak benih yang berusia dua minggu telah dipindahkan ke dalam palung dan dibekalkan dengan larutan formulasi nutrisi Cooper (Cooper, 1979) yang mengandungi (mg L^{-1}): 236 N, 60 P, 300 K, 185 Ca, 50 Mg, 68 S, 12 Fe (EDTA), 2.0 Mn, 0.1 Zn, 0.1 Cu, 0.3 B dan 0.2 Mo, dengan menggunakan teknik nutrisi cetek (NFT). Paras pH dikekalkan pada 6 dan kekonduksian electrik (E.C) daripada 1.5-2.5 dS m^{-1}. Varieti BBL dan Itali 167 telah dipilih dan dirawat dengan kepekatan Cd^{2+} 0, 0.5, 1, 2, dan 4 mg L^{-1} untuk menentukan morfologi, anatomi dan perubahan fisiologi yang berlaku kesan daripada ketoksikan Cd. Walau bagaimanapun, tiada tanda keracunan kelihatan sepanjang tempoh pertumbuhan pada dua tanaman berkenaan. Kepekatan Cd tinggi 0, 3, 6, 9 dan 12 mg L^{-1} telah digunakan untuk mengkaji semula kesan keracunan pada kedua-dua jenis varieti salad ini. Kesan negatif yang ketara telah dicatatkan dalam ciri morfologi, fisiologi, biokimia dan kandungan nutrisi dalam tanaman. Pada tahap Cd yang telah digunakan, varieti BBL adalah lebih tinggi dari segi jumlah daun, luas daun, berat segar dan berat kering akar daripada varieti Itali 167.

Terdapat peningkatan yang lebih tinggi dalam aktiviti antioksidan dan vitamin C dengan peningkatan kepekatan Cd berbanding kawalan; tetapi lebih tinggi pada varieti 167 berbanding BBL. Kedua-dua varieti tersebut memaparkan simptom ketoksikan Cd yang sama iaitu klorosis daun dan pertumbuhan yang terbantut dari tahap kepekatan 6 mg L^{-1} ke 12 mg L^{-1} Cd berbanding tanaman tanpa rawatan. Elemen nutrien makro dalam akar salad telah menurun dengan signifikan masing-masing berbanding tanaman kawalan pada tahap kepekatan kadmium yang paling tinggi iaitu 12 mg L^{-1}. Terdapat perbezaan yang signifikan dalam kandungan unsur mineral mikro dalam akar antara varieti salad, bagaimanapun, varieti 167 didapati menyerap Cd lebih banyak berbanding varieti BBL. Varieti 167 mempunyai kandungan Fe, Mn, Cu dan Zn yang lebih tinggi, menunjukkan kesan sinergi Cd dan elemen mikro dalam akar dan pucuk varieti salad.

Kalsium (Ca) tambahan pada tahap 0, 6 dan 9 mg L^{-1} pada air yang telah dicemari Cd 0, 6, dan 9 mg L^{-1} mengurangkan kesan negatif Cd secara signifikan terhadap semua parameter yang dikaji. Kalsium menambahbaik morfologi, fitokimia, anatomi dan ciri fisiologi salad. Disamping itu meningkatkan kecekapan pigmen fotosintesis, proline dan kandungan flavonoid; meningkatkan elemen nutrien mineral dan hasil serta mengurangkan MDA. Penambahan 6 mg L^{-1} Ca yang mengandungi 6 mg L^{-1} Cd dan 9 mg L^{-1} Ca yang mengandungi 6 mg L^{-1} Cd memberikan keputusan yang hampir sama dengan keputusan rawatan kawalan. Kajian ini menunjukkan bahawa aplikasi Ca^{2+} memberikan kesan yang signifikan dan antagonis terhadap Cd dengan menambah baik pertumbuhan dan perkembangan pokok. Varieti Italian 167 adalah tidak digalakkan untuk ditanam di persekitaran yang dicemari Cd memandangkan ia menyerap lebih banyak Cd berbanding variety BBL. Kalsium dengan ini boleh disyorkan untuk digunakan dalam media pertumbuhan sebagai strategi untuk mengurangkan kesan bahaya logam berat, terutamanya Cd, untuk meningkatkan metabolisma tanaman dan memastikan prestasi yang lebih baik dalam persekitaran yang dicemari Cd.

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I certify that a Thesis Examination Committee has met on 26 July 2017 to conduct the final examination of Sani Ahmad Jibril on his thesis entitled "Growth of Lettuce (*Lactuca sativa* L.) Affected by Cadmium Concentrations and Toxicity Alleviation by Calcium Supplement" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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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This is to confirm that:
- the research conducted and the writing of this thesis was under our supervision;
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AAS	Atomic Absorption Spectrometer
ASC	Ascorbic acid
ANOVA	Analysis of variance
BBL	Bombilasta
CT	Cytoplasm
Ca	Calcium
CAT	Catalase
CCFAC	Committee on food additives and contaminants
Cd	Cadmium
Ch	Chloroplast
Chl.	Chlorophyll
cm^2	Centimeter square
CO_2	Carbon dioxide
CV	Coefficient of variation
CW	Cell wall
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DF	Degrees of freedom
DMRT	Duncan's Multiple Range Test
DW	Dry weight
E	Transpiration rate
EC	Electrical conductivity
FAO	Food and Agriculture Organization
FRAP	Ferric ion reducing antioxidant reducing power
G	Grana
g	Gram

Gs	Stomatal conductance
H_2O_2	Hydrogen peroxide
HSD	Honest Significant Difference
JECFA	Joint External Committee on Food Additives
LSD	Least Significant Difference
MDA	Malondialdehyde
NFT	Nutrient Film Technique
PT	Plastoglobuli
Pn	Net photosynthesis
POD	Peroxidase
PS I	Photosystem I
PS II	Photosystem II
RCBD	Randomized Complete Block Design
ROS	Reactive Oxygen Species
S	Starch grain
SAS	Statistical Analyses System
SLA	Specific Leaf Area
SOD	Superoxidase
TEM	Transmission Electron Microscope
WHO	World Health Organization
*	Significant at 0.05 probability
**	Significant at 0.01 probability

CHAPTER 1

INTRODUCTION

1.1 Background

Global increase in population and industrialization are escalating the demand on fresh water resources. Insufficient safe water for domestic use leads to growing water shortage and pressure, thus, resulting in the utilization of contaminated sources, such as unprocessed metropolitan sewage effluents for growing of crops. Lazarova and Bahri (2008), made an estimation of about 10% of total land mass used in irrigation are supplied with untreated effluents. Generally, untreated effluent is a combination of liquid wastes from industries, commercial sources, households, resulting from daily uses, production and consumption activities. These anthropogenic activities are linked with environmental pollution of worldwide concern as it results in polluting underground water sources for lack of unplanned waste disposal of untreated domestic sewage and industrial effluents into water channels. Ultimately, this polluted water is drained into agricultural fields where it is utilized in the irrigation of food crops including vegetables. Wastewater offers consistently good resource for irrigation to growers and has advantageous addition of essential macro and micronutrients to agricultural fields, thereby creating opportunities for agriculture. Use of wastewater in agriculture could be beneficial to plants and animals on one hand and disadvantageous in the other as it contain minerals essential for growth and development as well as excessive amount of heavy metals, bacteria and viruses (UNU-INWEH, 2011). Naturally, plants take up essential micronutrients like Cu^{2+}, Fe^{2+}, Mo^{2+}, Mn^{2+}, and Zn^{2+} which are beneficial, at the same time assimilate others like Hg^{2+}, Cd^{2+}, Cr^{2+} and Pb^{2+} that are toxic to their systems. Though, Liu et al. (2001) explained that the toxic effect differs from genotype to genotype of the same crop plants, Memon and Schröder (2009) added that it is influenced by the nature of ion and ion concentration, type of plant, and age of plant. Itanna (2002), reiterated that differences in heavy metal absorption by vegetable crops are credited to plant resilience to the metals; while Alexander et al. (2006) stated that leafy vegetables are high accumulators of metal ions compared to root vegetables and legumes.

Certainly, cadmium turns out to be a common occurrence in wastewater of industries sited along the waterways in view of which the United States Environmental Protection Agency (USEPA) has enforced stringent regulations on cadmium levels in industrial discharges. As a toxic substance, small quantities of Cd are usually found in agricultural environments. Wang and Song (2009) reported polluted fields might contain 600 mg kg^{-1} Cd, but unpolluted areas could be 0.01 to 5 µg kg^{-1}. Use of sewage sludge as fertilizer in agriculture, zinc mining, combustion of fossil fuels, pesticides, phosphate fertilizer application, increase the naturally low amounts of Cd available to plants leading to accumulation in nature (Lombi et al. 2000).

According to Schutzendubel et al. (2001), Cd can restricts shoot and root development, disorderliness of the grana assemblies and decrease in chlorophyll synthesis. Sandalio et al. (2001) added that Cd can also inhibit the action of some sets of enzymes like that of the light fixing of Calvin cycle, nitrogen fixation, sugar metabolic processes and sulphate integration (Lee and Leustek, 1999) and acceleration of leaf senescence (Siedlecka and Krupa, 1999). Cadmium toxicity was associated with disruption in the absorption and transportation of macro and micronutrients in flora (Sandalio et al., 2001).

Oxidative degradation of lipids in the cells is caused by over production of ROS and their reaction with unsaturated fatty acids. Demiral and Turkan (2005) explained that the building block and efficiency of membrane tissues resulted in the rise of protoplasmic permeability, leading to escape of K^+ ions and other solutes and finally cause cell death which is a result of the ROS production process as well as some of its by-products.

Lettuce (*Lactuca sativa* L.) is a cosmopolitan important leafy vegetable that is consumed worldwide with annual production of about 18 million metric tons (Davey et al., 2007). Vitamins A and C in addition to iron and phosphorus are some of the important minerals found in lettuce. Many workers (Mensch and Baize, 2004; Kim et al., 1988) have reported lettuce as a good accumulator of Cd in its leaves. According to Cox, (2000) lettuce, spinach, cereals, and cabbage accumulates high content of heavy metals more than tomatoes, corn or sweet pea. In another work by Hibben et al. (1984), lettuce accumulated more lead than other vegetables when grown in contaminated soils. Lettuce was therefore suggested to be identified as measure for health status of vegetable plants cultivated in heavy metal polluted areas (Brown et al., 1996).

As a macro-essential nutrient, calcium serves an important part in plant life processes. Functions of membrane structure is insured by Ca for fixing to lipid bilayers, stabilizing phospholipids and finally offer essential structure to biological cells. Wang and Wang (2007), reported Ca regulated the actions of some important proteins separately or through proteins attached to calcium, such as calmodulin, which afterwards, initiate many protein kinases and additional proteins in cells. Certain vital elements like Ca, P, Zn, Cu, Mn and Fe acts antagonistically, limiting or inhibiting heavy metals uptake depending upon species of the plant and variety (Krupa et al., 1999). In another report by Zorrig et al. (2012), Ca can hinder undesirable changes induced by environmental factors via the control of free radicals and osmoregulation. Cadmium toxicity alleviation using Ca supplement, therefore, happens either by reducing Cd uptake or by declining its harmful effect. Little attention was given to assess the toxicity of Cd concentrations on physiological, biochemical and structural traits of vegetable crops, especially lettuce cultivated in Cd polluted environments and its alleviation by fertilization with calcium chloride salt.

Nutrient Film Technique (NFT) system was opted for in this research over soil because apart from being less labor intensive and its ability to a better control of pest infestations and prevent plant diseases, it offers the precise control of plant nutrient supply, water conservation comparative to soil cultures. It is easier to test, check electrical conductivity (EC), change and manipulate pH which is practically unthinkable in soil culture, along these lines guaranteeing upkeep over plant's development and improvement.

1.2 Objectives

This research was conducted to assess the effects of Cd concentrations on lettuce varieties and determine the appropriate level of calcium chloride required in the alleviation of cadmium uptake in polluted environments.

Specifically, the objectives of this research work were:

i. To identify two to three varieties of lettuces that may be planted under high temperature and humidity environment,
ii. To determine the morphological, physiological, biochemical and structural changes that may occur in lettuce varieties due to cadmium stress,
iii. To determine effects of calcium on the uptake of essential nutrient elements and cadmium, and
iv. To determine the most resistant variety and fertilization level of Ca to be recommended for planting on potentially Cd polluted environments.

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