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ชื่อโครงการ ผลของปุ๋ยมูลไส้เดือนต่อปริมาณซิลิคอนที่ใช้ได้ การดูดธาตุซิลิคอน และการสร้างมวลชีวภาพของพริกขี้หนูใหญ่

ชื่อนิสิต นางสาวลักษณา คชาบาล

ภาควิชา พฤกษศาสตร์

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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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Effects of vermicompost on available silicon content, silicon uptake and biomass production of Bird's eye chili

Miss Laksana Kachaban

This Senior Project Submitted in Partial Fulfillment of the Requirements
For the Degree of Bachelor of Science in Botany
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Project Title	Effects of vermicompost on available silicon content, silicon uptake and biomass production of Bird's eye chili
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บทคัดย่อ

การปรับปรุงดินด้วยปุ๋ยมูลไส้เดือนอาจมีผลเปลี่ยนแปลงคุณสมบัติทางกายภาพและเคมีของดินที่ทำให้ธาตุเสริมประโยชน์ เช่น ซิลิคอน (Si) อยู่ในรูปใช้ได้เพิ่มขึ้น เพื่อตรวจสอบผลดังกล่าว จึงทดลองปลูกพริกขี้หนูใหญ่ใน 4 สภาวะ ได้แก่ ดินที่ไม่มีการปรับปรุง (ชุดควบคุม) ดินที่ปรับปรุงโดยการเติมปุ๋ยมูลไส้เดือน (300 กรัม/กิโลกรัม) ปุ๋ยเคมี สูตร 15-15-15 (5 กรัม/กิโลกรัม) และปุ๋ยโซเดียมไตรซิลิเกต (1 mM SiO_2) เป็นเวลา 49 วัน ผลการทดลองพบว่า ปุ๋ยมูลไส้เดือนมีผลเพิ่มความสูง จำนวนใบ และน้ำหนักแห้งต้นและรากของพริกขี้หนูใหญ่อย่างมีนัยสำคัญเมื่อเทียบกับชุดควบคุม สำหรับการดูดธาตุซิลิคอนของพริกขี้หนูใหญ่ที่ปลูกด้วยดินที่ไม่มีการปรับปรุง พบว่ามีการสะสมซิลิคอนในส่วนยอดประมาณ 672 ppm และในราก 1,613 ppm อย่างไรก็ตาม ปุ๋ยมูลไส้เดือนไม่มีผลต่อปริมาณซิลิคอนใช้ได้ในดิน แต่มีผลเพิ่มปริมาณซิลิคอนต่อต้นของพริกขี้หนูใหญ่อย่างมีนัยสำคัญ นอกจากนี้ จากการตรวจสอบคุณสมบัติของดินพบว่า ปุ๋ยมูลไส้เดือนเพิ่มค่าการนำไฟฟ้า (EC) และค่าความเป็นกรดด่าง (pH) ของดิน อีกทั้งสังเกตพบว่าดินมีความหนาแน่นลดลง จึงอาจเป็นไปได้ว่าปุ๋ยมูลไส้เดือนปรับปรุงคุณสมบัติทางกายภาพและเคมีของดิน ทำให้มีการสร้างมวลชีวภาพของพริกขี้หนูใหญ่ที่เพิ่มขึ้น

คำสำคัญ: ปุ๋ยมูลไส้เดือน, การดูดธาตุซิลิคอน, การสร้างมวลชีวภาพ, พริกขี้หนูใหญ่

Project Title	Effects of vermicompost on available silicon content, silicon uptake and biomass production of Bird's eye chili
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Abstract

Soil amendment by vermicompost may modify soil physicochemical properties leading to enhancing available form of beneficial element, such as silicon (Si). To prove that, Bird's eye chili plants were grown under 4 conditions: soils without amendment (control), with vermicompost (300 gram/kilogram), with chemical fertilizer (15-15-15, 5 gram/kilogram) and with sodium trisilicate fertilizer (1 mM SiO_2) for 49 days. The results showed that vermicompost significantly increased height, leaf number and dry mass of shoots and roots, when compared to control. For Si uptake, the chili plants grown under the control condition accumulated Si approximately 672 ppm in shoots and 1,613 ppm in roots. Nonetheless, vermicompost did not affect soil available Si but significantly increased total Si contents per plant. Moreover, soil property analysis showed that vermicompost increased electrical conductivity (EC) and pH of soils and seemed to decrease soil compaction. Thus, it may be that vermicompost improved physicochemical properties of soils leading to increased biomass production of Bird's eye chili.

Keywords: Vermicompost, Silicon uptake, Biomass production, Bird’s eye chili

Acknowledgements

After collecting all the lovely chilies on 9th March 2019, I was so tired from laboratory and somehow could not sleep. So I stayed up late and thought about all the kindness I have received.

I remembered the moment when I talked to my professor, teacher Anchalee about this project for the first time. I was interested in organic fertilizer and vermicompost, so I decided to do this project immediately.

At first, I thought it would not be so hard to finish my project, but unexpected events happened along the way. This project could not have been finished without the help from everyone around me. Firstly, I must thank the Department of Botany, Faculty of Science, Chulalongkorn University for a good opportunity to develop my research skills. Thanks to my mom and my family for both mental and material support. A bunch of thanks to Assistant Professor Dr. Anchalee Chaidee for teaching and training me patiently, and also for the priceless advice. I cannot find the words more than thanks to say to her.

I need to thank to Mr. Prapan Saelee and Mrs. Junrung Cherdchoo for the material support. I would like to thank everyone in the Department of Botany: P' Prawes, P’ Bell, P’ Pla and P' Nut for every precious guidance. The Plant Ecology Laboratory also kindly let me borrow several equipment. Also, I would like to thank my friends; Penny, who always cheered me up when I was feeling down; Palm, Sorn, Valve, Plai and James, who stayed late in the lab with me, so I did not have to walk home alone. I feel really grateful to my CU Chorus friends, P’Pao, P’Toto and P’Bosh, for working outside together, and the people in Plerng Pra Jess for all the funny moment, especially Pao, who proofread this acknowledgement. Lastly, I want to say thanks to everyone at TURFPAG5 who was interested in my project, all of your questions inspired me to search for more answers. From this senior project, I have learned a lot from my mistakes, I gained a lot of experience, and I hope I could learn more in the future.

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Chapter I

Introduction

Chilli is used widely as a spice in food seasoning in a household and also has some nutrients which are good for health, e.g. anthocyanin (Govindarajan, Rajalakshmi and Chand, 1987). Thailand cultivates chili for domestic uses and exports chili products to other countries at the top range among ASEAN countries, which brought the income to Thailand about 2,521 million baht in 2014 (Bowichean, 2014: Online). Thai farmers can cultivate chili in every region and a chili cultivation report in 2016 showed that the chili's price selling in Chanthaburi Province was the second highest in the eastern region of Thailand. Moreover, 90 percent of planted chilies were spicy chilies which were composed of many cultivars, e.g. Bird's eyes chili which is extremely hot (50,000 – 80,000 SHU) (Kraikruan et al., 2008). In addition, a chili cultivar that gains an attention of farmers nowadays is "Si Sa Ket 1", which has high yield and is suitable chili product for customer demand. All advantages described above led to ubiquitous chili cultivation in Thailand. However, there are many problems in a cultivation process, for example, a wilting disease caused by bacteria, mosaic virus, and anthracnose, that ruined the chili production around 1,259 acres or 2.5 percent of all cultivation areas (Boonchuay, 2017: Online).

Research on the effects of silicon on chili's anthracnose resistance showed that chili plants applied with silicon had less injury from *Colletotrichum gloeosporrioides* about 64 – 75 percent (Jayawardana, Weerahewa and Saparamadu, 2014; 2015). Moreover, there was a study showing that chili took up silicon applied by root better than foliar spraying and silicon accumulated in its tissue as high as 2.5 percent per dry weight (Dogramaci et al., 2013). Therefore, this shows that chili is a silicon accumulating species. In general, plants can take up available silicon directly. However, a traditional cultivation that uses chemical fertilizer for a long time changes nutrition balance and chemical properties of the soil. This cultivation method also causes essential elements and beneficial elements (e.g. silicon) being in an unavailable form for plants. There are many ways to solve this problem, for example, adding a fertilizer which contains beneficial elements or improving soil property to enhance nutrient solubility. The latter can be done by adjusting soil pH to 6.0 – 6.5 or using soil conditioner such as organic fertilizer and/or earthworm (Cleyet – Marcel et al., 2001). Vermicompost, or earthworm compost, can increase beneficial microorganisms in plant rhizosphere, resulting in increased beneficial element uptake efficiency and solving micronutrient deficiency (Geiklooi and Shirmohammadi, 2013).

Furthermore, vermicompost can increase seed germination rate and seedling growth of mung bean, okra and cucumber (Hussain and Aabbasi, 2016). A few pieces of research in last 150 years have shown that vermicompost significantly benefits plant growth, however, there is less mechanistic evidence including its influence on nutrient solubility (Braga et al., 2016). So, this study aims to unravel the effects of vermicompost on available silicon content, silicon uptake and biomass production of Bird's eye chili. This study will provide some information about vermicompost efficiency in improving soil property, emphasizing on silicon element that benefits plant growth.

1.1 Objectives

To study the effects of vermicompost on available silicon content, silicon uptake and biomass production of Bird's eye chili.

1.2 Expected beneficial outcomes

Knowledge in soil amendment by vermicompost focusing on silicon availability will widen our understanding in plant nutrient relationship for better application in organic agriculture.

Chapter II

Literature review

2.1 Bird's eye chili

Bird's eye chili is one of the vegetables that has high – value trading in the global market. In Thailand, chili is used widely in the household and has a high capacity for export (Bowichean, 2014: Online). There are many cultivars of Bird's eye chili that are preferred by Thai farmers; 'Si Sa Ket 1 chili' is one of Bird's eye chili cultivar that has been bred from a big Bird's eye chili. The taxonomic characteristics of this cultivar are shrub with green stems and a presence of purple strip hairs, green lanceolate leaf blade with entire margins, moderate leaf density, white wheel shape flower with purple color at petal border, 2.7 – centimeter – long calyx, green raw fruit with slightly skin and changed to red when mature, slender fruit, point up and length around 7.6 centimeters with 2.2 grams in weight, the width of the fruit's widest part 0.8 centimeters. Agricultural characters of Si Sa Ket 1 chili shrub is 75.8 – 86.0 centimeters in height. First fruit sets at 65 days after flowering (120 days after germination), 50 percent of fruit set at 95 days after germination. Total yield in the Northeastern and Northern areas are 1.03 – 1.87 kg per m^2 in a dry season and 0.32 – 0.34 kg per m^2 in a rainy season. This cultivar has anthracnose resistant. (Chokpacchuen C., personal communication).

2.2 Earthworm

In agricultural practices, soil quality is important for growing plants to obtain a high yield. One parameter that can imply soil quality is a biological indicator, such as soil microorganisms, macrofauna, and microfauna. Microfauna composed of protozoa, nematodes, small – sized collembolan and mites (Fusaro et al., 2018). Soil organisms are directly related to soil quality that they decompose organic materials into organic matter which is important for plant growth. Earthworm is classified into Phylum Annelida that lives in soil and plays an important role as a decomposer in ecosystems. Therefore, the earthworm is widely used in vermicompost production but not all types of earthworms can produce the compost. There is a report that only six to seven species of 6,000 species can be used in vermicompost production, for example, African nightcrawler (*Eudrilus euginiae*) (Biernbaum, 2014).

African nightcrawlers (*E. eugeniae*) is a native species of a tropical area in West Africa. A habitat of African nightcrawler earthworm is underground in a shallow soil. The earthworms come up to the ground surface but then live in the dark place mostly in loamy soil with high organic matter content. Optimal temperature for them is between 15 – 25°C, and the temperature of earthworm habitat should not reach 32°C. The temperature in a range of 29.5 – 30°C is suitable for their mating. The relative humidity is between 70 – 80 percent and should not be less than 60 percent. This type of earthworm requires good ventilation, with concentrations of CO_2 in a range of 0.01 – 11.5 percent and NH_3 lower than 0.5 mg/L. Salt concentration should not be more than 0.5 percent, and soil pH is neutral to slightly basic with the optimal pH values of 5 – 8. The taxonomic characteristics of African nightcrawler earthworm are cold – blooded, segmented worm and hermaphrodites. An adult earthworm is a bit smaller than a straw with length of 1 foot and weighs 4 grams. A body of earthworm is retractable with purple grayish color skin with blue scintillation when exposed to sunlight; one end of the body is a mouth and another end is a tail with an anal cavity. Earthworm mates by fusing the 14^{th} – 16^{th} segments with the other and produces egg cocoon of 162 – 188 eggs per year. African nightcrawler earthworm ingests organic wastes faster than other species, thus it is used widely in vermicompost production (Boonwinit, 2016).

2.3 Vermicompost

Vermicompost is one of the soil amendments, a source of nutrients that is suitable for plant growth. Vermicomposting is a low – temperature process of decomposing diverse fresh organic matter by microorganisms and earthworms into a stable compost (Biernbaum, 2014). Vermicompost application improves soil quality by increasing nitrogen – fixing and phosphate – solubilizing bacteria, resulting in sufficient nutrients for plant growth requirement (Glolami et al., 2018).

There are a few studies on the effects of vermicompost on plants. For example, the application of vermicompost in strawberry by mixing vermicompost and soil at the different ratios. The results showed that adding vermicompost can obviously improve the growth, biomass production, average fruit weight, plant height, and leaf area. Furthermore, vermicomposting significantly improved enzyme activity of soil microbes and cation exchange capacity of soil (Zuo et al., 2018).

2.4 Essential and beneficial elements for plants: Silicon

Essential elements are the elements that plants need to complete their life cycle. There are 92 naturally occurring elements on earth with 17 elements which are essential to all plants (Kaur et al., 2016). They are divided into macronutrients and micronutrients. Moreover, the elements that promote growth and may be essential to particular taxa but are not required by all plants are called beneficial elements (Pilon – Smits et al., 2009). Beneficial elements such as aluminum (Al), sodium (Na), selenium (Se), cobalt (Co) and silicon (Si) are not critical for all plants but may improve plant growth and yield (Kaur et al., 2016).

Silicon (Si) is one of the earth's most prevalent elements, comprising more than 25 percent of the earth's crust (Marchner, 2012). Si is a main content in cell wall; Si interacts with pectins and polyphenols and enhances cell wall rigidity and strength (Currie and Perry, 2007). Si accumulation is different among species and varies by the density of Si transporters in root, as well as by the distinct mechanisms of Si loading into the xylem (Ma and Yamaji, 2008). An ability of a plant to accumulate Si is varied between 0.1 – 10% dry weight. So, plants species were classified by mechanisms of silicon uptaking (Tubaña and Heckman, 2015). The most extensively studied beneficial effect of Si on plant health is its role to reducine plant susceptibility to fungal diseases (Fauteux et al., 2006; Cai et al., 2008). Si polymerization can reinforce the cell wall by physically inhibiting fungal germ tube penetration through the epidermis, thereby impeding infections (Hayasaka, Fujii and Ishiguro, 2008). Moreover, Si promotes plant sturdiness and resistance to a variety of biotic and abiotic stresses.

For the roles of Si in *Capsicum annuum L*., a study investigated Si effects when applying soluble Si via root and foliar applications. The results showed that the firmness and thickness of *Capsicum* fruits were enhanced by both methods. Furthermore, the disease induced by *Colletotrichum gloeosporioides* was delayed for two days by both treatments, with significantly greater influence by root treatment than that of foliar treatment (Jayawardana, Weerahewa, and Saparamadu, 2014). Moreover, Si application can significantly reduce the lesion areas caused by *C. gloeosporioides* in chili pepper fruits and increased the thickness of cuticle and concentration of the phenolic compound in the cell wall when compared to Si – free control (Jayawardana, Weerahewa and Saparamadu, 2015).

Although silicon is easy to be found, there are various forms of silicon in soil and it affects silicon uptake of plants. Silicon in soil is divided into two phases which are solid and liquid phases (Figure 2.1). The solid – phase silicon is found the most in soil, e.g. crystalline forms, poorly crystalline/ microcrystalline and amorphous forms. By the way, these silicon forms are not available for plant to uptake. Another form is the liquid – phase silicon which is in available form and can be absorbed by plant root; an example of liquid – phase silicon is monosilicic acid (Matichenkov and Bochanikova, 2001; Tubaña and Heckman, 2015).

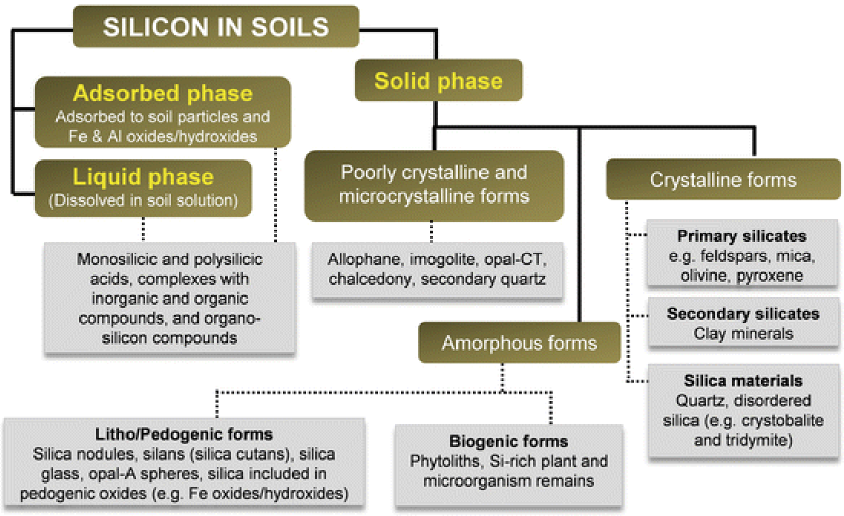

Figure 2.1 Fractions of silicon in soil (Tubaña and Heckman, 2015)

However, there are many factors that affect Si dissolution in the soil. Robinson, Brooks and Clothier (1999) have used *Berkheya coddii* in phytoremediation study. This research was composed of 4 treatments consisting of $MgCO_3$, $CaCO_3$, sulfur and chelating agent (an organic substance that can attach metal ions, e.g. NTA, DTPA, EDTA). For the results, both $MgCO_3$ and $CaCO_3$ decreased the metal uptake by affecting soil reduction and soil pH; sulfur and chelating agent could improve Ni and Co uptake significantly by increasing the dissolution of these elements (Robinson, Brooks and Clothier, 1999). So we can conclude that soil pH is an important factor for the element dissolution in soils. However, there are a few studies on the effects of vermicompost on silicon dissolution. Therefore, this study aims to investigate whether vermicompost would enhance soil available silicon content, silicon uptake and biomass production of Bird's eye chili.

Chapter III
Methodology

3.1 Materials

3.1.1 Plant materials

This experiment used Bird's eye chili ‘Si Sa Ket 1' which is courtesy of Si Sa Ket Horticultural Research Center, Department of Agriculture, Thailand.

To plant the Bird's eye chili, commercial soil was put in a seed tray, Bird's eye chili 2 seeds were planted in each hole of the seed tray and watered once every day. Chili plants that had 5 – 7 leaves were used in an experiment.

3.1.2 Vermicompost

Vermicompost was carried out in a greenhouse at Department of Botany, Faculty of Science, Chulalongkorn University. Firstly, cow manure was immersed in tap water with a ratio of 10: 14.3 kilograms for three days. Then, the water was discarded and the cow manure was ready for processing in a further step. Secondly, an earthworm bin was set up by putting the cow manure in four plastic containers (36.0 centimeters wide, 53.5 centimeters long and 14.0 centimeters tall), two kilograms per container. Thirdly, two grams of African nightcrawler earthworm (*Eudrilus eugeniae*) (from Loong Deaun’s farm in Pak Chong District at Nakhon Ratchasima Province) were cast in each container. Fourthly, dried soybean meal was soaked in tap water for five minutes before having been added 10 grams per container to feed the earthworms. Finally, each container was stacked as multi – layers, watered and covered with a mosquito net. The vermicompost bin was placed in the shade with the air temperature between 20 – 28 °C and relative humidity of 70 – 80 percent. The pH of the vermicompost bed was between 7.0 – 8.0. The vermicompost was collected every seven days for further analysis.

3.1.3 Soil

Soil sample was collected from a Bird's eye chili farm in Tha Mai District, Chanthaburi Province. Three areas in the farm were randomly assigned, soils within the depth of 0 – 30 centimeters were collected about 75 kilograms. The soils were mixed well and shoveled until they became evenly crumbly. Hundred to two hundred grams of soil were collected for physical and chemical property measurement. The soil samples were air – dried at room temperature, sieved through a 2 – mm sieve before being further analyzed.

3.1.4 Chemicals

The chemical solutions in the experiment were prepared as the following:

Si standard stock

To prepare Si standard stock (50 mM), sodium trisilicate (1.2112 grams) was dissolved in 100 milliliters of ddH_2O and adjusted the volume to 100 milliliters. The solution was autoclaved for 30 minutes then filtered to obtain the insoluble part. The insoluble part was dried in an incubator (N – Biotek, NB – 205) at 60°C until getting a constant dry weight. Sodium trisilicate did not dissolve completely, the dry weight of the insoluble part was used to recalculate the concentration of Si standard stock. Only 0.9334 grams of sodium trisilicate were dissolved, the actual concentration of Si standard stock solution was 38.53 mM, instead of 50 mM. This Si stock solution was used to perform Si standard curve.

Acetate buffer (1 N, pH 4)

To measure an available Si, 1 N acetate buffer (pH 4) was used. To prepare the acetate buffer, the solution A was prepared by dissolved 68.04 grams of sodium acetate ($CH_3COONa \bullet 3H_2O$) in 500 milliliters of ddH_2O. The solution B was prepared by dissolved 30 milliliters of acetic acid (CH_3COOH) to 0.5 liters. Then, the A solution was mixed with B solution in a ratio of 164 milliliters of solution A per 36 milliliters of solution B to get 1 N acetate buffer, pH 4.

Hydrochloric acid (0.6 N)

Hydrochloric acid was used to extract Si from samples (as acetate – soluble Si). Concentrated hydrochloric acid (HCl) (24.3053 milliliters) was dissolved in ddH_2O and adjusted to 0.5 liters to get 0.6 N hydrochloric acid.

Ammonium molybdate solution

To determine Si in samples by molybdenum blue assay, ammonium molybdate solution is the most important reagent. Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) (5.1 grams) was dissolved with ddH_2O and adjusted to 0.5 liters to get 10.2% (w/v) ammonium molybdate.

Sodium sulfate

Sodium sulfate (Na_2SO_4) was used in Si content determination. Sodium sulfate (8.5 grams) was dissolved with ddH_2O and adjusted to 0.5 liters to get 17.0% (w/v) sodium sulfate.

3.2 Experimental design

The experiment was designed as a randomized complete block design (RCBD) with four replications, using replication as a block. Treatments were as the following: (i) Control, soils with no added fertilizer, (ii) Positive control, the addition of silicate fertilizer (0.031 grams of sodium trisilicate per pot), (iii) Vermicompost, the addition of vermicompost (600 grams per pot), and (iv) Chemical fertilizer, the addition of 15 – 15 – 15 fertilizer for 10 grams per pot.

3.3 Plant growth measurement

Fifteen chili plants that had 5 – 7 leaves were selected and transplanted in 11 – inch pots containing two kilograms of soils. After that, all chili pots were placed in a greenhouse with a slight shade for seven days to let the chili plants recover from transplantation. Then, the chili plants were moved to an area with more sunlight in the greenhouse. They were watered once a day and grown under a natural light. Bird's eye chili growth was measured on 1^{st}, 4^{th}, 7^{th}, 14^{th} day and every 14 days after transplanted. The growth parameters have consisted of leaf number, plant height and bush width, measured with a 50 – centimeter stainless ruler. Data were shown as a growth curve.

3.4 Physicochemical properties of chili plants

After 49 days of planting, chili plants were collected and divided into four parts: old leaf, young leaf, stem and root. Chili samples were air – dried in a hot air oven at 60 °C for two days. Then, the dried samples were grounded in the mortar with liquid nitrogen and the chili powder was kept in a desiccator for subsequent analysis.

To determine silicon content in chili plants, dried powder was dissolved by autoclave – induced digestion method (Elliot and Snyder, 1991) with some modification. In brief, dried powders (0.1 grams) were added with 3 milliliters of 50% NaOH. The mixture was autoclaved at 1.5 lb/squared inch and 121 °C for one hour. Then, the extract was filtered and adjusted to a volume of 50 milliliters with distilled water. Subsequently, the extract was determined for silicon content with molybdenum blue colorimetric method (Imaizumi and Yoshida, 1958). Silicon content was calculated using silicon standard curve.

3.5 Physicochemical properties of soil and vermicompost

The physical and chemical properties of vermicompost and soil samples were determined before being used in the experiment. The physical and chemical properties consisted of pH, EC, moisture content, available silicon (water – soluble silicon and acetate – soluble silicon), amorphous silicon and amorphous silicon. The methodology of measurement and extraction of samples was as the following:

3.5.1 pH measurement

Based on the method previously described (Anderson and Ingram, 1993), samples (20 ± 0.1 grams) were added with 50 milliliters of water, mixed and stirred for 10 minutes then incubated for 30 minutes. After that, the mixture was stirred again for 2 minutes and pH was measured by using SevenCompact^{TM} pH/ Ion conductivity meter S220 (Mettler Toledo Group, Switzerland).

3.5.2 Electrical conductivity (EC)

Electrical conductivity was measured by following Anderson and Ingram (1993). Samples (20 ± 0.1 grams) were added with 50 milliliters of water and stirred for 10 minutes. After incubating for 30 minutes, it was stirred again for 2 minutes, following with 1 hour of incubation. Then, electrical conductivity was measured by using a conductivity tester (Hanna® instruments, USA).

3.5.3 Moisture content

According to Association of Official Analytical Chemists (1990), five grams of sample were dried in an incubator (N – Biotek, NB – 205) setting at 60°C for 2 days, or until getting a constant weight, and weighed to get dry weight. Then moisture content was calculated using the following equation:

$$\text{Moisture content (percent)} = [(W_1 - W_2) \times 100] / W_1$$

when W_1 is fresh weight and W_2 is dry weight.

3.5.4 Available Silicon

Water – soluble silicon was measured according to Nonaka and Takahashi (1988). Briefly, air – dried samples (10 grams) were sieved through a 2 – mm sieve. Then the samples were incubated with 60 milliliters of distilled water in a 50 – millimeter polycarbon bottle at 40°C for 1 week. After that, supernatant was taken for silicon measurement by using molybdenum blue colorimetric method (Dai et al, 2005). The absorbance at was measured using a microplate reader (SpectraMax® M3 Microplate Reader, Molecular Devices, USA).

For acetate – soluble silicon, air – dried samples (5 grams) were sieved through a 2 – mm sieve. Then the samples were incubated with 50 millimeters of 1 N acetate buffer (pH 4) in a 50 – millimeter polycarbon bottle at 40°C for 5 hours. The supernatant was collected and determined for silicon concentration (Imaizumi and Yoshida, 1958) using a standard curve of known silicon concentration (See Appendix).

3.5.5 Amorphous silicon

To measure amorphous silicon, samples (300 grams) were oven – dried at 40 °C for about two days. After dry weight was measured, amorphous silicon was extracted by following the method previously described (Georgiadis et al., 2015). In brief, the conditions for sample extraction were sample solution ratio of 1: 400, at room temperature. The extraction time was 168 hours with a horizontal shaker at 250 rpm.

After extraction, the extract was centrifuged at 3000 rpm for five minutes. In case the supernatant was not clear, additional filtration through a paper filter (1–2 μm) or a membrane filter (0.2 μm) was applied. Concentrations of silicon in the solution were determined by molybdenum blue assay in duplicates (Dai et al., 2005). Silicon content was calculated using a standard curve of silicon (Appendix).

3.6 Data and statistical analysis

The absorbance data from a microplate reader was determined with SoftMax® Pro Microplate Data Acquisition and Analysis Software Version 6 (Molecular Devices, USA). To investigate effects of vermicompost on available silicon content, silicon uptake and biomass production of Bird's eye chili, One – way ANOVA and Duncan’s multiples range test (DMRT) were used to analyze the variances and tested mean differences between treatments. Statistical analyses and graph preparation were done by using SPSS 22 for Windows (IBM Corp., Armonk, NYC, USA) and Microsoft Excel 2013 (Microsoft, USA).

Chapter IV
Results and Discussion

4.1 Chili plants

4.1.1 Growth of chili plants

After the chilies plants were transplanted, some growth parameters were measured to know how vermicompost affects chili growth. The growth parameters, which were leaf number, plant height and bush width, were measured on 1^{st}, 4^{th}, 7^{th}, 14^{th} day and every 14 days (28^{th} and 42^{nd} day). Then, the chili plants were collected for fresh and dry weight determination on the 49^{th} day; the results were shown as the following:

Leaf number

At the beginning, the average leaf numbers of chili plants were between 4.5 – 5.3 leaves. On the 7^{th} day, leaf production of the control and vermicompost treatments were increased, while that of chemical fertilizer treatment was remained the same. In contrast, chili plants supplemented with silicate fertilizer lost the first pair of true leaf, resulting in decrease of total leaf number (Figure 4.1). After 7 days, chili leaf production increased in all treatments. Furthermore, it was clearly seen that the vermicompost – treated chilies had the highest growth rate based on production of leaf number (Figure 4.1). Likewise, there was no clear difference in growth rate among the control, chemical fertilizer and silicate fertilizer at the early growth phase. Interestingly, leaf number of chilies increased dramatically between days 42 to 49. Based on an observation, it was assumed that a branching of chili bush affected the rapid of chili leaf number of all treatments during this growing period. On the 49^{th} day, the vermicompost – treated chilies had the highest leaf production, the second rank was control, followed by the chemical fertilizer and silicate fertilizer treatments.

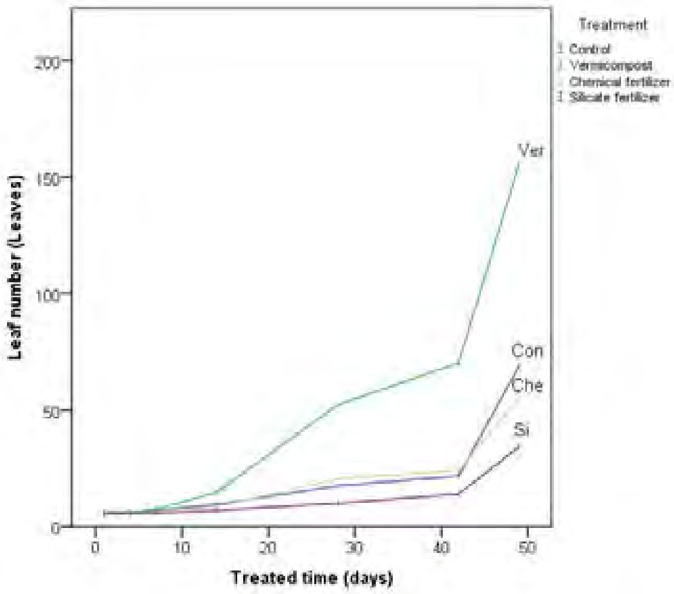

Figure 4.1 Time course of total leaf number during 49 days after transplanting.

At the end of experiment (49 days), there was significant difference of leaf number between vermicompost treatment and the control (Figure 4.2). Vermicompost increased leaf number of chili about 226 percent. Furthermore, the chemical fertilizer significantly enhanced leaf number of chili compared to the control. However, there was no difference between the silicate fertilizer treatment and the control. These findings imply that vermicompost and chemical fertilizer might enhance the chili growth.

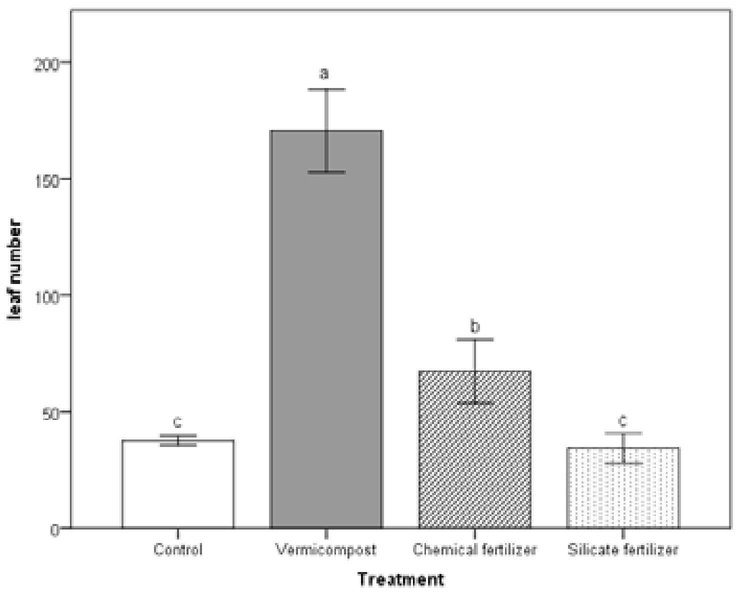

Figure 4.2 Time course of total leaf number during 49 days after transplanting. A graph shows mean ± SE (n=4). Significant mean difference according to Duncan's multiple range test ($P \leq 0.05$) was shown with the different lowercase letters.

<u>**Height**</u>

Chili height during 49 days was shown as in figure 4.3. The average chili height was between 3.3 – 3.9 centimeters at the beginning of experiment. After 14 days, chilies applied with vermicompost had higher height increment than other treatments. On the 49th day, chilies applied with vermicompost had the highest height, followed by the control, chemical fertilizer and silicate fertilizer, respectively. The increase in height did not cease yet at this cultivation time in all growing conditions.

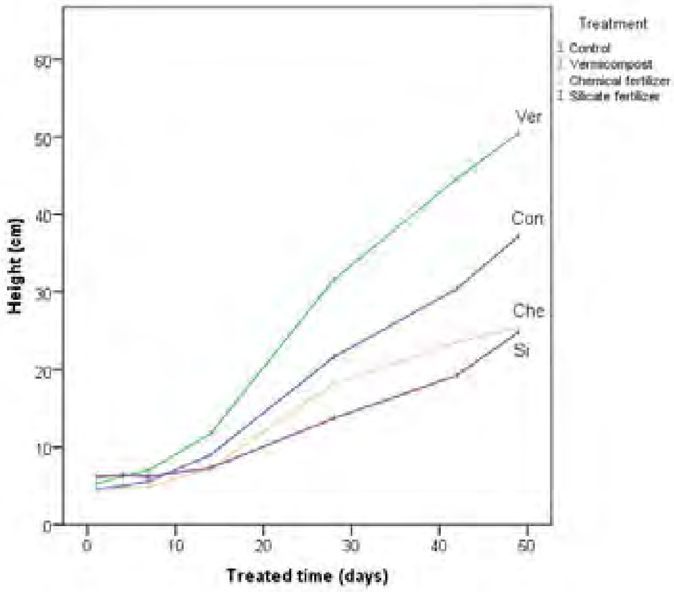

Figure 4.3 Time course of total height during 49 days after transplanting.

At the end of experiment, the height of chili plants applied with vermicompost was about 136 percent of control without fertilizer (Figure 4.4). However, there was no difference of height among other three treatments. Like chili leaf production, vermicompost application increments shoot height.

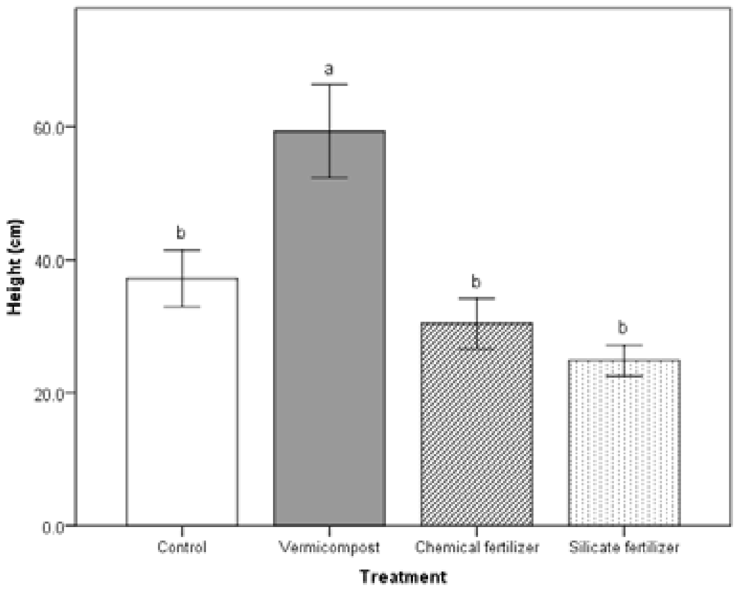

Figure 4.4 Time course of total height during 49 days after transplanting. Data shows mean ± SE (n=4). Significant mean difference according to Duncan's multiple range test (*P*≤0.05) was shown with the different lowercase letters.

<u>Bush width</u>

The growth curve based on bush width data was shown in figure 4.5. In the beginning, the average width of chilies were between 3.45 – 3.75 centimeters. Then, bush width values of chilies in all treatments increased dramatically. On the 49th day, vermicompost – treated chilies had the highest bush width, followed with chilies in the control, chemical fertilizer and silicate fertilizer treatments.

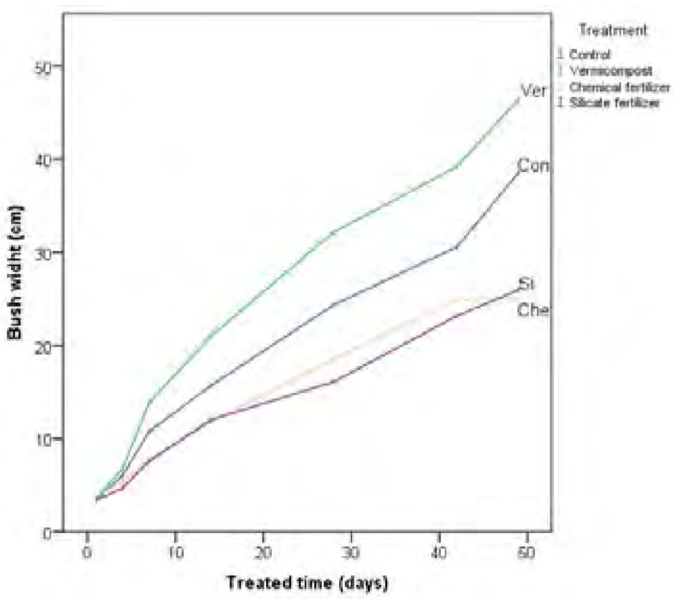

Figure 4.5 Time course of total bush width during 49 days after transplanting.

After 49 days of growing, bush width of vermicompost – applied chilies was similar to the control plants but significantly higher than the chilies applied with chemical fertilizer and silicate fertilizer (Figure 4.6). In total, it was clearly seen that vermicompost had high potential to be used for chili growth enhancement.

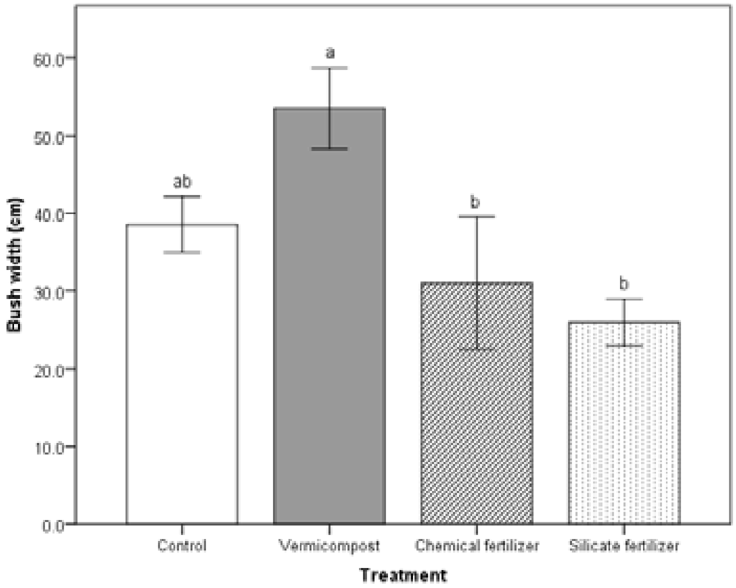

Figure 4.6 Time course of total bush width during 49 days after transplanting. Data shows mean ± SE (n=4). Significant mean difference according to Duncan's multiple range test ($P \leq 0.05$) was shown with the different lowercase letters.

<u>Dry weight</u>

Dry weight of chili plants was determined at the end of experiment (Figure 4.7). With vermicompost application, chili leaf dry weight was 463 percent of control (Figure 4.7). Furthermore, it was clearly seen that vermicompost was superior to other kinds of fertilizer in this experimental condition. Also, there was no difference in dry weight among other three treatments.

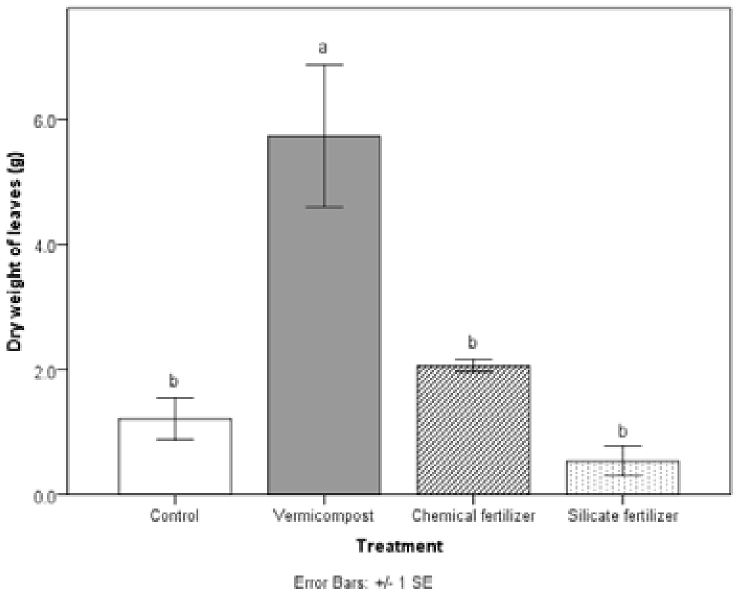

Figure 4.7 Time course of dry weight width during 49 days after transplanting. Data show mean ± SE (n=4). Significant mean difference according to Duncan's multiple range test ($P \leq 0.05$) was shown with the different lowercase letters.

From the results, vermicompost significantly increased chili leaf number, height, bush width and dry mass of shoots and roots, when compared to other conditions. These findings corresponded with the study of Lazcano et al. (2009) reporting that vermicompost addition obviously improved the biomass production of shoot and root in tomato plants. Vermicompost may benefit chili growth by various ways, such as increasing micronutrient content and stimulating enzymes that involve with growth and chlorophyll synthesis (Grusak and DallaPenna, 1999). Moreover, vermicompost consists of available form of nutrients, e.g. available phosphorus, soluble potassium, magnesium and calcium (Edwards and Burrows, 1988; Orozco et al., 1996), which plant can take up easily and sufficiently. In total, the data confirm the validity of vermicompost in enhancing plant growth, including chili.

However, the chili plants got a lot of diseases regardless of fertilizer application. Superior growth performance did not restrict the risk of pathogenic disease. The environment was the most important factor that impacted on disease infection of chili; the hot weather in a growing period of chili helped the dissemination and invasion of pathogens. During experimental period there were many pathogenic diseases that was hard to control. For examples, thrips (*Stenchaetohrips sp.*), green aphid (*Myzus persicae*), leafminer (*Liriomyza brassicae*) and other viruses. In order to solve this problem, the insecticide, Carbaryl S – 85 (3 grams per liters) was used to eliminate thrips and green aphids by spraying on chili leaves every 7 – 10 days.

4.1.2 Silicon content in chili plants

Silicon content measurement can indicate the ability to take up silicon of chili. Here, silicon contents in different parts of chili plants were shown as the following:

<u>Old leaf</u>

After 49 days of cultivation, old leaves of chili accumulated silicon about 374.67 ± 33.47 ppm under control condition (Figure 4.11, Table 4.1). However, there was no significant difference in silicon content among four treatments. These findings imply that vermicompost has no effect on silicon accumulation in chili old leaves.

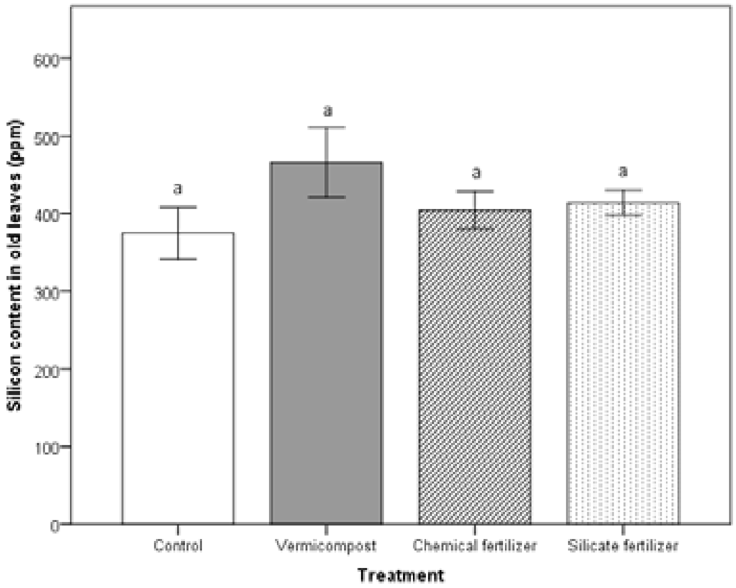

Figure 4.8 Silicon content in chili old leaves. Data shows mean ± SE (n=4). There was no significant difference ($P \leq 0.05$).

<u>Young leaf</u>

After 49 days of cultivation, silicon content in young leaves of chili was about 3 folds higher than in old leaves (Figures 4.11 and 4.12). The content was approximately 1296.49 ± 53.37 ppm in control plants (Figures 4.12, Table 4.1). Likewise, there was no significant difference of silicon contents among four treatments (Figure 4.12). Therefore, this shows that vermicompost has no effect on silicon accumulation of chili young leaf.

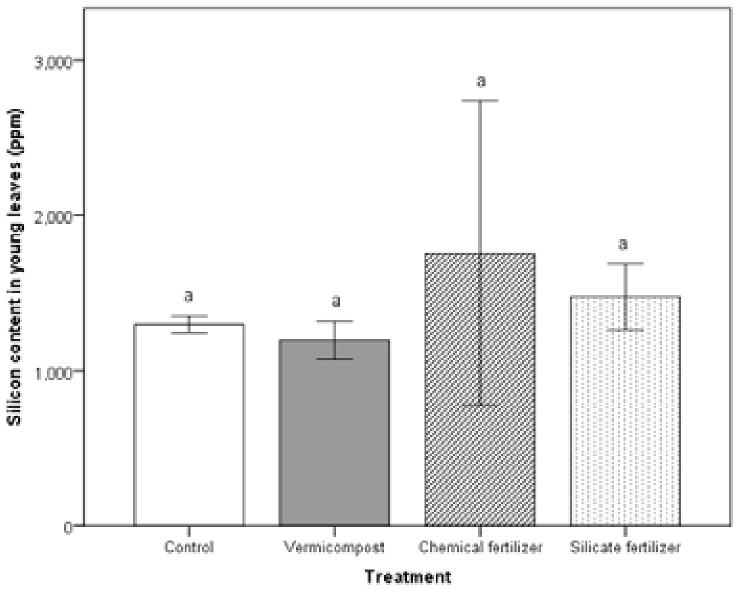

Figure 4.9 Silicon content in chili young leaves. Data shows mean ± SE (n=4). There was no significant difference (*P*≤0.05).

Stem

For stem parts, silicon content was at the same level as accumulated in young leaves; the content was approximately 1061.27 ± 26.70 ppm under control condition (Figures 4.12 and 4.13, Table 4.1). Also, the values were in a similar range in all treatments (Figure 4.13). These findings imply that vermicompost has no influence on silicon accumulation in chili stem.

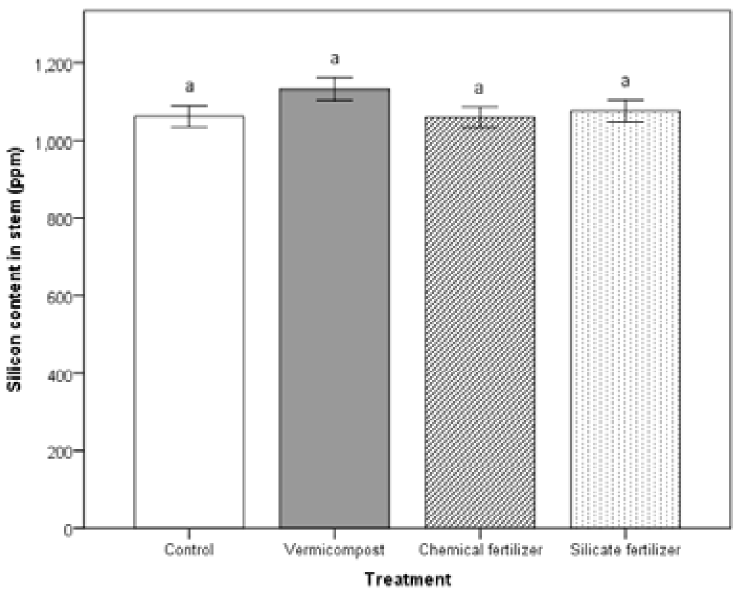

Figure 4.10 Silicon content in chili stem. Data shows mean ± SE (n=4). There was no significant difference (*P*≤0.05).

Root

The results showed that silicon also accumulated highly in root parts, with silicon content about 1549.23 ± 122.37 ppm under control condition (Figure 4.13, Table 4.1). With different kinds of fertilizer application, no change in silicon accumulation in root was found (Figure 4.13). Therefore, vermicompost did not influence the accumulation of silicon in chili root under this experimental condition.

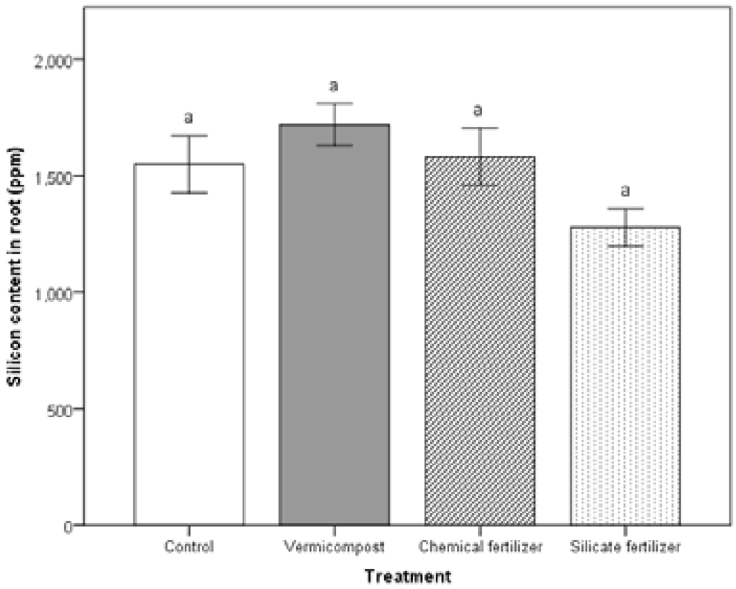

Figure 4.11 Silicon content in chili root. Data shows mean ± SE (n=4). There was no significant difference ($P \leq 0.05$).

From the results, vermicompost had no effect on silicon content in all parts of chili plants after 49 days of application. However, the data demonstrate that chili plants accumulated silicon in shoot about 0.07% dry weight. An ability of a plant to accumulate silicon varies highly between 0.1 – 10% dry weights. Overall, plants species were classified by mechanisms of silicon uptake into three classes (Tubaña and Heckman, 2015) as the following: (i) high silicon accumulation (4 – 10% dry weight), (ii) intermediate silicon accumulation (2 – 4% dry weight), and (iii) low silicon accumulation (0.1 – 2% dry weight).

In details, high silicon accumulation species had high – ability to take up silicon. Active mechanisms have an important role in silicon uptake of these plants. High silicon accumulation species are in Poaceae, Equisetaceae and Cyperaceae family. For intermediate silicon accumulation species, active and passive mechanisms have a role. Mostly, plants in intermediate silicon accumulation class are in Cucurbitales, Urticales and Commelinaceae family. In contrast, low silicon accumulation species lack of specific transporters. So, passive diffusion mechanism takes performance in silicon uptake and silicon transportation between cells. Examples of low silicon accumulation species are plants in Solanaceae and Fabaceae family.

Chili is a plant in Solanaceae family and in this experiment it accumulated silicon in shoot in a range of 0.1 – 2% dry weight. Therefore, chili is classified as a low silicon accumulating species. Lacking specific transporters and having passive transport mechanism may effect low silicon content accumulated in all parts of chili plants. This implication should be researched for more information in future.

Tables 4.1 Silicon content in different parts of chili under control condition.

Chili parts	Silicon content of control		
	ppm	ppm	%DW
Old leaves	374.67 ± 33.47^{NS}	672	0.07
Young leaves	1296.49 ± 53.37^{NS}		
Stems	1061.27 ± 26.70^{NS}		
Roots	1549.23 ± 122.37^{NS}	1,613	0.16

* F value: Analysis of variance shows no significant difference (NS) among four treatments.

In total, it was shown in this study that chili was the species that did not take up silicon a load of quantity. That is, chili accumulated silicon approximately 672 ppm in shoots and 1,613 ppm in roots (Table 4.1). It was also shown that vermicompost did not affect silicon accumulation in chili tissues in term of concentration (ppm). However, total silicon content in weight basis of vermicompost – applied chili (shoot: 7.85 mg/plant and root: 2.24 mg/plant) surely was higher than control (shoot: 1.34 mg/plant and root: 0.71 mg/plant). This information show that vermicompost might effect silicon co – localization in chili plants. This implication should be researched for more information in future.

4.2 Physicochemical properties of vermicompost and soil before the experiment

4.2.1 Vermicompost

After casting for one month, vermicompost was collected approximately 20 liters from every container and kept in a dry place, ready for being used in the experiment. The compost had a brown color, crumbly texture with earthy odor. During the composting process, there were some difficulties caused by uneven light intensity and moisture in each container. That is, the lowest floor of the container stack obtained higher moisture and less light intensity than the upper floor. This led to higher amounts of vermicompost produced by earthworms in the upper floor than on the ground floor. However, this had no impact on total compost yield.

Before being applied in the experiment, some physical and chemical properties of vermicompost were measured as shown in table 4.2. For pH, vermicompost was nearly neutral with pH 6.49. Vermicompost had electrical conductivity about 6.77 mS/cm, which is rather high, and moisture content of vermicompost was 81.69 percent.

For silicon analyses, vermicompost had amorphous silicon of about 2399.121 ppm (Table 4.2). For available silicon, water – soluble silicon was 11.840 ppm and acetate – soluble Si was 7.718 ppm.

4.2.2 Soil

For physicochemical properties, soil pH was quite acidic, about 5.04. For electrical conductivity, soil sample had a low value of about 0.48 mS/cm. Furthermore, the moisture content of the soil sample was 15.26 percent. In addition, silicon analyses showed that soil had water – soluble silicon of 7.325 ppm and acetate soluble Si content of 8.251 ppm. For amorphous Si, soil sample contained 1824.028 ppm. Besides, organic matter percentage is still in the progress of analysis.

Tables 4.2 Physicochemical properties of vermicompost and soil at the beginning of experiment

Physicochemical properties	Vermicompost	Soil
pH	6.49	5.04
Electrical conductivity (mS/cm)	6.77	0.48
Moisture content (percent)	81.69	15.26
Available silicon – water soluble (ppm)	11.840	7.325
Available silicon – acetate soluble (ppm)	7.718	8.251
Amorphous silicon (ppm)	2399.121	1824.028

4.3 Physicochemical properties of soil after the experiment

4.3.1 Soil

After the experiment, soils from four treatments were collected and air – dried to determine for physical and chemical properties: the results were shown as the following:

pH

After 49 days of experiment, soil samples were determined for pH. There was significant difference of pH in vermicompost treatment and control (Figure 4.17). Vermicompost increased pH of soil 123 percent (compared with control), silicate fertilizer also enhanced soil pH (110 percent of control) while chemical fertilizer treatment did not influence soil pH. These findings imply that vermicompost enhances soil pH. At first, the soil sample from the farm was quite acidity (pH=5.04). Typically, the proper pH for growing chili is around 6.0 – 6.5. Applying vermicompost to acidic soil can increase pH because pH of vermicompost was nearly neutral (Albanell et al., 1988). So, the results suggested that vermicompost can be used to improve acidic soil by increasing pH, so the chili can grow effectively (Eo and Park. 2019).

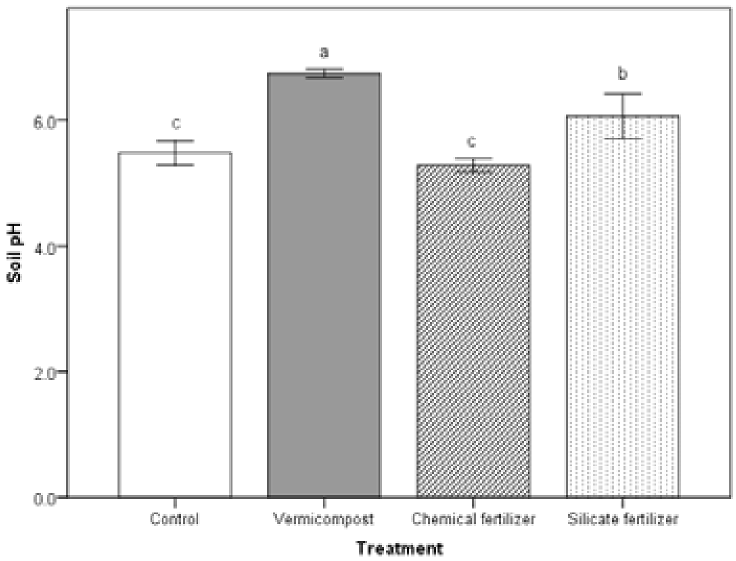

Figure 4.12 Soil pH after the experiment. Data show mean ± SE (n=4). Different lowercase letters indicate significant mean difference ($P \leq 0.05$).

Electrical conductivity

Electrical conductivity (EC) is a measure of the amount of salt in soil. Soil EC can illustrate the soil health. Soil EC also affect biomass production of plants, availability of nutrients and activity of microorganism in soil (United States Department of Agriculture, 2001: Online). The data show that soil EC of chemical fertilizer treatment was the highest in (Figure 4.18). There, the EC value was about 1.5 mS/cm which was about 272 percent of control value. Moreover, vermicompost treatment tended to enhance soil EC (161 percent of control), while silicate fertilizer treatment did not alter soil EC. In latter case, insolubility of silicate fertilizer might be the reason. However, the results have shown that applying vermicompost and chemical fertilizer increased soil EC to be higher than the initial levels. This information reveals that the addition of manure and nutrients has an important role in raising soil EC (Karmegam et al., 2019). Because increase of soil EC depends on the salt releasing and inorganic ions purging, ammonium based compound which was released tend to boost soil EC (Fang et al., 1999; Negi and Suthar. 2018). Moreover, vermicompost is organic fertilizer that releases nutrient slowly. That is a reason why chemical fertilizer can increase EC faster and greater than vermicompost. In total, application of vermicompost and chemical fertilizer truly nourished the soil as seen by the increase of soil EC.

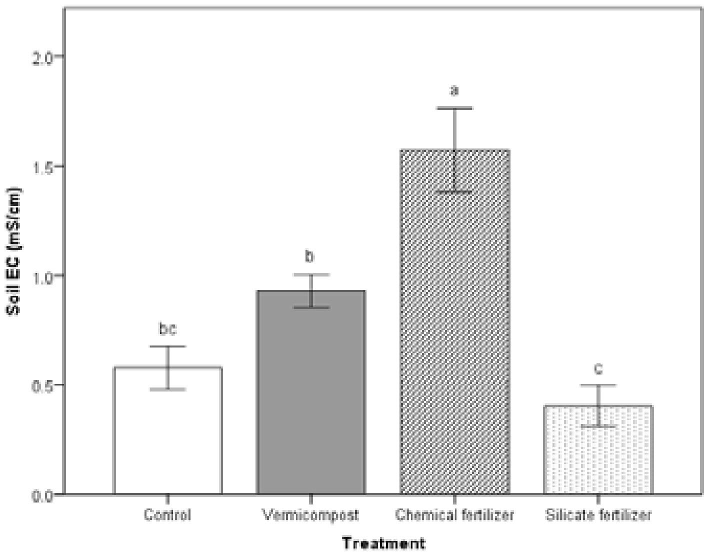

Figure 4.13 Soil EC after the experiment. Data show mean ± SE (n=4). Different lowercase letters indicate significant mean difference ($P \leq 0.05$).

<u>Available silicon</u>

Available silicon was a silicon form that plants can take up and use directly, there are two types of available silicon which had to be determined in this experiment; the results were shown as the following:

- **Acetate – soluble silicon**

From figure 4.14, vermicompost – treated soil had the highest of acetate – soluble silicon content, the second was silicate fertilizer treatment, followed by the control and chemical fertilizer. However, there was no significant difference among four treatments. These findings imply that vermicompost has no effect on acetate – soluble silicon in soil.

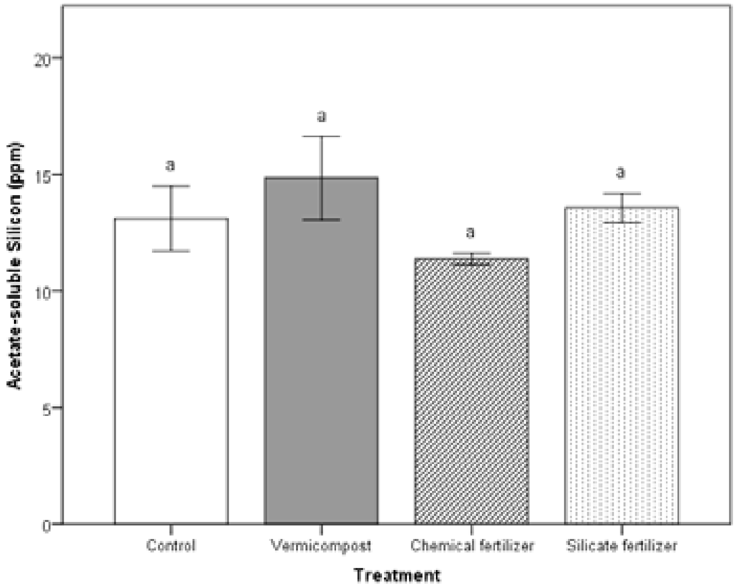

Figure 4.14 Available silicon content: acetate – soluble silicon in soil after the experiment. Data shows mean ± SE (n=4). Significant mean difference according to Duncan's multiple range test ($P \leq 0.05$) was shown with the different lowercase letters.

- **Water – soluble Silicon**

Figure 4.15 shows the water – soluble silicon content in the soil. Silicate fertilizer treatment had the highest of water – soluble silicon content, the second was chemical fertilizer treatment, followed by vermicompost and control. By the way, there was no significant difference between four treatments. Therefore, vermicompost has no effect on water – soluble silicon in soil.

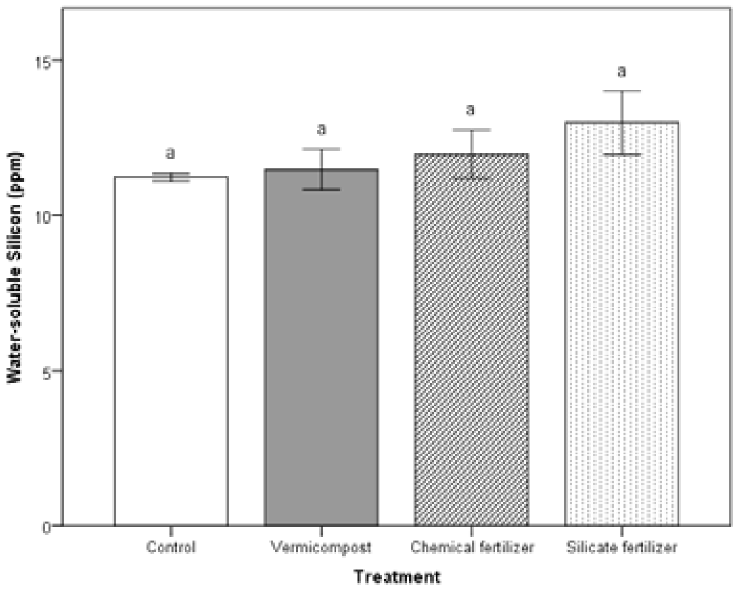

Figure 4.15 Available silicon content: water – soluble silicon in soil after the experiment. Data shows mean ± SE (n=4). Significant mean difference according to Duncan's multiple range test (*P*≤0.05) was shown with the different lowercase letters.

<u>Amorphous silicon</u>

From figure 4.16, chemical fertilizer and silicate fertilizer treatments had higher amorphous silicon in soil than control. In addition, the highest amorphous silicon content was found in silicate fertilizer treatment and there was no significant difference of amorphous silicon among vermicompost treatment and control. These findings imply that vermicompost has no effect on amorphous silicon in soil.

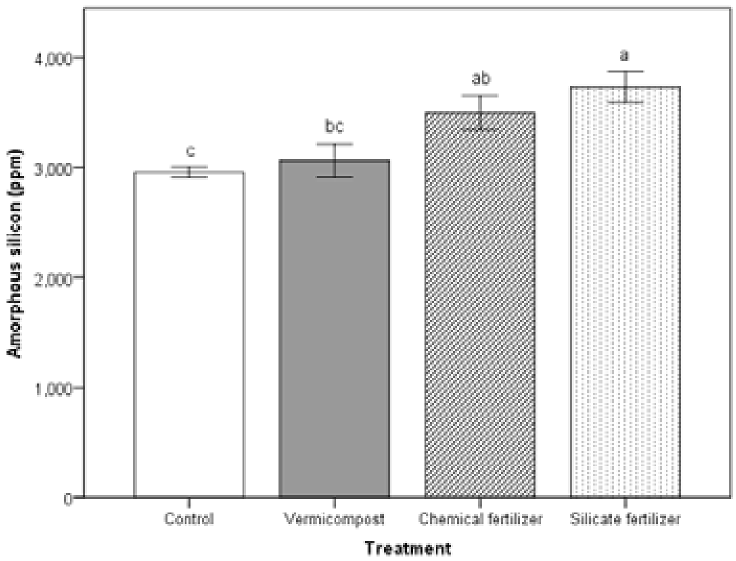

Figure 4.16 Amorphous silicon content in soil after the experiment. Data show mean ± SE (n=4). Significant mean difference according to Duncan's multiple range test (*P*≤0.05) was shown with the different lowercase letters.

From results, it was clearly shown that vermicompost had no effect on enhancing available silicon and amorphous silicon in soil. Up to now, there has been a few studies on vermicompost and silicon availability. Previous report on soil amendment by inoculating earthworm directly has shown that earthworm can instigate mobility and availability of silicon in soil (Bityutskii, Kaidun and Yakkonen, 2016). So, it may be that using vermicompost may lack of specific function provided by earthworm resulting in different outcomes in this study.

Chapter V
Conclusion

This project aims to study the effects of vermicompost on available silicon content, silicon uptake and biomass production of Bird's eye chili. To do so, chili plants were grown under four conditions: soils without amendment (control), with vermicompost (300 gram/kilogram), with chemical fertilizer (15 – 15 – 15, 5 gram/kilogram) and with sodium trisilicate fertilizer (1 mM SiO_2) for 49 days. For effects on available silicon and amorphous silicon in soil, the results showed that vermicompost did not affect soil available silicon and amorphous silicon contents. However, vermicompost slightly elevated soil EC and pH. For chili growth determination, vermicompost highly enhanced the growth rate during 49 days of planting and shoot and root biomass. However, the beneficial effect on chili growth was not corresponded with silicon uptake of chili plants, which are a silicon low accumulating species. Besides EC, pH and soil silicon, vermicompost may stimulate chili growth and development via other means. That is, vermicompost may provide high amount of other nutrients such as available nitrogen, phosphorus, potassium and organic matter. In total, it was concluded that vermicompost application significantly enhanced biomass production of Bird's eye chili without modifying soil silicon availability and silicon uptake.

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Appendix

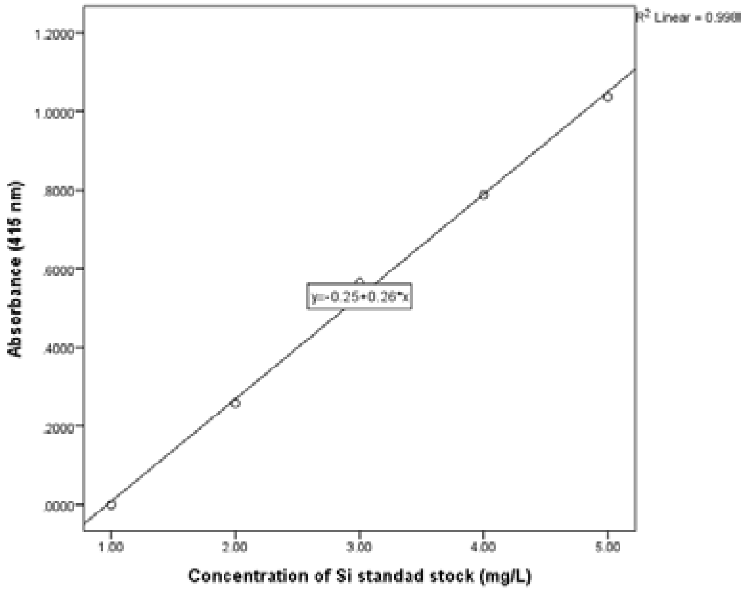

Figure A1. A standard curve of determination of Si concentration

Vita

Miss Laksana Kachaban was born on 5th September, 1996 in Chanthaburi, She has been interested in plants since she was 4 years old. So she decided to apply in Botany major at Department of Botany, Faculty of Science, Chulalongkorn University after she had finished her high school.