ผลของวัสดุชีวภาพและกึ่งชีวภาพต่อการเติบโตและคุณภาพหลังการเก็บเกี่ยวของผัก
สลัด *Lactuca sativa* L. พันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊ก

นางสาวไพบูลย์ หมุ่ยมาศ

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EFFECTS OF BIOMATERIAL AND SEMI-BIOMATERIAL ON GROWTH AND POSTHARVEST QUALITY OF BUTTERHEAD AND RED OAK LETTUCES *Lactuca sativa* L.

Miss Paiboon Muymas

A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	EFFECTS OF BIOMATERIAL AND SEMI-BIOMATERIAL ON GROWTH AND POSTHARVEST QUALITY OF BUTTERHEAD AND RED OAK LETTUCES *Lactuca sativa* L.
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ไพบูลย์ หมุ่ยมาศ : ผลของวัสดุชีวภาพและกึ่งชีวภาพต่อการเติบโตและคุณภาพหลังการเก็บเกี่ยวของผักสลัด *Lactuca sativa* L. พันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊ก (EFFECTS OF BIOMATERIAL AND SEMI-BIOMATERIAL ON GROWTH AND POSTHARVEST QUALITY OF BUTTERHEAD AND RED OAK LETTUCES *Lactuca sativa* L.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. กนกวรรณ เสรีภาพ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ศุภจิตรา ชัชวาลย์, ดร. ธีรดา หวังสมบูรณ์ดี, หน้า.

การศึกษานี้มีจุดประสงค์เพื่อเพิ่มผลผลิตผักสลัดด้วยการใช้วัสดุชีวภาพจากเปลือกกุ้ง (SS) และวัสดุกึ่งชีวภาพจากกากไคติน (FCM) ทั้งในกระถางและแปลงปลูกทดลองของเกษตรกร โดยปลูกผักสลัด *Lactuca sativa* L. พันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊กในกระถางในช่วงเวลา 3 ฤดู และในแปลงปลูกทดลองเกษตรกรอีก 2 ฤดู การปลูกในกระถางใส่เปลือกกุ้งและกากไคตินผสมกับวัสดุปลูกดังนี้ ดินผสมมูลวัวในอัตราส่วน 10:1 (T1) เปลือกกุ้ง 0.5% (T2) เปลือกกุ้ง 0.5% ร่วมกับแบคทีเรียผลิตไคติเนส *Bacillus licheniformis* SK-1 (T3) เปลือกกุ้ง 0.25% ร่วมกับกากไคติน 0.25% (T4) กากไคติน 2% (T5) และ แบคทีเรีย SK-1 10 มิลลิลิตร (T6) พบว่า ทุกชุดการทดลองที่ใส่เปลือกกุ้งและกากไคตินมีการเพิ่มขึ้นของผลผลิตอย่างมีนัยสำคัญของผักสลัดพันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊กทั้ง 3 ช่วงฤดูการปลูก เมื่อทำการปลูกในช่วงฤดูที่ 1 และ 3 พบว่า ชุดการทดลอง T5 มีการเพิ่มขึ้นของผลผลิตมากที่สุด คือ น้ำหนักสด น้ำหนักแห้ง จำนวนใบ ความกว้างและความยาวใบ ผักสลัดพันธุ์บัตเตอร์เฮดที่ปลูกในช่วงฤดูที่ 2 ชุดการทดลอง T5 มีการเพิ่มขึ้นของผลผลิตมากที่สุด ยกเว้นจำนวนใบ ที่มีอันดับที่ 2 รองจากชุดการทดลอง T3 ผักสลัดพันธุ์เรดโอ๊กที่ปลูกในช่วงฤดูที่ 2 พบว่า ชุดการทดลอง T5 มีการเพิ่มขึ้นของน้ำหนักสด และจำนวนใบมากที่สุด ขณะที่น้ำหนักแห้ง และความกว้าง มีอันดับที่ 2 รองจากชุดการทดลอง T2 การสูญเสียน้ำหนักสดหลังการเก็บรักษาที่อุณหภูมิ 8 องศาเซลเซียส ที่ความชื้นสัมพัทธ์ 60% พบว่า ทุกชุดการทดลองที่ใส่เปลือกกุ้งหรือกากไคตินมีการลดลงของการสูญเสียน้ำหนักสดอย่างมีนัยสำคัญทั้งผักสลัดพันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊ก การปลูกผักสลัดพันธุ์บัตเตอร์เฮดในช่วงฤดูที่ 1 และ 3 พบว่า ชุดการทดลอง T5 มีการสูญเสียน้ำหนักสดน้อยที่สุด การปลูกผักสลัดในช่วงฤดูที่ 2 พบว่า T5 มีอันดับที่ 2 รองจาก T3 ในผักสลัดพันธุ์เรดโอ๊กมีการสูญเสียน้ำหนักสดหลังการเก็บรักษาน้อยที่สุดทั้ง 3 ช่วงฤดูการปลูก การประเมินคุณลักษณะภายนอกโดยรวมของผักสลัดพันธุ์บัตเตอร์เฮด พบว่า ในช่วงฤดูที่ 1 และ 3 ชุดการทดลอง T5 มีคะแนนคุณลักษณะภายนอกโดยรวมมากที่สุด ในช่วงฤดูที่ 2 ชุดการทดลอง T2 และ T5 มีคะแนนคุณลักษณะภายนอกโดยรวมเท่ากันซึ่งสูงกว่าชุดการทดลองอื่นๆอย่างมีนัยสำคัญ ในผักสลัดพันธุ์เรดโอ๊ก พบว่า ทั้ง 3 ช่วงฤดูการปลูก T5 มีคะแนนคุณลักษณะภายนอกโดยรวมมากที่สุด นอกจากนี้ยังพบว่า ในช่วงฤดูที่ 1 และ 2 ชุดการทดลอง T4 และ T2 มีคะแนนคุณลักษณะภายนอกโดยรวมสูงเท่ากับชุดการทดลอง T5

จากการทดลองในกระถางพบว่า ชุดการทดลองที่ใส่กากไคติน 2% (T5) ดีที่สุด ดังนั้นจึงเลือกมาศึกษาในแปลงปลูกทดลองเกษตร โดยปลูกผักสลัดพันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊กเป็นเวลา 2 ช่วงฤดู ดังนี้ ใช้กากไคติน 20 กรัม ผสมกับวัสดุปลูกก่อนและหลังปลูก 1 สัปดาห์ เปรียบเทียบกับชุดการทดลองที่ไม่ใส่กากไคติน (ชุดการทดลองควบคุม) จากผลการทดลองพบว่า ทั้ง 2 ช่วงฤดูการปลูกของผักสลัดพันธุ์บัตเตอร์เฮด ชุดการทดลองที่ใส่กากไคติน มีการเพิ่มขึ้นของผลผลิตอย่างมีนัยสำคัญ โดยมีการเพิ่มขึ้นของพื้นที่ใบและน้ำหนักสด การปลูกผักสลัดพันธุ์บัตเตอร์เฮดในช่วงฤดูการปลูกที่ 1 พบว่า มีการเพิ่มขึ้นของจำนวนใบ เส้นผ่าศูนย์กลางหัว น้ำหนักสดและน้ำหนักแห้งอย่างมีนัยสำคัญ ในผักสลัดพันธุ์เรดโอ๊ก ทั้ง 2 ช่วงฤดูการปลูก พบว่า มีการเพิ่มขึ้นของ จำนวนใบ ความกว้าง ความยาว น้ำหนักสดและน้ำหนักแห้งอย่างมีนัยสำคัญ การปลูกผักสลัดพันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊กทั้ง 2 ช่วงฤดู พบว่า ชุดการทดลองที่ใช้กากไคตินมีการสูญเสียน้ำหนักสดหลังการเก็บรักษาที่อุณหภูมิ 8 องศาเซลเซียส ที่ความชื้นสัมพัทธ์ 60% มีการลดลงของการสูญเสียน้ำหนักสดอย่างมีนัยสำคัญเมื่อเปรียบกับชุดการทดลองควบคุม การปลูกผักสลัดพันธุ์บัตเตอร์เฮดในช่วงฤดูการปลูกที่ 1 พบว่า ชุดการทดลองที่ใช้กากไคตินมีคะแนนคุณลักษณะภายนอกโดยรวมดีกว่าชุดควบคุมอย่างมีนัยสำคัญ นอกจากนี้ยังพบว่า การปลูกผักสลัดพันธุ์เรดโอ๊กทั้ง 2 ช่วงฤดูการปลูกในชุดการทดลองที่ใช้กากไคตินมีคะแนนคุณลักษณะภายนอกโดยรวมดีกว่าชุดควบคุมอย่างมีนัยสำคัญ จากการศึกษาพบว่า กากไคตินที่มีคุณสมบัติของไคโตซานและมีปริมาณไนโตรเจนสูงทำให้สามารถกระตุ้นการเติบโตและเพิ่มผลผลิตผักสลัดพันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊กได้ในทุกสถานที่ปลูก สำหรับการวัดอัตราการสังเคราะห์แสง อัตราการคายน้ำ และค่าการชักนำการเปิดปากใบ พบว่า ผักสลัดพันธุ์เรดโอ๊ก มีค่าอัตราการสังเคราะห์แสง อัตราการคายน้ำ และการชักนำการเปิดปากใบสูงกว่าชุดการทดลองควบคุมอย่างมีนัยสำคัญ ในผักสลัดพันธุ์บัตเตอร์เฮด ค่าอัตราการสังเคราะห์แสง อัตราการคายน้ำ และการชักนำการเปิดปากใบไม่มีความแตกต่างกันระหว่างชุดการทดลองทั้ง 2 ช่วงฤดูการปลูก จากการศึกษาในครั้งนี้พบว่า การใช้กากไคติน 2% สามารถเพิ่มผลผลิตและรักษาคุณภาพหลังการเก็บรักษาของผักสลัดพันธุ์บัตเตอร์เฮดและพันธุ์เรดโอ๊กได้ดีที่สุดในทุกฤดูการปลูก

ภาควิชา พฤกษศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา พฤกษศาสตร์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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PAIBOON MUYMAS: EFFECTS OF BIOMATERIAL AND SEMI-BIOMATERIAL ON GROWTH AND POSTHARVEST QUALITY OF BUTTERHEAD AND RED OAK LETTUCES *Lactuca sativa* L.. ADVISOR: ASST. PROF. KANOGWAN SERAYPHEAP, CO-ADVISOR: ASSOC. PROF. SUPACHITRA CHADCHAWAN, DR. TEERADA WANGSOMBOONDEE, pp.

This study aimed to increase the production of lettuce through the application of biomaterial from shrimp shell (SS) and semi-biomaterial from fermented chitinous material (FCM) both in a test plot and in a local farm. 'Butterhead' lettuce (*Lactuca sativa* L. cv. 'Butterhead') and 'Red Oak' lettuce (*Lactuca sativa* L. cv. 'Red Oak') were cultivated during three crop seasons in a test plot and two crop seasons in a local farm. In the test plot, SS and FCM were supplemented to the 10:1 soil/cow manure growing medium (T1) as following: 0.5% SS (T2), 0.5% SS with chitinase-producing *Bacillus licheniformis* SK-1 (T3), 0.25% SS and 0.25% FCM (T4), 2% (T5) and 10 mL of SK-1 alone (T6). The supplementations of SS and/or FCM resulted in significant increases in yield of 'Butterhead' and 'Red Oak' lettuces in all three crop seasons. When applied in a test plot during the first and third crop seasons, lettuces grown with the presence of T5 showed the highest increase in yield as evaluated in terms of fresh weight, dry weight, leaf number, and leaf width and length. Supplementation of T5 during the second crop season of 'Butterhead' lettuce also resulted in the highest increases in yield with the exception of leaf numbers which was second to those treated with T3. During the second crop season of 'Red Oak', the cultivation with the presence of T5 resulted in the highest increase in fresh weight and leaf numbers, while dry weight, leaf width was second to those treated with T2. Weight losses after storage at 8°C and 60% relative humidity (RH) for 2 weeks were significantly reduced in all treatments treated with SS or FCM in both 'Butterhead' and 'Red Oak' lettuces. During the first and third crops, T5 treatment of 'Butterhead' lettuce resulted in the lowest fresh weight loss except during the second crop season which was second to those treated with T3. In 'Red Oak', the lowest of fresh weight loss was found in the T5 treatment in all three crop seasons. The best overall appearance of 'Butterhead' lettuce was observed when T5 was applied during the first and third crop seasons, while during the second crop, T2 and T5 treatments showed a significantly better overall quality than those treated with other treatments. The best overall appearance of 'Red Oak' lettuce was observed when T5 was applied during all three crop seasons. In addition, during the first and second crop season both T4 and T2 treatments showed significantly higher overall quality as in the T5 treatment.

FCM, the most outstanding treatment from the test plot experiment was used to test in a local farm. 'Butterhead' and 'Red Oak' lettuces were cultivated during two crop seasons. Twenty grams of the FCM per plant were supplemented to growing medium one week before and after transplantation compared to untreated soil (control). The supplementation of FCM resulted in significant increases in yield of 'Butterhead' lettuce as evaluated in terms of fresh weight in both crop seasons. During the first crop season 'Butterhead' lettuce showed a significant increase in leaf numbers, diameter of the lettuce head and fresh and dry weights. FCM treatment resulted in significant increases in yield of 'Red Oak' lettuce in terms of leaf numbers, leaf width, leaf length, and fresh and dry weights in both crop seasons. During both crop seasons, weight losses after storage at 8°C and 60% RH for 2 weeks were significantly reduced in FCM treatment in both 'Butterhead' and 'Red Oak' lettuces. 'Butterhead' lettuce treated with FCM showed significantly better overall visual quality than the control treatment during the first crop season. The finest overall lettuce appearance was observed in the FCM treated 'Red Oak' in both crop seasons. Our findings indicate that FCM with chitosan properties and high N content can promote growth and yield of 'Butterhead' and 'Red Oak' lettuces by affecting mineral allocation. Photosynthesis, transpiration rate and stomatal conductance of 'Red oak' lettuce in the FCM treatment were significantly higher than the control treatment during the second crop season. There was no significant difference in photosynthesis, transpiration rate and stomatal conductance of 'Butterhead' lettuce during both crop seasons. The results indicate that the application of 2% FCM is the best all-year-round supplement for 'Butterhead' and 'Red Oak' lettuce cultivation.

Department: Botany
Field of Study: Botany
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Student's Signature .....
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Co-Advisor's Signature .....
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CHAPTER I
INTRODUCTION

A concern for alternative food is now increasing which results in a high demand for environmentally safe production of food crops focusing on salad vegetable. Worldwide, lettuce (*Lactuca sativa* L.) is the most popular vegetable among salad vegetable crops (FOSTAT 2011). The lettuce growing processes usually typically apply high inputs of chemical substances to stimulate plant growth and yield (Shibuya and Minami 2001); (Hernández, Castillo et al. 2010) however, there many alternative production practices, researchers use various biomaterial nutrient sources to replace chemical fertilizers. Chitin, a natural polysaccharide which is present in a variety of species including shells of crustaceans, cuticles of insects, and cell wall of fungi and some algae (Nge, Nwe et al. 2006) can be used as organic growth stimulator to obtain higher crop yield (Sharp 2013).

Over the last 20 years, a rapid expansion in the culture of seafood industry has caused high amounts of waste materials rich in chitin. The byproducts of seafood processing containing high percentage of chitin which are pre-treated by to reduce size, deprotein and demineralize thus obtaining a chitin that can be used in several processes (Aye, Karuppuswamy et al. 2006, He, Chen et al. 2006). Microbial chitinase production have been using pre-treated chitins as a substrate (Wang and Chang 1997). Best of all, fermented chitinous materials are environmental safe, biocompatible, and biodegradable with plant tissues and display unique properties that are suitable for agriculture application (Shibuya and Minami 2001).

Fermented chitinous materials were shown to increase plant growth and behaved as useful agents that elicit defense reactions in plants and reduce the growth of pathogenic fungi and bacteria (Shibuya and Minami 2001). Plant cells can perceive chitin fragments resulting in increased plant metabolism and defense responses (Wan, Zhang et al. 2008). Ha et al. (2008) reported that supplementation of soils with seafood-wasted powder and *Bacillus subtilis* strain PMB-034 were effective in controlling *Fusarium* wilt of asparagus bean. Shoot dry weight also

increased from theses treatments. In addition, *Bacillus licheniformis* has been also reported to used in the production of extracellular chitinolytic enzymes (Kudan and Pichyangkura 2009) and can be an efficient plant stimulator (Brunetti, Farrag et al. 2012). It is implied that *B. licheniformis* is a good candidate for soil supplementation together with fermented chitinous material.

However, there is a few reports on the effect of chitin and *Bacillus* spp. amendment on plant growth and yield of vegetable. This research developed soil supplements using chitin in order to increase lettuce production. Effects of shrimp shell powder (SS), and fermented chitinous material (FCM), and *Bacillus licheniformis* strain SK-1 (SK-1) on growth, yield and postharvest quality of lettuces were investigated. The effect of these supplement on soil microbial populations and on lettuce physiology were also examined. Preliminary studies carried out during 2 months period in 2009 showed that when different amounts of SS or FCM were added to the growing medium twice on weeks 3 and 4, yield of the treated lettuces were increased. The best treatments of each material (0.5% SS and 2% FCM) were used as the basis of SS/FCM treatments in the present study

Objectives

1. To investigate the effects of biomaterial and semi-biomaterial on growth, yield and postharvest quality of lettuces in a test plot during three successive crop seasons.
2. To investigate the effects of biomaterial and semi-biomaterial on growth, yield, postharvest quality and selected soil microbial populations of lettuces in a local farm.

Expecting benefits

This study can be applied to assist farmers in increasing lettuce yield quality while reducing production cost. The results will increase postharvest quality and the nutritional value of lettuce. Finally, the integrated and organic product will promote the health of farmers and consumers in the future.

Content of the thesis:

1. Literature review.

2. Determination of effects of biomaterial and semi-biomaterial on growth, yield and postharvest quality of lettuces in a test plot during three successive crop seasons.
3. Determination of effects of biomaterial and semi-biomaterial on growth, yield, postharvest quality and selected soil microbial populations of lettuces in a local farm.
4. Results and discussion.
5. Conclusions.

CHAPTER II

LITERATURE REVIEWS

2.1 Lettuce

Lettuce (*Lactuca sativa* L.) is a member of the family Asteraceae (Compositae) (Still 2007). In Thailand, the total area under lettuce and chicory in 2010 was 3,637 hectare with a production of 8,613 Kg/hectare. The growing area was 3,750 in 2012 with a production of 8,533 Kg/hectare. It is mainly produced near big cities such as Nonthaburi and Bangkok.

‘Butterhead’ lettuce

‘Butterhead’ lettuce (Figure 1) is the most popular type of lettuce grown in Europe. The inside leaves, because of their lack of light, are cream or butter colored. The outer leaves are darker green (Rindels. 1994) Butterhead varieties are very tolerant to soil and weather conditions. Varieties are also not bitter in flavor, slow bolting, and mature in 55 to 65 days (Miles 2003).

‘Red Oak’ lettuce

‘Red Oak’ lettuce (Figure 2) is the easiest lettuces to grow. Leaves hav widevariety of shapes and colors. The leaves are tender, and not bitter, and plants are slow bolting. Varieties tend to mature in 30-55 days (Miles 2003).

Figure 1 ‘Butterhead’ lettuce (*Lactuca sativa* L. cv. ‘Butterhead’)

Figure 2 ‘Red Oak’ lettuce (*Lactuca sativa* L. cv. ‘Red Oak’)

Cultural requirements

Lettuce is relatively tolerant to a wide range of climatic and soil conditions It needs well-drained sandy loams, with a pH of 6.0-6.8. Many are tolerant to high day temperatures, although the most suitable temperature is 15-20°C. High temperatures will result in premature flowering, slow growth and bitter taste.

Seeds require a period of dry storage before sowing. The optimum germination temperature is 25°C; above this temperature, germination percentage falls rapidly due to an inhibition of gaseous diffusion and a consequent shortage of oxygen (Tindall 1983).

Irrigation is required at frequent intervals, particularly at transplanting and until the seedlings are established. Dry conditions are likely to induce premature flowering (Tindall 1983). Lettuce prefers a sandy-loam soil high in organic matter for growth and deverlopment (George Kuepper and Raeven Thomas 2002). Optimal fertilizer management and efficient use of N, P and K are necessary to improve yield and quality and to reduce production cost (Hoque, Ajwa et al. 2010).

Growth and development of lettuce

Most heading cultivars mature within 60-85 days from transplanting but the loose leaf types may be ready for harvesting within 35-50 days from planting (Tindall 1983). Lettuce passes through six distinct development stages: from seed to heading periods. The seedling stage occurs when the first true leaf develops a distinct circular cluster of leaves known as a rosette. Head formation will occur until the crop is ready for harvest (Kerns 1999).

Harvest and post-harvest

Harvesting lettuce during the early part of the day is preferable, particularly in hot weather. Lettuce is cut near the soil surface with a long knife then trims unwanted leaves usually leaving 4 to 5 wrapper leaves. After harvest, the lettuce is transported to a cooling storage room (Kerns 1999).

Use and nutritional composition

Lettuce is normally used in the raw state in salads but also as a cooked vegetable, particularly in South-East Asia. Loose-headed forms have higher vitamin A content than heading cultivars (Tindall 1983). The nutritional value of lettuce is given in Table 1.

Table 1 Nutritional value of 'Butterhead' lettuce, per 100 g.

Nutritional value per 100 g	
Water	96 mL
Protein	1.0 g
Fat	0.4 g
Carbohydrate	2.0 g
Fiber	0.4 g
Calcium	18 mg
Phosphorus	22 mg
Iron	0.4 mg
β-carotene	885 μg
Thiamine	0.04 mg
Riboflavin	0.04 mg
Niacin	0.2 mg
Ascorbic acid	4.0 mg

(Tindall 1983).

2.2 Chitin and chitosan

Chitin is a natural polysaccharide composed of β(1⟶4)-linked 2-acetamido-2-deoxy-β-D-glucose (*N*-acetylglucosamine). It is structurally identical to cellulose, but it has acetamide grops (-$NHCOCH_3$) at the C-2 positions. The derivative of chitin, chitosan is a linear polymer of α(1⟶4)- 2-amino-2-deoxy-β-D-glucopyranose and is easily derived by *N*-deacetylation. It can be characterized by the degree of deacetylations (Dutta, Dutta et al. 2004). The structures of cellulose, chitin and chitosan are shown in Figure 3.

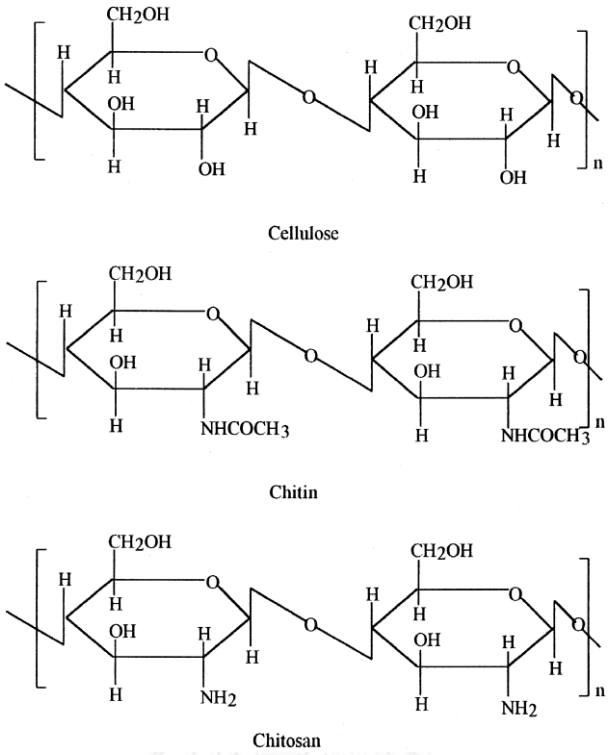

Figure 3 Structures of cellulose, chitin and chitosan (Rajkumar, Lee b et al. 2008).

There are several industries that produce chitin-rich materials as waste, the seafood industry being the most important source of chitin-rich materials in Thailand. Purified chitin can be obtained by the demineralization and deproteinization of crustacean shells and squid bones. These chitin-rich residues have unique properties that can be used in agriculture production enhancement (Shibuya and Minami 2001, Suresh and Anil Kumar 2012).

2.2.1 Shrimp shell

Shrimp shell (Figure 4), containing high chitin content, is usually pre-treated by the process of size reduction, deproteination and demineralization thus acquiring a chitin that can be of various uses (Aye, Karuppuswamy et al. 2006). The application of chitin was found to reduce the growth of pathogen *Streptomyces scabies,* which causes disease on potato tubers (Vruggink 1970). Previous studies have indicated that the amendment of soil with chitin could increase the development of the microbial population and microbial activity (Ha and Huang, 2007). Ha et al. (2008) reported that the application of chitin and *Bacillus subtilis* strain PMB-034 could control *Fusarium*

and increased seedling uptake of nutrients and growth of seedling. The shoot dry weight of theses seedlings were also found to be enhanced.

Figure 4 Shrimp shell (SS)

2.2.2 Fermented chitinous material

The fermented chitinousmaterial (FCM) (Figure 5) are derived from the method of chitinase preparation using the *Bacillus licheniformis* strain SK-1 (Kudan and Pichyangkura 2009) and shrimp shells as a chitin source. FCM was reported to have chitosan production left-over materials and microbes. Preliminary test of FCM adding to ornamental plant grown in pot led to a significant increase in plant growth (Rath Pichyangkura, personal communication). This may also be used to develop a useful semi-organic growing material having chitin and chitosan functions thus providing a good source of nitrogen.

Figure 5 Fermented chitinous material (FCM)

2.3. Chitin and chitosan on plant response

2.3.1 Effects of chitin and chitosan on plant growth and development

Plant growth improvements have been reported after the application of chitin-based treatment to a range of crops, which are thought to be independent of the effects on pest and disease control. For some horticultural and ornamental commodities, chitosan increases harvest yield. Effects of chitosan on the growth of soybean, mini-tomato, upland rice and lettuce seedlings were investigated by incorporating it into soil before planting. The early stages of growth of these crops were improved by the application and their dry matter weights were increased. Maximum growth improvements were observed in 0.5% chitosan treated soybean and upland rice and in 0.1% chitosan treated mini-tomato and lettuce (Chibu and Shibayama, 1999). Significant improvements in growth have also been reported in fruit and vegetable crops including daikon radishes (*Raphanus sativus* L.) (Tsugita et al.1993), cabbage (*Brassica oleracea*) (Hirano, Kitaura et al. 1996), soybean sprouts (*Vigna radiate* L.) (Lee, Kim et al. 2005), sweet basil (*Ocimum basilicum* L.)(Kim, Chen et al. 2005), grapevine (*Vitis vinifera)* (Ait Barka et al.2004), as well as ornamental crops, such as *Gerbera* (Wanichpongpan et al.2000) and *Dendrobium* orchids

(Chandrkrachang, 2002). Chitosan's effects on plant growth have also been shown in *Eustoma grandiflorum* (Raf.) Shinn (Ohta, Taniguchi et al. 1999). Chitosan application to the soil mix at sowing time remarkably enhanced plant growth and the treated plants flowered 15 days earlier than the controls. Moreover, a greater number and weight of flowers was produced by chitosan-treated plants. Chitosan application in soil mixture also promoted seedling growth of *Torenia fournieri* Linden ex E. Fourn., *Exacumaffine* Balf., *Begonia hiemalis* Fotsch, *Sinningia speciosa* (Lodd*.), Lobelia erinus* L. and *Mimulus hybridus* hort.ex A. Siebert et Voss (Ohta, Morishita et al. 2004). Chitosan O-80 at 1, 10, 50 and 100 ppm could induce early flowering and increase the inflorescence number of orchid *Dendrodium* 'EISKUL' (Limpanavech, Chaiyasuta et al. 2008).

Chitin and all its derivatives, have a high nitrogen content of 6.1%-8.3% (Yen and Mau 2007). Chitin can quickly be utilized as both a nitrogen source and energy source by plants and microbes when added to crops. Plants can access the nitrogen in chitin via a microbial breakdown and the release of inorganic nitrogen, or directly taking up monomers as organic nitrogen (Roberts and Jones 2012); (Spiegel, Kafkafi et al. 1988). Spiegel et al. (1988) clearly demonstrated that Chinese cabbages treated with chitin-based products grew faster than plants treated with a standard mineral fertilizer.

Chitosan was also reported to be involved with stomatal response. The stomatal aperture of tomato and *Commelina communis* was reduced when the epidermis was treated with chitosan (Lee S 1999). The result showed that foliar application of chitosan could decrease transpiration in pepper plants, resulting in a reduction in water use by 26–43%, while their biomass production and yield still remained unchanged (Marco Bittelli 2001), suggesting that chitosan could be an effective soil supplement.

2.3.2 Effects of chitin and chitosan on postharvest response

Recently, the method of using chitin and chitosan to control postharvest diseases of fruits was developed. Chitosan at low molecular weight (LMWC) has been reported to control postharvest diseases of citrus fruit (Chien, Sheu et al. 2007). The report discovered that pre-harvest chitosan sprays effectively inhibited the postharvest decay of strawberry fruit caused by *Botrytis cinerea* during storage at 3 and 13°C (Bhaskara-Reddy M V and J 2000) and the decay decreased with increasing

chitosan concentration (Chien, Sheu et al. 2007). Furthermore, fruits from chitosan sprayed plants were firmer and ripened at a slower rate as indicated by anthocyanin content and titratable acidity than berries from non-treated plants (Bhaskara-Reddy M V and J 2000).

Preharvest chitosan spray and postharvest chitosan coating treatments also changed the activities of polyphenol oxidase, peroxidase and phenylalamine ammonia-lyase (Meng X 2008). Applications of chitosan and chitin oligomers increase the activities of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL) resulting in the modification of phenylpropanoid pathways in which precursors of secondary metabolites including lignin, flavonoid pigments, and phytoalexins are produced. Such metabolites play an importance role in plant-pathogen interactions (Morrison 1993). Chitosan treatment also increases polyphenol oxidase (PPO) activity in disease resistant cultivars of pearl millet (*Pennisetum glaucum*) (Raj 2006). Oxidation of phenolic compounds associated with enhanced resistance to pathogens may involve PPO which could generate reactive oxygen species (Mayer 2006). Kim (2005) reported that chitosan increased antioxidant activity assayed by the DPPH free radical scavenging test at least 3.5-fold in sweet basil.

Due to the positive charge on the C2 of the glucosamine monomer below pH 6, chitosan is more soluble and has a better antimicrobial activity than chitin (Chen, Liau et al. 1998). The exact mechanism of the action of chitin, chitosan, and their derivatives in promoting growth is still not clear, but different mechanisms have been discussed (Rabea, Badawy et al. 2003).

In addition, previous studies have indicated that chitin and chitosan could effectively inhibit postharvest diseases of fruits by direct inhibition on growth of phytopathogens and indirect stimulus of defense-related enzyme activities. The enzymes include peroxides (POD), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL) and β-1, 3-glucanase (GLU). Nevertheless, it is significant to reiterate that the mode of action for chitin and its derivatives on controlling diseases of fruits is still limited and unclear (Zhang, Li et al. 2011).

2.3.3 Effects of chitin and chitosan on microbes

Chitin-containing microorganisms (both beneficial and pathogenic) use chitinases to control their growth and development by controlling the synthesis and degrade of cell walls and skeletons. Chitinases are usually produced in organisms that do not produce chitin themselves (Ayes 1994). Chitin added to the soil can help

beneficial antagonists by provoking the production of chitinases which can be used to destroy pests and pathogens. It can also be used as a nitrogen-rich polysaccharide source that increased the population. Then other control mechanisms are induced to fight plant pathogens. Chitosan supplemented to soil enhances plant-microorganism symbiotic interactions beneficial to plants as in the case of mycorrhizas. It also enhances the action of plague-controlling biological organisms such as *Trichoderma* sp. *and Bacillus* sp. (Schisler 2004). As with other responses to chitin-based treatments, chitin supplement together with a beneficial chitinolytic microbial agent augment may amplify the positive effects on germination. In addition to its role in protecting plants against pathogens, the chitinolytic bacterium *B. subtilis* AF 1 was found to promote seed germination and subsequent plant growth in pigeon peas even under pathogen pressure (Manjula and Podile 2001).

Various studies showing a significant capacity of chitosan in plant defense against diseases (Kurzawiǹska 2007). Chitosan is an exogenous elicitor of response mechanisms and has been shown to induce plant defences in tomato (Benhamou 1994) cucumber (Ben-Shalom 2003) and strawberry fruits (El Ghaouth 1992). Various studies have reported the defenses mechanism in plant activated by chitin through the production, release, and/or activation of phytoalexins (Kuchitsu, Kikuyama et al. 1993), phenolics (El Hassni and I. 2004) and reactive oxygen species (Kuchitsu, Kosaka et al. 1995). Several studies have been shown that chitosan stimulates other systems involved in resistance of plants to infection (Bohland 1997). Chitosan induces the accumulation of phytoalexins resulting in antifungal responses and enhances protection from further infections (Vasyukova, Zinov'eva et al. 2001).

Though chitin added to soil around cultivated crops may promote the growth of antagonistic microbes, it can be extremely difficult to observe accurately. As a consequence, the mainstream of trials have examined the effect of chitin on isolated and growing antagonists applied to plants (Sharp 2013).

Bacillus subtilis secretes chitinases into the growing medium (Chen 2009). Recently, it was found that the addition of chitin improved the reproduction of *B. subtilis*, and bacteria's fungicidal act. It also enhanced the control of *Fusarium* wilt in pigeon peas caused by *Aspergillus niger* (Manjula and Podile 2001).

Among many factors deteriorating the lettuce growth and crops are fungal diseases affected by soil fungi (Kurzawiǹska 2007). Chemical control are most frequently used for plant defense against phytopathogens. There is a global tendency to use chitosan as an option as its fungicidal effects leading to elicitation of protection mechanisms (Obsuwan 2007). As a safe biodegradable compound as well

as an elicitor, chitosan can be a prospective material as plant protectant helping agriculture production (Bautista-Ban˜osa 2006).

2.4. Nitrate in vegetable crop

Nitrate levels in vegetables are controlled by various factors including variety and seasonal factors. Nitrate levels are monitored in relation to lettuce (see Table 2). The European Commission has created an Acceptable Daily Intake (ADI) of 0 - 5 mg of nitrate per kg body weight (expressed as sodium nitrate) and a temporary acceptable daily intake of 0 - 0.1 mg of nitrite per kg body weight (expressed as sodium nitrite).

Mean levels of nitrate are shown in Table 2.

Table 2 Average concentrations of nitrate in vegetables

Vegetable	Nitrate (NO_3^-) mg/kg
Asparagus	13
Beans: green	
broad	195-450
Beetroot	21
Broccoli	1,560-2,588
Cabbage: green	125-471
white	150-1,600
Carrot	93-530
Cauliflower	115-270
Chicory	37-715
Cucumber	9
Fennel	23-242
Lettuce: open leaf	2,000
iceberg	907-4,674
Mushroom	140-1,750
Onion	70
Peas	80-210
Pepper	15-57
Radish	10-78
Spinach	110-1,510
	390-3,383

(Commission 1997)

2.5. Antioxidant compounds

Lettuce is an essential salad vegetable that can eat fresh cooked (Liu, Ardo et al. 2007). It relates to health benefits because of the presence of antioxidant components (Nicolle, Cardinault et al. 2004). There are several important antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). Some ate involved in ascorbate glutathione cycle (Halliwell-Asada cycle) (Mittova, Volokita et al. 2000, Michalak 2006). APX uses ascorbic acid as a reductant in the first step of the ascorbate-glutathione cycle. This is the most important peroxidase in H_2O_2 detoxification operating both in cytosol and chloroplasts (Mittova, Volokita et al. 2000, Smirnoff 2000).

Non-enzymatic scavengers are necessary in cellular components protection from ROSs (Chaudière and Ferrari-Iliou 1999); Ferrari-Iliou, 1999). The main antioxidants are ascorbic acid, glutathione, α-tocopherol, and phenolic compounds The pigments such as carotenoids also play an important roles (Babbar 2011);(Vijayakumar 2008); (Inzé and Montagu 1995, Rama Devi and Prasad 1998, Jimenez, Creissen et al. 2002, Tausz, Wonisch et al. 2003).

Previous studies showed high levels of antioxidant contents in lettuce (Cao, Sofic et al. 1996, Vinson, Hao et al. 1998, Caldwell 2003). The antioxidant in lettuce had high oxygen radical absorbance capacity (ORAC) (Cao, Sofic et al. 1996, Caldwell 2003). It can inhibit the effects of ethylene formation induced and had high activity against protein oxidation (Cao, Sofic et al. 1996).

Reports suggested that genotype and also growing conditions can have an effect on the antioxidant contents in many crops. The day/night temperature also showed an influence on the phenolic content and antioxidant activities (Wang and Zheng 2001).

The DPPH-radical scavenging method is alos used to measure antioxidant capacity in lettuce (Kang and Saltveit 2002). The technique used the 1,1-diphenyl-2-picrylhydrazyl free radical ($DPPH^{\bullet}$), which shows a UV-vis spectrum with a maximum of absorbance about 515 nm in methanol (Villano, Fernández-Pachón et al. 2007).

2.6. Soil microbial populations

The diversity of microorganisms in soil seems to be critical to the maintenance of soil health and quality. One of their important activities in the soil is breaking down the organic matter to inorganic forms. A perfect example of this is the

microbial release of NH_4^+, which is in turn xidized by *Nitrosomonas* bacterial to NO_2^- (nitrite). NO_2^- whichis subsequently oxidized to NO_3^- (nitrate) by the Nitrobacter group of bacteria. Similarly, other nutrients such as sulfur and phosphorus become available to plants as a result of this microbial activity (Preece and Read 1993).

Plant bacterial interactions are long known and have three well-differentiated manifestations. The first relation is between plants and pathogenic bacteria (for instance, *Agrobacterium* spp., *Erwinia* spp., *Ralstonia* spp., etc.), which causes a state of disease. Consequences for the plant are negative. A second manifestation is a direct interaction between plants and non-pathogenic bacteria leading to a beneficial association for both partners. This interaction is a mutualistic symbiosis, yielding positive effects for the plant. These two types of interactions arise as a consequence of a more findly tuned molecular signaling between the bacteria and the plants. However, the ultimate boundaries between a mutualistic and a pathogenic interaction can be unclear and the recognition and signal-transduction processes can be similar for both interactions (Baron 1995); (Soto, Sanjuán et al. 2006). The third type of interaction that numerous bacterial genera (e.g. *Alcaligenes* spp., *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp., etc.) establish with plants in principle could be considered as neutral for the plant (Mercado-Blanco and Bakker 2007). Some impotant microflora fungi and bacteria in agriculture describe below.

2.6.1 *Bacillus* spp.

Gram positive, rod-shaped, aerobic and endospore forming are the characteristics of *Bacillus* spp. (Rao, Tanksale et al. 1998). All *Bacillus* spores share a common architecture consisting in a set of concentric layers with a cortex and a coat surrounding the inner core. An additional loose balloon-like envelope called exosporium is observed around spores of *B. cereus* strains and of the closely-related species forming the *B. cereus* group, e.g. *B. thuringiensis, B. anthracis* or *B. mycoides*(Faille 2010)This structure has also been observed in other *Bacillus* species, such as *B. alvei, B. brevis*, or *B. sphaericus* but it has not been observed in spores of *B. subtilis* or *B. licheniformis*(Hachisuka, Kozuka et al. 1984).

Bacillus inoculants are especially interesting (at least theoretically) as through the use of gram-positive spore forming PGPR. They can persist in fields for long periods and can also be produced and stored for commercial purposes (Probanza, Lucas Garcıa et al. 2002). *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides* and *B. sphaericus strains* elicit significant reductions

in the incidence or severity of various diseases on a diversity of hosts (Choudhary and Johri 2009). Protection resulting from induced systemic resistance (ISR) is elicited by *Bacillus* spp. It has been reported against leaf-spotting fungal, bacterial pathogens, systemic viruses, a crown-rotting fungal pathogen, root-knot nematodes, and a stem-blight fungal pathogen as well as damping-off, blue mold and late blight diseases (Choudhary and Johri 2009).

B. subtilis strains are the most widely used as plant growth promoting rhizobacteria (PGPR) due to their disease reducing and antibiotic producing capabilities when applied as seed treatments (Kokalis-Burelle et al. 2006). Another important strain is *B. licheniformis*, a Gram-positive, spore-forming soil bacterium, classified as generally recognized as safe (GRAS), which shown to be an efficient PGPR (Brunetti, Farrag et al. 2012). Previous studies have indicated that various strains of *B. licheniformis* are also able to improve the growth and development of the host plant in heavy metal contaminated soils by mitigating the toxic effects of the heavy metals located on the plants (McLean, Beauchemin et al. 1990, McLean, Beauchemin et al. 1992, Yakimov, Timmis et al. 1995, Ramos, García et al. 2003).

2.6.2 Fluorescent *Pseudomonas* spp.

A diverse group, Fluorescent *Pseudomonas* spp. is a bacterium that can generally be visually distinguished from other pseudomonads by their ability to produce a water-soluble yellow-green pigment (fluorescent under ultraviolet irradiation (**λ** = 366 nm)). They are typically gram-negative with chemoheterotrophic motile rods and polar flagella that are grouped in RNA homology I as defined by Palleroni et al. (1973).

Known as PGPR (Mercado-Blanco and Bakker 2007), several *Pseudomonas* strains have been used to control many fungal, bacterial, viral and insect pests (Shanmugam, Senthil et al. 2002). Fluorescent pseudomonads have been extensively used for plant growth promotion and disease control. Several mechanisms have been suggested for disease control by fluorescent pseudomonad*s* involving production of siderophores, hydrogen cyanide (HCN), ammonia, antibiotics and volatile compounds etc. or by competing with pathogens for nutrients or colonization space (Thomashow and Weller 1996). Flourescent pseudomonads can trigger a plant-mediated resistance mechanism called induced systemic resistance (ISR) (Pieterse, Van Pelt et al. 2000) and are among the most effective rhizosphere bacteria. In addition to disease control, they exert beneficial effect on plant growth

promotion(Dubeikovsky 1993);(Raupach and Kloepper 1998). Fluorescent pseudomonads are also known to suppress soilborne fungal pathogens by producing antifungal metabolites and by sequestering iron in the rhizosphere through the release of iron-chelating siderophores, rendering it unavailable to other organisms (Schippers, Bakker et al. 1987); (Loper 1988)(Paulitz 1991)(Dwivedi 2003).

Recent reports by Ryu et al. (2004) indicated the identification of several volatile organic compounds produced by a variety of bacteria that promote plant growth and induce systemic resistance in Arabidopsis (*Arabidopsis thaliana*). One of the best studied examples is *P. fluorescens* WCS365. This strain controls tomato foot and root rot caused by *Fusarium oxysporum* f. sp. *Radicislycopersici*(Dekkers, Mulders et al. 2000). Howell and Stipanovic (1980) showed an inhibition by a fluorescent pseudomonads on *Pythium ultimum* in cotton seedlings (Gaber 1979)(Johnson 1978).

2.6.3 *Fusarium* spp.

Fusarium species may produce three types of spores, those being macroconidia, microconidia and chlamydospores. Part of a widespread cosmopolitan group of fungi *Fusarium* spp. will commonly colonize aerial and subterranean plant parts, either as primary or secondary invaders (El-Kazzaz, El-Fadly et al. 2008). Some *Fusarium* strains cause disease in many plants. Pathogens causing root and crown rot, and wilt are the main yield-limiting factors in food production.

Fusarium oxysporum causes intense damage in many crops (Correll 1991). The effects of *Fusarium* wilt are serious, which is caused by *Fusarium oxysporum*. Zhao et al. (2014) reported that all *Fusarium oxysporum* isolates were found to cause disease symptoms in the host plant.

2.6.4 *Pythium* spp.

The genus *Pythium* belongs to the family Pythiaceae, order Pythiales, class Oomycetes, phylum Oomycota, and kingdom Chromista(Kirk PM 2008); (Uzuhashi, Kakishima et al. 2010). The genus *Pythium* (van der Plaats-Niterink 1981) have hyphae that are hyaline and coenocytic without cross septa. Two types of sporangia are filamentous and globose. Zoospores develop in a vesicle and formed at the tip of a discharge tube. Oospores are formed in smooth or ornamented oogonia after fertilization with paragynous or hypogynous antheridia. The formation of zoospores are unlike from morphologically similar genera (Uzuhashi, Kakishima et al. 2010).

Pythium species are distributed from tropical to temperate sites. Many species of the genus are common and important pathogens of important crops where they may cause seed rot, seedling damping off and root rot (Agrios 2005)(Agrios 2005); (Le 2014). *Pythium* species are also important pathogens of wheat, which is one of the world's major crops.

2.6.5 *Trichoderma* spp.

Trichoderma is a fungal genus found in many regions of the world (Chaverri, Castlebury et al. 2003). These fungi appear in the form of colonies of mold, turning white or yellowish over time. One of the most important functions of *Trichoderma* involves the mold’s tendency to develop symbiotic relationships with plants (Akladious 2014). The application of *Trichoderma* spp. as biological control agents has been used against several soil-borne phytopathogenic fungi (Verma, Brar et al. 2007). *Trichoderma* spp. can be applied as spores which are very tolerant to adverse environmental conditions field use (Amsellem 1999).

Trichoderma species have been widely praised for their capacity to enhance plant growth, produce antibiotics, parasitize other fungi and compete with deleterious plant microorganisms. This enables the species to be used as bio fertilizers and biocontrols (Akladious 2014). In addition, certain strains induce systemic and localized resistance to several plant pathogens. Certain strains may improve plant growth and development (Ha 2010)..

Plants treated with *T. harzianum* resulted in large root area and cumulative root length (Howell 2003). Harman (2000) has reported that highly rhizosphere competent strains of *Trichoderma* increase root growth of a wide range of plants. Recently, *Trichoderma* spp. Are reported to promote seedling establishment, enhance plant growth and elicit plant defense reaction in many crops (Shanmugaiah, Balasubramanian et al. 2009), vegetables (Celar 2005), beans (Hoyos-Carvajal, Orduz et al. 2009) and corn (Windham, Windham et al. 1989). In addition, the increased growth response induced by *Trichoderma* species has also been reported in many types of crops (Lo, Lin et al. 2002).

CHAPTER III

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Plant materials

'Butterhead' (*Lactuca sativa* L. cv. 'Butterhead') and 'Red Oak' (*Lactuca sativa* L. cv. 'Red Oak') lettuce seeds (Super Green™) were purchased from ACK Hydro Farm Company Limited, Bangkok, Thailand.

3. 1.2 Instruments

1.2.1 Equipment for plant growing

- Seed starter trays
- Plastic pots(13.0 x 17.5 x 14.5 cm)
- Prong
- Cultivator
- Shovels
- Racks
- Coconut husk
- Soil
- Cow manure
- Ground shrimp shell
- Fermented chitinous material
- Plastic buckets
- Plastic beakers
- Plastic sheet for row cover

1.2.2 Equipment for growth and yield analysis

- Measuring tape
- Knife
- Basket boxes (14 x 19 x 10 inch)
- Plastic bags (20 x 30 and 10 x 15 cm)

- Balances
- Growth chambers
- Hot air oven (60°C)

1.2.3 Equipment for postharvest quality analysis

- Plastic bags (10 x 15 cm)
- Bag sealing machine
- Balances
- Growth chambers

1.2.4 Equipment for net photosynthesis, transpiration rateand stomatal conductance analysis

-A portable photosynthesis (LI-6400XT Version 6; LI-COR Inc., Lincoln, NE, USA)

1.2.5 Equipment forNitrate analysis

- Knife and cutting board
- Liquid nitrogen
- Aluminium foil
- Deep freezer (-80°C)
- Mortars and pestles
- Spatula
- Flasks (50 and 125 mL)
- Cylinders
- Filter paper (Whatman No. 1)
-Vortex mixture
- Spectrophotometer (Agilent Technology, USA)

1.2.6 Equipment for antioxidants extraction analysis

- Knife and cutting board
- Liquid nitrogen
- Aluminium foil
- Deep freezer (-80°C)
- Mortars and pestles
- Spatula
- Eppendorf tubes (1.5 mL)

- Flasks (50 and 125 mL)
- Centrifuge tube (15 and 50 mL)
- Cylinder
-Vortex mixture
- Refrigerated centrifuge (Universal 32R, Hettich, Germany)
- Spectrophotometer (Agilent Technology, USA)

1.2.7 Equipment for soil microbial populations

- Erlenmeyer flasks(25, 50, 250, 500 and 1000 mL)
- Centrifuge tubes (15 and 50 mL)
- Eppendorf tubes (1.5 mL)
- Pipettes
- Cylinders
- Beakers
- Laboratory bottles
- Petri dishes
- Petri dish can
- Plastic bags
- Plastic bands
- Glass dropper bottle, Amber
- Racks
- Blades
- Slides
- Transfer or inoculating needles
- Loops
- Alcohol burners
- Fluorescence generator (BH2-RFL-T3, Olympus, Japan)
- Refrigerated centrifuge (Universal 32R, Hettich, Germany)
- Spectrophotometer (Agilent Technology, USA)
- Vortex mixture
- Digital water bath (Daihan Labtech Co., LTD)
- Thermometer
- Hotair oven (180°C) (Memmert, Germany)
- Shaker (Biosan, USA)
- Lamina Flow (Class I, Microflow, UK)
- Automatic Autoclave (TC-459, Taiwan)

- Incubator(Memmert, Germany)
- Incubator shaker(Model IN-666, K Germmyco, Taiwan)

1.2.8 Equipment for soil chemical parameters

1.2.8.1 Equipment for pH analysis

- Beakers (50 mL)
- Stirring rods
- Cylinders
- Glass electrode pH meter (Eutech, Singapore)

1.2.8.2 Equipment for electrical conductivity (EC)analysis

- Erlenmeyer flasks (125 mL)
- Glass funnels
- Filter paper (Whatman No. 5)
- Laboratory bottles
- Filtering flasks (500 mL)
- Cylinders (50 mL)
- Thermometer
- Digital conductivity meter (NTST, USA)

1.2.8.3 Equipments for organic matter (OM), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in soil analysis

- Erlenmeyer flask (50 and 250 mL)
- Pipettes
- Beaker Glass (25 and 50 mL)
- Burette (10 and 50 mL)
- Balances
- Micro-kjeldahl tubes (100 mL)
- Distillation apparatus
- Digestion system
- Test tubes
- Filter paper (Whatman No. 5)
- Auto dilutor
- Volumetric flasks (50 and 1,000 mL)

- Dispenser (25 mL)
- Shaker
- pH meter
- UV-Vis Spectrophotometer (PerkinElmer Lamda 35 UV/VIS, USA) for P
- Flame Photometer (Corring 410)er (Coring 410) for K
- Atomic AAsorption Spectrophotometer GBC Model Sens AA for Ca and Mg

3.1.3 Chemicals and reagents

3.1.3.1 Chemical for nitrate analysis

- Deionized water
- Activated charcoal
- Nitrate 5 nitrate reagent power pillow
- Standard nitrate

3.1.3.2 Chemical for chlorophyll *a*, chlorophyll *b* and carotenoids content analysis

- 80% acetone

3.1.3.3 Chemicals for ascorbic acid content analysis

- 2% Dinitrophenylhydradzine (DNPH) in 4.5 M sulfuric acid
- 6% metaphosphoric acid in 2 M acetic acid
- 2% 2, 6-dichlorophenolindolphenol (DCIP)
- 2% thiourea in 5% metaphosphoric acid
- 90% sulfuric acid
- Standard ascorbic acid

3.1.3.4 Chemicals for phenolic compound analysis

- 80% Ethanol
- 4 N NaOH
- 6 N HCl
- Ethyl acetate
- Distilled water

- Folin-Ciocalteu's phenol reagent (Fluka, Switzerland)
- Standard gallic acid

1.3.5 Chemicals for flavonoid content analysis

- 5% $NaNO_2$
- 10% $AlCl_3$
- 1 M NaOH
- (+)-catechin

1.3.6 Chemicals for total antioxidant activity analysis

- 0.2 mM of DPPH (1, 1-diphenyl-2-picrylhydrazyl) ethanolic solution
- 80% Ethanol

1.3.7 Chemicals for malondialdehyde (MDA) analysis

- 5% and 15% (w/v) trichloroacetic acid (TCA)
- 0.5% thiobarbituric acid (TBA)

1.3.8 Chemicals for soil microbial population analysis

1.3.8.1 Tryptic Soy agar (TSA) selective medium for *Bacillus* spp. (Bashan et al. 1993)

- 40 g TSA
- 1,000 mLdistilled water
- 50 µg/mL nystatin
- 50 µg/mL cycloheximide

1.3.8.2 King agar B selective medium (1 Litre) for Fluorescent *Pseudomonads* spp. (Sand and Rovira., 1970)

- 20 g peptone
- 1.5 g dipotassium hydrogen phosphate
- 1.5 g magnesium sulfate
- 10 g agar
- 10 mL glycerol
- 1,000 mLdistilled water
- 100 mg penicilin G
- 45 mg/mL novobiocin

- 75 mg/mL cycloheximide
- 3 mL 95% ethanol

1.3.8.3 Malachite green agar 2.5 mg (MGA 2.5) a selective medium (1 Litre) for *Fusarium* spp. (Castellá et al. 1997)

- 15 g peptone
- 1 g Potassium di-hydrogen phosphate (KH_2PO_4)
- 0.5 g $MgSO4 \cdot 7H_2O$
- 20 g agar
- 1,000 mL distilled water
- 2.5 mg malachite green
- 100 mg chloramphenicol
- 50 mg streptomycin

1.3.8.4 Potato dextrose agar (PDA) selective medium (1 Litre) for *Pythium* spp. (Masago et al. 1977)

- 39 g PDA
- 1,000 mLdistelled water
- 10 mg benomyl
- 25 mg nystatin
- 25 mg pentachloronitrobenzene
- 10 mg rifampicin
- 500 mg ampicillin

1.3.8.5 Trichoderma medium E (TME) selective medium (1 Litre) for *Trichoderma* spp. (Papavizas and Lumsden, 1982)

- 200 mL V-8 Juice
- 1 g glucose
- 20 agar
- 700 mLdistelled water
- 100 mg neomycin sulfate
- 100 mg bacitracin
- 100 mg penicilin G
- 100 mg folpet

- 25 mg chlorotracycline hydrochloride
- 20 mg nystatin
- 500 mg sodium propionate

1.3.9 Chemicals for soil chemical parameter

1.3.9.1 Chemicals for organic matter (OM)analysis

(Walkley and Black, 1947)

- 1.0 $NK_2Cr_2O_7$
- H_2SO_4
- Distilled water
- O-phenanthroline
- 0.5 N $FeSO_4.7H_2O$

1.3.9.2 Chemicals for availability phosphorus (P) analysis

(Bray and Kurtz, 1945)

- 0.03 N NH_4F
- 0.1 N HCl

1.3.9.3 Chemicals for availability potassium (K), calcium (Ca) and magnesium (Mg) analysis

(Jackson, 1958)

- 1 N NH_4OAc

3.2. METHODS

3.2.1 Determination of the effects of biomaterial and semi-biomaterial on growth, yield and postharvest quality of lettuces during three successive crop seasons grown in a test plot

3.2.1.1 Chitin-rich materials

Two different sources of chitin were used: shrimp shell powder (SS) and fermented chitinous material (FCM). FCM was derived from the process of chitinase preparation using *Bacillus licheniformis* strain SK-1 (SK-1) (Kudan and

Pichyangkura 2009) and shrimp shells as chitin source. The cultures were composed of shrimp shells, SK1, 0.25% yeast extract, 0.5% $(NH_4)_2SO_4$, 0.03% $MgSO_4$, 1.0% KH_2SO_4 and 0.2% K_2HPO_4. The cultures were incubated with shaking for 7 days at 37°C. Afterward, FCM residues (solid phase) were separated by centrifugation at 8,000 rpm (9,820 × g) and air dried.

3.2.1.2 Plant cultivation practices

'Butterhead' (*Lactuca sativa* L. cv. Butterhead) and 'Red Oak' lettuce cultivation was grown during three successive crop seasons (March-April 2010, July-September 2010 and December 2010-February 2011) at Chulalongkorn University, Bangkok, Thailand. During the second and third crop seasons, a transparent polyethylene was used to cover the plants. Each crop season, a randomized complete block design (RCBD) was conducted with eight plants per replicate and four replicates per treatment. A light meter (LI-250A, Li-cor, Lincoln, USA) was used to measure light intensity. Air temperature and air relative humidity at plant height were recorded by a Thermo-Hygrograph (Isuzu Seisakusho Co., Ltd., Tokyo, Japan). Cultivation, irrigation practices, and climatic conditions during the growing period are summarized in Table 5.

Lettuce seeds were sown on top of a growing medium consisting of 2:1 ground coconut husk/soil that was packed in plastic trays and covered with a 10 mm layer of soil. Three weeks after sowing, the seedlings were move into pots and filled with 1 kg of growing medium (10:1 soil/cow manure) supplemented with 0.5% SS, 0.5% SS and 10 mL of SK-1 suspension (A600nm = 1.0) (0.5% SS +SK-1), 0.25% SS and 0.25% FCM (0.25% SS+0.25% FCM), 2% FCM and SK-1 alone. SK-1 was prepared according to Kudan and Pichyangkura (2009). The addition of bacterial suspension was assumed to have no contribution to the supplement mass. Control treatment was performed without any supplement.

FCM and SK-1 were applied to lettuce seedlings twice. The SS/FCM treatment was applied at week 4. At Week 6, 50 g of cow manure were added again. The plants were cultured for 8 weeks. Watering was applied in the morning and in the afternoon.

3.2.1.3 Plant growth and yield analysis

Lettuces were harvested and leaf number of each lettuce was counted. The width and length of the biggest leaf of each lettuce were analyzed.

The fresh weight of each lettuce was investigated and used for the weight loss calculation. A drying chamber at 60°C was used to dry lettuce for 7 days

Statistical analysis

Four replicates per treatment were done for all measurements. Data was analyzed using one-way analysis of variance (One-way ANOVA). Mean differences were performed using Duncan's multiple range test (DMRT). Differences with $p \leq 0.05$ were considered significant.

3.2.1.4 Postharvestquality analysis

Lettuces were stored in a chamber at 8°C and 60% RH for 14 days. Fresh weight loss and overall visual quality were investigated after removal from storage. Fresh weight loss was calculated as follows:

$$\text{Fresh weight loss (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)} * 100}{\text{Initial weight (g)}}$$

Overall visual quality of each lettuce was evaluated after storage by using a quality index scale from 4 to 0, where 4 = excellent (essentially free from defects), 3 = good (minor defects; not objectionable), 2 = fair (slight to moderate objectionable defects; lower limit of sales appeal), 1 = poor (excessive defects; limited salability), 0 = extremely poor (not useable). Visual quality evaluation scales were modified from Rennie et al. (2001).

Statistical analysis

Statistical analyses for overall visual quality were performed with a Kruskal-Wallis test ($p<0.05$). Data analyses were performed using SPSS 14.0 for Windows Evaluation Version.

3.2.1.5 Determination of the effects of biomaterial or semi-biomaterial on selected soil chemical parameters

Soil samples were collected for soil chemical and physical properties analysis before planting and on harvesting day. Soil samples were collected from the root zone and kept in bags and stored at 4°C for analysis.

3.2.1.6 pH analysis

The soil pH analysis was investigated by using a 1:1 soil/water aqueous extract. The mixture was left to stand and the pH was read with a pH meter using a glass electrode (Peech, 1965).

3.2.1.7 Electrical conductivity (EC) of soil analysis

Soil EC was analyzed by using a 1:5 soil/water aqueous extract. EC of the supernatant was read with a digital conductivity meter (Digital conductivity meter, Fisher Scientific) (Lee, Park et al. 2004).

3.2.1.8 Soil organic matter (OM) analysis

Soil OM was analyzed by taking 1.0 g soil into a 250 mL Erlenmeyer flask. O-phenanthroline was used as an indicator. Contents were titrated against 0.5 N $FeSO_4.7H_2O$ for green to red-brown end point (Walkley 1947).

3.2.1.9 Total nitrogen (N) in soil analysis

Total nitrogen (Kjeldahl method) was analyzed and bromocresol green-methyl red were added as the indicator. The titration used 0.1 N standard hydrochoric acid solutions for the green to red-brown end point. Blank was prepared in the same manner without adding a soil sample (Bremner 1965).

Calculation:

$$\frac{\text{mL } H_2SO_4 \text{ used (soil sample} - \text{blank titratation)} \times N \times 0.14 \times 100}{\text{weight of soil sample (g)}} = \% \text{ nitrogen}$$

3.2.1.10 Availability phosphorus (P) in soil analysis

Soil available P was analyzed (Bray 1945). The absorbance of the filtrate was investigated by spectrophotometer at 882 nm (Lambda 35 UV/VIS Spectrometer, PerkinElmer).

Calculation:

Available P = $\frac{B \times df\ (sample) \times R}{A \times df\ (standard)}$ mg kg^{-1}

A = Soil sample (g)

B = 0.03 N NH_4F, 0.1 N HCl (mL)

R = standard set

df = dilution factor

3.2.1.11 Availability potassium (K) in soil analysis

Soil available K was analyzed (Jackson 1958). The absorbance of the filtrate was examined by flame photometer at 383 nm (Corning 410).

Calculation:

Available K = $\frac{D \times df\ (sample) \times R}{A \times df\ (standard)}$ mg kg^{-1}

A = Soil sample (g)

B = 1 N ammonium acetate solution of pH 7 (mL)

df = dilution factor

D = standard solution for KCl mg kg^{-1}

3.2.1.12 Chemicals for availability of calcium (Ca) and magnesium (Mg) in soil analysis

Soil available Ca and Mg was analyzed (Jackson 1958). The absorbance of the filtrate was examined by atomic absorption spectrophotometer at 422 nm and 285 nm (GBC, AA S/N 6360), respectively.

Calculation:

Available Ca = $\frac{D \times df \times B}{A}$ mg kg^{-1}

A = Soil sample (g)

B = 1 N ammonium acetate solution of pH 7 (mL)

df = dilution factor

D = standard solution for Ca (mg mL^{-1})

Calculation:

Available Mg = $\frac{D \times df \times B}{A}$ mg kg^{-1}

A = Soil sample (g)

B = 1 N ammonium acetate solution of pH 7 (mL)

df = dilution factor

D = standard solution for Mg (mg mL^{-1})

Statistical analysis

Five replicates per treatment were used for all measurements. Data was analyzed using one-way analysis of variance (One-way ANOVA) test. Mean differences were performed using Duncan's multiple range test (DMRT). Differences with $p<0.05$ were considered significant.

3.2.2 Determination of the effects of biomaterial and semi-biomaterial on growth, yield, postharvest quality and selected soil microbial populations of lettuces in a local farm

3.2.2.1 Plant materials

Plant materials were the same as 1.1.

3.2.2.2 Treatments of biomaterial or semi-biomaterial and growing condition

The best treatment from the test plot was applied as a supplement to the experimental plot. The experiments were carried out in 2 crop seasons: 14 January- 3 March and 23 May- 21 July 2012 in a local farm at Supan Buri province, Thailand. Light intensity, photoperiod, minimum and maximum air temperatures, and relative humidity were recorded for each season.

Each experimental plot consisted of 90 lettuces placed in three rows (30 cm apart) of 30 plants (30 cm apart). The two outer rows and the first and last lettuces of the middle row were kept as guard plants.

Germination, acclimatization and watering of the seedling were carried out as described in 2.1.2.At the beginning of week 4, the experimental plots were amended with 51 kg of cow manure/1 m^2 and SS or FCM as stated in 2.1.2. Three weeks after sowing, the seedlings were transplanted to the experimental plots. At the beginning of week 5, the same amount of SS or FCM was added along with cow

manure to make up a total of 100 g supplement to each lettuce. At the beginning of week 7, only 50 g of cow manure were added to each lettuce. The plants were grown for a further 2 weeks and harvested in the morning at the beginning of week 9.

3.2.2.3 Growth, yield and postharvest quality of lettuces

3.2.2.3.1 Plant growth and yield analysis

Plant and growth yield were measured according to 2.1.3.

Statistical analysis

Twenty plants per treatment were used for all measurements. The means were compared by the independent sample *t-test* at a significant level of 0.05 ($P<0.05$) using SPSS software version 14. The data were shown as means ± SE (standard error).

2.1.1.1 Postharvest quality analysis

Postharvest quality was measure according to 2.1.4.

2.1.1.2 Determination of net photosynthesis, transpiration rateand stomatal conductance

Seven weeks after planting, net photosynthesis, transpiration rate and leaf stomatal conductance were measured using a portable photosynthesis measurement system (LI-6400XT Version 6; LI-COR Inc., Lincoln, NE, USA). Ten plants were used for each treatment. The measurement was performed within the time period from 8.00 am to 12.00 am and 13.00 pm to 16.00 pm maintaining the air temperature, relative humidity, CO_2 concentration and approximate photosynthetic photon flux density at 25°C, 80-90%, 400 µmol mol^{-1} and 1000 µmol $m^{-2}s^{-1}$, respectively. From each subplot, five plants were randomly selected and the measurements were taken on the terminal leaflets of the three youngest fully expanded leaves.

Statistical analysis

Ten replicates per treatment were done for all measurements. The means were compared by the independent sample T-Testat a significant level of 0.05 ($P<0.05$) using SPSS software version 14. The data were shown as means ± SE (standard error).

2.1.1.3 Determination of nitrate assimilation in lettuce leaves

Nitrate (NO_3-N) was measured by the cadmium reduction method (Do et al. 2010). Samples (2.5 g) were ground with liquid nitrogen to fine powder, and then 100 mL of deionized water and activated charcoal were added. The extracted was filtered through #1 Whatman filter paper. After that, 25 mL of supernatant and NitraVer 5 Nitrate Reagent Powder Pillow (Hach Co. USA.) were mixed. The homogenate were vortexed for 2 minutes and incubatedat room temperature for 5 minutes. Then, the absorbance of the solution was determined by spectrophotometer at 882 nm to afford NO_3-N (in mg/L).

2.1.1.4 Determination of non-enzymatic antioxidants

In order to analyze the content of antioxidants, including ascorbic acid (AA) (vitamin C), chlorophyll *a*, chlorophyll *b*,carotenoids, total phenolics, flavonoids, malondialdehyde and total antioxidant activity, lettuce leaves were collected on day's 0 and 14 and then stored at -80°C until analysis.

2.1.1.4.1 Determination of chlorophyll *a*, chlorophyll *b* and carotenoids content

Total pigments were extracted from leaves (0.5 g) with 30 mL of 80% acetone. Then, the extract was centrifuged for 5 minutes at 4,000 g. the absorbance of the supernatant was determined at 480, 645, 663 and 710 nm. The chlorophyll *a*, chlorophyll *b* carotenoid concentrations were calculated from the following equation:

Chlorophyll *a* = $12.7(A_{663} - A_{710}) - 2.69(A_{645} - A_{710})*30*1.119/FW$

Chlorophyll *b* = $22.9(A_{645} - A_{710}) - 4.68(A_{663} - A_{710})*30*1.119/FW$

Carotenoids = $(A_{480}+ 0.114(A_{663} - A_{710}) - 0.638(A_{645} - A_{710}))*30*1000)/112.5*FW$

The chlorophyll *a*, chlorophyll *b* and carotenoid contents were expressed as μmol/g FW (Kirk 1965).

2.1.1.4.2 Determination of total ascorbic acid (AA) content

Total AA content was determined using the dinitrophenylhydradzine (DNPH) method with some modifications (Shin 2007). Total AA was quantified by measurement of the absorbance at 540 nm and compared to the

standard curve. The concentration was expressed as ascorbic acid on a fresh weight basis, mg g^{-1}.

2.1.1.4.3 Determination of total phenolics content

Phenolic contents in leaf lettuce were determined according to the Folin-Ciocalteu colorimeter method with some modifications (Ju-Hee 2006). The absorbance at 750 nm was measuredand compared to the standard curve. Phenolic contents were expressed as mg gallic acid equivalents per 1 g of leaf.

2.1.1.4.4 Determination of flavonoid contents

Flavonoid contents in lettuceleaf were determined by a colorimeter method with some modifications (Ju-Hee 2006). The absorbance at 510 nm was measuredand compared to the standard curve. The flavonoid contents were expressed as mg (+)-catechin equivalents per 1 g of leaf.

2.1.1.4.5 Determination of DPPH radical scavenging activity

The scavenging activity of sample extracts was analyzed by the determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity effect on DPPH radical according to the method of Ju-Hee et al. (2006) with some modifications. The absorbance of the sample was recorded with spectrophotometer at 520 nm against a blank of ethanol without DPPH. The DPPH radical scavenging activity (%) was calculated by the following equation:

$$\text{Radical scavenging activity (\%)} = (1 - A_{sample}/A_{control}) * 100$$

Where A_{sample} is the absorbance in the presence of sample extract and $A_{control}$ is the absorbance in the absence of sample extract.

2.1.1.4.6 Malondialdehyde content determination

(Ríos, Rosales et al. 2008)

After harvesting, lettuces were stored in a chamber at 8°C for 14 days. The samples were then stored at -80°C until used for the analysis of lipid peroxidation. Lipid peroxidation was measured by estimating the concentration of malondialdehyde (MDA), using thiobarbituric acid-reactive substances (TBARS) assay according to Zhang et al. (2005) with some modifications. The supernatant was used to measure the absorbance at 450, 532 and 600 nm.

The MDA concentration (μmol/g FW) was calculated according to the equation: $6.45x (A_{532} – A_{600}) – 0.56xA_{450}$ (Zhang et al. 2005).

2.1.1.5 Statistical analysis

Five replicates per treatment were done for 2.2.3.4-2.2.3.5.6 measurements. The means were compared by the independent sample T-Testat a significant level of 0.05 ($P<0.05$) using SPSS software version 14. The data were shown as means ± SE (standard error).

3.2.3 Determination of the effects of biomaterial or semi-biomaterial on selected soil microbial populations

2.3.1. Soil sampling

Soil samples were collected from each plot on the day of plantation and the day of harvesting for analysis of microbial populations. Ten soil cores were removed from the center row of each plot. Samples were removed from the root zone around plants in the rows. Three samples per plot were collected and placed in plastic bags then stored at 4°C for analysis within 3 months. Numbers of selected bacteria and fungi were quantified using selective media.

2.3.2. Propagule densities of selected soil microorganisms

Soil samples were analyzed for selected soil microorganisms using 10-fold serial dilutions of soil and five different selective media. Numbers of *Bacillus* spp., Fluorescent *Pseudomonad* spp., *Fusarium* spp., *Pythium* spp.and *Trichoderma* spp. were quantified.

Each soil sample (10 g) was suspended with 90 mL of sterile distilled water. Soil suspensions were shacked at 250 rpm for 15 minutes. Serial 10-fold dilutions were made to 10^{-3} (Table 3). Triplicate plates for each medium were used for each sample, and several media required different soil dilutions for statistically accurate propagule estimation (Table 3). Colonies were counted from plates containing 1-200 colonies. Data are expressed as number of colony forming units (CFUs)/g of dry soil.

Table 3 Media, dilution factors, organisms, and incubation conditions for microorganisms isolated from soils in experiment plots.

Medium	Dilution Factor*	Organisms cultured	Temperature (°C)	Incubation (days)	Light conditions	Reference
TSA	10^{-2},10^{-3}	*Bacillus* spp.	28	1-2	Dark	Bashan et al. 1993
King's medium B	10^{-2},10^{-3}	Fluorescent *Pseudomonad* spp.	25	1-2	Dark	Sand and Rovira, 1970
MGA 2.5	10,10^{-1}	*Fusarium* spp.	28	5	Dark	Castellá et al. 1997
PDA	10^{-2},10^{-3}	*Pythium* spp.	28	3	Dark	Masago et al. 1977
TME	10^{-2},10^{-3}	*Trichoderma*spp.	25	5	Light	Papavizas and Lumsden, 1982

*Dilution factor number is the 1:10 serial dilution from each sample which was plated in triplicate.

2.3.3 Statistical analysis

Nine replicates per treatment were done for all measurements. The means were compared by the independent sample T-Testat a significant level of 0.05 (P<0.05) using SPSS software version 14. The data were shown as means ± SE (standard error).

3.2.4 Determination of the effects of biomaterial or semi-biomaterial on selected soil chemical parameters

2.1.2 Soil sample analysis

Soil samples were measure according to 2.2.

2.1.3 Statistical analysis

Five replicates per treatment were done for all measurements. The means were compared by the independent sample T-Testat a significant level of 0.05 (P<0.05) using SPSS software version 14. The data were shown as means ± SE (standard error).

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Effects of biomaterial and semi-biomaterial on growth, yield and postharvest quality of lettuces grown in a test plot

4.1.1 Effects of biomaterial and semi-biomaterial on growth and yield

1.1.1. Leaf number

'Butterhead' lettuce

The most significant and highest leaf number was observed in T5 (2% FCM) during the first crop season. In the second crop season, there were no significant differences between T3 (0.5% SS+SK-1), T4 (0.25% SS+0.25% FCM) and T5. All treatments with SS/FCM (T2, T3, T4 and T5), showed significantly higher leaf numbers than T1 (control) and T6 (SK-1) treatments during all three crop seasons. Leaf numbers of T6 treatment were slightly lower than T1 treatment during the first crop season. No significant differences in leaf numbers were observed between T1 and T6 treatments during the second and third crop seasons (Figure 10).

'Red Oak' lettuce

The most significant and highest leaf number was observed in T5 during first and second crop seasons. However, lettuces in T4 and T5 treatments showed no significant difference of leaf numbers during the first crop season. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly higher leaf numbers than T1 and T6 treatments during all three crop seasons. No significant differences in leaf numbers were observed between T1 and T6 treatments during all three crop seasons (Figure 11).

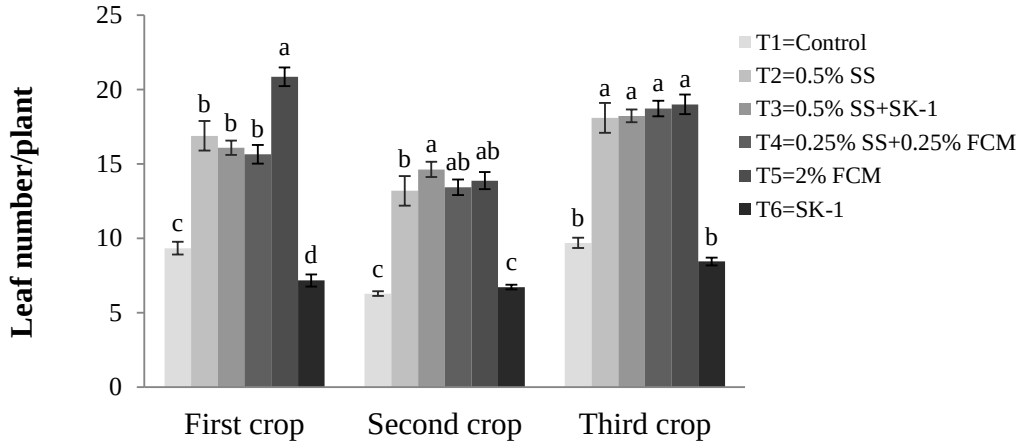

Figure 10 Effects of biomaterial and semi-biomaterial on leaf numbers of 'Butterhead' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

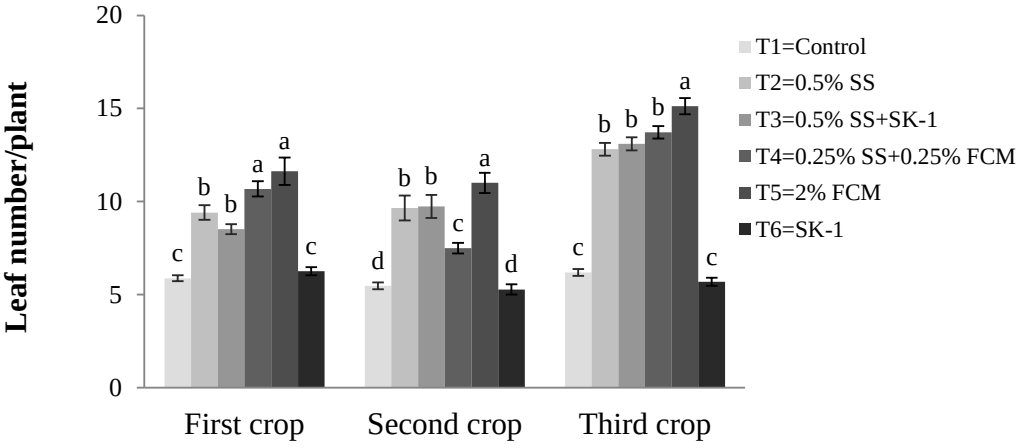

Figure 11 Effects of biomaterial and semi-biomaterial on leaf numbers of 'Red Oak' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

1.1.2. Leaf width

'Butterhead' lettuce

The width and length of the largest leaf in each lettuce plant were measured, as indicators of plant growth. Lettuces in T5 treatment resulted in the largest and most significant increases in the leaf width of lettuce planted during the first and third crop seasons. There were no significant difference in leaf width among T2, T3, T4 and T5 during the second crop season. Leaf width in T2, T3, T4 and T5 treatments were also significantly larger than that in T1 and T6 treatments, whereas the largest leaf width was observed in T5 treatments during all three crop seasons. In the third crop, in T2, T3, T4 and T5 treatments were also significantly larger than that in T1 and T6. The most significant and largest leaf width was observed in T5, where the lowest leaf width was observed in T6 treatment (Figure 12).

'Red Oak' lettuce

During the first crop season, T5 treatment resulted in the largest and most significant increase in leaf width of the lettuces. During the second and third crop seasons, all treatments with SS/FCM (T2, T3, T4 and T5) showed significantly higher leaf width than T1 and T6 treatments. There were no significant differences in leaf width among T2, T3, T4 and T5 treatments during the second and third crop seasons. No significant differences in leaf width were observed between T1 and T6 treatments in all three crop seasons (Figure 13).

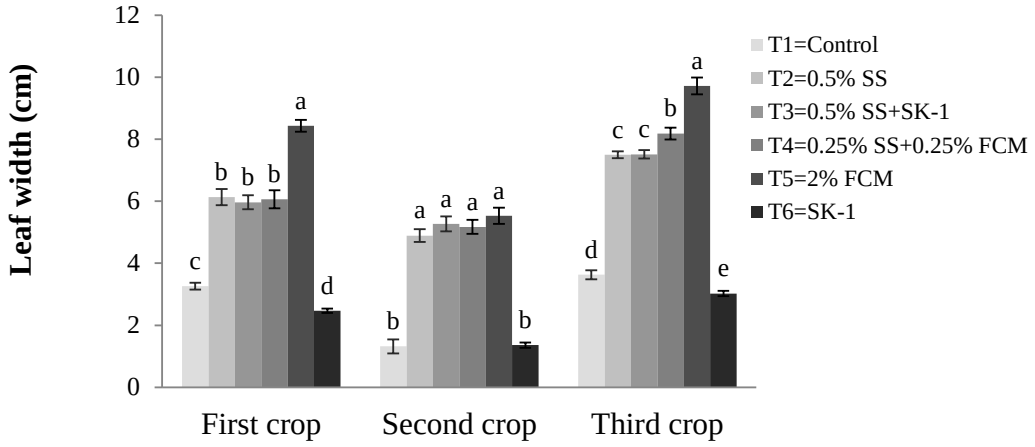

Figure 12 Effects of biomaterial and semi-biomaterial on leaf width of 'Butterhead' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

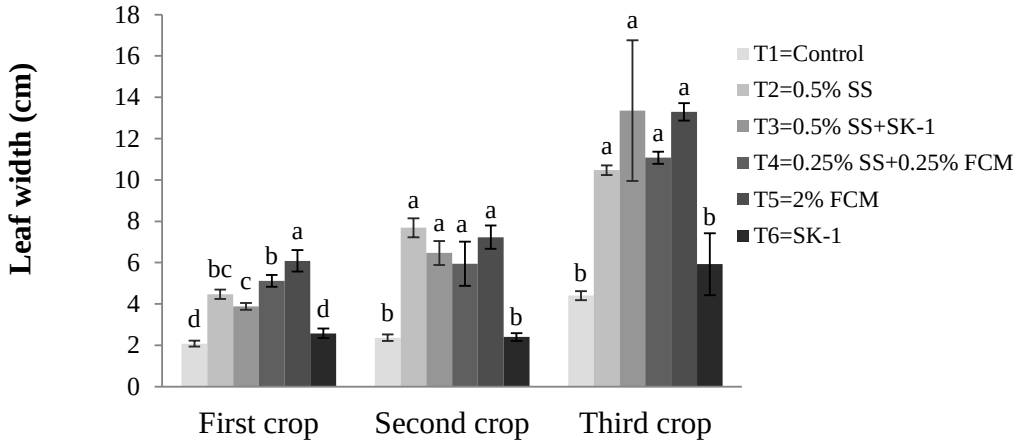

Figure 13 Effects of biomaterial and semi-biomaterial on leaf width of 'Red Oak' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

1.1.3. Leaf length

‘Butterhead’ lettuce

The most significant and highest leaf length was observed in T5 treatment during all three crop seasons. There were no significant differences in leaf length among T2, T3 and T4 treatments during all three crop seasons. No significant differences in leaf length were observed between T1 and T6 treatments during the first and second crop seasons. Leaf length in the T6 treatment was slightly lower than T1 treatment during the third crop season (Figure 14).

‘Red Oak’ lettuce

Lettuces in the T5 treatment showed the highest leaf length during the first and third crop seasons, whereas T4 treatment showed the highest leaf length during the second crop season. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly higher leaf length than T1 and T6 treatments during all three crop seasons. No significant differences in leaf length were observed between T1 and T6 treatments during all three crop seasons (Figure 15).

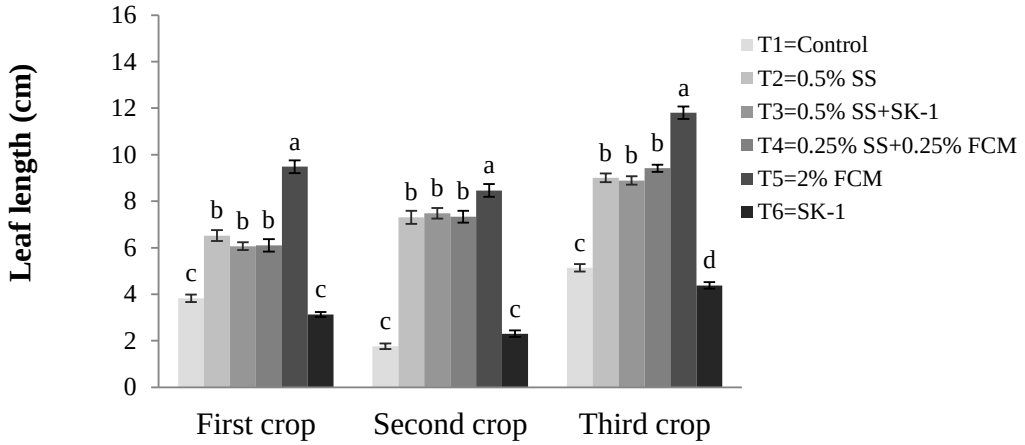

Figure 14 Effects of biomaterial and semi-biomaterial on leaf length of 'Butterhead' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

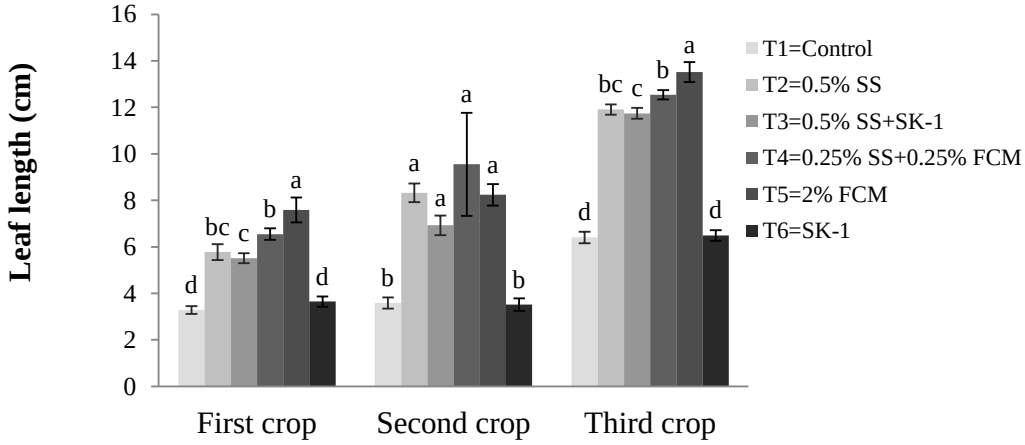

Figure 15 Effects of biomaterial and semi-biomaterial on leaf length of 'Red Oak' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

1.1.4. Fresh weight

'Butterhead' lettuce

Lettuces in T5 treatment resulted in the highest and most significant increase in the fresh weight of lettuce during the first and third crop seasons. Such increase was remarkable during the first crop season when fresh weight of lettuces grown with the presence of T5 treatment was approximately doubled when compared with other SS/FCM (T2, T3 and T4) treatments. There were no significant differences in fresh weight among T2, T3, T4 and T5 treatments during the second crop season. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly higher fresh weight than T1 and T6 treatments during all three crop seasons. No significant differences in fresh weight were observed between T1 and T6 treatments in all three crop seasons (Figure 16).

'Red Oak' lettuce

The most significant and highest fresh weight was observed in T5 treatment during all three crop seasons. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly higher fresh weight than T1 and T6 treatments during the first and third crop seasons, whereas T2, T3 and T5 treatments showed significantly higher fresh weight than T1 and T6 treatments during the second crop season. There were no significant differences in fresh weight among T2, T3 and T5 treatments in the second crop season. No significant differences in fresh weight were observed between T1 and T6 treatments during the first and third crop seasons (Figure 17).

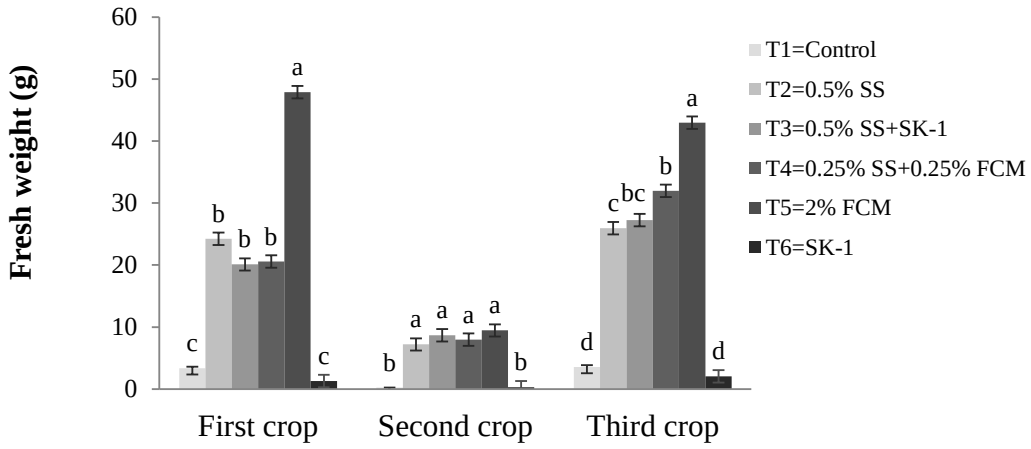

Figure 16 Effects of biomaterial and semi-biomaterial on fresh weight of 'Butterhead' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

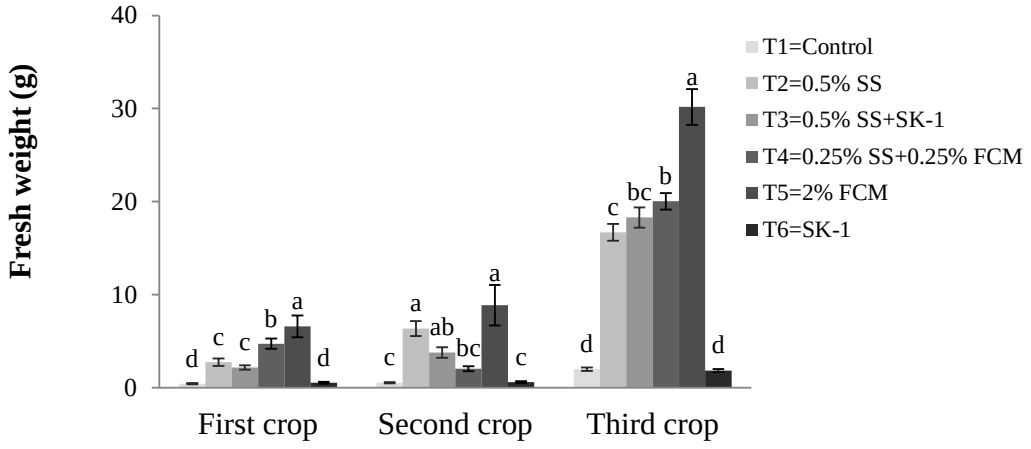

Figure 17 Effects of biomaterial and semi-biomaterial on fresh weight of 'Red Oak' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

1.1.5. Dry weight

'Butterhead' lettuce

The most significant and highest dry weight was observed in T5 treatment during the first and second crop seasons. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly higher dry weight than T1 and T6 treatments during the first and second crop seasons. There were no significant differences in dry weight among T2, T3, T4 and T5 treatments during the third crop season. No significant differences in dry weight were observed between T1 and T6 treatments during all three crop seasons (Figure 18).

'Red Oak' lettuce

Lettuces in T5 treatment resulted in the highest and most significant increase in the dry weight of the lettuce during the first and third crop seasons, whereas T2 treatment resulted in the highest and most significant increase in dry weight of lettuce during the second crop seasons. There were no significant differences in dry weight among T2 and T5 treatments during the second crop season. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly higher dry weight than T1 and T6 treatments during the first and third crop seasons. No significant differences in dry weight were observed between T1 and T6 treatments during all three crop seasons (Figure 19).

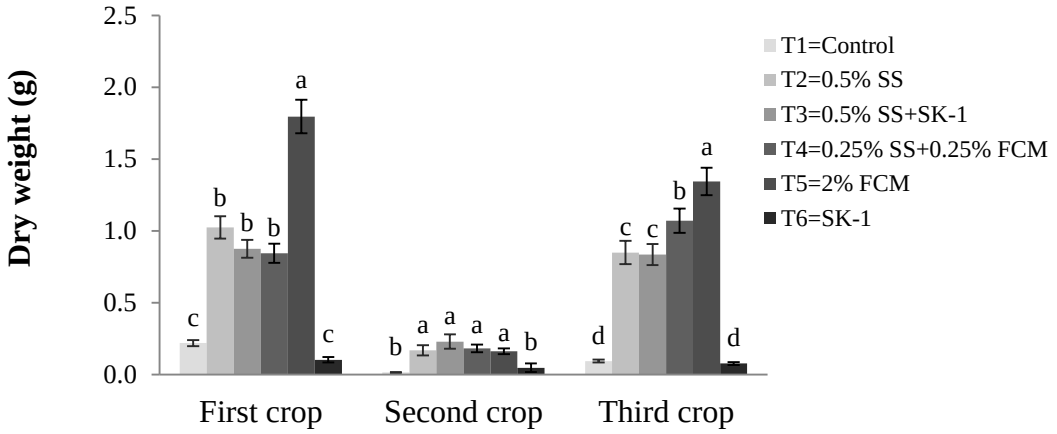

Figure 18 Effects of biomaterial and semi-biomaterial on dry weight of 'Butterhead' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

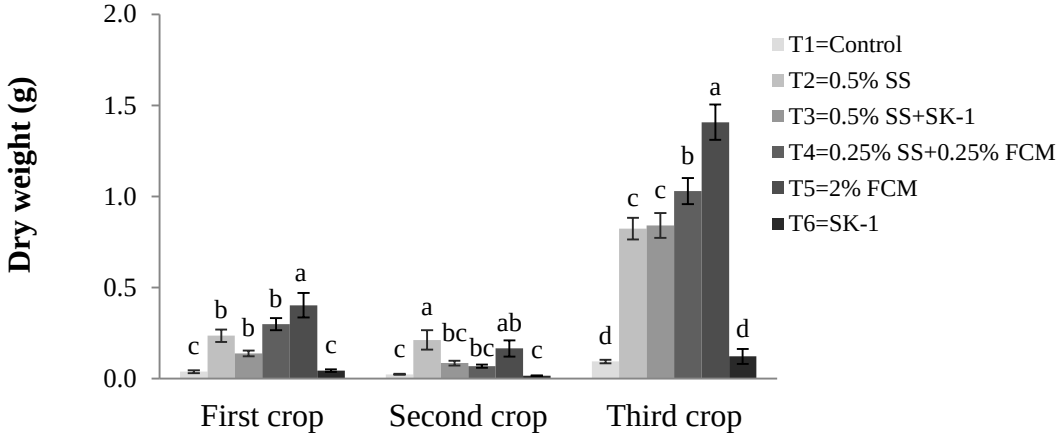

Figure 19 Effects of biomaterial and semi-biomaterial on dry weight of 'Red Oak' lettuce in a test plot after 8 weeks of cultivation. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

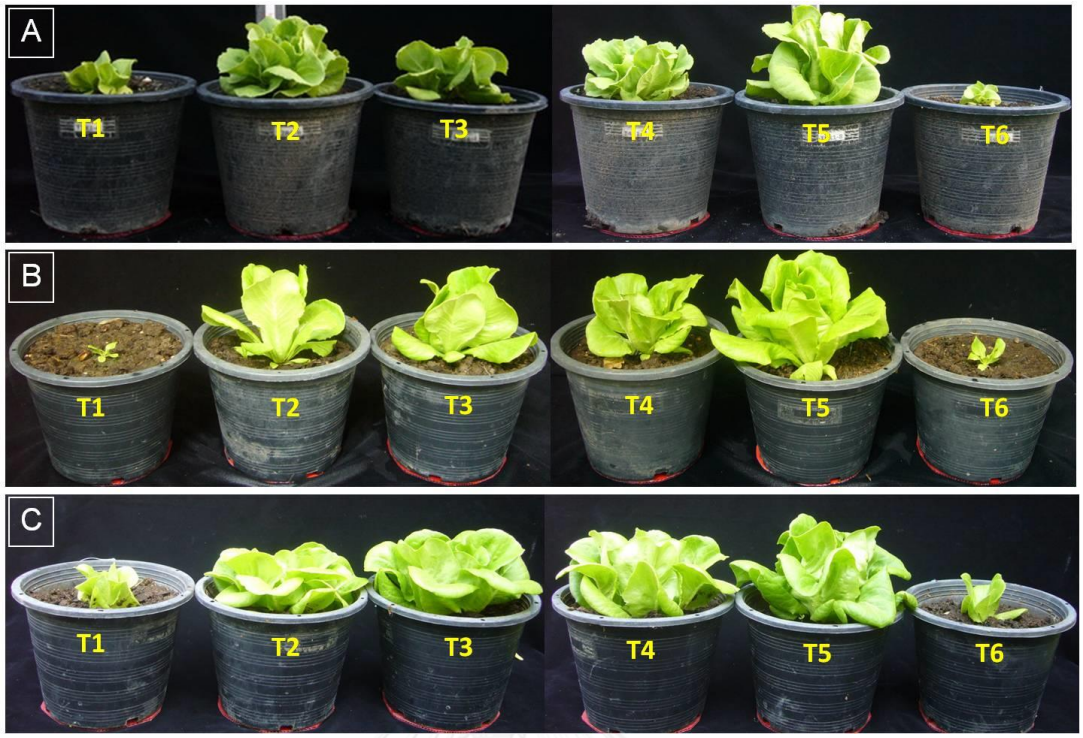

Figure 20 Effects of biomaterial and semi-biomaterial on growth of 'Butterhead' lettuce in the first (A) second (B) and third (C) crops after 8 weeks of cultivation in a test plot.

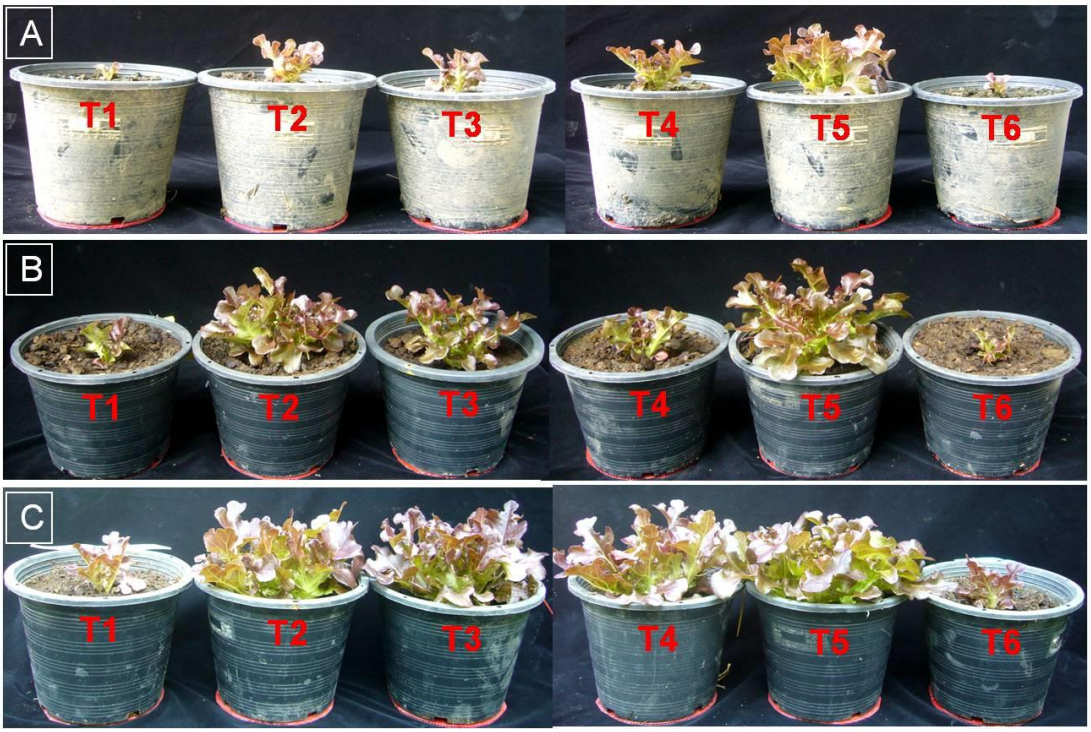

Figure 21 Effects of biomaterial and semi-biomaterial on growth of 'Red Oak' lettuce in the first (A) second (B) and third (C) crops after 8 weeks of cultivation in a test plot.

4.1.2 Effects of biomaterial and semi-biomaterial on postharvest quality

1.1.6. Percentage of fresh weight loss

'Butterhead' lettuce

After harvesting, 32 plants per treatment were packed in sealed plastic bags and stored in a chamber at 8°C for 14 days. Weight losses of lettuces after storage at 8°C and 60% RH for 2 weeks were significantly reduced in control (T1) treatments during all three crop seasons. During the first and third crop seasons, the application of SS/FCM treatmentsresulted in the lowest fresh weight loss. During the second crop season, T3 treatment resulted in the lowest fresh weight loss. However, there were no significant differences in fresh weight loss among T3, T4 and T5 treatments during the second crop season. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly lower fresh weight loss than T1 and T6 treatments during all three crops (Figure 22).

'Red Oak' lettuce

The weight losses of the lettuces in T5 treatment resulted in the lowest fresh weight loss during all three crop seasons. During the first crop season, there were significant differences in fresh weight loss among T5 and, T1, T4 and T6 treatments. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly lower fresh weight loss than T1 and T6 treatments during the second crop season. There were no significant differences in fresh weight loss among T2, T3, T4 and T5 treatments during the second crop season. During the third crop season, T4 treatment resulted in the lowest fresh weight loss. There were no significant differences in fresh weight loss among T2, T4 and T5 treatments during the third crop season (Figure 23).

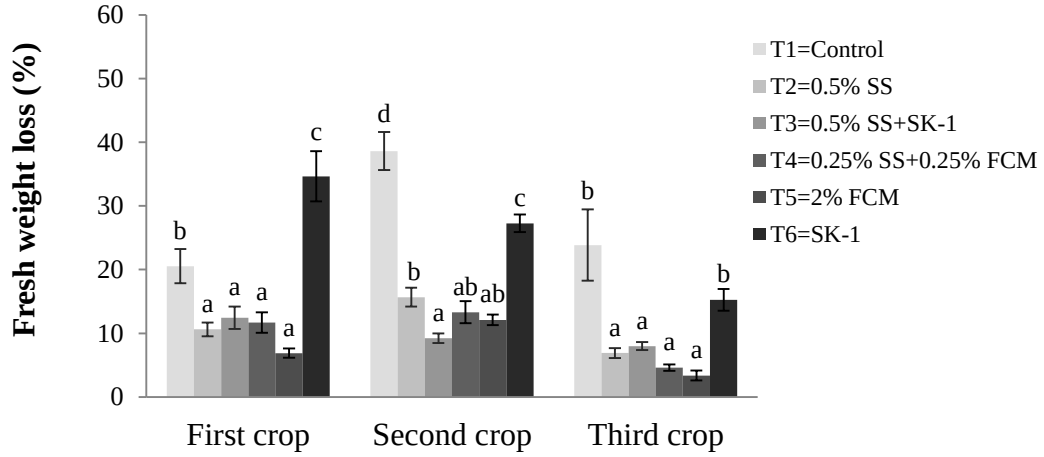

Figure 22 Effect of biomaterial and semi-biomaterial on fresh weight loss of 'Butterhead' lettuce following storage at 8°C and 60% RH for 14 days. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

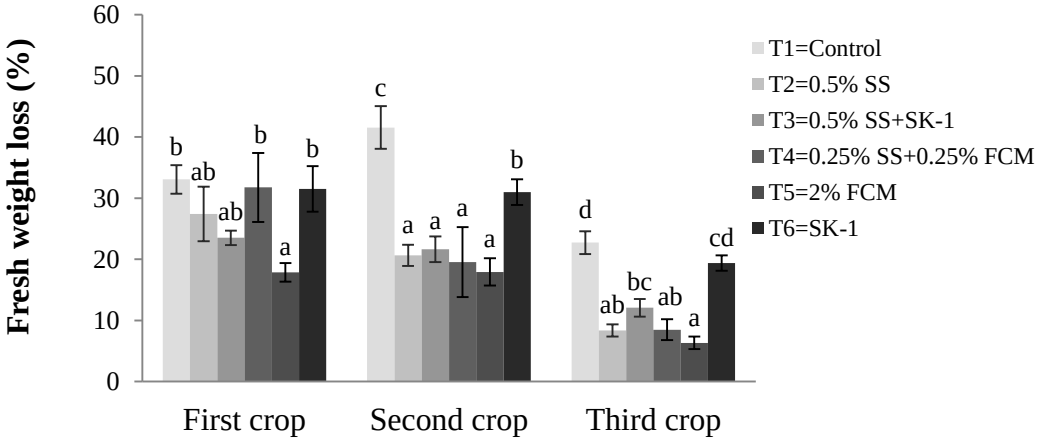

Figure 23 Effect of biomaterial and semi-biomaterial on fresh weight loss of 'Red Oak' lettuce following storage at 8°C and 60% RH for 14 days. Data are means of 32 heads. Data with the different letters are significantly different at *p*=0.05level of significance. The vertical bars represent the standard error (SE) of the means.

1.1.7. Overall visual quality score

'Butterhead' lettuce

After storage at 8°C and 60% RH for 2 weeks, better overall visual quality was observed among the SS/FCM-treated lettuces in all three crop seasons. Lettuces in T5 treatment showed the best overall visual quality during the first and third crop seasons, while lettuces of the other SS/FCM treatments showed insignificant differences in overall visual quality in the first and second crop seasons. In the second crop season, lettuces in the T2 and T5 treatments showed significantly better in overall visual quality than other treatments. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly better in overall visual quality than T1 and T6 treatments in the third crop seasons (Figure 24).

'Red Oak' lettuce

Lettuces in T4 and T5 treatments showed significantly better in overall visual quality than T1, T2, T3 and T6 treatments in the first crop season. In the second crop season, the T2 and T5 treatments showed significantly better in overall visual quality than T1, T3, T4 and T6 treatments. All treatments with SS/FCM (T2, T3, T4 and T5) showed significantly better in overall visual quality than T1 and T6 treatments in the third crop seasons. There were no significant differences in overall visual quality between T1 and T6 treatments in the third crop season (Figure 25).

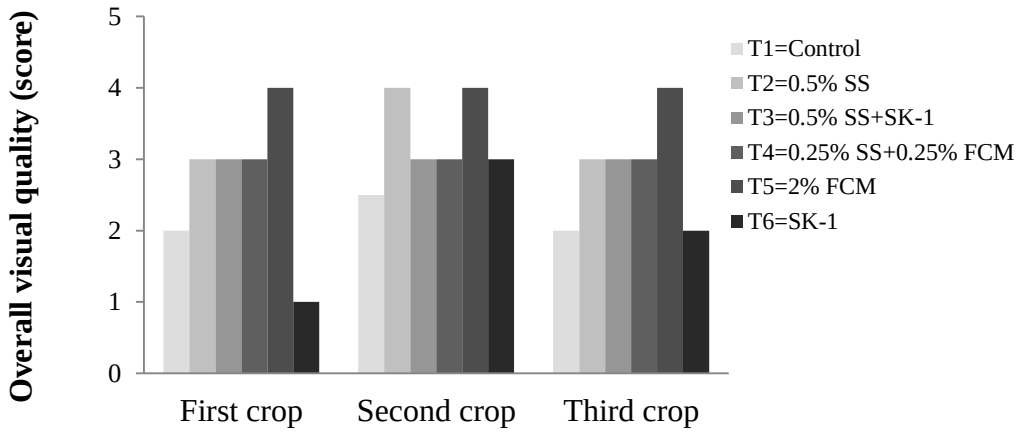

Figure 24 Effect of biomaterial and semi-biomaterial on overall visual quality of 'Butterhead' lettuce following storage at 8°C and 60% RH for 14 days. Data are means of 32 heads. A quality index scale from 4 to 0, where 4 = excellent (Essentially free from defects), 3 = good (Minor defects; not objectionable), 2 = fair (Slightly to moderately objectionable defects; lower limit of sales appeal), 1 = poor (Excessive defects; limited salability), 0 = extremely poor (Not useable).

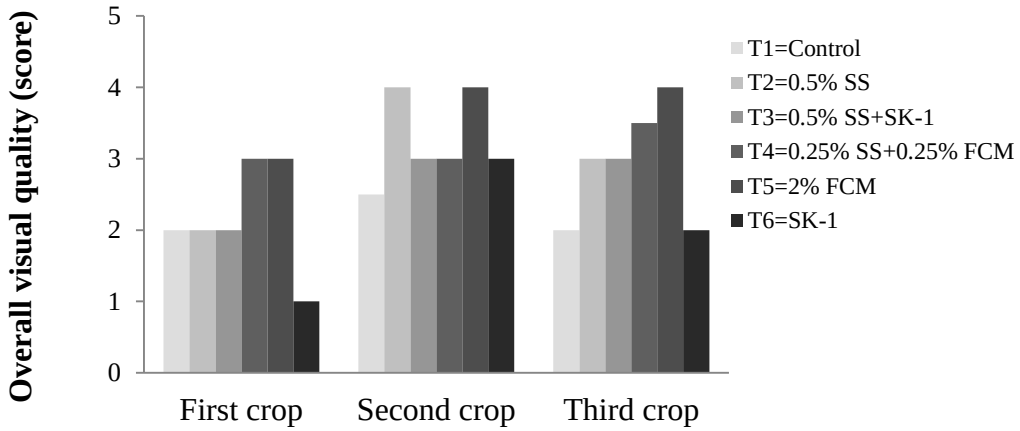

Figure 25 Effect of biomaterial and semi-biomaterial on overall visual quality of 'Red Oak' lettuce following storage at 8°C and 60% RH for 14 days. Data are means of 32 heads. A quality index scale from 4 to 0, where 4 = excellent (Essentially free from defects), 3 = good (Minor defects; not objectionable), 2 = fair (Slightly to moderately objectionable defects; lower limit of sales appeal), 1 = poor (Excessive defects; limited salability), 0 = extremely poor (Not useable).

4.1.3 Effects of biomaterial and semi-biomaterial on selected soil chemical and physical parameters of lettuces grown in a test plot

Soil chemical properties

The chemical characteristics of the growing media before seedling transplantation did not significantly vary in pH, EC and OM among different treatments (Table 3). The T5 treatment showed the highest increase in total N and P availability in soil. Soil K and Ca availability showed the highest increase in the T4 treatment. Soil Mg availability showed the highest increase in the T2 treatment. All treatments supplemented with SS and/or FCM (T2, T3, T4 and T5) showed higher increases in the Mg availability in the soil than the control treatment. Following the cultivation of lettuces after all three crop seasons, the total N, soil P and K availability remained high with the application of SS/FCM. During all three crop seasons, the pH and EC in soil level ranged from 6.9-7.2 and 0.94-1.65 dS m^{-1} respectively.

Table 3 Chemical characteristics of the growing media before planting in the first crop season and after harvesting in three successive crop seasons in a test plot

	Treat.	pH	EC (dS/m)	OM (%)	Total N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
Before planting	T1	7.5	1.56	5.24	0.25	156	3232	5521	157
	T2	7.5	1.88	4.46	0.21	252	2525	6137	663
	T3	7.1	1.88	4.19	0.18	147	2525	6846	539
	T4	7.3	2.34	4.77	0.25	256	3838	7001	488
	T5	7.0	2.34	4.41	0.30	520	3434	5367	500
	T6	7.3	2.03	4.90	0.18	161	2727	5306	511
First crop after harvesting	T1	7.0	0.94	3.40	0.17	115	1000	6380	419
	T2	7.2	1.49	6.10	0.28	327	1100	3180	339
	T3	7.2	1.20	3.92	0.22	280	1100	3100	299
	T4	7.2	1.27	5.62	0.31	390	1200	3420	329
	T5	7.0	1.65	5.62	0.35	533	1500	2900	299
	T6	7.0	1.07	4.64	0.19	163	1000	3180	229
Second crop after harvesting	T1	7.0	1.41	5.94	0.35	404	2727	4876	408
	T2	7.0	1.09	6.57	0.35	428	2222	6287	490
	T3	7.0	1.25	5.91	0.21	347	2323	5917	531
	T4	7.1	1.09	5.38	0.21	408	2929	5331	507
	T5	6.9	1.25	7.11	0.39	696	2424	6436	933
	T6	7.0	1.41	7.33	0.45	452	2525	6392	1118
Third crop after harvesting	T1	7.1	1.41	5.62	0.31	302	2626	5453	748
	T2	7.1	1.56	6.13	0.35	367	2525	8955	1405
	T3	7.1	1.56	4.70	0.28	352	2626	8044	1429
	T4	7.2	1.09	4.82	0.31	398	2525	7405	1124
	T5	7.0	1.09	6.72	0.34	549	2222	6526	1389
	T6	7.0	1.09	5.74	0.35	315	1919	6506	1089

Table 4 Cultivation, irrigation practices and climatic conditions during the three crop seasons in a test plot

	First crop season	Second crop season	Thirdcrop season
Lettuce transplanting	March 1, 2010	July 25, 2010	December 4, 2010
Date of harvesting	April 26, 2010	September 19, 2010	February 4, 2011
Crop duration (days)	56	56	63
Irrigation water (l plant^{-1}) total	62	31	86
Minimum light intensity (μmol m^{-2} s^{-1})	817	76	136
Maximum light intensity (μmol m^{-2} s^{-1})	1,155	1,225	1,700
Minimum air temperature (°C)	25	28	29
Maximum air temperature (°C)	45	41	39
Minimum airRH (%)	32	39	30
Maximum air RH (%)	47	96	53

4.2 Effects of the FCM on growth, yield, postharvest quality and selected soil microbial populations of lettuces in a local farm

In period studies on the effects of biomaterial and semi-biomaterial on growth, yield and postharvest quality of lettuces grown in a test plot, the results clearly showed that T5 (2% FCM) was the finest supplement as it increased lettuces yield and postharvest quality. The number one treatment from the period study was used in a local farm.

4.2.1 Effects of FCM on growth and yield

1.1.8. Leaf number

‘Butterhead’ lettuce

Lettuce treated with 2% FCM showed significantly higher leaf numbers than in the control treatment during the first crop season. There were no significant differences between control and FCM treatments during the second crop season (Figure 26).

‘Red Oak’ lettuce

During both crop seasons, the FCM treatment showed significantly higher leaf numbers than the control treatment (Figure 26).

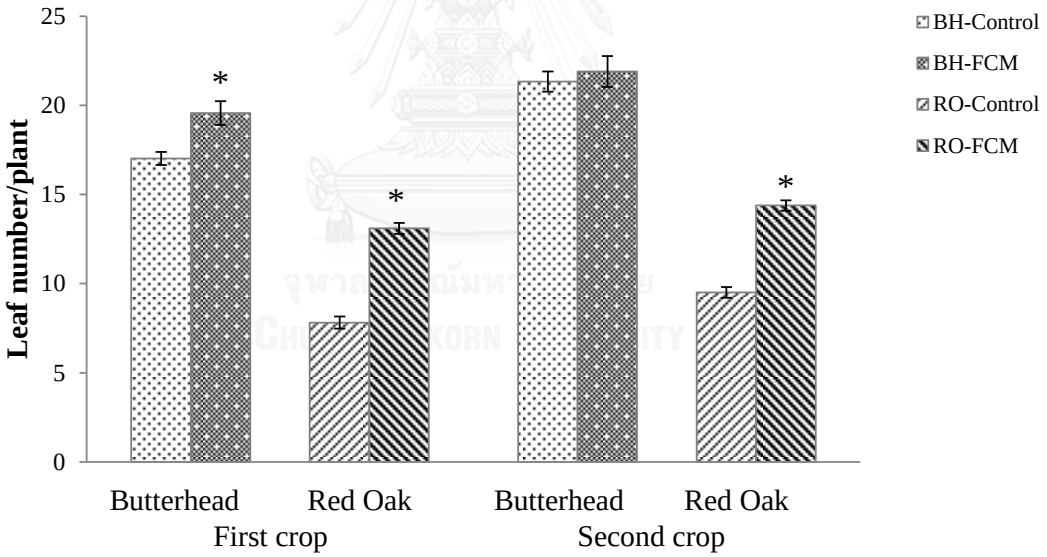

Figure 26 Effects of 2% FCM on leaf number of ‘Butterhead’ and ‘Red Oak’ lettuces in a local farm after 8 weeks of cultivation. ***** indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.9. Leaf width

‘Butterhead’ lettuce

There were no significant differences in the leaf width of ‘Butterhead’ lettuce between the control and FCM treatments during both crop seasons (Figure 27).

‘Red Oak’ lettuce

Lettuce in the FCM treatment showed significantly larger leaf width than in the control treatment during both crop seasons (Figure 27).

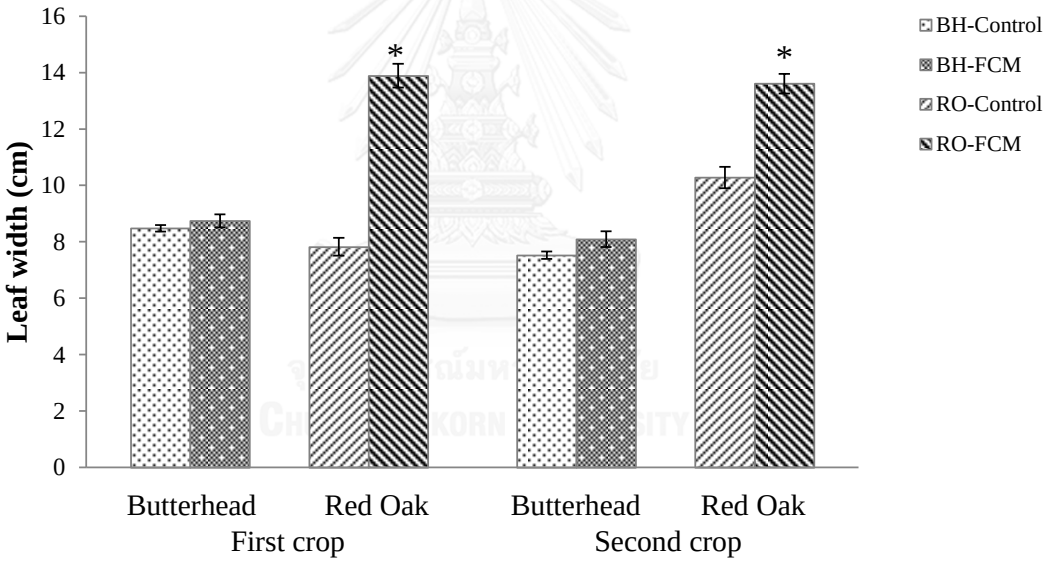

Figure 27 Effects of 2% FCM on leaf width of ‘Butterhead’ and ‘Red Oak’ lettuces in a local farm after 8 weeks of cultivation. * indicates the significant difference between control and FCM treatments.Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.10. Leaf length

‘Butterhead’ lettuce

There were no significant differences in leaf length between the control and FCM treatments during both crop seasons (Figure 28).

‘Red Oak’ lettuce

The FCM treatment showed significantly higher leaf length than in the control treatment during both crop seasons (Figure 28).

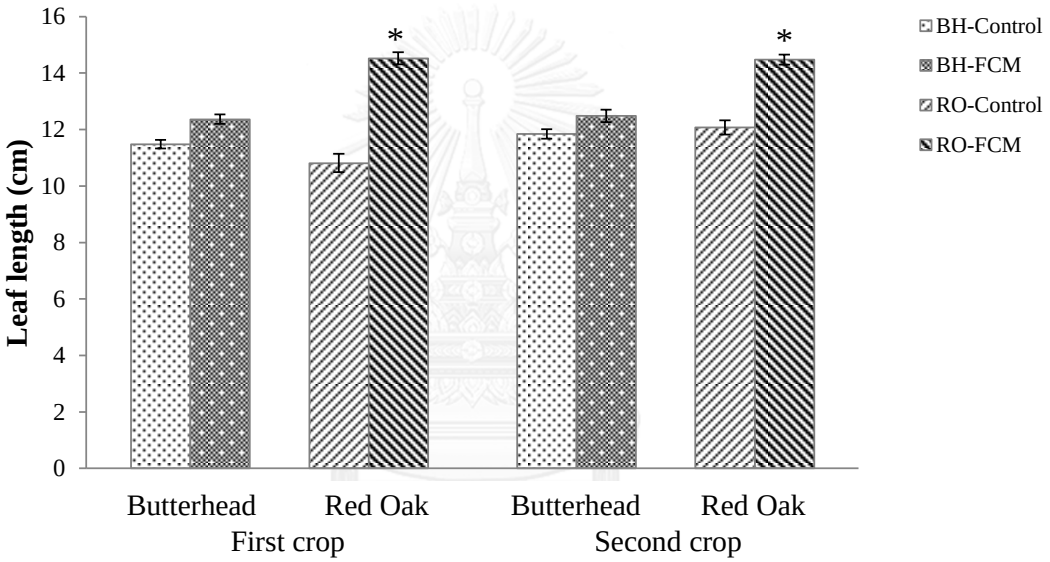

Figure 28 Effects of 2% FCM on leaf length of ‘Butterhead’ and ‘Red Oak’ lettuces in a local farm after 8 weeks of cultivation. ***** indicates the significant difference between control and FCM treatments.Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.11. Diameter of lettuce head

‘Butterhead’ lettuce

Lettuce treated with FCM showed significantly larger diameter of lettuce head than in the control treatment during the first crop season. There were no significant differences in diameter of the lettuce head between the control and FCM treatments during the second crop season (Figure 29).

‘Red Oak’ lettuce

The FCM treatment showed significantly higher diameter of lettuce head than in the control treatment during the first crop season. There were no significant differences in diameter of the lettuce head between the control and FCM treatments during the second crop season (Figure 29).

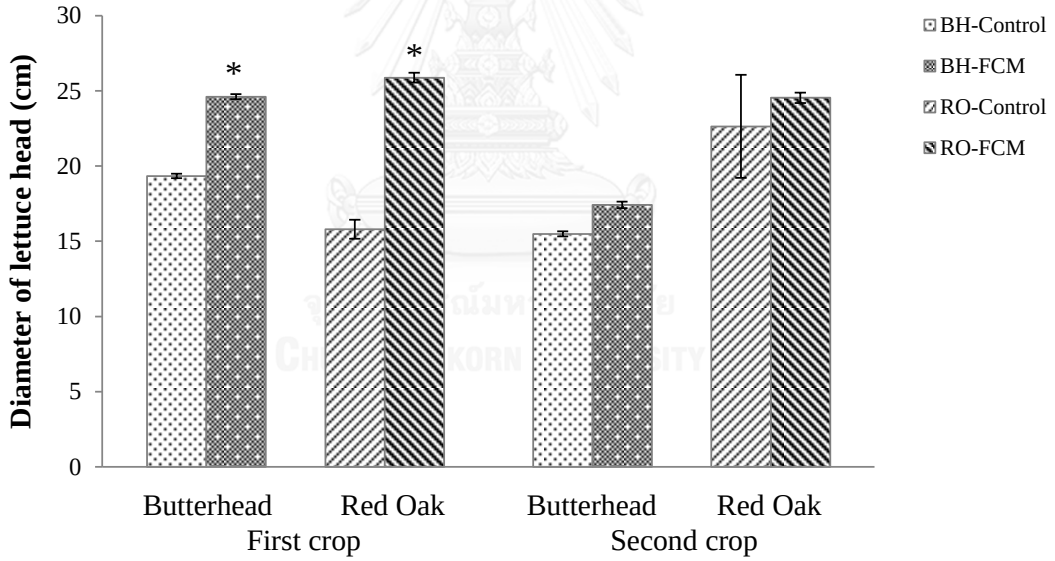

Figure 29 Effects of 2% FCM on diameter of lettuce head of ‘Butterhead’ and ‘Red Oak’ lettuces in a local farm after 8 weeks of cultivation. ***** indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.12. Fresh weight

‘Butterhead’ lettuce

Lettuce treated with FCM showed significantly higher fresh weight than in the control treatment during both crop seasons. During the first crop season, the fresh weight of the ‘Butterhead’ lettuce was 53 g plant^{-1} in the FCM treatment compared to the control treatment, which was approximately 2.4 times higher than in the control treatment (Figure 30).

‘Red Oak’ lettuce

The FCM treatment showed significantly higher fresh weight than in the control treatment in both crop seasons. In the first crop season, the fresh weight of ‘Red Oak’ lettuce was 42 g plant^{-1} in the FCM treatment compared to the control treatment, which was approximately 14 times higher than in the control treatment. In the second crop season, the fresh weight of lettuce was 30 g plant^{-1} in the FCM treatment compared to the control treatment, which was approximately 2.3 times higher than in the control treatment (Figure 30).

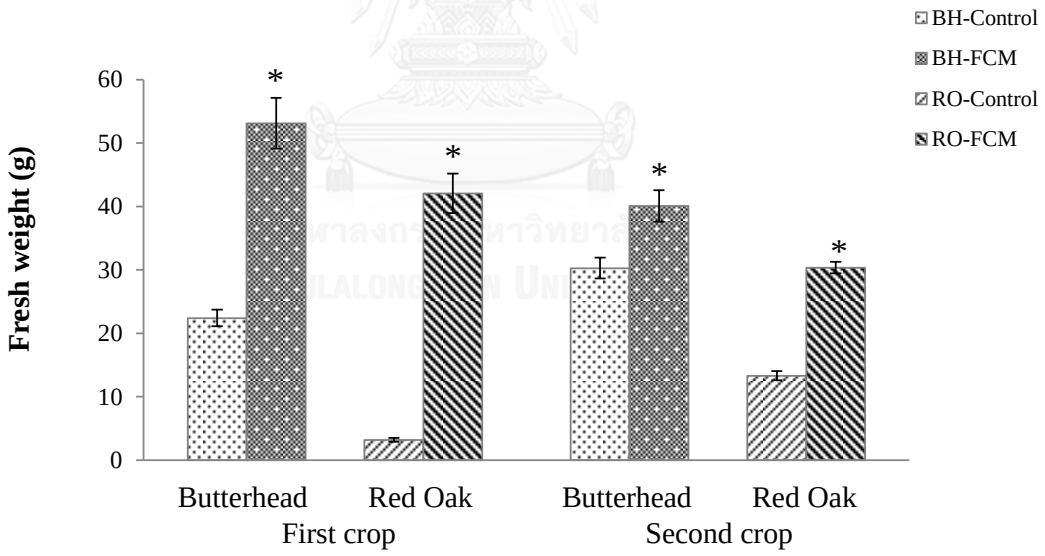

Figure 30 Effects of 2% FCM on fresh weight of ‘Butterhead’ and ‘Red Oak’ lettuces in a local farm after 8 weeks of cultivation. * indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.13. Dry weight

‘Butterhead’ lettuce

The FCM treatment showed significantly higher dry weight than in the control treatment during the first crop season. There were no significant differences in dry weight between the control and FCM treatments during the second crop season. During the first crop season, the dry weight of ‘Butterhead’ lettuce was 1.38 g plant^{-1} in the FCM treatment compared to the control treatment, which was approximately 8.6 times higher than in the control treatment (Figure 31).

‘Red Oak’ lettuce

Lettuce in the FCM treatment showed significantly higher dry weight than in the control treatment during both crop seasons. In the first crop season, the dry weight of ‘Red Oak’ lettuce was 1.38 g plant^{-1} in the FCM treatment compared to the control treatment, which was approximately 8 times higher than the control treatment. During the second crop season, the dry weight of lettuce was 1.22 g plant^{-1} in the FCM treatment compared to the control treatment, which was approximately 4.5 times higher than in the control treatment (Figure 31).

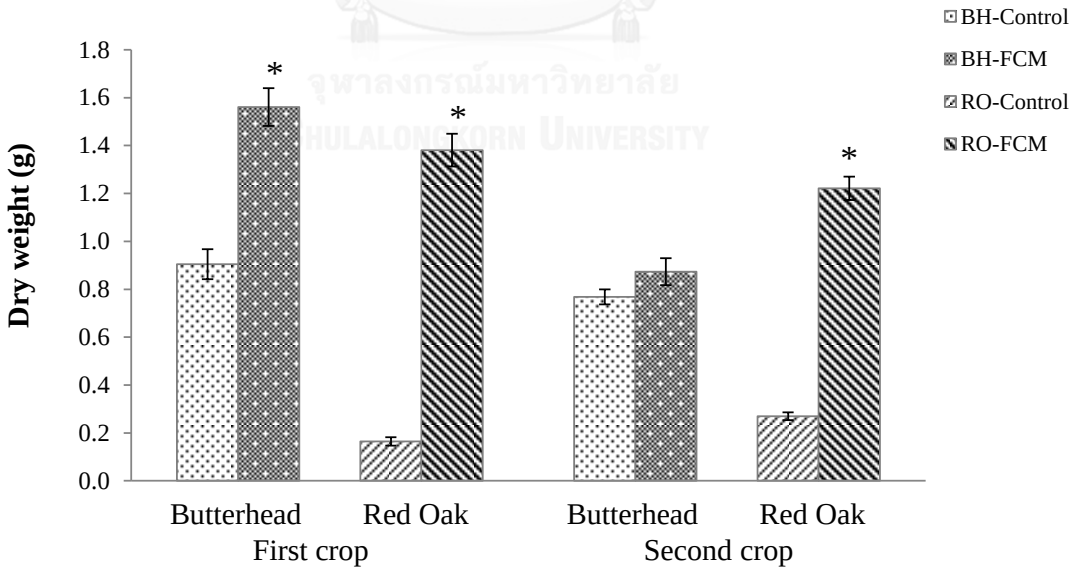

Figure 31 Effects of 2% FCM on dry weight of ‘Butterhead’ and ‘Red Oak’ lettuces in a local farm after 8 weeks of cultivation. * indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

Control treatment FCMtreatment

Figure 32 Effects of 2% FCM on growth yield of ‘Butterhead’ lettuces during the first crop in a local farm after 8 weeks of cultivation. (A) The control treatment. (B) The FCM treatment.

Control treatment FCMtreatment

Figure 33 Effects of 2% FCM on growth yield of ‘Butterhead’ lettuces during the second crop in a local farm after 8 weeks of cultivation. (A) The control treatment. (B) The FCM treatmen

Cntrol treatment FCMtreatment

Figure 34 Effects of 2% FCM on growth yield of 'Red Oak' lettuces during the first crop in a local farm after 8 weeks of cultivation. (A) The control treatment. (B) The FCM treatment.

Control treatment FCMtreatment

Figure 35 Effects of 2% FCM on growth yield of 'Red Oak' lettuces during the first second crop in a local farm after 8 weeks of cultivation. (A) The control treatment. (B) The FCM treatment.

4.2.2 Effects of FCM on postharvest quality

1.1.14. Fresh weight loss

'Butterhead' lettuce

After harvest, 20 plants per treatment were packed in sealed plastic bags and stored in a chamber at 8°C for 14 days. Weight losses of lettuces after storage at 8°C and 60% RH for 2 weeks were significantly reduced in the FCM treatment during the first crop season. During the second crop season, the control treatment showed significantly lower fresh weight loss than the FCM treatment (Figure 36).

'Red Oak' lettuce

Lettuces treated with FCM showed significantly lower fresh weight loss than the control treatment during both crop seasons (Figure 36).

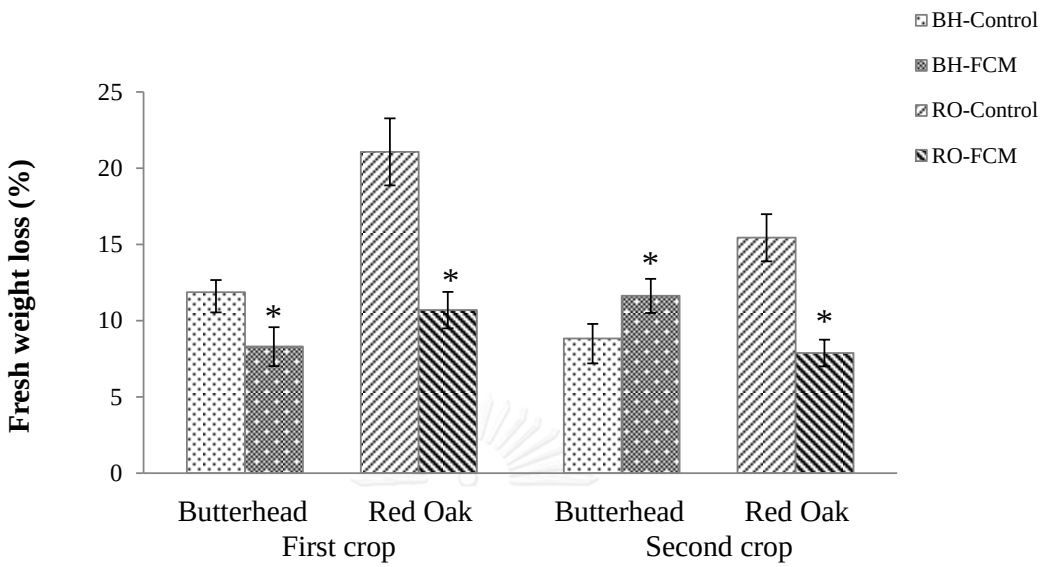

Figure 36 Effects of 2% FCM on fresh weight loss of 'Butterhead' and 'Red Oak' lettuces following storage at 8°C and 60% RH for 14 days. Data are means of 16 heads. Data with the different letters are significantly different at p=0.05 level of significance. The vertical bars represent the standard error (SE) of the means. * indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with p<0.05 were considered significant.

1.1.15. Overall visual quality score

‘Butterhead’ lettuce

After storage at 8°C and 60% RH for 14 days, better overall visual quality was observed in the FCM treatment during the first crop season. While lettuces of the control and the FCM treatments showed insignificant differences in overall visual quality during the second crop season (Figure 37).

‘Red Oak’ lettuce

Lettuces treated with FCM showed significantly better in overall visual quality than in the control treatment during both crop seasons (Figure 37).

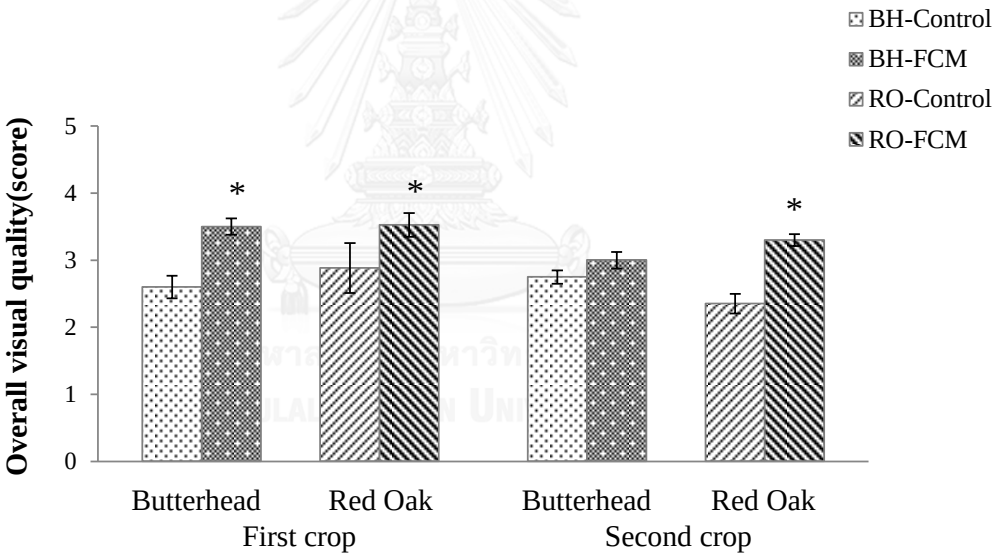

Figure 37 Effects of 2% FCM on overall visual quality of ‘Butterhead’ and ‘Red Oak’ lettuces following storage at 8°C and 60% RH for 14 days. Data are means of 16 heads. A quality index scale from 4 to 0, where 4 = excellent (Essentially free from defects), 3 = good (Minor defects; not objectionable), 2 = fair (Slightly to moderately objectionable defects; lower limit of sales appeal), 1 = poor (Excessive defects; limited salability), 0 = extremely poor (Not useable).

4.2.3 Effects of FCM on net photosynthesis, transpiration rate and stomatal conductance of lettuce leaf

1.1.16. Net photosynthesis

'Butterhead' lettuce

There were no significant differences in the net photosynthesis rate between the control and FCM treatments during both crop seasons (Figure 38).

'Red Oak' lettuce

Lettuces treated with FCM showed significantly higher net photosynthesis rate than in the control treatment during both crop seasons (Figure 38).

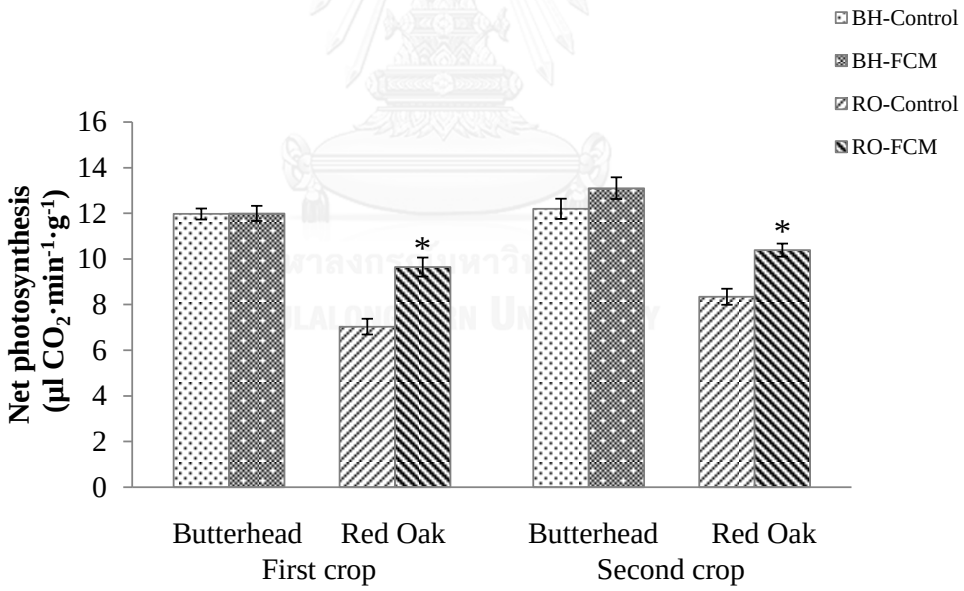

Figure 38 Effects of 2% FCM on the net photosynthesis rate of 'Butterhead' and 'Red Oak' lettuces in a local farm after 7 weeks of cultivation. * indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.17. Transpiration rate

'Butterhead' lettuce

There were no significant differences in the transpiration rate between the control and FCM treatments during both crop seasons (Figure 39).

'Red Oak' lettuce

There were no significant differences in the transpiration rate were observed between the control and FCM treatments during the first crop season. The FCM treatment showed a significantly higher transpiration rate than in the control treatment during the second crop season (Figure 39).

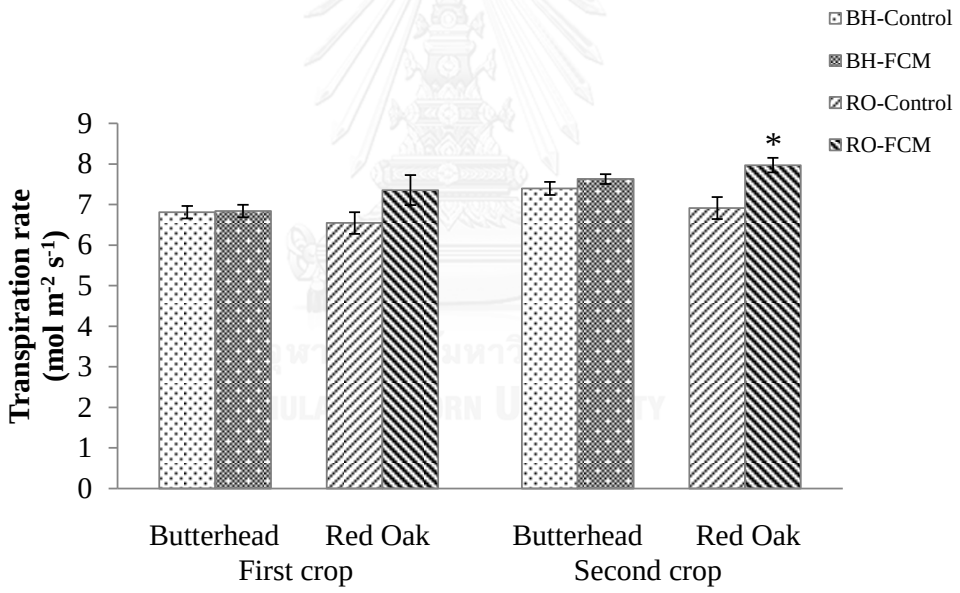

Figure 39 Effects of 2% FCM on transpiration rate of 'Butterhead' and 'Red Oak' lettuces in a local farm after 7 weeks of cultivation. * indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.18. Stomatal conductance of lettuce leaf

‘Butterhead’ lettuce

There were no significant differences in stomatal conductancebetween the control and FCM treatments during both crop seasons (Figure 40).

‘Red Oak’ lettuce

Lettuces treated with FCM showed a significantly higher stomatal conductancethan in the control treatments during both crop seasons (Figure 40).

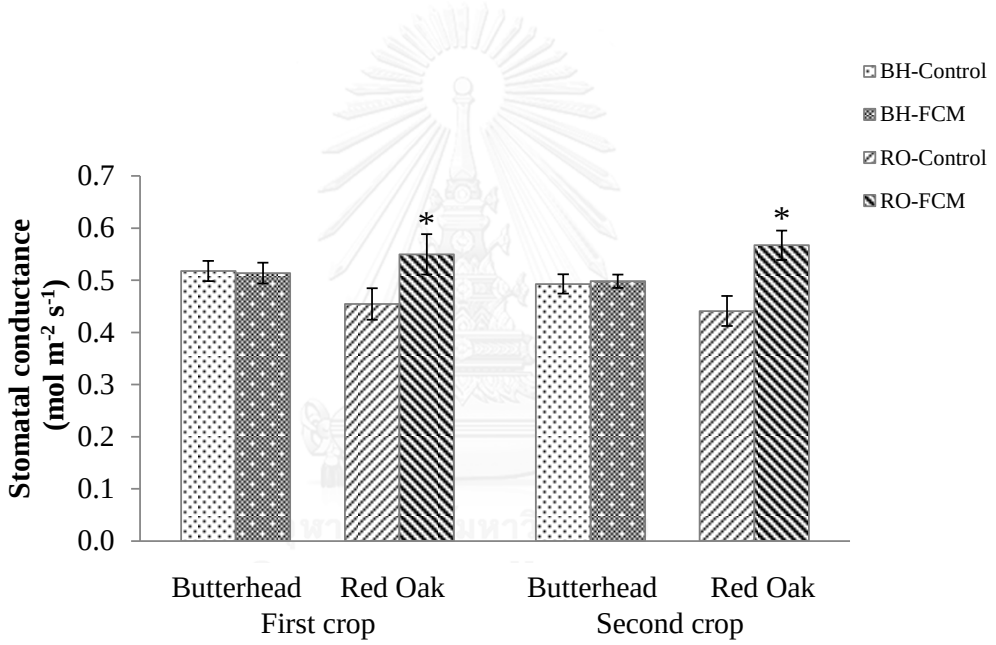

Figure 40 Effects of 2% FCM on leaf stomatal conductance of ‘Butterhead’ and ‘Red Oak’ lettuces in a local farm after 7 weeks of cultivation.* indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

4.2.4 Effects of FCM on nitrate contents

‘Butterhead’ lettuce

The FCM treatment tended to have higher nitrate contents than the control treatment during the first crop season. However, there were no significant differences in the nitrate contentsbetween the control and FCM treatments during both crop seasons (Figure 41).

‘Red Oak’ lettuce

The FCM treatment during the first crop season showed significantly higher nitrate contentsthan in the control treatment on day 14 after storage. While during the second crop season, the control treatment showed significantly higher nitrate contentsthan in the FCM treatment on day 0 of low temperature storage (Figure 42).

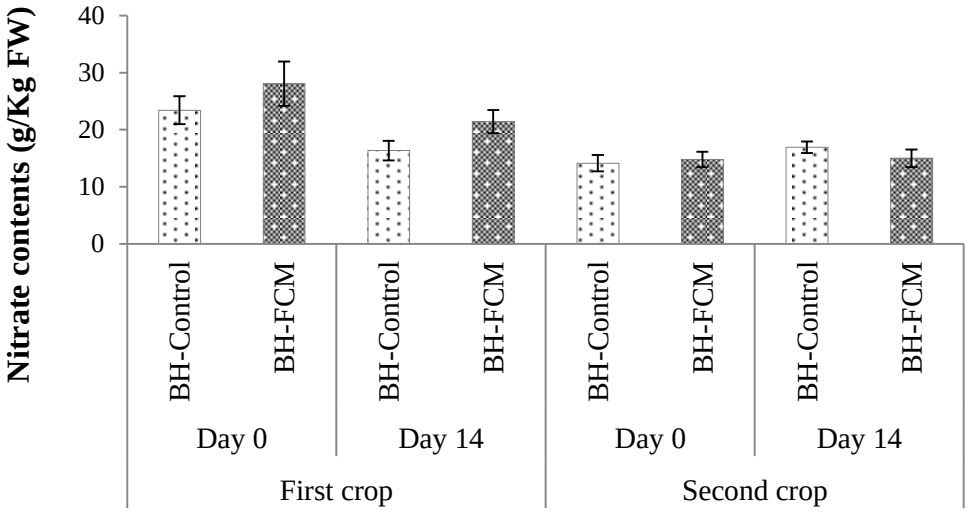

Figure 41 Effects of 2% FCM on nitrate contentsof 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

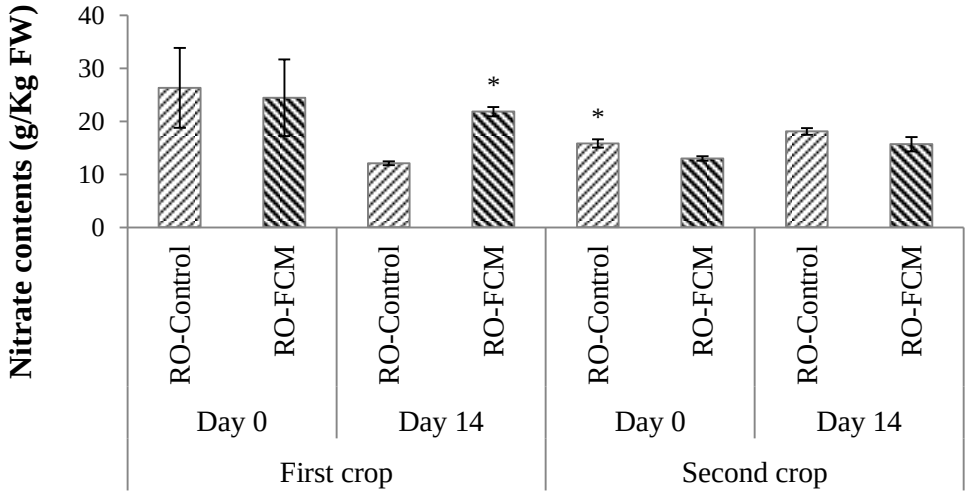

Figure 42 Effects of 2% FCM on nitrate contentsof 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days.* indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

4.2.5 Effects of FCM on chlorophyll *a*, chlorophyll *b* and carotenoid contents

1.1.19. Chlorophyll *a* contents

'Butterhead' lettuce

During both crop seasons, there were no significant differences in the chlorophyll *a* contents in both control and FCM treatments before and after storage at 8°C and 60% RH for 14 days (Figure 43).

'Red Oak' lettuce

The FCM treatment during the first crop season showed significantly higher chlorophyll *a* contents than in the control treatment on day 14 after storage. While during the second crop season, the control treatment showed significantly higher chlorophyll *a* contentsthan in the FCM treatment on day 0 of low temperature storage (Figure 44).

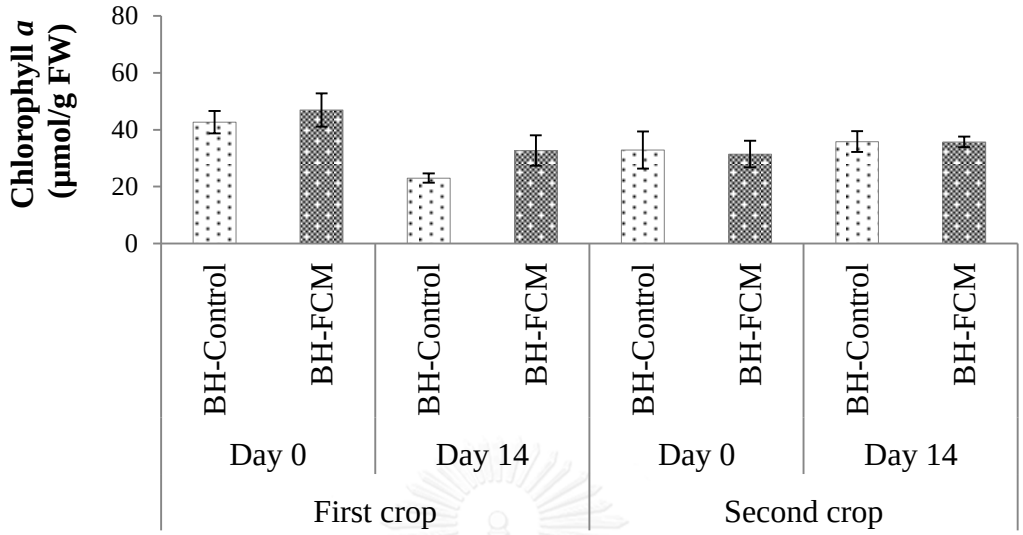

Figure 43 Effects of 2% FCM on chlorophyll *a* contents of 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

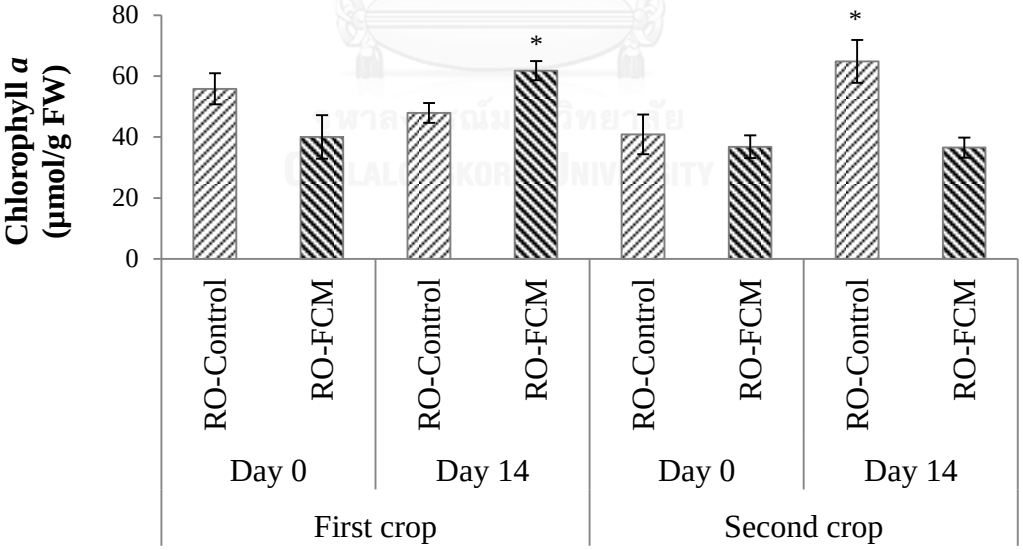

Figure 44 Effects of 2% FCM on chlorophyll *a* contents of 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.20. Chlorophyll *b* contents

'Butterhead' lettuce

During the first season, the highest level of chlorophyll *b* contents was found on day 0 of the storage. There were no significant differences in chlorophyll *b* contents between the control and FCM treatments before and after storage at 8°C and 60% RH for 14 days during crop seasons (Figure 45).

'Red Oak' lettuce

The FCM treatment during the first crop season showed significantly higher chlorophyll *b* contents than in the control treatment on day 14 after storage. While during the second crop season, the control treatment showed significantly higher chlorophyll *b* contentsthan in the FCM treatment on day 0 of low temperature storage (Figure 46).

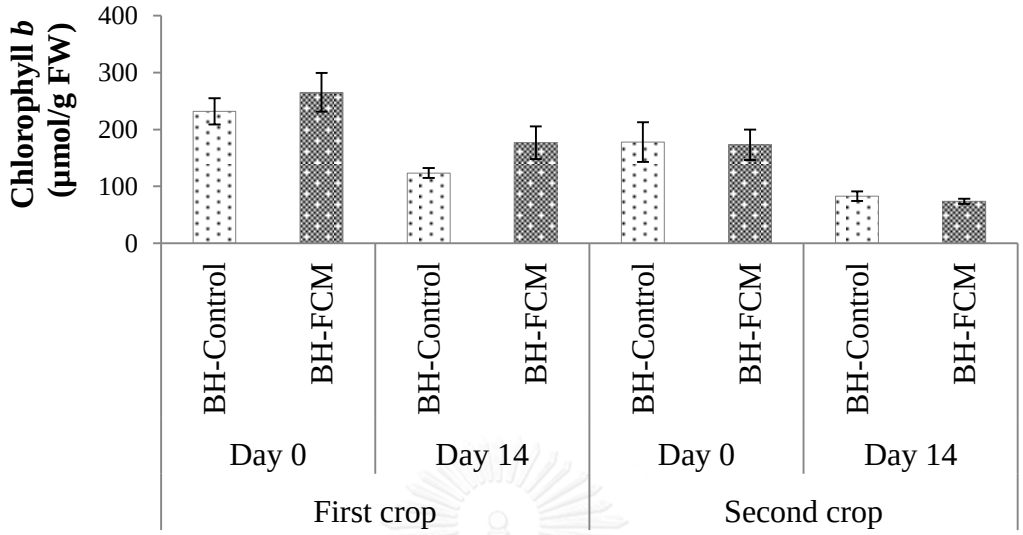

Figure 45 Effects of 2% FCM on chlorophyll *b* contents of 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

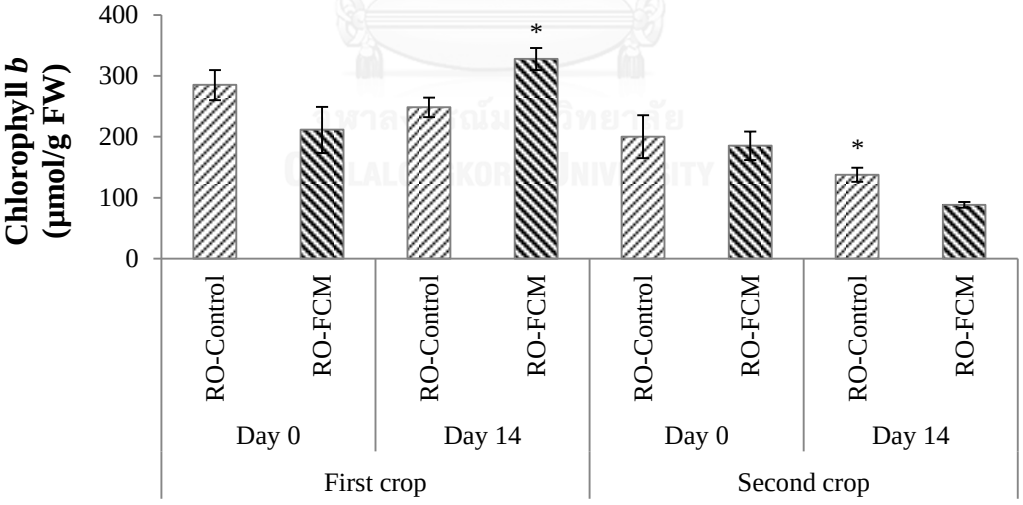

Figure 46 Effects of 2% FCM on chlorophyll *b* contents of 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.21. Carotenoid contents

'Butterhead' lettuce

There were no significant differences in the carotenoid contents between the control and FCM treatments before and after storage at 8°C and 60% RH for 14 days during both crop seasons (Figure 47).

'Red Oak' lettuce

During the first crop season, lettuces treated with FCM showed significantly higher carotenoid contents than the control treatment on day 14 of the storage. During the second crop season, the control treatment showed significantly higher carotenoid contents than the FCM treatment on day 14 (Figure 31). During both crop seasons, there were no significant differences in carotenoid contents between the control and FCM treatments on day 0 of the storage (Figure 48).

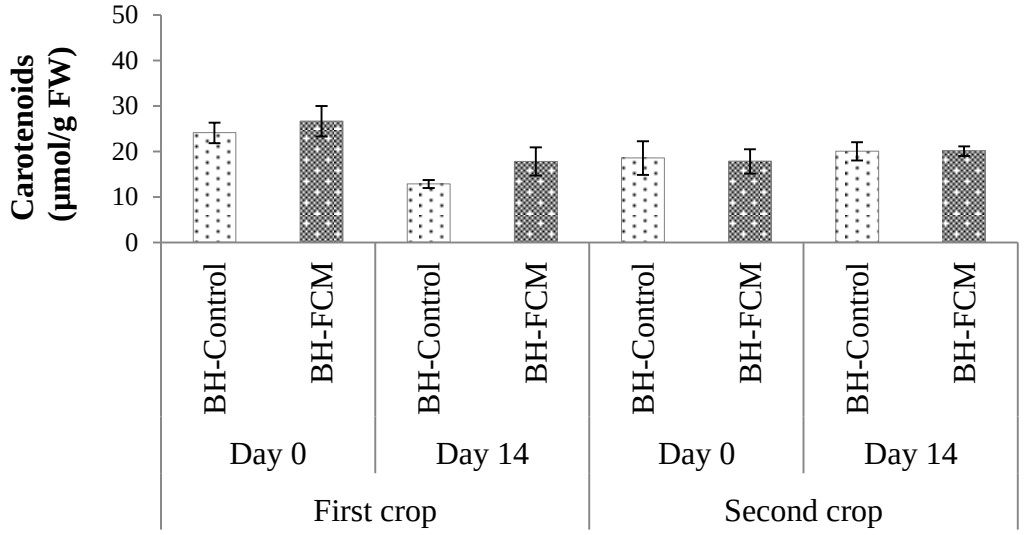

Figure 47 Effects of 2% FCM on carotenoid contents of 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

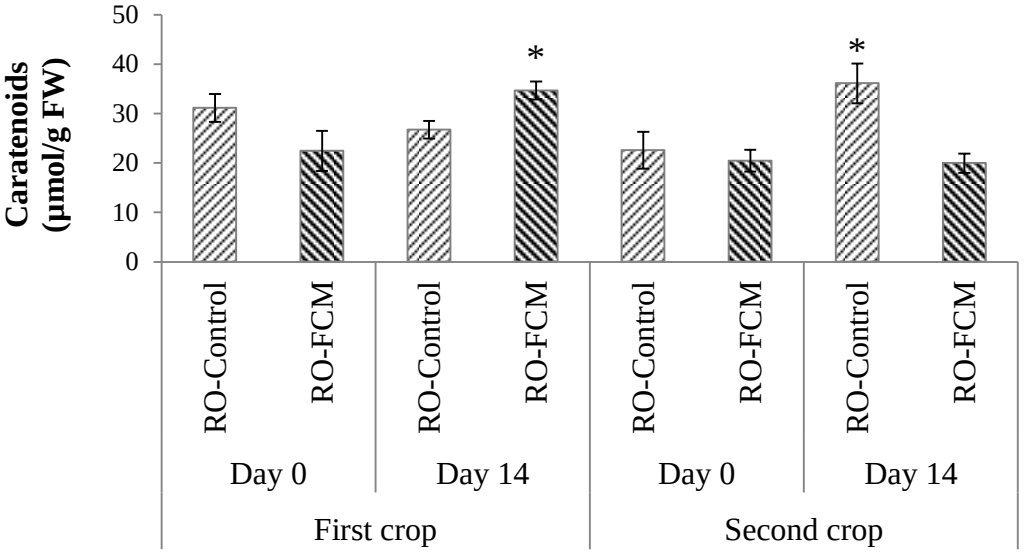

Figure 48 Effects of 2% FCM on carotenoid contents of 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days.* indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

4.2.6 Effects of FCM on antioxidant contents

1.1.22. Ascorbic acid contents

'Butterhead' lettuce

There were no significant differences in the ascorbic acid contents between the control and FCM treatments during both crop seasons. During the first crop season, the control and the FCM treatments showed the highest ascorbic acid content on day 14 after storage (Figure 49).

'Red Oak' lettuce

During the first crop season, the control treatment tended to have a higher ascorbic acidcontents than the FCM treatment. During the second crop season, the ascorbic acidwas low on day 0. However, there were no significant differences in the ascorbic acidcontents between the control and FCM treatments during both crop seasons (Figure 50).

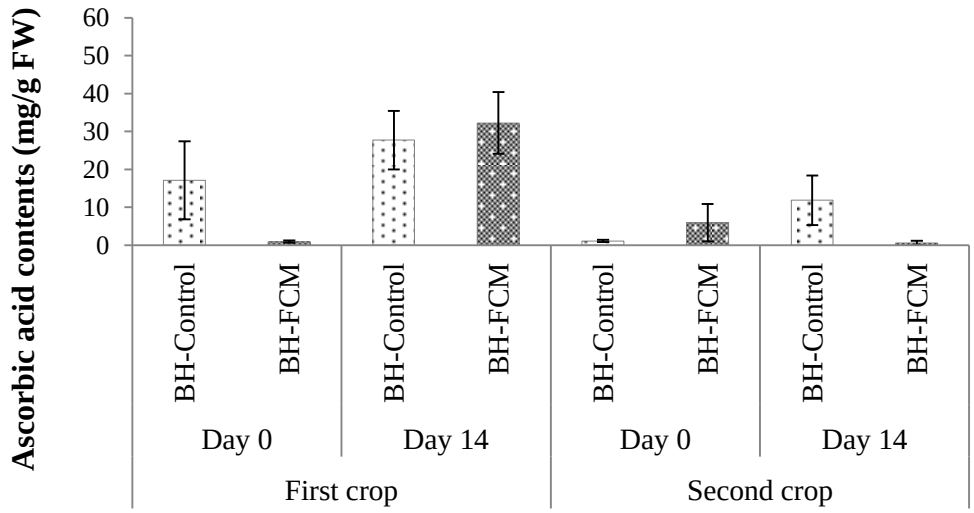

Figure 49 Effects of 2% FCM on ascorbic acid contents of 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

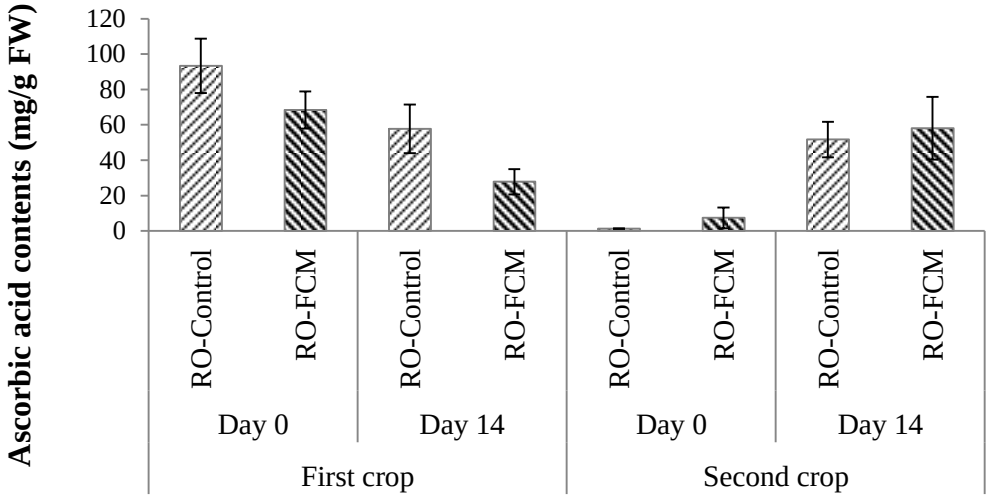

Figure 50 Effects of 2% FCM on ascorbic acidcontents of 'Red Oak' grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.23. Total phenolic contents

‘Butterhead’ lettuce

There were no significant differences in the total phenolic contents between the control and FCM treatments during both crop seasons. The control and the FCM treatments during the second crop season showed the lowest of total phenolic content on day 0 of the storage (Figure 51).

‘Red Oak’ lettuce

The control treatment was observed to have higher total phenolic contents than in the FCM treatment on days 0 and 14 during the first crop season. Both treatments during the second crop season had the lowest of total phenolic content on day 0 of the storage. However, there were no significant differences in the total phenolic contents between the control and FCM treatments in both crop seasons (Figure 52).

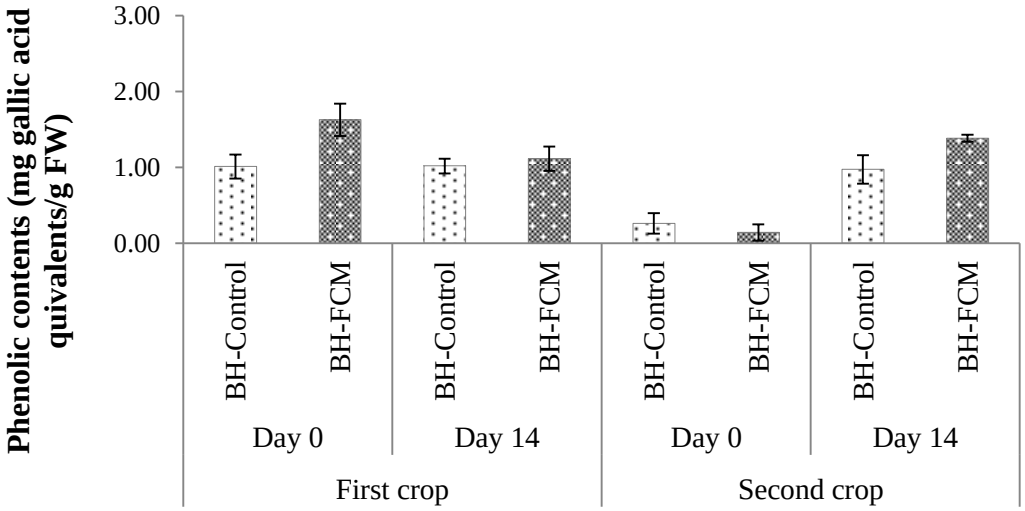

Figure 51 Effects of 2% FCM on total phenolic contents of 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

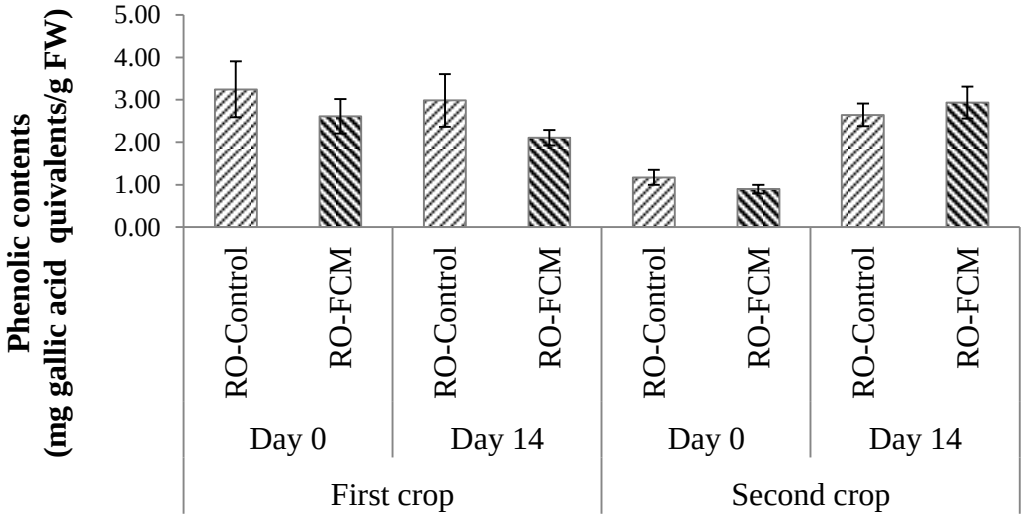

Figure 52 Effects of 2% FCM on total phenolic contents of 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.24. Flavonoid contents

‘Butterhead’ lettuce

There were no significant differences observed in flavonoid contents between the control and FCM treatments during the first crop season. Lettuces treated with FCM during the second crop season showed a significantly higher flavonoid contents than in the control treatment on day 14 after storage (Figure 53).

‘Red Oak’ lettuce

No significant differences in flavonoid contents were observed between the control and FCM treatments during both crop seasons on day 0 and day 14 after storage (Figure 54).

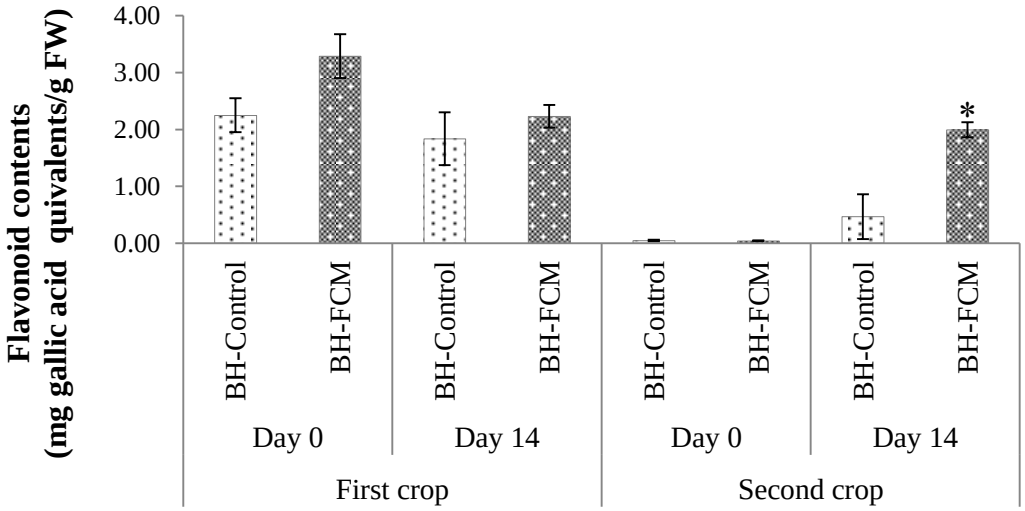

Figure 53 Effects of 2% FCM on flavonoid contents of 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days.* indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

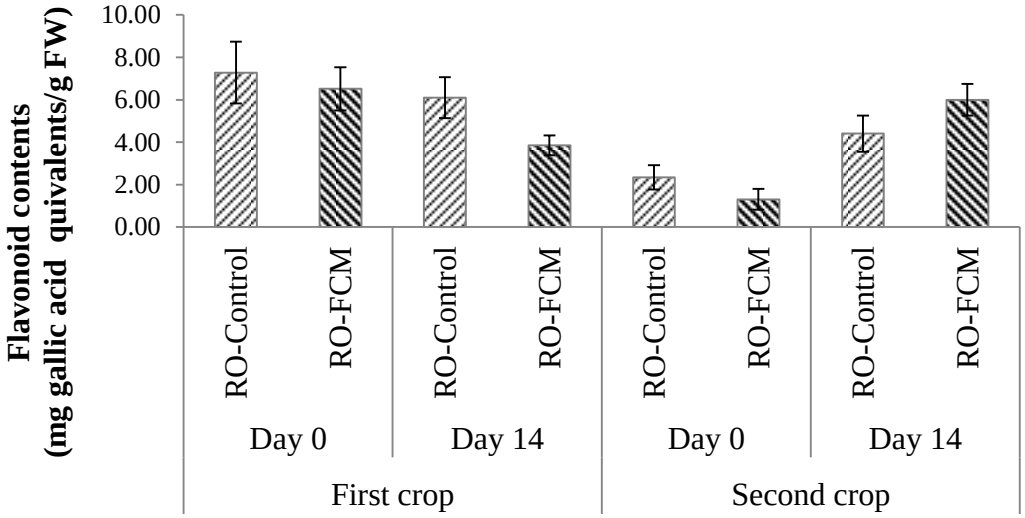

Figure 54 Effects of 2% FCM on flavonoid contents of 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.25. DPPH radical scavenging activities

'Butterhead' lettuce

There were no significant differences in DPPH radical scavenging activities between the control and FCM treatments during the first crop season. The control treatment showed significantly higher DPPH radical scavenging activity than in the FCM treatment during the second crop season on day 14 after storage (Figure 55).

'Red Oak' lettuce

During both crop seasons, no significant differences in DPPH radical scavenging activities were observed between the control and the FCM treatments on day 0 and day 14 after storage (Figure 56).

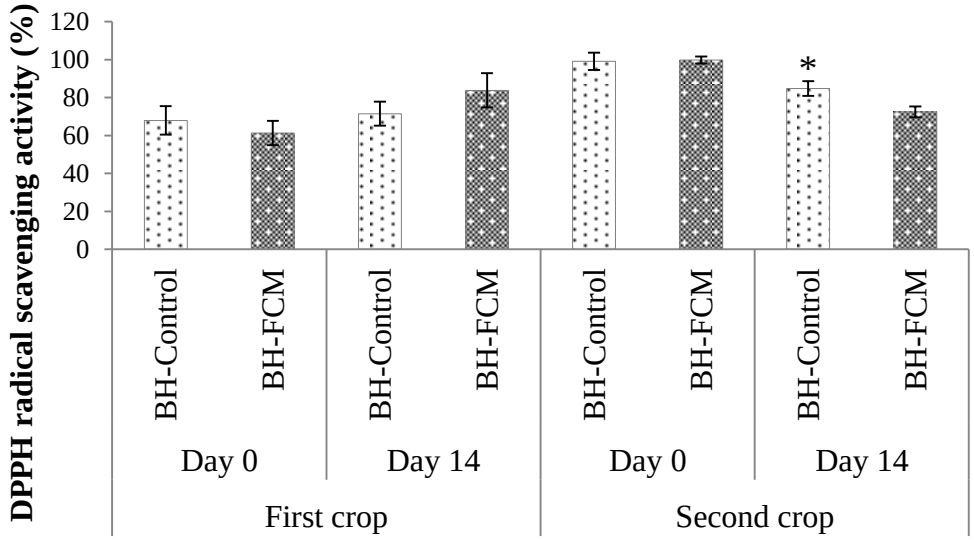

Figure 55 Effects of 2% FCM on DPPH radical scavenging activities of 'Butterhead' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days.* indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

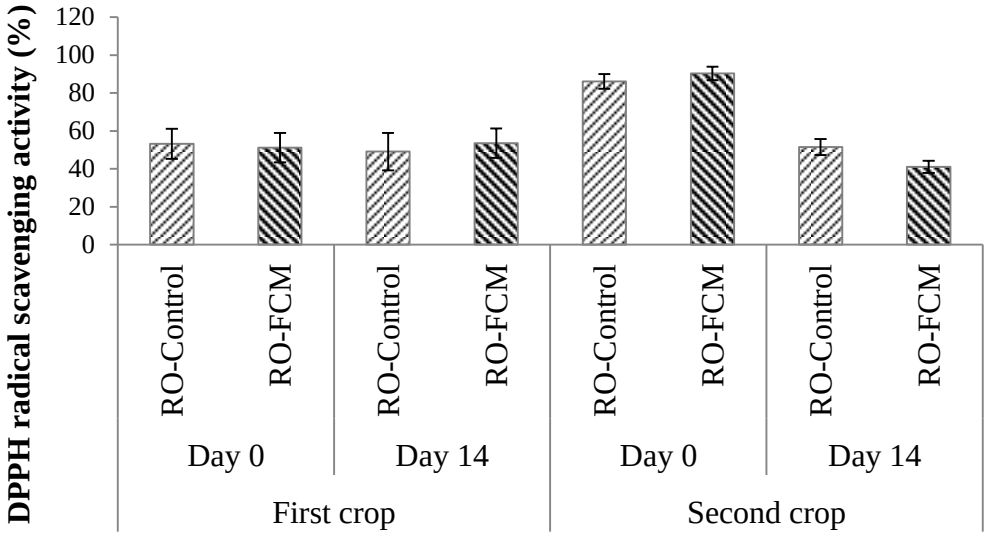

Figure 56 Effects of 2% FCM on DPPH radical scavenging activities of 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

1.1.26. MDA contents

'Butterhead' lettuce

During the first crop season, the FCM treatment showed higher MDA contents than the control treatment on day 0 of the storage. During the second crop season, the FCM treatment tended to have higher MDA contents on day 14 (Figure 57).

'Red Oak' lettuce

During the first crop season, the control treatment showed significantly higher MDA contents than in the FCM treatment on day 0 of the storage. No significant differences in MDA contents were observed between the control and the FCM treatments during the second crop seasons (Figure 58).

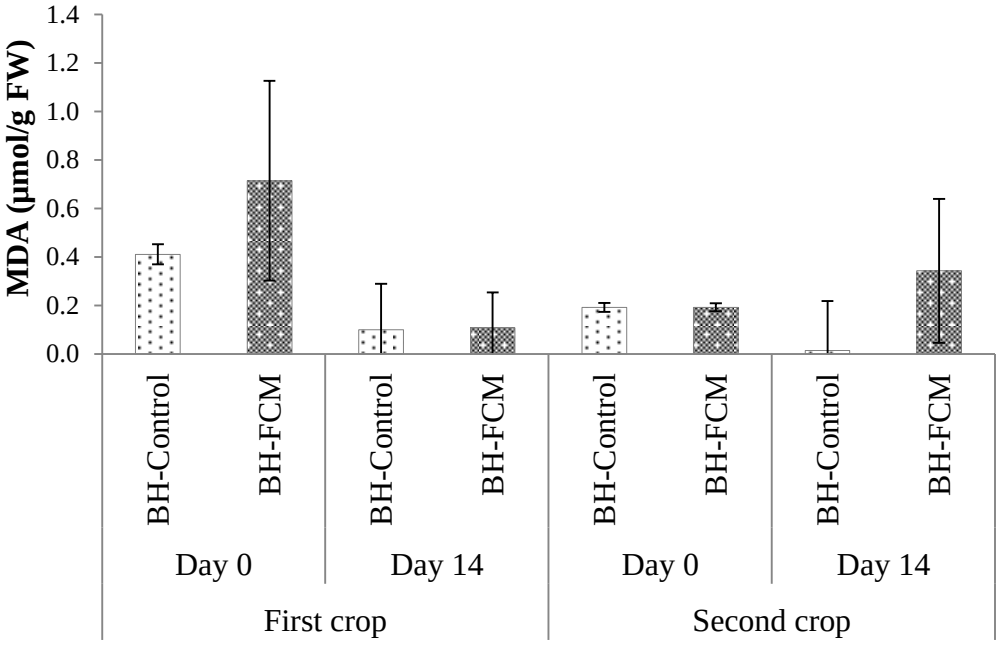

Figure 57 Effects of 2% FCM on MDA contents of 'Butterhead' grown in a local farm following storage at 8°C and 60% RH for 14 days. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

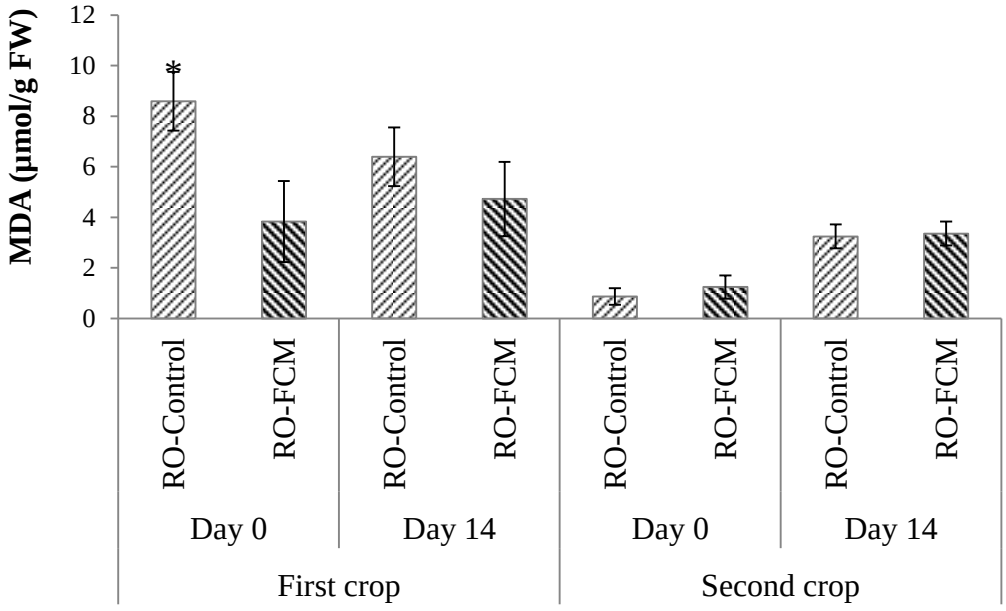

Figure 58 Effects of 2% FCM on MDA contents of 'Red Oak' lettuce grown in a local farm following storage at 8°C and 60% RH for 14 days. * indicates the significant difference between control and FCM treatments. Mean differences were performed using T-Test. Differences with $p<0.05$ were considered significant.

4.2.7 Effects of FCM on selected soil microbial populations of lettuces grown in a local farm

1.1.27. 'Butterhead' lettuce

Prior to planting, Butterhead lettuce in both the control and FCM treatments had significantly higher microbial populations than after planting for Fluorescent *Pseudomonas* spp., *Fusarium* spp. and *Trichoderma* spp. in the FCM treatments.After planting for 5 weeks, there were significantly higher numbers of *Bacillus* spp. in both the control and FCM treatments as well as *Pythium* spp. in the FCM treatment. There was no significant differences in *Pythium* spp. and *Trichoderma* spp. populations in the control treatment obvered before and after planting (Table 5).

1.1.28. 'Red Oak' lettuce

Fluorescent *Pseudomonas* spp. and *Fusarium* spp. populations in both control and FCM treatments before planting had significantly higher numbers than after planting. *Trichoderma* spp. population in the control treatment before planting also had significantly higher numbers than after planting.

After planting for 5 weeks, there was an increase in microbial populations of *Bacillus* spp. in both the control and FCM treatments and the increase was also observed on *Pythium* spp. in the control treatment compared to before planting. However, these evated number of populations were not significant differences (Table 6).

Table 5 Effect of FCM on selected soil microbial populations of 'Butterhead' lettuce before and after planting in a local farm.

Organisms cultured	Treatment	Colony forming units (CFU) per g of soil	
		Before planting	After planting
1.*Bacillus* spp.	BH-Control	$1.0 \times 10^{5} \pm 9.3 \times 10^{3NSb}$	$4.2 \times 10^{5} \pm 3.2 \times 10^{4Ba}$
	BH-FCM	$1.7 \times 10^{5} \pm 3.5 \times 10^{4NSb}$	$4.6 \times 10^{6} \pm 1.7 \times 10^{6Aa}$
2.Fluorescent	BH-Control	$2.0 \times 10^{6} \pm 2.6 \times 10^{5Ba}$	$1.9 \times 10^{5} \pm 1.4 \times 10^{4NSb}$
Pseudomonas spp.	BH-FCM	$3.2 \times 10^{6} \pm 1.9 \times 10^{5Aa}$	$1.7 \times 10^{5} \pm 1.9 \times 10^{4NSb}$
3.*Fusarium* spp.	BH-Control	$4.1 \times 10^{5} \pm 6.4 \times 10^{4Ba}$	$1.4 \times 10^{4} \pm 1.6 \times 10^{3NSb}$
	BH-FCM	$1.1 \times 10^{6} \pm 2.8 \times 10^{5 Aa}$	$7.0 \times 10^{4} \pm 2.0 \times 10^{4NSb}$
4.*Pythuim* spp.	BH-Control	$9.6 \times 10^{4} \pm 2.2 \times 10^{4NSns}$	$1.3 \times 10^{4} \pm 5.0 \times 10^{4NSns}$
	BH-FCM	$9.2 \times 10^{4} \pm 2.4 \times 10^{4NSb}$	$3.3 \times 10^{5} \pm 4.3 \times 10^{3NSa}$
5.*Trichoderma* spp.	BH-Control	$9.0 \times 10^{3} \pm 1.4 \times 10^{3Bns}$	$7.0 \times 10^{3} \pm 2.4 \times 10^{3NSns}$
	BH-FCM	$1.8 \times 10^{4} \pm 3.7 \times 10^{3Aa}$	$3.2 \times 10^{3} \pm 5.1 \times 10^{2NSb}$

Mean followed by different letters in each row are significantly different by T-Test at $P<0.05$. Data are mean values ± SE.Capital letter compared between the control and FCM treatments. Small letter compared between before and affer planting.

Table 6 Effect of FCM on selected soil microbial populations of 'Red Oak' lettuce before and after planting in a local farm.

Organisms cultured	Treatment	Colony forming units (CFU) per g of soil	
		Before planting	After planting
1.Bacillus spp.	BH-Control	$2.0 \times 10^{5} \pm 6.4 \times 10^{4NSns}$	$5.4 \times 10^{5} \pm 2.6 \times 10^{5NSns}$
	BH-FCM	$1.2 \times 10^{5} \pm 1.3 \times 10^{4NSns}$	$2.4 \times 10^{5} \pm 9.9 \times 10^{4NSns}$
2.Fluorescent	BH-Control	$3.2 \times 10^{6} \pm 1.8 \times 10^{5Ba}$	$2.0 \times 10^{5} \pm 1.3 \times 10^{4NSb}$
Pseudomonas spp.	BH-FCM	$2.1 \times 10^{6} \pm 2.2 \times 10^{5Aa}$	$2.1 \times 10^{5} \pm 1.0 \times 10^{4NSb}$
3.Fusarium spp.	BH-Control	$6.3 \times 10^{5} \pm 1.4 \times 10^{5Ba}$	$8.0 \times 10^{4} \pm 1.7 \times 10^{4\ NSb}$
	BH-FCM	$1.3 \times 10^{6} \pm 2.4 \times 10^{5Aa}$	$7.9 \times 10^{4} \pm 2.1 \times 10^{4NSb}$
4.Pythuim spp.	BH-Control	$6.9 \times 10^{4} \pm 1.0 \times 10^{4NSns}$	$7.3 \times 10^{4} \pm 1.7 \times 10^{4NSns}$
	BH-FCM	$6.6 \times 10^{4} \pm 1.5 \times 10^{4NSns}$	$4.3 \times 10^{4} \pm 4.0 \times 10^{3NSns}$
5.Trichoderma spp.	BH-Control	$8.7 \times 10^{3} \pm 1.0 \times 10^{3NSa}$	$3.1 \times 10^{3} \pm 6.9 \times 10^{2NSb}$
	BH-FCM	$9.8 \times 10^{3} \pm 6.7 \times 10^{2NSns}$	$8.8 \times 10^{3} \pm 3.5 \times 10^{3NSns}$

Mean followed by different letters in each row are significantly different by T-Test at $P<0.05$. Data are mean values ± SE. Capital letter compared between the control and FCM treatments. Small letter compared between before and affer planting.

4.2.8 Effects of FCM on selected soil chemical parameters of lettuces grown in a local farm

Soil chemical properties

'Butterhead' lettuce

There were no significant differences in pH, EC, OM, the total N and Mg availability in the soils between control and FCM treatments before and after planting. Soil P, K and Ca availability showed higher increases in the FCM treatment than in the control treatment before planting. The FCM treatment showed higher increases in the P and K availability in the soil than the control treatment before and after planting. Soil Ca availability showed higher increases in the FCM treatment than in the control treatment before planting. During all two crop seasons, the pH and EC in soil level ranged from 5.80-6.67 and 0.43-1.18 dS m^{-1} respectively (Table 7).

'Red Oak' lettuce

No significant differences in pH, EC, OM, the total N and Ca availability in soils between the control and FCM treatments before and after planting.

During the second crop season, the FCM treatment showed higher increases in soil P availability than the control treatment after planting. Soil K availability showed higher increases in the FCM treatment than in the control treatment before and after planting. Soil Mg availability showed higher increases in the FCM treatment than in the control treatment before and after planting during second season. During all two crop seasons, the pH and EC in soil level ranged from 5.60-6.85 and 0.36-1.17 dS m^{-1} respectively (Table 7).

Table 7 Chemical characteristics of the growing media before and after lettuce cultivation in a local farm

Crop season	Soil	Treatment	pH	EC (dS/m)	OM (%)	Total N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
		Manure	8.26	6.76	-	1.39	28	279	134	46
		Shrimp shell	7.49	4.29	-	8.15	153	40	884	40
		FCM	6.12	8.09	-	5.69	147	309	3	5
	Original soil	BH-Control	5.60	0.16	1.34	0.10	109	193	1696	220
		BH-FCM	5.50	0.13	1.25	0.10	103	180	1486	222
		RO-Control	5.65	0.12	1.04	0.09	107	138	132	162
		RO-FCM	5.60	0.09	0.83	0.07	113	100	932	152
After	Before planting	BH-Control	5.90	0.43	2.71	0.14	154	776	1592	270
supplement		BH-FCM	5.80	0.72	3.26	0.14	307	1089	1708	294
		RO-Control	5.75	0.36	2.14	0.12	155	721	1558	133
		RO-FCM	5.75	0.63	2.81	0.22	114	1056	1554	176
First crop	After harvest	BH-Control	6.50	0.06	1.63	0.09	144	539	1947	263
		BH-FCM	6.45	0.07	1.76	0.14	227	688	2017	266
		RO-Control	6.30	0.10	1.06	0.07	140	490	1173	222
		RO-FCM	5.60	0.17	1.28	0.03	194	395	1020	196
Second crop	After harvest	BH-Control	6.50	1.03	2.80	0.23	263	2406	1915	331
		BH-FCM	6.67	1.18	4.28	0.22	433	2934	1597	366
		RO-Control	6.85	1.09	2.19	0.19	200	2678	1433	259
		RO-FCM	6.78	1.05	2.96	0.12	456	2737	1138	274

Table 8 Cultivation and climatic conditions during the two crop seasons in a local farm

	First crop season	Second crop season
Lettuce transplanting	January 14, 2012	May 23, 2012
Date of harvesting	March 3, 2012	July 21, 2012
Crop duration (days)	50	60
Minimum air temperature (°C)	19.2	24.6
Maximum air temperature (°C)	35.7	35.4
Minimum air RH (%)	40	51
Maximum air RH (%)	81	76

DISCUSSION

1. Effects of biomaterial and semi-biomaterial on growth, yield and postharvest quality of lettuces grown in a test plot

1.1. Effects of biomaterial and semi-biomaterial on growth and yield of lettuces

The applications of chitin-rich residues can increase growth and yield of 'Butterhead' and 'Red Oak' lettuces during all crop seasons. In addition, application of SS and FCM resulted in an improved soil structure and plant nutrient contribution. The degradation of chitin in SS and FCM could supply helpful nutrient elements and plant growth stimulators. Chitosan has been shown to support the growth of prairie gentian *Eustoma grandiflorum* (Raf.) Shinn (Ohta, Taniguchi et al. 1999). Adding 0.1% chitosan into soil help increase growth of lettuce (Chibu 1999). Chitosan released from chitin-rich residues may lead to many positive effects which were observed in lettuce production during this study.

Increased lettuce growth may cause by the accessibility of amino compounds that releasing from chitin after the decomposition in the soil. Besides, high nutrient contents of the supplement can enhance growth of lettuces in terms of higher leaf numbers and increments in leaf width and leaf length. Increased number of lettuce leaves and leaf expansion resulted in high photosynthetic production thus increasing higher fresh and dry weight of lettuces. Considerably elevated leaf growth could be bcause of the amino component in chitin and capacity of lettuces which absorb nitrogen from the soil when chitin was degraded. The results obtained with FCM treatment showed the achievability of applying a local substance in enhancing agriculture production.

Although 2% FCM treatment cause increases in production during all crop seasons, its effects on lettuces growth is season-specific. Growth data proposes that increased yield of lettuces during the second and third crop seasons was mainly due to an increase in leaf size. Therefore, addition of SS/FCM to the growing medium can

lead to the increase in yield. Moreover, irrigated water during the second crop season could decrease soil moisture and lettuce growth. In the third crop season, maximum air temperature was the lowest. Under these conditions, nutrient elements, temperature and available light are the most significant factors scheming growth of the cropping round (Glenn 1984). On the other hand, control treatments that added only cow manure appeared to show considerably low fresh weight due to limited supply of nutrient.

Previous studies have demonstrated that various microbial strains of *Bacillus* (*B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105) promote the growth of *Pinus pinea* L. seedlings, but this biological effect was not found with both strains in combination. This implies a possible competitive effect (Probanza, Lucas Garcıa et al. 2002). Nevertheless, rhizosphere bacteria can have negative, neutral or positive effects on plant growth. The present results indicate that application of SK1 alone did not promote growth of lettuce in any crop season.

Effects of biomaterial and semi-biomaterial on postharvest quality

There are limited references related to the effect of chitin-rich residues application on increased postharvest quality at harvest. Pre-harvest chitosan sprays showed a beneficial effect on flesh firmness and titratable acidity in strawberries stored at low temperature (Reddy 2006). This could be due to the formation of a chitosan film on fruit which can act as a barrier for O_2 uptake thereby reducing the metabolic activity. Also, chitosan was reported to reduce pepper plant transpiration resulting in a reduction in water use while maintaining biomass production and yield (Bittelli 2001). The positive effects of SS/FCM treatments could be the effect influenced from the growth period through to postharvest storage of lettuces.

Effects of the FCM on growth, yield, postharvest quality and selected soil microbial populations of lettuces in a local farm

Effects of FCM on growth and yield

The applications of FCM enhanced the growth and yield of 'Butterhead' and 'Red Oak' lettuces during all crop seasons. The effects of FCM on growth and yield of lettuce such as leaf number per plant, leaf width and length, fresh weight and dry weight were significantly increased during both crop seasons. These results indicated that application of FCM had tremendous effects on growth and yield in lettuce. Our results showed that growth and yield of lettuce were greater in a local farm than in a test plot.The supplementations of FCM to the growing medium resulted in a significant, season-specific increase in yield. In addition, irrigation water was limited during the second crop season which reduced soil moisture and lettuce growth. Between the two crop seasons, the maximum air temperature was lower in the second crop season as well as the variation in air temperatures (Table 9). Under these conditions, nutrient elements and temperature are the most important factors controlling growth and length of the cropping cycle (Glenn 1984). However, control treatments which received only cow manure as fertilizer appeared to have significantly lower fresh weight than FCM treatment due to limited supply of nutrient.

During the microbial breakdown of chitin, several substances are liberated. Characterization of these products had revealed the presence of N-acetyl glucosamine, glucosamine, glucosamine, acetic acid and ammonia (Muzzarelli 1997). Accordingly, a mechanism for the degradation can be postulated. The polymer is probably hydrolyzed to yield N-acetylglucosamine, which is then converted to acetic acid and glucosamine, and the ammonia is liberated from the latter compound or one of its subsequent derivatives (Muzzarelli 1997). The amendment with chitin alone (without antagonists) moderately increased the plant growth (Rajkumar, Lee b et al. 2008). There are very few studies on the effect of chitosan on plant growth, development and productivity, which is mainly attributed to stimulation of plants immunity against microorganisms(ChunYan L 2003); (Sereih, Neven et al. 2007);(No, Meyers et al. 2007); (Gornik 2008). Recently, some researchers reported that chitosan enhanced plant growth and development (Khan 2002);(Chibu H 2003); (Gornik 2008). Application of chitosan affected key enzymes activities of nitrogen metabolism (nitrate reductase, glutamine synthetase and protease) and enhanced plant growth

and development in rice. Study on rice and soybean, the results also showed that application of chitosan at early growth stages increased plant growth and development; as a result increased seed yield (Chibu 2002). Moreover, chitosan can promoted the growth of various crops such as cabbage (*Brassica oleracea* L. var *Capitata*) (Hirano 1996), sweet basil (Kim, Chen et al. 2005) and soya bean sprouts (Lee, Kim et al. 2005).

Effects of FCM on postharvest quality

Loss of fresh weight in vegetables during storage is caused by water exchange between the internal and external atmospheres, the transpiration rate being accelerated by cellular breakdown (Woods 1990). Many indicators of lettuce quality, including color, texture and flavor may be influenced by abiotic and biotic factors (Kleinhenz 2003). An added positive effect of FCM treatment could maintain postharvest quality of lettuces during low temperature storage which clearly shown in 'Red Oak' lettuce.In addition, it was found that 'Red Oak' lettuce had higher chlorophyll pigments and antioxidant contents than in 'Butterhead' lettuce during both crop seasons. It is possible that increased chlorophyll pigments and antioxidant contents in 'Red Oak' lettuce could play a critically protective role in maintaining postharvest quality during low temperature storage of lettuces. The superior overall postharvest quality was found in the FCM treatment in 'Red Oak' lettuce during both crop seasons compared to those in the control treatment. The same results were shown from both test plot and local farm. As mentioned above**,** chitosan can reduce pepper plant transpiration resulting in a reduction in water use while maintaining biomass production and yield (Bittelli 2001). The beneficial effects of FCM treatments could be extended from the growth period in a local farm through the low temperature storage of lettuces after harvest.

Effects of FCM on net photosynthesis, transpiration rate and stomatal conductance of lettuce leaf

Net photosynthesis, transpiration rates and stomatal conductance of lettuces grown with FCM were significantly higher than the control treatment in 'Red Oak' lettuce in all crop seasons. As a result, 'Red Oak' lettuce in the FCM treatment had a higher growth and yield than the control treatment in both crop seasons. Increased number of lettuce leaves and leaf expansion provided greater photosynthetic production which resulted in a higher fresh and dry weight of the lettuce plants. Khan et al. (2002) reported that application of chitosan increased photosynthesis in leaves of maize and soybean. In Dendrobium orchid, chloroplasts in the young leaves of the plants treated with chitosan O-80 treatment was found to be significantly larger than those of the non-chitosan-treated ones (Limpanavech, Chaiyasuta et al. 2008). However, according to Mondal et al. (2012), they reported that the effects of different concentrations of chitosan application on photosynthesis were significant but no significant influence on chlorophyll content of leaves was observed in okra. No significant differences in net photosynthesis, transpiration rate and stomatal conductance of 'Butterhead' lettuce between the FCM and control treatments during both crop seasons may also be dependent on cultivar types which have different pigment compositions such as 'Butterhead' and 'Red Oak' lettuces.

Light conditions also have an important effect on the quality and yield of vegetables. Light intensity needed for the maximum rate of photosynthesis is quite distinct, depending on vegetable cultivars and ambient condition (Yang et al. 2012).Higher light maximum intensity was observed during the first crop season. The optimum of light intently cloud provides greater photosynthetic production which resulted in higher growth and yield of the lettuce plants. This correlated with increases in stomatal conductance and transpiration rate (Bittelli 2001).Stomatal conductance increased once with increasing transpiration rate. Acatrinei. (2010) observed that in protected spaces a relation between transpiration rate and stomatal conductance, which implied a humidity factor. Mechanism of opening-closure stomata played a very central role in carbon assimilation and water elimination. A decreasing of substomatic CO_2 with increasing photosynthesis rate as well as

increasing the stomatal conductance and transpiration rate is observed in this study in lettuce treated with FCM.

Misra et al. (2000) also suggested that chitosan might be an effective antitranspirant to preserve water resources use in agriculture. In their investigation, they examined the potential of foliar applications of chitosan on pepper plants transpiration in the growth room and in the field. Irit et al. (2009) unveiled some of the aspects through which chitosan was able to reduce transpiration in bean plants after being used as a foliar spray. However, FCM did not induce stomatal closure as chitosan did.

Effects of FCM on nitrate contents

Since several studies have been directed toward the effects of nitrate intake on human health especially nitrate from lettuce leaves. In many countries, the maximum level of nitrates allowed for the consumption of lettuce is 4000 ppm, although it can be restricted to 3500 ppm in some countries (e.g., Germany and Switzerland) (Marouane 2011). In our study, the lower levels of nitrate content were shown in both cultivars of lettuce. In 'Butterhead' lettuce showed no significant differences in nitrate content between the FCM and control treatments in both crop seasons whereas 'Red Oak' lettuce the FCM treatment showed significantly higher nitrate contents than the control treatment on day 14 during the first crop season. Ozgen et al. (2014) reported that green and red varieties of lettuce responded differently to the fertilizer sources. Their results indicated that organic and inorganic fertilizers showed the different effects on chlorophylls and nitrate concentration. The cultivar that had the highest anthocyanin content accumulated higher nitrate concentration than the others. FCM which considered being similar to organic fertilizer also showed the higher accumulated content of nitrate in 'Red Oak' lettuce which is a red variety. However, the control treatment of 'Red Oak' showed significantly higher nitrate contents than in the FCM treatment on day 0 in the second crop season.

Mondal et al. (2012) found that the effects of different concentrations of chitosan application on nitrate reductase activity in leaves were significant. As a

result, sources and dose of nitrogen given to soil can affect the nitrate accumulation of soil-grown crops. The content of nitrate and sugar had the opposite trend, that is, increase of blue light enhanced the accumulation of sugar and simultaneously degraded the nitrate content (Chen et al. 2014). It may be because sugar can elicit an increase in nitrate reductase messenger RNA accumulation (Lillo, 1994). The high light intensity normally promotes the growth of lettuce and decreases nitrate concentration in lettuce(Blom-Zandstra 1988); (Gaudreau 1995). Moreover, beyond the light intensity and nitrogen source, genetic effects also play an important role in nitrate accumulation(Behr 1988);(Escobar-Gutiérrez 2002); (Dzida, Jarosz et al. 2012). For instance, many wild types have been shown to accumulate very large amounts of nitrate, whereas genotypes with quite low concentrations were found among cultivated lettuces, particularly 'Butterhead' varieties(Behr 1988);(Drews 1996); (Escobar-Gutiérrez 2002).

Effects of FCM on chlorophyll *a*, chlorophyll *b* and carotenoid contents

Chlorophylls and carotenoids are crucial plant pigments for photosynthesis and their abundances results in greater assimilation of solar radiations into consumable sugars (Ladhari 2014). Carotenoids are pivotal accessory pigments playing major roles in photosynthesis (Demmig-Adams 1990)by collecting light and transferring the excitation energy to the chlorophyll (Siefermenn-Harms 1987)and by stabilizing proteins of the light-harvesting complex (Plumley 1987). In addition, these pigments are responsible for quenching of singlet oxygen (Knox 1985). Carotenoids play an antioxidant molecules role, capable of scavenging the harmful singlet oxygen(Mikkelsen 1995); (Telfer 1994)and of de-exciting chlorophyll a (Senser 1990). A decrease in chlorophylls and carotenoids can increase lipid peroxidation as much as the higher level of MDA in Valladolid leaves under the non-filtered air with additional ozone (O_3) treatment (Barreno 2004). Application of chitosan could improve chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents in wheat grass (*Agropyron repens*) (Nasibeh Tourian 2013). In our study, during the first crop season, 'Red oak' lettuces treated with FCM showed significantly higher

carotenoid contents than the control treatment on day 14 of the storage. In contrast, 'Butterhead' lettuce showed no significant differences in the chlorophyll *a*, chlorophyll *b* and carotenoid contents between the control and FCM treatments during both crop seasons.It is possible that FCM could help maintaining the carotenoid content of red color leave cultivar of lettuces while chlorophyll started to degrade during low temperature storage.

Takagi et al. (1990) observed that the most abundant carotenoid present in green leaves of lettuce was β-carotene in summer and lutein in other seasons. Similarly, lutein was main carotenoid in several varieties and cultivars of lettuces (two butter lettuces, two Batavia lettuces, one oak leaf lettuce and one pigmented oak leaf variety) harvested in winter or spring (Nicolle, Cardinault et al. 2004). In our study, 'Red Oak' lettuce showed significant differences in carotenoid contents between the FCM and the control treatment in the both crop seasons on day 0. There were no significant differences in carotenoid contents observed in 'Butterhead' lettuce between the FCM and the control treatments in both crop seasons. Organic and inorganic fertilizer also had effects on pigments, phytochemicals and nitrate concentrations of red and green lettuce (Ozgen, Sekerci et al. 2014). The biotic factors such as growth stage and disease and abiotic factors such as temperature, light and nutrient which affect lettuce growth also influence pigment concentration (Shaked-Sachray, Weiss et al. 2002)whereas genotype could act alone or together with temperature and light in shifting lettuce pigment levels (Crozier, Lean et al. 1997).

Effects of FCM on antioxidant contents

The health benefits of lettuce have been attributed to the presence of antioxidant compound, including phenolics and high-fiber and vitamin C contents (Ozgen, Sekerci et al. 2014). Recently, some researchers reported that a regular intake of antioxidant compounds from lettuce is useful to improve the lipid status and to prevent lipid peroxidation in tissues (Nicolle, Cardinault et al. 2004).Increased antioxidant content, especially in 'Red Oak' lettuces are desirable because of their

health enhancing properties.Significant differences in antioxidant contents were observed in ‘Red Oak’ lettuce grown in the FCM treatments on day 0 after harvest during the first crop season.

Some authors have demonstrated that chitosan could act as an exogenous elicitor in plant tissue inducing different responses such as the *de novo* biosynthesis of phenolic compounds (Benhamou N 1998)and (Benhamou 1994). A high content of some phenolic compounds for example anthocyanins and flavonoids, particularly in red-leafed cultivars, in agreement with previous studies(Nicolle, Cardinault et al. 2004); (Llorach 2008). These phenolic compounds, including ascorbic acid play a key role in plant resistance and adaptation to environmental stress in lettuce (Oh 2009). Oh. (2009) observed that activation of secondary metabolism and antioxidants protected lettuce plants when transferred from a protected environment to normal growing conditions.

However, there were no significant differences in ascorbic acid, total phenolic contents and DPPH radical scavenging activity in ‘Butterhead’ lettuce between the control and the FCM treatments in both crop seasons.Liu Ardo et al. (2007) reported that cultivar, type, and color may influence the change of total phenolic content and antioxidant activities of lettuce. The red lettuce cultivars generally contained greater phenolic compounds and had stronger antioxidant activities than the green lettuce within the same type under the same growing conditions (Liu, Ardo et al. 2007). Caldwell (2003) also reported that red leaf lettuce generally contains larger amounts of phenolics than romaine or butterhead lettuce.In our study, ‘Red Oak’ lettuce had higher antioxidant compounds than in ‘Butterhead’ lettuce.

Anthocyanins were reported to be one of the primary phenols in red lettuce leaf tissue (DuPont, Mondin et al. 2000, Caldwell 2003). The higher phenolic content of red lettuce observed in this study might be attributed to higher anthocyanin content and the high antioxidant capacity of red lettuce could be due to the strong antioxidant capacity anthocyanins. Literature data show that many plant phenolic compounds are characterized by a bitter taste (Lesschaeve 2005). Phenolic compound have been reported to be highly correlated with antioxidant activity in lettuce (Kim 2007). Liu et al. (2007) found that the level of total phenolic content in

red lettuce was 2.4-fold that of green lettuce, whereas the DPPH· scavenging activity was 1.2-fold. This discrepancy may be attributed to color interference of anthocyanin with DPPH· (Arnao 2000). The significantly different total phenolic content and DPPH· scavenging activity between red and green lettuce observed in this study suggests that red lettuce would be a good choice for consumers interested in beneficial foods for health (Liu, Ardo et al. 2007). Liu et al. (2007) found that lettuce harvested in July, which was grown at higher temperatures and greater light intensity, possessed significantly higher antioxidant capacity than lettuce harvested in September, however, the total phenolic content of each harvest was not significantly different. These data also suggest that it is important for the lettuce producer to consider environmental factors when selecting lettuce cultivars for enhanced antioxidant capacity (Liu, Ardo et al. 2007).Moreover, besides phenolic compound, there are various other compounds with significant antioxidant activity in plant, including carotenoids, terpenoids and some vitamins. Numerous studies have reported that vitamin C and carotenoids participate in the creation of antioxidant properties of a plant (Sun 2012).

Significant differences of MDA were observed in 'Red Oak' lettuce grown in the control and FCM treatments on day 0 during the first crop season. However,there were no significant differences in the levels of MDA between the control and the FCM treatments of 'Butterhead' lettuce. As a product of membrane lipid peroxidation, MDA is used to assess the extent of oxidative stress in plants, and its level is increased under stress conditions (Liu 2009.). Reactive oxygen species such as $O_2^{\cdot-}$ and endogenous H_2O_2 can be overproduced in plants under stress conditions and will thereby increase MDA content(Alscher et al. 2002).The stimulation of MDA level by *Chrysanthemum morifolium* aqueous extract in *Chrysanthemum morifolium* leaves was recorded by Zhou et al. (2009), which disturbed the balance between the activity of anti-oxidative enzymes and peroxidation of membrane lipids and accordingly affected the structure and functions of membranes, the main mechanisms of allelopathy (Singh et al. 1999). The initiation phase of lipid peroxidation is the abstraction of hydrogen atoms from lipid molecules. Several free radicals are responsible for this, one being hydroxyl radical (Gutteride (1988).

Peroxidation of lipids is particularly damaging because the products of this process lead to the spread of further free radical reactions (Catala 2006). Dependence between enzymatic activity and lipid peroxidation was also observed in soybeans subjected to allelopathic stress by treatment with phenolic extract from *Brassica napuse*(Haddadchi 2009).

Effects of FCM on selected soil microbial populations of lettuces grown in a local farm

Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality (Jeffries 2003). Soil microorganisms also show interaction to plant in several directions. In this particular study, *Bacillus* spp. and flourescent *Pseudomonas* spp. were representative of PGPR which may produce some antimicrobial substances and *Trichoderma* spp. represented a parasitic fungus of plant fungal pathogens. *Fusarium* spp. and *Pytium* spp. are common and important pathogen of fruit, vegetable and ornamental crops where they can cause seed rot, seedling damping off and rot (Weller, Raaijmakers et al. 2002);(Agrios 2005); (Le 2014).

The result of this study showed that soil amended with FCM may not have significant effects on microbial populations in the soil. Although, increasing of *Bacillus* spp. populations 5 weeks after planting 'Butterhead' and 'Red Oak' lettuces was observed but the increase occurred in both control and FCM treatments which may not be only the effect of FCM. However, higher numbers of *Bacillus* spp. after planting in FCM treatment may derive from multiplication of left-over *B. licheniformis* SK-1 in FCM. FCM treatment did not affect populations of other beneficial microbes, flourescent *Pseudomonas* spp. and *Trichoderma* spp. in this study. The tendency of these two microbial populations was decrease after planting. There were no previous reports about the effect of FCM on microbial populations. However, its related material, chitin and chitosan, could improve multiplication of *B. subtilis* and bacteria's fungicidal action (Manjula and Podile 2001).

Decrease in *Fusarium* spp. population was observed after planting ‘Butterhead’ and ‘Red Oak’ lettuces in both control and FCM treatments. Increasing of *Bacillus* spp. population in this study might be a factor that affected *Fusarium* spp. population by their antimicrobial activity (Hariprasad 2011). In addition to FCM related materials, chitin and chitosan, their effects showed an efficiency to reduce disease and populations of *Fusarium oxysporum* in field (Ashley 1998). Chitin amendment to soil could also suppress plant parasitic nematodes and resulted in changes in the bacterial communities of soil, rhizosphere and endorhiza (Hallmann, Rodrıguez-Kábana et al. 1999). It has been reported that chitin might stimulate the growth of antagonists and/or the plants which might also facilitate plant protection (Rajkumar, Lee b et al. 2008). Therefore, the application of chitin and its derivatives in agriculture was to modify plant-microbial interactions and improve crop yields (Sharp 2013). However, in this study increase of *Pythium* spp. populations in the FCM treatment had no effect to limit growth in both ‘Butterhead’ and ‘Red Oak lettuces. It’s possible that these species of *Pythium* may not be a pathogen of lettuces.

CHAPTER V
CONCLUSION

Effects of biomaterial and semi-biomaterial on growth, yield and postharvest quality of lettuces grown in a test plot

1.1. Effects of biomaterial and semi-biomaterial on growth and yield

The incorporations of SS/FCM as the chitin-rich residues to a growing medium promoted growth and yield of 'Butterhead' and 'Red Oak' lettuces during all three crop seasons. This study clearly suggests that 2% FCM (T5) was the best supplement since it increased lettuces' fresh weight, leaf width and leaf length.

1.2. Effects of biomaterial and semi-biomaterial on postharvest quality

The best overall appearance in all three crops was observed in both 'Butterhead' and 'Red Oak' lettuces treated with 2% FCM. In 'Butterhead' lettuce, weight losses after storage at 8°C and 60% RH for 14 days were significantly reduced with the treatments of SS and FCM in all three crop seasons. In 'Red Oak', weight losses were significantly reduced with the treatments of SS and FCM during all three crop seasons, and using 2% FCM resulted in the lowest fresh weight loss compared to other treatments during the second crop season.

Effects of the FCM on growth, yield, postharvest quality and selected soil microbial populations of lettuces in a local farm

1.3. Effects of FCM on growth and yield

The results clearly show that, application of 2% FCM to the soil gave better results for cultivating lettuce. It showed that 2% FCM can increase the growth and yield in the two crop seasons in a local farm. The effect of FCM on growth and yield of 'Butterhead' and 'Red Oak' lettuces indicated that 2% FCM significantly enhanced the growing factors and improved the average values of fresh weight.

1.4. Effects of FCM on postharvest quality

Weight losses of 'Butterhead' lettuce were significantly reduced with the treatment of FCM only during the first crop season, and using of FCM resulted in the lowest fresh weight loss compared to the control treatment. Better overall appearance was observed in 'Red Oak' lettuces treated with 2% FCM in both crop seasons. Moreover, in 'Red Oak' lettuces, weight losses were significantly reduced in the FCM treatment in both crop seasons.

1.5. Effects of FCM on net photosynthesis, transpiration rate and stomatal conductance of lettuce leaf

For 'Butterhead' lettuce, there were no significantly differences in the net photosynthesis, transpiration rate and stomatal conductance between the control and FCM treatments during both crop seasons. 'Red Oak' lettuce treated with FCM had higher net photosynthesis, transpiration rate and stomatal conductance than the control treatment during the second crop season.

1.6. Effects of FCM on nitrate contents

The content of nitrates in 'Butterhead' lettuce leaves of the FCM treatment was not significant different from the control treatment during both crop seasons. During the first crop season, in 'Red Oak' lettuce, the FCM treatment had higher nitrate contents than in the control treatment on day 14 after storage. While during the second crop season, the control treatment had higher nitrate contentsthan in the FCM treatment on day 0. The accumulation of nitrates in the lettuce leaves were below the maximum limits set by the European Commission Regulation for lettuce fresh product.

1.7. Effects of FCM on chlorophyll *a*, chlorophyll *b* and carotenoid contents

Chlorophyll *a*, *b* and carotenoid contents data were not statistically significant in 'Butterhead' lettuce during both crop seasons. In 'Red Oak' lettuces, there were significantly higher in chlorophyll *a*, *b* and contents in FCM treatment after storage at 8°C and 60% RH for 14 days during the first crop season.

1.8. Effects of FCM on antioxidant contents

Regarding antioxidant compounds, FCM treatment had no significant effect on ascorbic acid and phenolic contents in 'Butterhead' lettuce. The increase in flavonoid contents in the FCM treatment was higher than those of control after storage at 8°C and 60% RH for 14 days during the second crop season. While, DPPH radical scavenging activity, which indicates antioxidant capacity, in the control treatment was higher than the FCM treatment on day 14 after storage during the second crop. Adding FCM to 'Red Oak' lettuce planting soil had no effect on ascorbic acids, phenolics, flavonoids and DPPH radical scavenging activity during both crop seasons. Regarding MDA contents, which reflect the level of lipid peroxidation, it was found that the control treatment had higher MDA contents than the FCM treatments on day 0 during the first crop season.

1.9. Effects of FCM on selected soil microbial populations of lettuces grown in a local farm

This study indicated that FCM treatment had no effect on microbial populations in soil. However, theoretical microbial populations exhitbited an influence on increasing in beneficial pathogenic *Fusarium* spp.

REFERENCES

Akladious, S.A.a.A., S.M. . 2014. Akladious, S.A. and Abbas, S.M. (2014). Application of TrichodermaharzianumT22 as a biofertilizer potential in maize growth. Journal of Plant Nutrition 37, 30-49. *Journal of Plant Nutrition*, **37**, 30-49.

Arora, A.B., T,M. Nari M.G. Strasburg, G. 2000. .Modulation of liposomal membranes fluidity by flavonoids and isoflavonoids. . *Archives of Biochemistry and Biophysic* **373**, 102.

Ayes, M.D., Howard, S.C., Kuzio, J., Lopez-Ferber, M. andPossee, R.D. 1994. The complete DNA sequence of Auto grap hacalifornica nuclear polyhedrosis virus. *Virology*, **202**, 586-605.

Babbar, N.O., H.S. Uppal, D.S. and Patil, R.T. . 2011. Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. . *Food Research International* **44**, 391-396.

Bautista-Ban˜osa, S., , A.N. Herna´ndez-Lauzardoa, M.G. Vela´zquez-del Vallea, M. Herna´ndez-Lo´peza, E. Ait Barkab, E. Bosquez-Molinac, C.L. Wilson. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection* **25**, 108-118.

Ben-Shalom, N., Ardi, R., Pinto, R., Aki, C., Fallik, E.,. 2003. Controlling gray mould caused by Botrytis cinerea in cucumber plants by means of chitosan. . *Crop Protection*, **22**, 285-290.

Benhamou, N.L., P.J. , Nicole, M. . 1994. Induction of systemic resistance to Fusarium crown and root rot in tomato plants by seed treatment with chitosan. *Phytopathology* **84**, 1432-1444.

Bhaskara-Reddy M V, B.K., Corcuff R, Castaigne F , J, a.A. 2000. Effect of pre-harvest chitosan sprays on post-harvest infection by Botrytis cinereaand quality of strawberry fruit. *Postharvest Biology Technology* **20**, 39-51.

Blokhina, O., Virolainen, E., Fagerstedt, K.V. 2003. Antioxidants, Oxidative Damage and Oxygen Deprivation Stress: a Review. *Annals of Botany*, **91**(2), 179-194.

Bohland, C.B., T. Loers, G. Feussner, I. Grambow, H.J. 1997. Differential induction of lipoxygenase isoforms in wheat upon treatment with rust fungus elicitor, chitin oligosaccharades, chitosan, and methyl jasmonate. *Plant Physiology*, **114**, 679-685.

Bryan, N.S., Alexander, D.D., Coughlin, J.R., Milkowski, A.L., Boffetta, P. 2012. Ingested nitrate and nitrite and stomach cancer risk: An updated review. *Food and Chemical Toxicology*, **50**(10), 3646-3665.

Caldwell, C.R. 2003. Alkylperoxyl Radical Scavenging Activity of Red Leaf Lettuce (Lactuca sativa L.) Phenolics. *Journal of Agricultural and Food Chemistry*, **51**(16), 4589-4595.

Camougrand, N., Rigoulet, M. 2001. Aging and oxidative stress: studies of some genes involved both in aging and in response to oxidative stress. *Respiration Physiology*, **128**(3), 393-401.

Cao, G., Sofic, E., Prior, R.L. 1996. Antioxidant Capacity of Tea and Common Vegetables. *Journal of Agricultural and Food Chemistry*, **44**(11), 3426-3431.

Carris, L. 2003. M.W. Dick, Straminipilous Fungi. Systematics of the Peronosporomycetes Including Accounts of the Marine Straminipilous Protists, the Plasmodiophorids and Similar Organisms. *Mycopathologia*, **156**(4), 385-386.

Celar, F.a.N.V. 2005. Effects of Trichoderma spp. and Gliocladium roseum culture filtrates on seed germination of vegetables and maize. Journal of Plant Diseases 112, 343-350. *Journal of Plant Diseases*, **112**, 343-350.

Chan, K.-G., Tiew, S.-Z., Ng, C.-C. 2007. Rapid isolation method of soil bacilli and screening of their quorum quenching activity. *As. Pac. J. Mol. Biol. Biotech*, **15**, 153-156.

Chang, W.-T., Chen, Y.-C., Jao, C.-L. 2007. Antifungal activity and enhancement of plant growth by Bacillus cereus grown on shellfish chitin wastes. *Bioresource Technology*, **98**(6), 1224-1230.

Chaudière, J., Ferrari-Iliou, R. 1999. Intracellular Antioxidants: from Chemical to Biochemical Mechanisms. *Food and Chemical Toxicology*, **37**(9–10), 949-962.

Chaverri, P., Castlebury, L.A., Overton, B.E., Samuels, G.J. 2003. Hypocrea/Trichoderma: species with conidiophore elongations and green conidia. *Mycologia*, **95**(6), 1100-1140.

Chen, C.-S., Liau, W.-Y., Tsai, G.-J. 1998. Antibacterial Effects of N-Sulfonated and N-Sulfobenzoyl Chitosan and Application to Oyster Preservation. *Journal of Food Protection*, **61**(9), 1124-1128.

Chen, F.W., M. Zheng, Y. luo,J. Yang, X. and Wang.X. 2009.
Quantitative changes of plant defense enzymes and phytohormone in
biocontrol of cucumber fusarium wilt by Bacillus subtilis B 57
World Journal of Microbiol Biotechol, **26**, 925-932.

Chien, P.-J., Sheu, F., Lin, H.-R. 2007. Coating citrus (Murcott tangor) fruit with low molecular weight chitosan increases postharvest quality and shelf life. *Food Chemistry*, **100**(3), 1160-1164.

Choudhary, D.K., Johri, B.N. 2009. Interactions of Bacillus spp. and plants – With special reference to induced systemic resistance (ISR). *Microbiological Research*, **164**(5), 493-513.

Ciha, A.J., Brun, W.A. 1975. Stomatal Size and Frequency in Soybeans1. *Crop Sci.*, **15**(3), 309-313.

Commission, E. 1997. Report of the Scientific Committee for Food (Ed.) 38, European Commission Directorate-General Industry.

Costigan, P.A. 1986. The effects of soil temperature on the response of lettuce seedlings to starter fertilizer. *Plant and Soil*, **93**(2), 183-193.

Crozier, A., Lean, M.E.J., McDonald, M.S., Black, C. 1997. Quantitative Analysis of the Flavonoid Content of Commercial Tomatoes, Onions, Lettuce, and Celery. *Journal of Agricultural and Food Chemistry*, **45**(3), 590-595.

Cuero, R.G.O., G. Washington, A. . 1991. N-carboxymethyl chitosan inhibition of afla toxin production: role of zinc. *Biotechnology Letters* **13**, 441-444.

Cushnie, T.P., Lamb, A.J. (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26, 343-356. 2005. Antimicrobial activity of flavonoids. . *International Journal of Antimicrobial Agents* **26**, 343-356.

D.W., S. 2007. *Genome mapping and molecular breeding in plant: Vegetables*

5ed. Springer-Verlag Berlin Heideberg, California: C. Kole

De Rijke E, O.P., Niessen WMA, Ariese F, Gooijer C, Brinkmann UAT. 2006. Analytical separation and detection methods for flavonoids. *Journal of Chromatography A*, **1112**, 31-63.

Dekkers, L.C., Mulders, I.H.M., Phoelich, C.C., Chin-A-Woeng, T.F.C., Wijfjes, A.H.M., Lugtenberg, B.J.J. 2000. The sss Colonization Gene of the Tomato-Fusarium oxysporum f. sp. radicis-lycopersici Biocontrol Strain Pseudomonas fluorescens WCS365 Can Improve Root Colonization of Other Wild-type Pseudomonas spp. Bacteria. *Molecular Plant-Microbe Interactions*, **13**(11), 1177-1183.

DuPont, M.S., Mondin, Z., Williamson, G., Price, K.R. 2000. Effect of Variety, Processing, and Storage on the Flavonoid Glycoside Content and Composition of Lettuce and Endive. *Journal of Agricultural and Food Chemistry*, **48**(9), 3957-3964.

Dzida, K., Jarosz, Z., Michałojc´, Z., Nurzyn´ska-Wierdak, R. 2012. The influence of diversified nitrogen and liming fertilization on the chemical composition of lettuce. *Acta Scientiarum Polonorum - Hortorum Cultus*, **11**(3), 247-254.

El-Kazzaz, M., El-Fadly, G., Hassan, M., El-Kot, G. 2008. Identification of some Fusarium spp. using molecular biology techniques. *Egypt. J. Phytopathol*, **36**(1-2), 57-69.

El-Tantawy, E.M. 2009. Behavior of tomato plants as affected by spraying with chitosan and aminofort as natural stimulator substances under application of soil organic amendments. *Pak J Biol Sci*, **12**(17), 1164-73.

El Ghaouth, A., Arul, J., Grenier, J., and Asselin, A. . 1992. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruits. *Phytopathology*, **82**, 398-402.

El Hassni, M., El Hdrami., A., Daayf, F., Ait Birka, E and El Hadrami,, I. 2004. Chitosan , antifungal product against Fusarium oxysporum

f.sp.albedinis and elicitor of defense reactions in date palm roots.

. *Phytopathologia Mediterranea*, **43**, 195-204.

FAOSTAT. 2014. The FAO (Food and Agriculture Organization of the United Nations) Statistical Database (September 16, 2014).

Ferreres, F., Gil, M.I., Castañer, M., Tomás-Barberán, F.A. 1997. Phenolic Metabolites in Red Pigmented Lettuce (Lactuca sativa). Changes with Minimal Processing and Cold Storage. *Journal of Agricultural and Food Chemistry*, **45**(11), 4249-4254.

FOSTAT. 2011. The FAO (Food and Agriculture Organization of the United Nations) Statistical Database. (September 16, 2014).

Frantz, J.M., Bugbee, B. 2005. Acclimation of Plant Populations to Shade: Photosynthesis, Respiration, and Carbon Use Efficiency. *Journal of the American Society for Horticultural Science*, **130**(6), 918-927.

Ge, H.-C., Luo, D.-K. 2005. Preparation of carboxymethyl chitosan in aqueous solution under microwave irradiation. *Carbohydrate research*, **340**(7), 1351-1356.

Geels, F.P., Schippers, B. 1983. Selection of Antagonistic Fluorescent Pseudomonas spp. and their Root Colonization and Persistence following Treatment of Seed Potatoes. *Journal of Phytopathology*, **108**(3-4), 193-206.

George Kuepper, J.B., and, Raeven Thomas. 2002. Specialty lettuce and Greens: Organic production., NCAT Agriculture specialists.

Gutteridge, J.M. 1994. Biological origin of free radicals, and mechanisms of antioxidant protection. *Chem Biol Interact*, **91**(2-3), 133-40.

Ha, M.T., Huang, J.-W. 2007. Control of Fusarium wilt of asparagus bean by organic soil amendment and microorganisms. *Pathol Bull* **16**, 169-180.

Ha, M.T., Huang, Y.-M., Huang, J.-W. 2008. Influence of organic amendment and Bacillus subtilis on mineral nutrient uptake of asparagus bean in two field soils. *Plant Pathol Bull* **17**, 289-296.

Ha, T.N. 2010. Using Trichoderma species for biological control of plant pathogens in Vietnam. *J. ISSAAS.* , **16**(1), 17-21.

Hachisuka, Y., Kozuka, S., Tsujikawa, M. 1984. Exosporia and Appendages of Spores of Bacillus Species. *Microbiology and Immunology*, **28**(5), 619-624.

Hallmann, J., Rodrıguez-Kábana, R., Kloepper, J. 1999. Chitin-mediated changes in bacterial communities of the soil, rhizosphere and within roots of cotton in relation to nematode control. *Soil Biology and Biochemistry*, **31**(4), 551-560.

Hamid, R., Khan, M.A., Ahmad, M., Ahmad, M.M., Abdin, M.Z., Musarrat, J., Javed, S. 2013. Chitinases: an update. *Journal of pharmacy & bioallied sciences*, **5**(1), 21.

Harman, G.E. 2000. Myths and dogmas of biocontrol changes in perceptions derived from research on Trichoderma harzinum T-22. *Plant disease*, **84**(4), 377-393.

Hartz, T.K. 2007a. Efficient nitrogen management for cool-season vegetable.

Hartz, T.K., Johnstone, P., Williams, E., Smith, R. 2007. Establishing lettuce leaf nutrient optimum ranges through DRIS analysis. *HortScience*, **42**(1), 143-146.

Hartz, T.K., Johnstone, P.R., Williams, E., Smith, R.F. 2007b. Establishing lettuce leaf nutrient optimum ranges through DRIS analysis. *HortScience*, **42**, 143-146.

He, H., Chen, X., Sun, C., Zhang, Y., Gao, P. 2006. Preparation and functional evaluation of oligopeptide-enriched hydrolysate from shrimp (< i> Acetes chinensis</i>) treated with crude protease from< i> Bacillus</i> sp. SM98011. *Bioresource technology*, **97**(3), 385-390.

He, S.Y., Feng, G.P., Yang, H.S., Wu, Y., Li, Y.F. 2004. Effects of pressure reduction rate on quality and ultrastructure of iceberg lettuce after vacuum cooling and storage. *Postharvest Biology and Technology*, **33**(3), 263-273.

Hernández, A., Castillo, H., Ojeda, D., Arras, A., López, J., Sánchez, E. 2010. Effect of vermicompost and compost on lettuce production. *Chilean Journal of Agricultural Research*, **70**(4), 583-589.

Hirano, S., Kitaura, S., Sasaki, N., Sakaguchi, H., Sugiyama, M., Hashimoto, K., Tanatani, A. 1996. Chitin biodegradation and wound healing in tree bark tissues. *Journal of environmental polymer degradation*, **4**(4), 261-265.

Holsteijn, H.M.C.v. 1981. Growth and photosynthesis of lettuce, Landbouwhogeschool te Wageningen.

Hopkins, W.G. 1999. *Introduction to plant physiology: Introduction to Plant Physiology.* . John Wiley & Sons, Inc, USA: The University of Western Ontario.

Hoque, M.M., Ajwa, H., Othman, M., Smith, R., Cahn, M. 2010. Yield and postharvest quality of lettuce in response to nitrogen, phosphorus, and potassium fertilizers. *HortScience*, **45**(10), 1539-1544.

Howell, C. 2003. Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. *Plant disease*, **87**(1), 4-10.

Howell, C., Stipanovic, R. 1995. Mechanisms in the biocontrol of Rhizoctonia solani-induced cotton seedling disease by Gliocladium virens: antibiosis. *Phytopathology*, **85**(4), 469-472.

Howell, C., Stipanovic, R. 1980. Suppression of Pythium ultimium induced damping off of cotton seedlings by Pseudomonas fluorescens and its antibiotic pyoluterin. *Phytopathology*, **70**, 712-715.

Hoyos-Carvajal, L., Orduz, S., Bissett, J. 2009. Growth stimulation in bean (< i> Phaseolus vulgaris</i> L.) by< i> Trichoderma</i>. *Biological control*, **51**(3), 409-416.

Inthichack, P., Nishimura, Y., Fukumoto, Y. 2012. Effect of potassium sources and rates on plant growth, mineral absorption, and the incidence of tip burn in cabbage, celery, and lettuce. *Horticulture, Environment, and Biotechnology*, **53**(2), 135-142.

Inzé, D., Montagu, M.V. 1995. Oxidative stress in plants. *Current Opinion in Biotechnology*, **6**(2), 153-158.

Jackson, L., Stivers, L., Warden, B., Tanji, K. 1994. Crop nitrogen utilization and soil nitrate loss in a lettuce field. *Fertilizer Research*, **37**(2), 93-105.

Jimenez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M., Mullineaux, P. 2002. Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta*, **214**(5), 751-758.

Jing, S.-B., Li, L., Ji, D., Takiguchi, Y., Yamaguchi, T. 1997. Effect of Chitosan on Renal Function in Patients with Chronic Renal Failure. *Journal of Pharmacy and Pharmacology*, **49**(7), 721-723.

Johnstone, P., Hartz, T., Cahn, M., Johnstone, M. 2005. Lettuce response to phosphorus fertilization in high phosphorus soils. *HortScience*, **40**(5), 1499-1503.

Joyce, E. 1988. Role of fluorescent siderophore production in biological control of Pythium ultimum by a Pseudomonas fluorescens strain. *Phytopathology*, **78**, 166-172.

Kader, A.A. 2002. Quality parameters of fresh-cut fruit and vegetable products. *Fresh-cut fruits and vegetables. Science, technology and market. CRC Press, Boca Raton, Florida, USA.[Links]*, 11-20.

Kang, H.-M., Saltveit, M.E. 2002. Antioxidant Capacity of Lettuce Leaf Tissue Increases after Wounding. *Journal of Agricultural and Food Chemistry*, **50**(26), 7536-7541.

Kerns, D.L., M.E. Matheron, J.C. Palumbo, C.A. Sanchez, D.W. Still, B.R. Tickes, K. Umeda and M.A. Wilcox. 1999. Guidelines for head lettuce production in Arizona, College of Agriculture and Life Sciences, University of Arizona, Tucson, . Arizona.

Kerns, D.L., M.E. Matheron, J.C. Palumbo, C.A. Sanchez, D.W. Still, B.R. Tickes, K. Umeda and M.A. Wilcox. 2001. Guidelines for Head Lettuce Production in Arizona Principles and Practice of Soil Science: The Soil as a Natural Resource. 4 ed, Blackwell. Oxford, UK.

Kim, D.O., Lee, C.Y. 2004. Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Crit Rev Food Sci Nutr*, **44**(4), 253-73.

Kim, H.-J., Chen, F., Wang, X., Rajapakse, N.C. 2005a. Effect of chitosan on the biological properties of sweet basil (Ocimum basilicum L.). *Journal of agricultural and food chemistry*, **53**(9), 3696-3701.

Kim, H.J., Chen, F., Wang, X., Rajapakse, N.C. 2005b. Effect of chitosan on the biological properties of sweet basil (ocimum basilicum L.). *Journal of Agricultural and Food Chemistry*, **53**(9), 3696-3701.

Kuchitsu, K., Kikuyama, M., Shibuya, N. 1993. N-Acetylchitooligosaccharides, biotic elicitor for phytoalexin production, induce transient membrane depolarization in suspension-cultured rice cells. *Protoplasma*, **174**(1-2), 79-81.

Kuchitsu, K., Kosaka, H., Shiga, T., Shibuya, N. 1995. EPR evidence for generation of hydroxyl radical triggered byN-acetylchitooligosaccharide elicitor and a protein

phosphatase inhibitor in suspension-cultured rice cells. *Protoplasma*, **188**(1-2), 138-142.

Kudan, S., Pichyangkura, R. 2009. Purification and characterization of thermostable chitinase from Bacillus licheniformis SK-1. *Appl Biochem Biotechnol*, **157**(1), 23-35.

Kumar, B.S., H.K. Prasher, S. Tiwari, P. Salhan, M. Sharma, P. . 2011. A Review of Phytochemistry and Pharmacology of Flavonoids. Internationale Pharmaceutica Sciencia 1 (1), 25-40. *1*, **1**(25-40).

Kurzawiń ska, H. 2007. Potential use of chitosan in the control of lettuce pathogens. Polish Chitin Society *Monograph XII*, 173-178.

Lawlor, D.W. 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of experimental Botany*, **53**(370), 773-787.

LeA, P., SmithB, M., AitkenA, E. 2010. Pythium spp. on ginger (zingiber officinale Roscoe) in Australia. *Major sponsors*, 62.

Lee, J.-J., Park, R.-D., Kim, Y.-W., Shim, J.-H., Chae, D.-H., Rim, Y.-S., Sohn, B.-K., Kim, T.-H., Kim, K.-Y. 2004. Effect of food waste compost on microbial population, soil enzyme activity and lettuce growth. *Bioresource Technology*, **93**(1), 21-28.

Lee, K.W., Lee, H.J., Kang, K.-S., Lee, C.Y. 2002. Preventive effects of vitamin C on carcinogenesis. *The Lancet*, **359**(9301), 172.

Lee S, C.H., Suh S, Doo IS, Oh KY, Choi EJ, Schroeder Taylor AT, Low PS, Lee Y. . 1999. Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and Commelina communis. *Plant Physiology*, **121**(1), 147-52.

Lee, Y.-S., Kim, Y.-H., Kim, S.-B. 2005a. Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. *HortScience*, **40**(5), 1333-1335.

Lee, Y.S., Kim, Y.H., Kim, S.B. 2005b. Changes in the respiration, growth, and vitamin C content of soybean sprouts in response to chitosan of different molecular weights. *HortScience*, **40**(5), 1333-1335.

Limpanavech, P., Chaiyasuta, S., Vongpromek, R., Pichyangkura, R., Khunwasi, C., Chadchawan, S., Lotrakul, P., Bunjongrat, R., Chaidee, A., Bangyeekhun, T. 2008. Chitosan effects on floral production, gene expression, and anatomical changes in the< i> Dendrobium</i> orchid. *Scientia horticulturae*, **116**(1), 65-72.

Liu, X., Ardo, S., Bunning, M., Parry, J., Zhou, K., Stushnoff, C., Stoniker, F., Yu, L., Kendall, P. 2007. Total phenolic content and DPPH< sup></sup> radical scavenging activity of lettuce (< i> Lactuca sativa</i> L.) grown in Colorado. *LWT-Food Science and Technology*, **40**(3), 552-557.

Lo, C.-T., Lin, C.-Y., **羅朝村**, **林俊義**. 2002. Screening strains of Trichoderma spp for plant growth enhancement in Taiwan. *Plant Pathology Bulletin*(4), 215-220.

Manjula, K., Podile, A. 2001. Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of Bacillus subtilis AF 1. *Canadian Journal of Microbiology*, **47**(7), 618-625.

Marco Bittelli, M.F., Gaylon S. Campbell , Everett J. Nichols. 2001. Reduction of transpiration through foliar application of chitosan. *Agricultural and Forest Meteorology* **107**, 167-175.

Mayer, A.M. 2006. Polyphenol oxidases in plants and fungi: Going places? A review. . *Phytochemistry*, **67**, 2318-2331.

McLean, R., Beauchemin, D., Beveridge, T. 1992. Influence of oxidation state on iron binding by Bacillus licheniformis capsule. *Applied and environmental microbiology*, **58**(1), 405-408.

McLean, R.J., Beauchemin, D., Clapham, L., Beveridge, T.J. 1990. Metal-binding characteristics of the gamma-glutamyl capsular polymer of Bacillus licheniformis ATCC 9945. *Applied and environmental microbiology*, **56**(12), 3671-3677.

Meng X, L.B., Liu J, Tian S. 2008. Physiology response and quality attributes of table grape fruits to chitosan preharvest spray and postharvest coating during of table storage. *Food Chemistry* **106**, 501-508.

Mercado-Blanco, J., Bakker, P.A. 2007. Interactions between plants and beneficial Pseudomonas spp.: exploiting bacterial traits for crop protection. *Antonie van Leeuwenhoek*, **92**(4), 367-389.

Michalak, A. 2006. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish J. of Environ. Stud* **15**, 523-530.

Miles, C. 2003. Winter lettuce variety trials: Lettuce types. , Mount Vernon Northwestern Washington Research and Extension Center. Washington.

Minh Thanh Ha, Y.-M.H., and Jenn-Wen Huang. 2008. Influence of organic amendment and *Bacillus subtilis* on mineral nutrient uptake of asparagus bean in two field soils. . *Plant Pathology Bulletin*, **17**, 289-296.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, **7**(9), 405-410.

Mittova, V., Volokita, M., Guy, M., Tal, M. 2000. Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative Lycopersicon pennellii. *Physiologia Plantarum*, **110**(1), 42-51.

Molassiotis, A., Sotiropoulos, T., Tanou, G., Diamantidis, G., Therios, I. 2006. Boron-induced oxidative damage and antioxidant and nucleolytic responses in shoot tips culture of the apple rootstock EM 9 (< i> Malus domestica</i> Borkh). *Environmental and Experimental Botany*, **56**(1), 54-62.

Mondal, M., Malek, M., Puteh, A., Ismail, M., Ashrafuzzaman, M., Naher, L. 2012. Effect of foliar application of chitosan on growth and yield in okra. *Australian Journal of Crop Science*, **6**(5), 918.

Morrison, T.A.a.D.R.B. 1993. Cell wall phenolic content in tissue types of developing maize internodes 13-16 February 1993 ed. New Zealand, pp. 1097-1099.

Negi, P., Roy, S. 2000. Effect of Blanching and Drying Methods on< i> β</i>-Carotene, Ascorbic acid and Chlorophyll Retention of Leafy Vegetables. *LWT-Food Science and Technology*, **33**(4), 295-298.

Nge, K.L., Nwe, N., Chandrkrachang, S., Stevens, W.F. 2006. Chitosan as a growth stimulator in orchid tissue culture. *Plant Science*, **170**(6), 1185-1190.

Nicolle, C., Cardinault, N., Gueux, E., Jaffrelo, L., Rock, E., Mazur, A., Amouroux, P., Rémésy, C. 2004. Health effect of vegetable-based diet: lettuce consumption improves cholesterol metabolism and antioxidant status in the rat. *Clinical Nutrition*, **23**(4), 605-614.

No, H., Meyers, S., Prinyawiwatkul, W., Xu, Z. 2007. Applications of chitosan for improvement of quality and shelf life of foods: a review. *Journal of food science*, **72**(5), R87-R100.

Noctor, G., Foyer, C.H. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annual review of plant biology*, **49**(1), 249-279.

Obsuwan, A.U.J.A.T.d.S.K. 2007. Chitosan for Improving Orchid Production and Quality. *Global Science Books*, **1**(1), 1-5.

Ohta, K., Morishita, S., Suda, K., Kobayashi, N., Hosoki, T. 2004. Effects of chitosan soil mixture treatment in the seedling stage on the growth and flowering of several ornamental plants. *Journal of the Japanese Society for Horticultural Science (Japan).*

Ohta, K., Taniguchi, A., Konishi, N., Hosoki, T. 1999. Chitosan treatment affects plant growth and flower quality in Eustoma grandiflorum. *HortScience*, **34**(2), 233-234.

Ozgen, S., Sekerci, S., Kaya, C. 2014. Nitrate and phytochemicals: may these vary in red and green lettuce by application of organic and inorganic fertilizers? *Biological Agriculture & Horticulture*(ahead-of-print), 1-10.

Page, A., Miller, R., Keeney, D. 1982. Total carbon, organic carbon, and organic matter. *Methods of soil analysis. Part*, **2**, 539-579.

Palleroni, N., Kunisawa, R., Contopoulou, R., Doudoroff, M. 1973. Nucleic acid homologies in the genus Pseudomonas. *International Journal of Systematic Bacteriology*, **23**(4), 333-339.

Patkowska, E., Pi**ę**ta, D., Pastucha, A. 21. THE EFFECT OF BIOCHIKOL 020 PC ON MICROORGANISM COMMUNITIES IN THE RHIZOSPHERE OF FABACEAE PLANTS.

Pieterse, C.M., Van Pelt, J.A., Ton, J., Parchmann, S., Mueller, M.J., Buchala, A.J., Métraux, J.-P., Van Loon, L.C. 2000. Rhizobacteria-mediated induced systemic resistance (ISR) in< i> Arabidopsis</i> requires sensitivity to jasmonate and

ethylene but is not accompanied by an increase in their production. *Physiological and Molecular Plant Pathology*, **57**(3), 123-134.

Preece, J.E., Read, P.E. 1993. *The biology of horticulture: an introductory textbook*. John Wiley & Son.

Probanza, A., Lucas Garcıa, J., Ruiz Palomino, M., Ramos, B., Gutiérrez Mañero, F. 2002. < i> Pinus pinea</i> L. seedling growth and bacterial rhizosphere structure after inoculation with PGPR< i> Bacillus</i>(< i> B. licheniformis</i> CECT 5106 and< i> B. pumilus</i> CECT 5105). *Applied Soil Ecology*, **20**(2), 75-84.

Rabea, E.I., Badawy, M.E.-T., Stevens, C.V., Smagghe, G., Steurbaut, W. 2003. Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules*, **4**(6), 1457-1465.

Raj, S.N.S., B.R. and Shetty, H.S. . 2006.
Induction and accumulation of polyphenol oxidase
activities as implicated in development of resistance
against pearl millet downy mildew disease. . *Functional Plant Biology* **33**, 563-571.

Rama Devi, S., Prasad, M. 1998. Copper toxicity in< i> Ceratophyllum demersum</i> L.(Coontail), a free floating macrophyte: Response of antioxidant enzymes and antioxidants. *Plant Science*, **138**(2), 157-165.

Ramos, B., García, J.A.L., Probanza, A.n., Barrientos, M.L., Gutierrez Mañero, F.J. 2003. Alterations in the rhizobacterial community associated with European alder growth when inoculated with PGPR strain< i> Bacillus licheniformis</i>. *Environmental and Experimental Botany*, **49**(1), 61-68.

Rao, M.B., Tanksale, A.M., Ghatge, M.S., Deshpande, V.V. 1998. Molecular and biotechnological aspects of microbial proteases. *Microbiology and molecular biology reviews*, **62**(3), 597-635.

Raschke, K. 1975. Stomatal action. *Annual Review of Plant Physiology*, **26**(1), 309-340.

Raupach, G.S., Kloepper, J.W. 1998. Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathology*, **88**(11), 1158-1164.

Ravi Kumar, M.N. 2000. A review of chitin and chitosan applications. *Reactive and functional polymers*, **46**(1), 1-27.

Rice-Evans, C., Miller, N., Paganga, G. 1997. Antioxidant properties of phenolic compounds. *Trends in plant science*, **2**(4), 152-159.

Richard Smith, M.C. 2010. Fine tuning nitrogen management for vegetable production, UC Cooperative Extension

Monterey County

1432 Abbott Street

Salinas. CA.

Rindels., S. 1994. *Lettuce varieties: Horticulture and home pest news*. Department of Horticulture, State University of Science and Technology, Iowa.

Roberts, P., Jones, D.L. 2012. Microbial and plant uptake of free amino sugars in grassland soils. *Soil Biology and Biochemistry*, **49**(0), 139-149.

Rock, C.L., Jacob, R.A., Bowen, P.E. 1996. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E, and the carotenoids. *Journal of the American Dietetic Association*, **96**(7), 693-702.

Romani, A., Pinelli, P., Galardi, C., Sani, G., Cimato, A., Heimler, D. 2002. Polyphenols in greenhouse and open-air-grown lettuce. *Food Chemistry*, **79**(3), 337-342.

Ryu, C.-M., Farag, M.A., Hu, C.-H., Reddy, M.S., Kloepper, J.W., Paré, P.W. 2004. Bacterial volatiles induce systemic resistance in Arabidopsis. *Plant Physiology*, **134**(3), 1017-1026.

Sakihama, Y., Cohen, M.F., Grace, S.C., Yamasaki, H. 2002. Plant phenolic antioxidant and prooxidant activities: phenolics-induced oxidative damage mediated by metals in plants. *Toxicology*, **177**(1), 67-80.

Schippers, B., Bakker, A.W., Bakker, P.A. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Annual review of Phytopathology*, **25**(1), 339-358.

Schisler, D.S., P. Behle, R. & Jackson, M. 2004. Formulation of Bacillus spp. for biological control of plant diseases. *Phytopathology* **94**, 1267-1271.

Sereih, A., Neven, A., Abd-El-Aal, S., Sahab, A. 2007. The mutagenic activity and its effect on the growth of Trichoderma harzianum and Fuzarium oxysporum F. *J Appl Sci Res*, **3**, 350-455.

Sgherri, C., Cosi, E., Navari-Izzo, F. 2003. Phenols and antioxidative status of Raphanus sativus grown in copper excess. *Physiologia Plantarum*, **118**(1), 21-28.

Shaked-Sachray, L., Weiss, D., Reuveni, M., Nissim-Levi, A., Oren-Shamir, M. 2002. Increased anthocyanin accumulation in aster flowers at elevated temperatures due to magnesium treatment. *Physiologia plantarum*, **114**(4), 559-565.

Shanmugaiah, V., Balasubramanian, N., Gomathinayagam, S., Manoharan, P., Rajendran, A. 2009. Effect of single application of Trichoderma viride and Pseudomonas fluorescens on growth promotion in cotton plants. *African Journal of Agricultural Research*, **4**(11), 1220-1225.

Shanmugam, V., Senthil, N., Raguchander, T., Ramanathan, A., Samiyappan, R. 2002. Interaction ofPseudomonas fluorescens withRhizobium for their effect on the management of peanut root rot. *Phytoparasitica*, **30**(2), 169-176.

Sharp, R.G. 2013. A review of the applications of chitin and its derivatives in agriculture to modify plant-microbial interactions and improve crop yields. *Agronomy*, **3**(4), 757-793.

Shibuya, N., Minami, E. 2001. Oligosaccharide signalling for defence responses in plant. *Physiological and Molecular Plant Pathology*, **59**(5), 223-233.

Shimshi, D., Ephrat, J. 1975. Stomatal behavior of wheat cultivars in relation to their transpiration, photosynthesis, and yield. *Agronomy Journal*, **67**(3), 326-331.

Simons, M., Van Der Bij, A.J., Brand, I., De Weger, L.A., Wijffelman, C.A., Lugtenberg, B.J. 1996. Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting Pseudomonas bacteria. *MPMI-Molecular Plant Microbe Interactions*, **9**(7), 600-607.

Smirnoff, N. 2000. Ascorbic acid: metabolism and functions of a multi-facetted molecule. *Current opinion in plant biology*, **3**(3), 229-235.

Sosa, A., Padilla, J., Ortiz, J., Etchevers, J.D. 2012. Biomass Accumulation and its Relationship with the Demand and Concentration of Nitrogen, Phosphorus, and Potassium in Lettuce. *Communications in Soil Science and Plant Analysis*, **43**(1-2), 121-133.

Soto, M.J., Sanjuán, J., Olivares, J. 2006. Rhizobia and plant-pathogenic bacteria: common infection weapons. *Microbiology*, **152**(11), 3167-3174.

Spiegel, Y., Kafkafi, U., Pressman, E. 1988. Evaluation of a protein-chitin derivative of crustacean shells as a slow-release nitrogen fertilizer on Chinese cabbage. *Journal of Horticultural Science (UK)*.

Stanghellini, M., Rasmussen, S. 1994. Hydroponics: a solution for zoosporic pathogens. *Plant disease*, **78**(12), 1129-1138.

Still, D.W. 2007. *Genome mapping and molecular breeding in plant: Vegetables*. *5 ed*. Springer-Verlag Berlin Heideberg, California: C. Kole.

Sun, J., Chu, Y.-F., Wu, X., Liu, R.H. 2002. Antioxidant and Antiproliferative Activities of Common Fruits. *Journal of Agricultural and Food Chemistry*, **50**(25), 7449-7454.

Tagliaferro, A., Heim, KE., Bobily, DJ. . 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. . *The Journal of Nutritional Biochemistry* **13**(10), 572-584.

Taiz, L., Zeiger, E. 2010. Plant physiology. *Sunderland, MA: Sinauer Associates*.

Tausz, M., Wonisch, A., Grill, D., Morales, D., Jiménez, M.S. 2003. Measuring antioxidants in tree species in the natural environment: from sampling to data evaluation. *Journal of experimental botany*, **54**(387), 1505-1510.

Thomashow, L.S., Weller, D.M. 1996. Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. in: *Plant-microbe interactions*, Springer, pp. 187-235.

Tindall, H.D. 1983. *Vegetables in the tropics.* , London: Macmillan. .

Toro, M., Azcon, R., Barea, J. 1997. Improvement of Arbuscular Mycorrhiza Development by Inoculation of Soil with Phosphate-Solubilizing Rhizobacteria To Improve Rock Phosphate Bioavailability ((sup32) P) and Nutrient Cycling. *Applied and environmental microbiology*, **63**(11), 4408-4412.

Uzuhashi, S., Kakishima, M., Tojo, M. 2010. Phylogeny of the genus< i> Pythium</i> and description of new genera. *Mycoscience*, **51**(5), 337-365.

van Bruggen, A.H., Brown, P.R., Greathead, A. 1990. Distinction between infectious and noninfectious corky root of lettuce in relation to nitrogen fertilizer. *Journal of the American Society for Horticultural Science*, **115**(5), 762-770.

van der Plaats-Niterink, A.J. 1981. *Monograph of the genus Pythium*. Centraalbureau voor Schimmelcultures Baarn.

Vasyukova, N.I., Zinov'eva, S.V., Il'inskaya, L.I., Perekhod, E.A., Chalenko, G.I., Gerasimova, N.G., Il'ina, A.V., Varlamov, V.P., Ozeretskovskaya, O.L. 2001. Modulation of plant resistance to diseases by water-soluble chitosan. *Prikladnaya Biokhimiya i Mikrobiologiya*, **37**(1), 121-122.

Verma, M., Brar, S.K., Tyagi, R., Surampalli, R., Valero, J. 2007. Antagonistic fungi,< i> Trichoderma</i> spp.: Panoply of biological control. *Biochemical Engineering Journal*, **37**(1), 1-20.

Verstraeten, S.V., Keen, C.L., Schmitz, H.H., Fraga, C.G., Oteiza, P.I. 2003. Flavan-3-ols and procyanidins protect liposomes against lipid oxidation and disruption of the bilayer structure. *Free Radical Biology and Medicine*, **34**(1), 84-92.

Vijayakumar, S.P., G. and Vijayalakshmi N.R. . 2008. Vijayakumar, S., Presannakumar, G. and Vijayalakshmi N.R. (2008). Antioxidant activity of banana flavonoids. Fitoterapia 79, 279-282. *Fitoterapia* **79**, 279-282.

Villano, D., Fernández-Pachón, M., Moyá, M., Troncoso, A., García-Parrilla, M. 2007. Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*, **71**(1), 230-235.

Vinson, J.A., Hao, Y., Su, X., Zubik, L. 1998. Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry*, **46**(9), 3630-3634.

Vruggink, H. 1970. The effect of chitin amendment on actinomycetes in soil and on the infection of potato tubers by Streptomyces scabies. *Netherlands Journal of Plant Pathology*, **76**(5), 293-295.

Wallace, T. 1962. The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms. *Soil Science*, **94**(5), 350.

Wan, J., Zhang, X.C., Neece, D., Ramonell, K.M., Clough, S., Kim, S.Y., Stacey, M.G., Stacey, G. 2008. A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. *Plant Cell*, **20**(2), 471-481.

Wang, S.-L., Chang, W.-T. 1997. Purification and characterization of two bifunctional chitinases/lysozymes extracellularly produced by Pseudomonas aeruginosa K-187 in a shrimp and crab shell powder medium. *Applied and environmental microbiology*, **63**(2), 380-386.

Wang, S.Y., Zheng, W. 2001. Effect of plant growth temperature on antioxidant capacity in strawberry. *Journal of Agricultural and Food Chemistry*, **49**(10), 4977-4982.

Wanichpongpan, P., Suriyachan, K., Chandrkrachang, S. 2001. Effect of chitosan on the growth of Gerbera flower plant (Gerbera jamesonii). *Chitin and chitosan: Chitin and Chitosan in Life Science, Yamaguchi, Japan*, 198-201.

Weindling, R. 1934. Studies on a lethal principle effective in the parasitic action of Trichoderma lignorum on Rhizoctonia solani and other soil fungi. *Phytopathology*, **24**(1), 153-151.

Weindling, R. 1932. Trichoderma lignorum as a parasite of other soil fungi. *Phytopathology*, **22**(8), 837-845.

Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., Thomashow, L.S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens 1. *Annual review of phytopathology*, **40**(1), 309-348.

White, R.E. 2009. *Principles and practice of soil science: the soil as a natural resource*. John Wiley & Sons.

Wien, H.C. 1997. *The physiology of vegetable crops*. Cab International.

Wilkinson, S., Davies, W.J. 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, cell & environment*, **25**(2), 195-210.

Windham, G., Windham, M., Williams, W. 1989. Effects of Trichoderma spp. on maize growth and Meloidogyne arenaria reproduction. *Plant Disease*, **73**(6), 493-495.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. *Biochemistry Journal*, **322**, 681-692.

Yakimov, M.M., Timmis, K.N., Wray, V., Fredrickson, H.L. 1995. Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface Bacillus licheniformis BAS50. *Applied and Environmental Microbiology*, **61**(5), 1706-1713.

Yang, X.-h., Lu, G., Zhao, Z.-h., Liu, L., Yao, X. 2007. Isolation and identification of Fusarium species from cucumber wilt diseased plants in vegetable greenhouses in northeastern China. *JOURNAL-SHENYANG AGRICULTURAL UNIVERSITY*, **38**(3), 308.

Yen, M.T., Mau, J.L. 2007. Selected physical properties of chitin prepared from shiitake stipes. *LWT - Food Science and Technology*, **40**(3), 558-563.

Zhang, H., Li, R., Liu, W. 2011. Effects of chitin and its derivative chitosan on postharvest decay of fruits: a review. *International journal of molecular sciences*, **12**(2), 917-934.

Zhao, B., Yan, J., Zhang, S., Liu, X., Gao, Z. 2013. Phylogeny and pathogenicity of< i> Fusarium</i> spp. isolated from greenhouse melon soil in Liaoning Province. *Saudi Journal of Biological Sciences*.

APPENDIX A

1. Culture media and growth conditions

1.1 Tryptic Soy agar (TSA) selective medium for *Bacillus* spp.

Culture media

Dissolved 40 g of TSA in 1,000 mL distilled water and added 10 mL glycerol. Sterilized by autoclaving at 121°C for 15 minutes. The antibiotics: 50 mg nystatin and 50 mg cycloheximide were added to the medium after autoclaved.

Procedure for media

Bacillus spp. are selected by heat-treating dilutions at 100°C for 15 minutes (Bashan et al. 1993). Each soil sample (10 g) was suspended with 90 mL of sterile distilled water. Soil suspensions were shacked at 250 rpm for 15 minutes. Serial 10-fold dilutions were made to 10^{-3}. Soil suspensions were performed at 100°C for 15 minutes. After heat treatment, heat-treated soil suspensions were incubated at room temperature for 20 minutes and serially diluted prior to plating on TSA agar for isolation of single colonies. Plates were incubated for 1-2 days at 28°C under dark light. Number of colonies of *Bacillus* spp. were counted and the results were expressed as CFU per gram of soil (Chan et al. 2007).

1.2 King agar B selective medium for Fluoresent *Pseudomonads* spp.

Culture media

Dissolved 20 g peptone, 1.5 g dipotassium hydrogen phosphate, 1.5 g magnesium sulfate and 10 g agar in 990 mL distilled water and added 10 mL glycerol. Shake until the solutes have dissolved. Sterilized by autoclaving at 121°C for 15 minutes. The antibiotics: 100 mg/mL penicilin G, 45 mg/mL novobiocin,75 mg/mL cycloheximide and 3 mL 95% ethanol were added to the medium after autoclaved.

Procedure for media

Each soil sample (10 g) was suspended with 90 mL of sterile distilled water. Soil suspensions were shacked at 250 rpm for 15 minutes. Serial 10-fold dilutions were made to 10^{-3} and serially diluted prior to plating on King agar Bfor isolation of single colonies.Plates were incubated for 1-2 days at 25°C under dark light. Colonies were counted of the fluorescing bacteria under the UV lamp. The results were expressed as CFU per gram of soil (Sand and Rovira., 1970).

1.3 Malachite green agar 2.5 ppm (MGA 2.5) a selective medium for *Fusarium* spp.

Culture media

Dissolved 15 g peptone, 1 g KH_2PO_4, 0.5 g MgSO4·$7H_2O$ and 20 g agar in 1,000 mL distilled water. Shake until the solutes have dissolved. Sterilized by autoclaving at 121°C for 15 minutes. The antibiotics: 2.5 ppm malachite green, 100 mg chloramphenicol and 50 mg streptomycinwere added to the medium after autoclaved.

Procedure for media

Each soil sample (10 g) was suspended with 90 mL of sterile distilled water. Soil suspensions were shacked at 250 rpm for 15 minutes. Serial 10-fold dilutions were made to 10^{-3} and serially diluted prior to plating on MGA 2.5 agar for isolation of single colonies. Plates were incubated for 5-7 days at 28°C under dark light. Number of colonies of *Fusarinm* spp. were counted and the results were expressed as CFU per gram of soil(Castellá et al. 1997).

1.4 Potato dextrose agar (PDA) selective medium for *Pythium* spp.

Culture media

Dissolved 39 g of PDA in distilled water. Shake until the solutes have dissolved and adjust the pH to 5.6. Adjust the volume of the medium to 1 litre with distilled water. Sterilized by autoclaving at 121°C for 15 minutes. The antibiotics: 10 mg benomyl, 25 mg nystatin, 25 mg pentachloronitrobenzene, 10 mg rifampicin and 500 mg ampicillin were added to the medium after autoclaved.

Procedure for media

Each soil sample (10 g) was suspended with 90 mL of sterile distilled water. Soil suspensions were shacked at 250 rpm for 15 minutes. Serial 10-fold dilutions were made to 10^{-3} and serially diluted prior to plating on PDA agar for isolation of single colonies. Plates were incubated for 3-5 days at 28°C under dark light. Number of colonies of *Pythium* spp. werecounted and the results were expressed as CFU per gram of soil(Masago et al. 1977).

1.5. *Trichoderma* medium E (TME) selective medium for *Trichoderma* spp.

Culture media

Selective medium for isolation *Trichoderma* spp. from soil. The medium were contains (per liter of liquid): 200 mL V-8 Juice, 1 g glucose, and 20 g agar. The agar was autoclaved separately in 500 mL of water and mixed with the diluted V-8 Juiceafter autoclaving. The antibiotics were added to the medium after autoclaved. This medium contained (per liter of V-8 Juice agar): 100 mg each of neomycin sulfate, bacitracin, penicilin G, andfolpet; 25 mg chlorotracycline hydrochloride; 20 mg; nystatin and 500 mg sodium propionate.

Procedure for media

Each soil sample (10 g) was suspended with 90 mL of sterile distilled water. Soil suspensions were shacked at 250 rpm for 15 minutes. Serial 10-fold dilutions were made to 10^{-3} and serially diluted prior to plating on TME agar for isolation of single colonies. Plates were incubated for 5-7 days at 25°C under continuous fluorescent light. Number of colonies of *Trichoderma* spp. were counted and the results were expressed as CFU per gram of soil(Papavizas and Lumsden, 1982).

APPENDIX B

Table B.1 Nitrate concentration measurement.

Nitrate concentrations (mM)	Absorbance (500 nm)
0	0
22.15	0.114
44.30	0.209
66.45	0.312
88.60	0.385

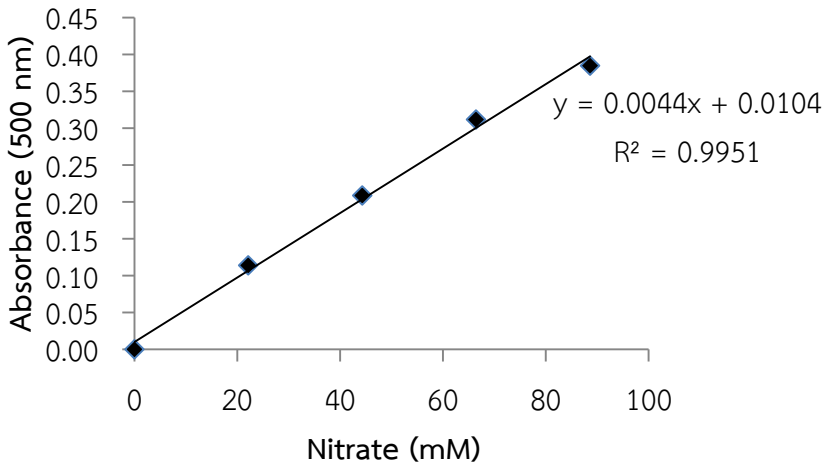

Figure B.1 Standard curve of standard nitrate.

Table B.2 Ascorbic acid concentration measurement.

Ascorbic acid concentrations (mM)	Absorbance (540 nm)
0	0
5	0.084
10	0.212
15	0.317
20	0.398
25	0.505
30	0.591
35	0.704
40	0.849

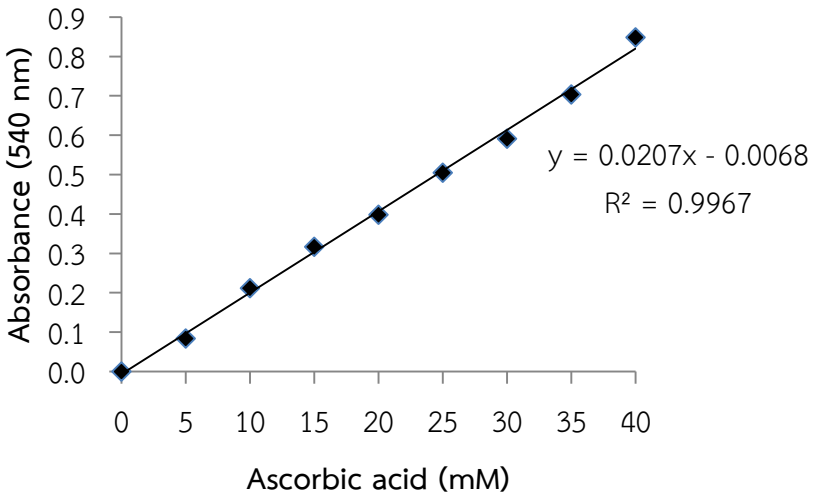

Figure B.2 Standard curve of standard ascorbic acid.

Table B.3 Phenolic concentration measurement.

Phenolic concentrations (µg/mL)	Absorbance (750 nm)
0	-0.03524
50	0.21765
100	0.38674
150	0.64963
200	0.91342
250	0.92078

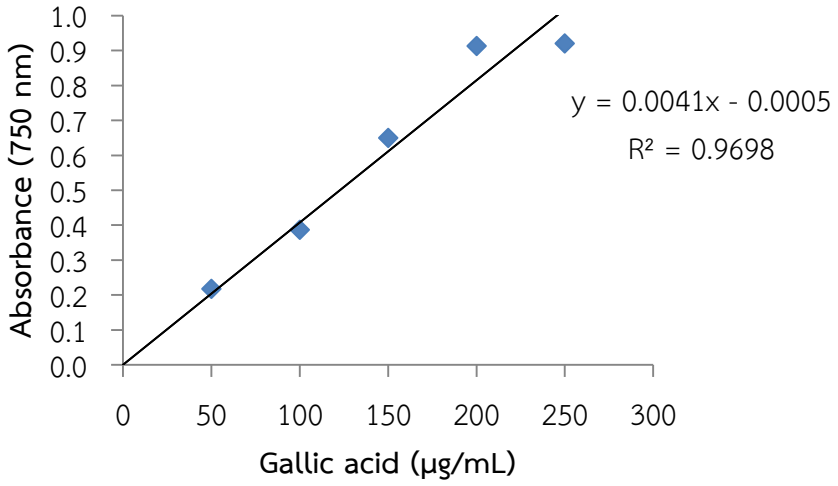

Figure B.3 Standard curve of standard phenolic.

Table B.4 Flavonoid concentration measurement.

Flavonoid concentrations (μg/mL)	Absorbance (510 nm)
0	-0.23349
50	-0.10066
100	0.10066
150	-0.44793
200	0.00194
250	0.12158
300	0.27546
350	0.43755
400	0.43954
450	0.40619
500	0.38826
550	0.7823
600	0.6626

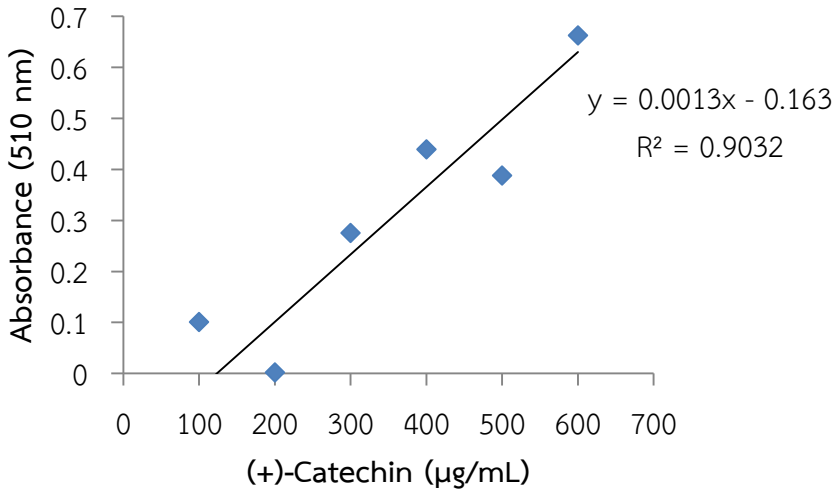

Figure B.4 Standard curve of standard flavonoid.

APPENDIX C

Table C.1 Leaf number, leaf width and leaf length of 'Butterhead' lettuce using SS and FCM at different combination during the three successive crop seasons: March-April, 2010 (first), July-September, 2010 (second), and December 2010-February 2011 (third) in a test plot. *

Crop	Treatment	Leaf number/	Leaf width	Leaf length
First	T1	9.33 ± 0.42 ^{c}	3.26 ± 0.11 ^{c}	3.83 ± 0.16 ^{c}
	T2	16.88 ± 0.61 ^{b}	6.13 ± 0.26 ^{b}	6.52 ± 0.24 ^{b}
	T3	16.08 ± 0.49 ^{b}	5.97 ± 0.23 ^{b}	6.07 ± 0.17 ^{b}
	T4	15.64 ± 0.62 ^{b}	6.06 ± 0.29 ^{b}	6.10 ± 0.27 ^{b}
	T5	20.85 ± 0.64 ^{a}	8.43 ± 0.19 ^{a}	9.48 ± 0.28 ^{a}
	T6	7.17 ± 0.41 ^{d}	2.47 ± 0.07 ^{d}	3.13 ± 0.11 ^{d}
Second	T1	6.28 ± 0.16 ^{c}	1.32 ± 0.23 ^{b}	1.76 ± 0.12 ^{c}
	T2	13.19 ± 0.42 ^{b}	4.89 ± 0.20 ^{a}	7.30 ± 0.28 ^{b}
	T3	14.63 ± 0.51 ^{a}	5.27 ± 0.24 ^{a}	7.48 ± 0.23 ^{b}
	T4	13.42 ± 0.52 ^{ab}	5.17 ± 0.23 ^{a}	7.34 ± 0.25 ^{b}
	T5	13.88 ± 0.58 ^{ab}	5.53 ± 0.26 ^{a}	8.46 ± 0.27 ^{a}
	T6	6.72 ± 0.15 ^{c}	1.36 ± 0.09 ^{b}	2.30 ± 0.14 ^{c}
Third	T1	9.69 ± 0.34 ^{b}	3.63 ± 0.14 ^{d}	5.13 ± 0.16 ^{c}
	T2	18.09 ± 0.39 ^{a}	7.50 ± 0.11 ^{c}	9.00 ± 0.19 ^{b}
	T3	18.22 ± 0.43 ^{a}	7.51 ± 0.14 ^{c}	8.89 ± 0.18 ^{b}
	T4	18.72 ± 0.53 ^{a}	8.18 ± 0.19 ^{b}	9.42 ± 0.16 ^{b}
	T5	19.00 ± 0.67 ^{a}	9.72 ± 0.27 ^{a}	11.80 ± 0.26 ^{a}
	T6	8.44 ± 0.26 ^{b}	3.03 ± 0.08 ^{e}	4.38 ± 0.14 ^{d}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to Duncan's multiple range test (p = 0.05). T1=Control, T2=0.5% SS, T3=0.5% SS+SK-1, T4=0.25% SS+0.25% FCM, T5=T2% FCM and T6=SK-1.

Table C.2 Fresh and dry weight, percentage of fresh weight loss and overall visual quality of 'Butterhead' lettuce using SS and FCM at different combination during the three successive crop seasons: March-April, 2010 (first), July-September, 2010 (second), and December 2010-February 2011 (third) in a test plot. *

Crop season	Treatment	Fresh weight (g)	Dry weight (g)	Fresh weight loss (%)	Overall visual quality (score)
First	T1	3.35 ± 0.25 ^{c}	0.22 ± 0.02 ^{c}	20.55 ± 2.68 ^{b}	1.62 ± 0.16 ^{c}
	T2	24.23 ± 2.69 ^{b}	1.02 ± 0.08 ^{b}	10.62 ± 1.08 ^{a}	3.19 ± 0.15 ^{b}
	T3	20.10 ± 2.01 ^{b}	0.87 ± 0.06 ^{b}	12.46 ± 1.76 ^{a}	3.04 ± 0.13 ^{b}
	T4	20.57 ± 2.09 ^{b}	0.84 ± 0.07 ^{b}	11.70 ± 1.60 ^{a}	2.93 ± 0.17 ^{b}
	T5	47.90 ± 3.05 ^{a}	1.80 ± 0.12 ^{a}	6.88 ± 0.71 ^{a}	3.63 ± 0.09 ^{a}
	T6	1.28 ± 0.09 ^{c}	0.10 ± 0.02 ^{c}	34.65 ± 3.94 ^{c}	1.29 ± 0.18 ^{c}
Second	T1	0.24 ± 0.03 ^{b}	0.09 ± 0.01 ^{d}	38.61 ± 2.99 ^{d}	2.63 ± 0.15 ^{b}
	T2	7.20 ± 0.88 ^{a}	0.85 ± 0.08 ^{c}	15.66 ± 1.48 ^{b}	3.65 ± 0.12 ^{a}
	T3	8.66 ± 0.89 ^{a}	0.84 ± 0.07 ^{c}	9.23 ± 0.74 ^{a}	3.73 ± 0.12 ^{a}
	T4	7.95 ± 0.96 ^{a}	1.07 ± 0.09 ^{b}	13.32 ± 1.75 ^{ab}	3.75 ± 0.11 ^{a}
	T5	9.46 ± 1.68 ^{a}	1.34 ± 0.10 ^{a}	12.11 ± 0.82 ^{ab}	4.00 ± 0.09 ^{a}
	T6	0.30 ± 0.03 ^{b}	0.08 ± 0.01 ^{d}	27.28 ± 0.38 ^{c}	2.94 ± 0.09 ^{b}
Third	T1	3.56 ± 0.31 ^{d}	0.02 ± 0.00 ^{b}	19.63 ± 3.87 ^{b}	1.89 ± 0.14 ^{d}
	T2	25.94 ± 1.14 ^{c}	0.17 ± 0.04 ^{a}	6.90 ± 0.77 ^{a}	2.71 ± 0.13 ^{c}
	T3	27.25 ± 1.77 ^{bc}	0.23 ± 0.05 ^{a}	8.00 ± 0.65 ^{a}	3.13 ± 0.19 ^{bc}
	T4	31.95 ± 2.01 ^{b}	0.18 ± 0.03 ^{a}	4.59 ± 0.50 ^{a}	3.38 ± 0.15 ^{b}
	T5	42.96 ± 3.89 ^{a}	0.16 ± 0.02 ^{a}	3.37 ± 0.79 ^{a}	4.23 ± 0.17 ^{a}
	T6	2.03 ± 0.18 ^{d}	0.05 ± 0.03 ^{b}	15.24 ± 1.70 ^{b}	2.06 ± 0.19 ^{d}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to Duncan's multiple range test (p = 0.05). T1=Control, T2=0.5% SS, T3=0.5% SS+SK-1, T4=0.25% SS+0.25% FCM, T5=T2% FCM and T6=SK-1.

Table C.3 Leaf number, leaf width and leaf length of 'Red Oak' lettuce using SS and FCM at different combination during the three successive crop seasons: March-April, 2010 (first), July-September, 2010 (second), and December 2010-February 2011 (third) in a test plot. *

Crop season	Treatment	Leaf number/ plant	Leaf width (cm)	Leaf length (cm)
First	T1	5.88 ± 0.16 ^{c}	2.08 ± 0.14 ^{d}	3.29 ± 0.16 ^{d}
	T2	9.41 ± 0.40 ^{b}	4.47 ± 0.22 ^{bc}	5.78 ± 0.34 ^{bc}
	T3	8.52 ± 0.27 ^{b}	3.88 ± 0.17 ^{c}	5.51 ± 0.21 ^{c}
	T4	10.68 ± 0.41 ^{a}	5.12 ± 0.29 ^{b}	6.55 ± 0.25 ^{b}
	T5	11.63 ± 0.74 ^{a}	6.09 ± 0.52 ^{a}	7.59 ± 0.54 ^{a}
	T6	6.25 ± 0.22 ^{c}	2.58 ± 0.24 ^{d}	3.65 ± 0.22 ^{d}
Second	T1	5.47 ± 0.19 ^{d}	2.37 ± 0.16 ^{b}	3.59 ± 0.24 ^{b}
	T2	9.66 ± 0.67 ^{b}	7.69 ± 0.45 ^{a}	8.33 ± 0.40 ^{a}
	T3	9.74 ± 0.62 ^{b}	6.47 ± 0.58 ^{a}	6.93 ± 0.42 ^{a}
	T4	7.50 ± 0.28 ^{c}	5.94 ± 1.07 ^{a}	9.55 ± 2.21 ^{a}
	T5	11.00 ± 0.54 ^{a}	7.23 ± 0.56 ^{a}	8.24 ± 0.46 ^{a}
	T6	5.28 ± 0.27 ^{d}	2.40 ± 0.19 ^{b}	3.51 ± 0.27 ^{b}
Third	T1	6.19 ± 0.18 ^{c}	4.40 ± 0.21 ^{b}	6.40 ± 0.25 ^{d}
	T2	12.81 ± 0.34 ^{b}	10.48 ± 0.23 ^{a}	11.91 ± 0.22 ^{bc}
	T3	13.09 ± 0.35 ^{b}	13.36 ± 3.40 ^{a}	11.74 ± 0.23 ^{c}
	T4	13.72 ± 0.33 ^{b}	11.08 ± 0.30 ^{a}	12.54 ± 0.21 ^{b}
	T5	15.13 ± 0.44 ^{a}	13.29 ± 0.42 ^{a}	13.52 ± 0.42 ^{a}
	T6	5.69 ± 0.21 ^{c}	5.93 ± 1.50 ^{b}	6.49 ± 0.22 ^{d}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to Duncan's multiple range test (p = 0.05). T1=Control, T2=0.5% SS, T3=0.5% SS+SK-1, T4=0.25% SS+0.25% FCM, T5=T2% FCM and T6=SK-1.

Table C.4 Fresh and dry weight, percentage of fresh weight loss and overall visual quality of 'Red Oak' lettuce using SS and FCM at different combination during the three successive crop seasons: March-April, 2010 (first), July-September, 2010 (second), and December 2010-February 2011 (third) in a test plot. *

Crop season	Treatment	Fresh weight (g)	Dry weight (g)	Fresh weight loss (%)	Overall visual quality (score)
First	T1	0.42 ± 0.06 ^{d}	0.04 ± 0.08 ^{c}	33.06 ± 2.33 ^{b}	1.33 ± 0.18 ^{b}
	T2	2.74 ± 0.40 ^{c}	0.24 ± 0.03 ^{b}	27.40 ± 4.46 ^{ab}	2.50 ± 0.18 ^{a}
	T3	2.17 ± 0.24 ^{c}	0.14 ± 0.02 ^{b}	23.51 ± 1.18 ^{ab}	2.36 ± 0.16 ^{a}
	T4	4.73 ± 0.57 ^{b}	0.30 ± 0.03 ^{b}	31.76 ± 5.64 ^{b}	2.65 ± 0.14 ^{a}
	T5	6.58 ± 1.16 ^{a}	0.40 ± 0.07 ^{a}	17.86 ± 1.54 ^{a}	2.67 ± 0.18 ^{a}
	T6	0.54 ± 0.11 ^{d}	0.04 ± 0.01 ^{c}	31.51 ± 3.71 ^{b}	1.46 ± 0.17 ^{b}
Second	T1	0.54 ± 0.07 ^{c}	0.09 ± 0.01 ^{d}	41.55 ± 3.48 ^{c}	2.38 ± 0.27 ^{b}
	T2	6.37 ± 0.80 ^{a}	0.82 ± 0.06 ^{c}	20.63 ± 1.74 ^{a}	3.47 ± 0.19 ^{a}
	T3	3.78 ± 0.58 ^{ab}	0.84 ± 0.07 ^{c}	21.65 ± 2.10 ^{a}	3.40 ± 0.24 ^{a}
	T4	2.03 ± 0.26 ^{bc}	1.03 ± 0.07 ^{b}	19.53 ± 5.73 ^{a}	3.12 ± 0.21 ^{a}
	T5	8.87 ± 2.18 ^{a}	1.41 ± 0.10 ^{a}	17.93 ± 2.23 ^{a}	3.67 ± 0.25 ^{a}
	T6	0.58 ± 0.10 ^{c}	0.12 ± 0.04 ^{d}	30.98 ± 2.10 ^{b}	3.06 ± 0.19 ^{a}
Third	T1	1.99 ± 0.17 ^{d}	0.02 ± 0.00 ^{c}	22.71 ± 1.86 ^{a}	2.56 ± 0.13 ^{c}
	T2	16.71 ± 0.91 ^{c}	0.21 ± 0.05 ^{a}	8.35 ± 1.01 ^{a}	3.07 ± 0.13 ^{b}
	T3	18.29 ± 1.09 ^{bc}	0.09 ± 0.01 ^{bc}	12.07 ± 1.44 ^{bc}	3.40 ± 0.13 ^{b}
	T4	20.03 ± 0.89 ^{b}	0.07 ± 0.01 ^{bc}	8.72 ± 1.62 ^{ab}	3.31 ± 0.20 ^{b}
	T5	30.17 ± 1.91 ^{a}	0.17 ± 0.04 ^{ab}	6.32 ± 1.03 ^{a}	3.93 ± 0.07 ^{a}
	T6	1.83 ± 0.18 ^{d}	0.02 ± 0.00 ^{c}	19.38 ± 1.26 ^{a}	2.46 ± 0.14 ^{c}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to Duncan's multiple range test (p = 0.05). T1=Control, T2=0.5% SS, T3=0.5% SS+SK-1, T4=0.25% SS+0.25% FCM, T5=T2% FCM and T6=SK-1.

Table C.5 Leaf number, leaf width and leaf length, diameter of lettuce head of 'Butterhead' and 'Red Oak' lettuces using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Treatment	Leaf number/ plant	Leaf width (cm)	Leaf length (cm)	Diameter of lettuce head (cm)
First	BH-Control	17.02 ± 0.37 ^{b}	8.48 ± 0.12 ^{ns}	11.48 ± 0.15 ^{ns}	19.34 ± 0.22 ^{a}
	BH-FCM	19.57 ± 0.67 ^{a}	8.74 ± 0.23 ^{ns}	12.37 ± 0.17 ^{ns}	24.62 ± 0.44 ^{b}
Second	BH-Control	21.33 ± 0.57 ^{ns}	7.53 ± 0.13 ^{ns}	11.85 ± 0.16 ^{ns}	15.49 ± 0.26 ^{ns}
	BH-FCM	21.90 ± 0.87 ^{ns}	8.09 ± 0.28 ^{ns}	12.48 ± 0.22 ^{ns}	17.41 ± 0.41 ^{ns}
First	RO-Control	7.81 ± 0.34 ^{b}	7.82 ± 0.32 ^{b}	10.81 ± 0.33 ^{b}	15.80 ± 0.64 ^{b}
	RO-FCM	13.10 ± 0.30 ^{a}	13.89 ± 0.42 ^{a}	14.52 ± 0.21 ^{a}	25.88 ± 0.33 ^{a}
Second	RO-Control	9.51 ± 0.30 ^{b}	10.28 ± 0.38 ^{b}	12.08 ± 0.25 ^{b}	22.64 ± 3.42 ^{ns}
	RO-FCM	14.39 ± 0.29 ^{a}	13.61 ± 0.35 ^{a}	14.48 ± 0.18 ^{a}	24.54 ± 0.34 ^{ns}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). BH = 'Butterhead' and RO = 'Red Oak' lettuce.

Table C.6 Fresh and dry weight, percentage of fresh weight loss and overall visual quality ‘Butterhead’ and ‘Red Oak’ lettuces using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Treatment	Fresh weight (g)	Dry weight (g)	Fresh weight loss (%)	Overall visual quality (score)
First	BH-Control	22.41 ± 1.32 [b]	0.90 ± 0.06 [b]	11.86 ± 0.81 [b]	2.60 ± 0.17 [b]
	BH-FCM	53.11 ± 4.01 [a]	1.56 ± 0.08 [a]	8.30 ± 1.28 [a]	3.50 ± 0.12 [a]
Second	BH-Control	30.28 ± 1.62 [b]	0.77 ± 0.03 [ns]	8.82 ± 0.96 [a]	2.75 ± 0.10 [ns]
	BH-FCM	40.09 ± 2.49 [a]	0.87 ± 0.06 [ns]	11.62 ± 1.11 [b]	3.00 ± 0.12 [ns]
First	RO-Control	3.21 ± 0.31 [b]	0.16 ± 0.02 [b]	21.07 ± 2.20 [b]	2.88 ± 0.37 [b]
	RO-FCM	42.06 ± 3.12 [a]	1.38 ± 0.68 [a]	10.69 ± 1.19 [a]	3.53 ± 0.18 [a]
Second	RO-Control	13.32 ± 0.71 [b]	0.27 ± 0.02 [b]	15.44 ± 1.55 [b]	2.35 ± 0.15 [b]
	RO-FCM	30.34 ± 0.92 [a]	1.22 ± 0.05 [a]	7.88 ± 0.87 [a]	3.30 ± 0.09 [a]

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). BH ‘Butterhead’ and RO = ‘Red Oak’ lettuce.

Table C.7 Net photosynthesis, transpiration rate and stomatal conductance of 'Butterhead' and 'Red Oak' lettuces using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Treatment	Net photosynthesis ($\mu l\ CO_2 \cdot min^{-1} \cdot g^{-1}$)	Transpiration rate ($mol\ m^{-2}\ s^{-1}$)	Stomatal conductance ($mol\ m^{-2}\ s^{-1}$)
First	BH-Control	11.97 ± 0.24 ^{ns}	6.81 ± 0.16 ^{ns}	0.52 ± 0.02 ^{ns}
	BH-FCM	11.99 ± 0.33 ^{ns}	6.84 ± 0.15 ^{ns}	0.51 ± 0.02 ^{ns}
Second	BH-Control	12.20 ± 0.44 ^{ns}	7.40 ± 0.16 ^{ns}	0.49 ± 0.02 ^{ns}
	BH-FCM	13.10 ± 0.47 ^{ns}	7.63 ± 0.12 ^{ns}	0.50 ± 0.01 ^{ns}
First	RO-Control	7.03 ± 0.34 ^{a}	6.54 ± 0.27 ^{ns}	0.45 ± 0.03 ^{ns}
	RO-FCM	9.64 ± 0.42 ^{b}	7.36 ± 0.37 ^{ns}	0.55 ± 0.04 ^{ns}
Second	RO-Control	8.35 ± 0.35 ^{a}	6.91 ± 0.27 ^{a}	0.44 ± 0.03 ^{a}
	RO-FCM	10.39 ± 0.29 ^{b}	7.97 ± 0.18 ^{b}	0.57 ± 0.03 ^{b}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). BH 'Butterhead' and RO = 'Red Oak' lettuce.

Table C.8 Nitrate contents of 'Butterhead' and 'Red Oak' lettuces using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Treatment	Nitrate contents (g/Kg FW)	
		Storage time (Days)	
		0 Day	14 Days
First	BH-Control	23.40 ± 2.44 ^{ns}	16.34 ± 1.69 ^{ns}
	BH-FCM	28.07 ± 3.87 ^{ns}	21.44 ± 2.04 ^{ns}
Second	BH-Control	14.14 ± 1.45 ^{ns}	16.95 ± 1.01 ^{ns}
	BH-FCM	14.79 ± 1.33 ^{ns}	15.01 ± 1.54 ^{ns}
First	RO-Control	26.32 ± 7.54 ^{ns}	12.08 ± 0.35 ^{b}
	RO-FCM	24.45 ± 7.22 ^{ns}	21.82 ± 0.89 ^{a}
Second	RO-Control	15.82 ± 0.76 ^{a}	18.09 ± 0.62 ^{ns}
	RO-FCM	12.99 ± 0.44 ^{b}	15.69 ± 1.35 ^{ns}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). BH 'Butterhead' and RO = 'Red Oak' lettuce.

Table C.9 Chlorophyll *a*, *b* and carotenoid contents of 'Butterhead' lettuce using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Storage time (Days)	Treatment	Chlorophyll *a* (μmol/g FW)	Chlorophyll *b* (μmol/g FW)	Carotenoid (μmol/g FW)
First	0	BH-Control	42.71 ± 3.95 ^{ns}	231.80 ± 22.96 ^{ns}	24.10 ± 2.25 ^{ns}
		BH-FCM	42.94 ± 5.90 ^{ns}	265.37 ± 34.30 ^{ns}	26.65 ± 3.36 ^{ns}
	14	BH-Control	22.99 ± 1.65 ^{ns}	123.51 ± 8.49 ^{ns}	12.84 ± 0.86 ^{ns}
		BH-FCM	32.70 ± 5.34 ^{ns}	176.82 ± 28.62 ^{ns}	17.80 ± 3.10 ^{ns}
Second	0	BH-Control	32.90 ± 6.55 ^{ns}	178.01 ± 34.92 ^{ns}	18.54 ± 3.69 ^{ns}
		BH-FCM	31.43 ± 4.70 ^{ns}	173.10 ± 26.58 ^{ns}	17.84 ± 2.67 ^{ns}
	14	BH-Control	35.85 ± 3.65 ^{ns}	82.50 ± 8.43 ^{ns}	20.02 ± 2.00 ^{ns}
		BH-FCM	35.69 ± 1.86 ^{ns}	73.79 ± 4.47 ^{ns}	20.08 ± 1.07 ^{ns}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). BH = 'Butterhead' lettuce.

Table C.10 Chlorophyll *a*, *b* and carotenoid contents of 'Red Oak' lettuce using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Storage time (Days)	Treatment	Chlorophyll *a* (μmol/g FW)	Chlorophyll *b* (μmol/g FW)	Carotenoid (μmol/g FW)
First	0	RO-Control	55.86 ± 5.13 ^{ns}	284.86 ± 24.85 ^{ns}	31.16 ± 2.81 ^{ns}
		RO-FCM	40.02 ± 7.17 ^{ns}	211.31 ± 37.75 ^{ns}	22.47 ± 4.03 ^{ns}
	14	RO-Control	47.90 ± 3.23 ^{a}	248.10 ± 16.04 ^{b}	26.76 ± 1.80 ^{b}
		RO-FCM	61.80 ± 3.17 ^{b}	327.62 ± 17.97 ^{a}	34.69 ± 1.80 ^{a}
Second	0	RO-Control	40.93 ± 6.52 ^{ns}	200.03 ± 35.25 ^{ns}	22.60 ± 3.71 ^{ns}
		RO-FCM	36.83 ± 3.77 ^{ns}	185.12 ± 23.30 ^{ns}	20.48 ± 2.19 ^{ns}
	14	RO-Control	64.80 ± 7.06 ^{a}	137.68 ± 11.38 ^{a}	36.14 ± 4.02 ^{a}
		RO-FCM	36.54 ± 3.32 ^{b}	88.31 ± 4.59 ^{b}	19.98 ± 1.93 ^{b}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). RO = 'Red Oak' lettuce.

Table C.11 Ascorbic acid, total phenolic and flavonoid contents of 'Butterhead' lettuce using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Storage time (Days)	Treatment	Ascorbic acid contents (mg/g FW)	Total phenolic contents (mg/g FW)	Flavonoid contens (mg/g FW)
First	0	BH-Control	17.13 ± 10.29 ^{ns}	1.01 ± 0.16 ^{ns}	2.25 ± 0.30 ^{ns}
		BH-FCM	0.95 ± 0.27 ^{ns}	1.63 ± 0.21 ^{ns}	3.29 ±0.38 ^{ns}
	14	BH-Control	27.73 ± 7.72 ^{ns}	1.02 ± 0.10 ^{ns}	1.84 ±0.46 ^{ns}
		BH-FCM	32.24 ± 8.15 ^{ns}	1.11 ± 0.16 ^{ns}	2.23 ±0.20 ^{ns}
Second	0	BH-Control	1.12 ± 0.31 ^{ns}	0.26 ± 0.13 ^{ns}	0.05 ±0.02 ^{ns}
		BH-FCM	5.95 ± 4.96 ^{ns}	0.14 ± 0.11 ^{ns}	0.04 ±0.01 ^{ns}
	14	BH-Control	11.84 ± 6.51 ^{ns}	0.97 ± 0.19 ^{ns}	0.47 ±0.39 ^{b}
		BH-FCM	0.57 ± 0.56 ^{ns}	1.38 ± 0.04 ^{ns}	1.99 ±0.13 ^{a}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). BH = 'Butterhead' lettuce.

Table C.12 Ascorbic acid, total phenolic and flavonoid contents of 'Red Oak' lettuce using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Storage time (Days)	Treatment	Ascorbic acid contents (mg/g FW)	Total phenolic contents (mg/g FW)	Flavonoid contens (mg/g FW)
First	0	RO-Control	93.39 ± 15.42 ^{ns}	3.25 ± 0.66 ^{ns}	7.28 ± 1.45 ^{ns}
		RO-FCM	68.44 ± 10.50 ^{ns}	2.61 ± 0.41 ^{ns}	6.52 ± 1.02 ^{ns}
	14	RO-Control	57.73 ± 13 .81 ^{ns}	2.99 ± 0.62 ^{ns}	6.10 ± 0.96 ^{ns}
		RO-FCM	27.83 ± 7.09 ^{ns}	2.10 ± 0.18 ^{ns}	3.85 ± 0.46 ^{ns}
Second	0	RO-Control	1.35 ± 0.21 ^{ns}	1.17 ± 0.18 ^{ns}	2.34 ± 0.57 ^{ns}
		RO-FCM	7.38 ± 5.95 ^{ns}	0.90 ± 0.10 ^{ns}	1.31 ± 0.48 ^{ns}
	14	RO-Control	51.69 ± 10.07 ^{ns}	2.64 ± 0.27 ^{ns}	4.41 ± 0.85 ^{ns}
		RO-FCM	58.09 ± 17.78 ^{ns}	2.94 ± 0.38 ^{ns}	6.00 ± 0.75 ^{ns}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). RO = 'Red Oak' lettuce.

Table C.13 DPPH radical svarvenging activity and contents of ‘Butterhead’ lettuce using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Storage time (Days)	Treatment	DPPH radical svarvenging activity (%)	MDA (μmol/g FW)
First	0	BH-Control	67.96 ± 7.56 ^{ns}	0.41 ± 0.04 ^{ns}
		BH-FCM	61.37 ± 6.41 ^{ns}	0.71 ± 0.41 ^{ns}
	14	BH-Control	71.48 ± 6.33 ^{ns}	0.10 ± 0.19 ^{ns}
		BH-FCM	83.80 ± 9.01 ^{ns}	0.11 ± 0.15 ^{ns}
Second	0	BH-Control	99.18 ± 4.56 ^{ns}	0.19 ± 0.02 ^{ns}
		BH-FCM	99.82 ± 1.83 ^{ns}	0.19 ± 0.02 ^{ns}
	14	BH-Control	84.75 ± 3.94 ^{a}	0.01 ± 0.20 ^{ns}
		BH-FCM	72.44 ± 2.91 ^{b}	0.34 ± 0.30 ^{ns}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). BH = ‘Butterhead’ lettuce.

Table C.14 DPPH radical svarvenging activity and contents of ‘Red Oak’ lettuce using FCM during the two crop seasons: January-March, 2012 (first) and May-July 2012 (second) in a local farm. *

Crop season	Storage time (Days)	Treatment	DPPH radical svarvenging activity (%)	MDA (μmol/g FW)
First	0	BH-Control	53.14 ± 7.93 ^{ns}	8.5823 ± 1.16 ^{a}
		BH-FCM	51.10 ± 7.77 ^{ns}	3.8285 ± 1.60 ^{b}
	14	BH-Control	49.08 ± 9.92 ^{ns}	6.3957 ± 1.16 ^{ns}
		BH-FCM	53.55 ± 7.72 ^{ns}	4.7231 ± 1.47 ^{ns}
Second	0	BH-Control	86.14 ± 3.87 ^{ns}	0.8676 ± 0.32 ^{ns}
		BH-FCM	90.40 ± 3.55 ^{ns}	1.2409 ± 0.46 ^{ns}
	14	BH-Control	51.57 ± 4.22 ^{ns}	3.2432 ± 0.47 ^{ns}
		BH-FCM	41.05 ± 3.15 ^{ns}	3.3548 ± 0.47 ^{ns}

*Values are means ± S.E. Means followed by different letters within each season/ parameter combination differ significantly according to T-Test (p<0.05). RO = ‘Red Oak’ lettuce.

VITA

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