CHAPTER 5

GROWTH OF CHINESE KALE IN RESPONSE TO LEONARDITE (HIGH HUMUS MATERIAL) AND BENEFICIAL MICROORGANISMS

5.1 Introduction

Many studies have shown that crop yield and nutritional quality were improved by organic fertilizers and/or beneficial microorganisms. Organic fertilizers usually promote the growth and activities of microorganisms. The value of organic fertilizer (humus) is created mainly by microorganisms. In a study by Hanane *et al*. (2008) inoculation of actinomycetes strains to wheat grown in from the soil supplemented with rock phosphate (RP) significantly promoted the growth of the wheat plants. It had the greatest stimulatory effect on plant growth with a 47-50 percent and 78-80 percent increase in shoot and root weight, respectively, in comparison with the sterile control.

Humic substance has been recognized as the factor in soil organic matter that directly affects the physical, chemical, and microbiological properties of the soil, and indirectly affects plant growth (Fernandez-escobar *et al*., 1996). The addition of humic substances to soils low in organic matter, or in nutrient solutions, have produced very significant responses in plant growth.

Leonardite contains high humic substances, which include humic acid, fulvic acid, and humins. It is the formed from the decomposition of a type of lignite coal.

Leonardite is typically used in agriculture as a fertilizer. Fernandez-escobar *et al.* (1996) reported that in young olive plants the foliar application of leonardite extracts stimulated shoot growth even without the addition of mineral elements to the irrigation water. Under field conditions the application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, B, Mg, Ca, and Fe in leaves.

Chinese kale (*Brassica oleracea* var. alboglabra) is one of the most commonly grown leafy vegetables in northern Thailand. Organic cultivation systems must exclude the use of synthetic fertilizers, pesticides, and insecticides. Improvement of the nutritional value of Chinese kale by bio-organic inputs in the production system is of interest and could lead to better consumer health. The humus content in organic fertilizer is a key component of its value in an agricultural system. Little consideration has previously been given to humus content in organic fertilizer, which plays an important role in crop yield and nutritional value improvement.

The objective of the present work was to study the effect of high humus materials (leonardite) added to organic substrates in various rates with and without beneficial microorganism to evaluate their effects on growth parameters and nutrient content of Chinese kale seedlings.

5.2 Materials and methods

5.2.1 Plant material and culture conditions

Screenhouse experiments were conducted at the Faculty of Agriculture, Chiang Mai University, Thailand. Randomized complete block (RCB) design was used in this experiment. Selected seedling media (SSM) composed of coconut husk

compost without leonardite and microbial addition was used as the control treatment. The selected seedling media was mixed with leonardite (humic acid = 57.96%) at various rates: 5%, 10% and 15% (w/v). After one week of germination, three selected isolates (*Azospirillum* VAs 2., *Beijerinckia* VBe 75. and actinomycetes Vac 77) were inoculated as a single isolate to Chinese kale (*Brassica oleracea* var. alboglabra) seedlings (10^7 CFU/plant) and raised in SSM with and without leonardite (16 treatments, 3 replications). All of the seedlings were raised in plastic seedling trays. Growth parameters such as shoot and root dry weight, and nutrient uptake were measured 20 days after inoculation. The treatments used in each experiment were as follows:

1) Control (selected seedling media, SSM)
2) SSM + VAs 2
3) SSM + VBe 75
4) SSM + VAc 77
5) SSM + 5% leonardite
6) SSM + 10% leonardite
7) SSM + 15% leonardite
8) SSM+ VAs 2 + 5% leonardite
9) SSM+ VBe 75 + 5% leonardite
10) SSM+ VAc 77 + 5% leonardite
11) SSM+ VAs 2 + 10% leonardite
12) SSM+ VBe 75 +10% leonardite

13) SSM+ VAc 77 + 10% leonardite

14) SSM+ VAs 2 + 15% leonardite

15) SSM+ VBe 75 +15% leonardite

16) SSM+ VAc 77 + 15% leonardite

5.2.2 Growth measurement and chemical analysis

At harvesting time, Chinese kale seedlings were carefully removed from each plastic seedling tray and the roots were washed thoroughly with tap water. The seedlings were then divided into roots and shoots, and were oven dried at 60 °C until constant dry weights were recorded. Dried shoots were analyzed for nutrient content as described by Bremner (1965) for total nitrogen (N), by Walinga *et al*. (1989) for total phosphorus (P), calcium (Ca), and magnesium (Mg) and by Kalra (1998) for total potassium (K).

Humic acid in leonardite was analysed. The extract solutions were the mixture of 0.5 M NaOH and 0.15 M $Na_4P_2O_7$ (pH 7) at a ratio of 1:1 (v/v). The sample (1.2 g) was transferred into a 50 mL centrifuge tube containing 25 mL of the extractant. All the samples were shaked for 24 hr at 120 rpm. After shaking all the tubes were centrifuged at 5,000 g for 12 min. Then 20 mL of the supernatant of each sample was collected and 4mL of 6N HCl was added. The mixture of each sample was again centrifuge. Then the precipitate of humic acid (Na-humate) was taken and oven-dried. The constant dry weigh of Na-humate was recorded (Deborah and Burbab, 1999).

Table 5.1 Chemical properties of leonardite and selected seedling media

Sample	pH	EC(mS/cm)	Humic acid (%)
leonardite	2.51	3.98	57.96
SSM	6.49	2.27	26.06
SSM + 5% leonardite	5.15	3.26	27.87
SSM + 10% leonardite	5.03	3.51	30.75
SSM + 15% leonardite	4.92	3.90	35.49

5.2.3 Statistical analysis and calculation

Analysis of variance for shoot and root dry weight as well as nutrient uptake (N, P, K, Ca, and Mg) was done by using Statistix 8.0 (Tallahassee, FL, USA).

5.3 Results and discussion

5.3.1 Effect of beneficial microorganisms and leonardite on the growth of Chinese kale seedlings

In general, the growth of Chinese kale seedlings appeared to be improved by application of beneficial microorganisms together with leonardite especially for shoot dry weight. The shoot dry weights of all of the treatments were higher than that of the control except for treatments 2, 3, and 9 (Table 5.1). The results demonstrated that SSM plus leonardite and microorganisms, specifically treatment 16 (SSM with actinomycetes + 15% leonardite), treatment 12 (SSM with *Beijerinckia* spp. + 10% leonardite), and treatment 10 (SSM with *Azospirillum* spp. + 5% leonardite), gave the highest shoot dry weights with values of 1.69, 1.65, and 1.62 g/10plants, respectively. Treatment 16 also gave the highest root dry weight with a value of 0.21 g/10plants. The next highest root dry weights were observed for treatment 8 (SSM +*Azospirillum* spp. + 5% leonardite) and treatment 14 (SSM with *Azospirillum* spp. + 15% leonardite) with values of 0.20 and 0.18 g /10plants, respectively. These results

indicated that application of beneficial microorganism together with high humus materials promoted seedling growth parameters. Shoot and root growth enhancement in response to inoculation with beneficial microorganisms was reported by other researchers. Khalid *et al.* (2004) found that inoculation of wheat with high IAA producing rhizobacteria gave up to 13.5% and 37.7% increases in root and shoot elongation, respectively. Furthermore, Jacoud *et al*. (1999) inoculated *Azospirillum lipoferum* to maize seedlings and suggested that it increased the root surface area of maize. Our results suggested that the application of actinomycetes plus 15% leonardite effectively promoted both shoot and root dry weights of Chinese kale seedlings.

Table 5.2 Shoot and root dry weight of Chinese kale seedlings grown in selected seedling media inoculated with beneficial microorganisms

Treatment	Dry weight (g/10plants)	
	Shoot	**Root**
1. Control (Selected Seedling Media, SSM)	1.48 bcd[/1]	0.165 abcd
2. SSM + *Azospirillum* sp. (VAs 2)	1.39 de	0.15 bcd
3. SSM + *Beijerinckia* sp. (VBe 75)	1.21 e	0.17 abcd
4. SSM + actinomycetes (VAc 77)	1.57 abcd	0.12 cd
5. SSM + 5% leonardite	1.55 abcd	0.17 abcd
6. SSM + 10% leonardite	1.56 abcd	0.16 abcd
7. SSM + 15% leonardite	1.53 abcd	0.15 abcd
8. SSM+*Azospirillum* sp. (VAs 2)+ 5% leonardite	1.56 abcd	0.20 ab
9. SSM+ *Beijerinckia* sp. (VBe 75)+ 5% leonardite	1.45 cd	0.16 abcd
10. SSM+ actinomycetes (VAc 77)+ 5% leonardite	1.62 abc	0.17 abcd
11. SSM+*Azospirillum* sp. (VAs 2)+ 10% leonardite	1.51 abcd	0.11 d
12. SSM+ *Beijerinckia* sp. (VBe 75)+10% leonardite	1.65 ab	0.14 bcd
13. SSM+ actinomycetes (VAc 77)+ 10% leonardite	1.53 abcd	0.13 cd
14. SSM+*Azospirillum* sp. (VAs 2)+ 15% leonardite	1.62 abc	0.18 abc
15. SSM+ *Beijerinckia* sp. (VBe 75)+15% leonardite	1.55 abcd	0.15 abcd
16. SSM+ actinomycetes (VAc 77)+ 15% leonardite	1.69 a	0.21 a
F – test	*	*
C.V (%)	7.81	23.64

* *indicates the effect is significant at P< 0.05*

[/1] *Values within each column followed by same letter are not significantly different at P < 0.05*

5.3.2 Effect of beneficial microorganisms and leonardite on nutrient uptake in Chinese kale seedlings

The application of beneficial microorganisms and leonardite to Chinese kale seedling not only enhanced growth, but also significantly increased nutrient uptake. Nutrient uptake in Chinese kale seedlings was measured for all of the treatments and is shown in Table 5.2. In general uptake of N, P, Ca, and Mg for all of the treatments appears to be higher or significantly higher than those of the control except for treatments 2 and 3. The results showed that higher N uptake was obtained with treatment 4 (SSM with actinomycetes), treatment 11 (SSM with *Azospirillum* + 10% leonardite) and treatment 6 (SSM with 10% leonardite) with the amounts of 60.42, 52.96, and 51.87 mg/10plants, respectively. The P uptake value was significantly higher than that of the control (10.09 mg/10plants) in treatment 16 (SSM with actinomycetes + 15% leonardite) with a value of 10.92 mg/10plants, treatment 5 (SSM + 5% leonardite) with a value of 10.58 mg/10plants, and treatment 6 (SSM + 10% leonardite) with a value of 10.39 mg/10plants. Inoculation with actinomycetes in treatment 4 (SSM with actinomycetes) gave the highest value of K uptake (132.73 mg/10plants). The highest value of Ca uptake was obtained in treatment 14 (SSM + *Azospirillum* spp. + 15% leonardite) with a value of 23.85 mg/10plants which was significantly higher than that of the control (13.57 mg/ 10plants). The maximum level of Mg uptake was achieved in treatment 16 (SSM with actinomycetes + 15% leonardite) with a value of 6.77 mg/10plants, which was significantly higher than that of the control (5.55 mg/10plants). Overall the amount of nutrient uptake was increased when actinomycetes was inoculated with a high rate of leonardite.

Table 5.3 Nutrient uptake by Chinese kale seedlings inoculated with microorganisms in selected seedling media.

Treatment No.	Nutrient uptake (mg/10plants)				
	N	P	K	Ca	Mg
1	41.97 efg[/1]	10.09 abc	108.33bc	13.57 gh	5.55 cdef
2	37.57 g	9.08 bc	99.87 cd	11.94 hi	4.78 fg
3	28.28 h	7.41 d	88.27 d	10.40 i	3.81 g
4	60.42 a	9.53 abc	132.73 a	17.26 cdef	6.56 abc
5	44.70 cdefg	10.58 a	115.10 b	16.42 efg	5.43 def
6	51.87 bc	10.39 ab	99.52 cd	21.33 ab	6.60 ab
7	42.31 defg	9.90 abc	90.69 bcd	20.45 b	5.84 abcde
8	41.12 efg	10.23 abc	100.53 bcd	15.95 fg	5.20 ef
9	44.37 cdefg	8.86 c	98.47 cd	16.66 defg	5.40 def
10	43.29 defg	10.38 ab	106.18 bc	16.71 def	5.53 cdef
11	52.96 ab	8.91 c	101.00 bcd	18.84 bcdef	5.68 bcdef
12	50.27 bcd	9.85 abc	107.78 bc	20.01 bc	6.28 abcd
13	46.99 bcdef	9.09 bc	99.84 cd	19.76 bcd	6.68 ab
14	48.30 bcde	9.79 abc	102.03 bcd	23.85 a	6.49 abc
15	40.09 fg	9.97 abc	97.16 cd	19.25 bcde	5.85 abcde
16	46.23 bcdef	10.92 a	110.69 bc	21.31 ab	6.77 a
F – test	**	**	**	**	**
C.V (%)	10.89	8.84	8.73	10.51	10.80

* *indicates the effect is significant at P< 0.01*

/1 *Values within each column followed by same letter are not significantly different at P < 0.01*

5.4 Conclusion

Growth parameters and nutrient uptake of Chinese kale seedlings were improved by microbial and leonardite application. Compared with the control, selected seedling media (SSM) with actinomycetes plus 15% leonardite was able to promote good plant growth and gave the highest shoot and root dry weight. The nutrient uptake (N, P, K, Ca, and Mg) of Chinese kale seedlings obtained by this treatment was also quite high compared with the rest of the treatments. Our results suggested that good growth and high nutrient uptake in the seedlings would therefore ensure good performance and high quality vegetables after transplanting.