CHAPTER 4

EFFECTIVENESS OF SINGLE AND MULTIPLE MICROBIAL INOCULATIONS ON GROWTH AND NUTRIENT UPTAKE OF CHINESE KALE SEEDLINGS

4.1 Introduction

Currently, the environmental pollution caused by the use of agricultural chemicals is a major concern. This is particularly true in areas such as watersheds where a plentiful amount of vegetable production uses agricultural chemicals in high amounts. The resulting environmental pollution is quickly developing into a cause of concern for human health. The problem can be minimized by switching from conventional agriculture to organic agriculture. By doing such a practice, the use of biotechnology, especially microbiology is important. In northern Thailand, Chiang Mai province is a main supplier of vegetables that are used both domestic and for export. This region of Thailand is suitable for vegetable production but has a high risk of pesticide contamination to the environment, especially the highland watersheds. Development of bio-organic seedling media for use in greenhouse vegetable production should be the first priority suitable seedling media help promoting the survival rate and growth of seedling while reducing the amount of chemical usage. As a result, the reduction of toxic contamination into the environment can ensure that seedlings will have a complete root system and grow well (Shutsrirung *et al*., 2009). In addition, the development of bio-organic seedling media could decrease imports from abroad, thus promoting the use of local materials. In addition, a study on the use of quality seedling media with beneficial microorganisms showed that inoculation increased crop yield in shoot and root weight as well as increased seedling survival rate after transplantation (Vestberg, 2004).

The use of beneficial microorganisms such as *Azospirillum*, *Beijerinkia* and *Azotobacter* is a way to increase the nutritional quality and yield of vegetable crops. It also helps promote growth and disease-resistance, which in turn allows a higher survival rate of the seedlings. This can be extended and applied to improve the soil and productivity, as well as reduce chemical fertilizer usage and the cost of production (Jacoud *et al*., 1999; Swaminathan and Srinivasan, 2006).

High quality seedling medias are the common factor in the good product system. Savitri (2002) mixed the sand with coconut husk compost and found that can promotes Crystal White growth and used as a peat. The development of seedling media is important because the use of bacteria has a beneficial effect on nutrient content and growth of Chinese kale seedlings. The use of seedling media containing bacteria would provide an alternative to using chemical fertilizer to produce high quality seedlings. Inoculation of seedlings with selected microorganisms would not only be beneficial to vegetable production but also decrease agricultural waste and help develop a system of sustainable agriculture (Shutsrirung, 2005).

This experiment studied the effects of using suitable seedling media containing beneficial microorganisms on the growth and nutrient uptake of Chinese kale seedlings based on previous experimental findings (Shutsrirung, 2005).

4.2 Materials and Methods

This study evaluated the effect of inoculation with coconut husk seedling media containing beneficial microorganisms on the growth and nutrients uptake of Chinese kale (*Brassica oleracea* var. alboglabra) seedlings. Culativation took place in the screenhouse of the Faculty of Agriculture, Chiang Mai University, Thailand. Randomized complete block design (RCBD) was used in this experiment. The control treatment was selected seedling media (SSM) alone, which was coconut husk compost, without beneficial microorganisms. In the treatments with beneficial microorganisms, three selected isolates *Azospirillum* sp. (VAs 2), *Beijerinckia* sp. (VBe 75), and actinomycetes (VAc 77) were inoculated as single and multiple isolates to Chinese kale seedlings (10^7 CFU per plant) after one week of germination. The treatments used in each experiment were as follows:

1) control (SSM),
2) SSM + VAs 2,
3) SSM + VBe 75
4) SSM + VAc 77
5) SSM +VAs 2 + VBe 75
6) SSM +VBe 75 + VAc 77
7) SSM +VAs 2 + VAc 77
8) SSM + VAs 2 + VBe 75 + VAc 77

The growth parameters shoot and root dry weight along with nutrient uptake were measured at 20 DAI.

4.2.1 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).

4.2.2 Statistical analysis and calculation

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

4.3 Results and discussion

4.3.1 Effect of beneficial microorganisms on the growth of Chinese kale seedlings

The shoot dry weight was significantly increased in treatment 8 with the three isolates combined (VAs 2 + VBe 75 + VAc 77) with a value of 2.93 g /10plants ($p<0.01$, Table 4.1). This value was 20 percent higher than the control, which had a shoot dry weight of 2.43 g /10plants. The treatments with one or two isolates did not exceed the dry weight of the control.

The root dry weight that resulted from treatment 4 (VAc 77) had the highest value with 0.36 g /10plants. It was significantly higher than control (0.27 g /10plants) by 32 percent ($p<0.01$). The next highest was treatment 6 (VBe 75 + VAc 77), which resulted in a root dry weight of 0.31 g /10plants that was 12 percent higher than the control treatment.

From these studies, only treatment 8 with the three isolates (VAs 2 + VBe 75+ VAc 77) resulted in a higher shoot dry weight than the control. According to Solans *et al*., 2009 when actinomycetes were co-inoculated with *S. meliloti*, nodulation and plant growth were significantly stimulated compared to plants inoculated with only *S. meliloti*. Greqor *et al*.(2003) inoculated of three mutant strains of *B. japonicum* with *S. kanamyceticus*, significant increases in nodule occupancy (up to 55%). Increases in shoot nitrogen composition (27.1%-40.9%) were also caused by co-inoculation with S. kanamyceticus. However, when the three selected actinomycete isolates colonized cucumber roots endophytically for 8 weeks, they promoted plant growth and suppressed pathogenic activities of *P. aphanidermatum* on seedling and mature cucumber plants (El-Tarabily *et al*., 2009). The combination of the three isolates resulted in significantly better suppression of diseases and plant growth promotion, than where the plants were exposed to individual strains. Cassa *et al*. (2009) reported that maize and soybean were inoculated with *Azospirillum brasilense* Az3 and *Bradyrhizobium japonicum* E109 the single and co-inoculations showed the capacity to promote seed germination, nodule formation, and early development of seedlings.

Gadagi *et al*. (2004) found that inoculation with *Azospirillum* strain OAD-2 on *Gaillardia pulchella* showed significantly increased plant height, number of leaves per plant, branches per plant, and total dry mass accumulation than other inoculations and/or uninoculated control. Fallik *et al*. (1989) studied the inoculation of maize seedlings with *Azospirillum* and found that it significantly increased the root surface area as compared to non-inoculated plants. Nuti *et al*. (2008) examined the inoculation of the tomato (*Lycopersiconesculentum*) with *Bacillus subtilis* strain 101 combined with *Azospirillum brasilense* Sp245. The co-inoculation enhanced root growth and development.

Table 4.1 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	2.43 b[/1]	(100%)	0.27 bc	(100%)
2. VAs 2	2.36 bc	(97%)	0.28 bc	(103%)
3. VBe 75	2.41 b	(99%)	0.25 c	(90%)
4. VAc 77	2.28 bc	(94%)	0.36 a	(132%)
5. VAs 2 + VBe 75	2.23 bcd	(92%)	0.28 bc	(103%)
6. VBe 75 + VAc 77	2.15 cd	(88%)	0.31 b	(112%)
7. VAs 2 + VAc 77	2.02 d	(83%)	0.25 c	(90%)
8. VAs 2 + VBe 75 + VAc 77	2.93 a	(120%)	0.26 bc	(95%)
F – test	**		**	
C.V. (%)	5.25		10.58	

** *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*

4.3.2 Effect of beneficial microorganisms on nutrient uptake in Chinese kale seedlings

The nutrient uptake of Chinese kale seedlings for all of the treatments are shown in Table 4.2 and all of the nutrients studied were significantly increased compared to control (p< 0.01), except for P uptake. A high N uptake was obtained in treatment 8 with the three isolates combined (VAs 2 + VBe 75+ VAc 77) with a value of 95.1 mg/10plants, which was increased by 61 percent compared to the control treatment of 59.2 mg/10plants. The treatments with single isolate followed by the treatments with two isolates, all had higher N uptake compared to control. The P uptake value was not significantly changed in any of the treatments with single or multiple isolates; however, the values were slightly increased except for treatment 6. Treatment 4 (VAc 77) gave the highest P uptake with a value of 17.2 mg/10plants, which was only 19 percent higher than control. Treatment 4 also gave the highest value for K uptake with 93.3 mg/10plants. This was significantly higher than control treatment (33.9 mg/10plants) by175 percent. The highest value of Ca uptake was obtained in treatment 8 (VAs 2 + VBe 75+ VAc 77) with a value of 12.9 mg/10plants which was significantly higher than the control (17.8 mg/10plants) by 65 percent. The Mg uptake of all of the treatments except treatment 7 was significantly higher than control. The maximum Mg value observed was in treatment 8 (VAs 2 + VBe 75+ VAc 77) with a value of 10.6 mg/10plants. It was 25 percent higher than the control treatment (8.5 mg/10plants).

Table 4.2 Nutrient uptake by Chinese kale seedlings grown in selected seedling media inoculated with beneficial microorganisms

Treatment	Nutrient uptake (mg/10plants)				
	N	**P**	**K**	**Ca**	**Mg**
1. Control	59.2 f (100%)	14.5 (100%)	33.9 c (100%)	7.8 d (100%)	8.5 d (100%)
2. VAs 2	81.1 c (137%)	15.8 (109%)	71.5 ab (211%)	9.4 bcd (121%)	10.2 ab (120%)
3. VBe 75	87.6 b (148%)	16.2 (112%)	83.7 ab (247%)	8.6 cd (110%)	10.4 ab (122%)
4. VAc 77	88.7 b (150%)	17.2 (119%)	93.3 a (275%)	10.9 ab (140%)	9.5 bc (112%)
5. VAs 2 + VBe 75	68.8 e (116%)	16.9 (117%)	77.2 ab (228%)	9.6 bcd (123%)	9.6 bc (113%)
6. VBe 75 + VAc 77	75.4 d (127%)	16.1 (111%)	82.8 ab (244%)	9.9 bcd (127%)	9.9 abc (116%)
7. VAs 2 + VAc 77	69.9 e (118%)	15.2 (105%)	83.2 ab (245%)	10.3 bc (132%)	9.2 cd (108%)
8. VAs 2 + VBe 75 + VAc 77	95.1 a (161%)	13.6 (94%)	61.9 b (183%)	12.9 a (165%)	10.6 a (125%)
F – test	**	ns	**	**	**
C.V (%)	2.28	9.16	21.83	12.41	5.90

*** 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*
ns indicates the effect is not significant at P< 0.01

This experiment showed that although the root dry weight increased only in treatments 2, 4, 5, and 6. In summary the inoculation of seedlings specifically with the combination of the three isolates significantly increased the uptake of all of the studied nutrients with the execption of phosphorus. This is consistent with the results from Gadagi *et al*. (2004) who found that the *Azospirillum* strain OAD-2 resulted in an increased uptake nitrogen in *Gaillardia pulchella* when compared to the control treatment. Teixeira *et al*. (2009) found that applying the bacteria *Azospirillum brasilense* REC3 (S1) to gain increased nitrogen uptake. The trial of Kapulnik *et al*. (1985) found that *Azospirillum brasilense* inoculation on wheat seeds (*Triticum aestivum* L.) positively affects the growth of roots. The uptake of nutrients was improved when the roots grew well and this resulted in a higher volume of growth and productivity in a study by Akbar *et al*. (2009) after inoculation of *Azospirillum basilense* (native or Sp7) and *Rhizobium meliloti* (native or DSMZ 30135) plus 2,4-D in wheat. The weight and yield of wheat increased by 26 and 22 percent, respectively. The levels of the nutrients nitrogen, phosphorus, and potassium were increased in the inoculated wheat. The inoculation with *Azospirillum basilense* (native or Sp7) resulted in an increase in productivity and a 29 percent increase in the amount of nitrogen, a 22.8 percent increase in phosphorus, and a 59.5 percent increase in potassium.

4.4 Conclusion

Single and multiple microbial isolates were used to study the effects on growth and uptake of nutrients. The inoculation treatments were very effective in promoting the growth of the dry weight of Chinese kale especially the multiple microbial inoculation with *Azospirillum* 2 (VAs 2), *Beijerinckia* 75 (VBe 75), and actinomycetes 77 (VAc 77). This resulted in the maximum shoot dry weight observed of 2.93 g /10plants, which was 20 percent higher than the control. For actinomycetes VAc 77, the root dry weight of 0.36 g /10plants was higher than the control by 32 percent and significantly increased the uptake of nutrients. Additionally, all of the elements (nitrogen, phosphorus, potassium, calcium and magnesium) had a higher amount than the control, with nitrogen showing an increase of 61 percent. The only mineral that did not have statistically significant increase in nutrient uptake was phosphorus.