CHAPTER 3

SCREENING OF BENEFICIAL MICROORGANISMS FOR CHINESE KALE SEEDLING PRODUCTION

3.1 Introduction

Beneficial microorganisms (BMs) include plant growth promoting rhizobacteria (PGPR) , associative and endophytic bacteria, actinomycetes, and fungi. These groups of BMs have been found to enchance the growth and health of various agricultural crops. Many studies have shown that the production of growth promoting substances such as gibberellins and auxins promote plant growth by stimulating adventitious roots and root hair formation and elongation (Strzelczyk and Pokojska-Burdziej, 1984; Finnie and Van Staden, 1985). Increased production of plant growth hormones stimulates nutrient uptake and results in a pronounced effect on plant growth and development. The most active auxin in plants is indole-3-acetic acid (IAA), which can be synthesized in culture media by various microorganisms when supplemented with L-tryptophan.Wheat seedlings that were treated with *Azospirillum brazilance* had an increased number and length of lateral roots (Barbieri *et al*., 1986.). A study on Florida's vegetable transplant industry looked at mixed inoculums of mycorrhiza (*Glomus intraradices*), actinomycetes (*Streptomyces griseovirdis*), and fungi (*Trichoderma harzianum*) that were used for tomato and pepper seedlings. The use of this microbial treatment resulted in a 26% increase in fruit size and yield over the control (Nemec, 1994). PGPR and other BMs are also known to fix nitrogen and solubilize insoluble and inorganic phosphates thus increasing nutrient availability for plant growth. It is quite obvious that inoculation with BMs promotes the growth of healthy roots and high quality seedlings, which are an important issue in the production of vegetable and other agricultural crops. Studies have shown that compost used as growing media reduces the requirement for fertilizer, improves crop vigour, and enhances root system growth (Chilembwe *et al.*, 2004). The use of compost blending with BMs as growing media has been studied as well. Bandara *et al*. (2006) suggested that plant growth could be promoted by endophytic bacteria based on an increase in N_2 fixation and phytohormone synthesis. BMs when used as a type of bio-organic fertilizer increase crop yield and quality by improving nutrient uptake (Fertilizer Act, 2009). Oken and Itzigsohn (1995) found that the phytohormones of *Azospirillum* can increase the respiration rate and metabolism of roots as well as increase the spreading of roots thereby improving water and nutrient uptake. Shutsrirung *et al*. (2009) studied the inoculation of BMs on the seedling media of tomato. The results showed that actinomycetes positively impacted the growth of tomato. Many microorganisms can also produce an enzyme that helps digest organic matter, which improves soil structure and releases nutrients hence promoting the growth of plants (Shutsrirung, 2010).

Objective of this study was to screen IAA producing microorganisms together with nitrogen fixing and phosphate solubilizing ones and blend them with finished compost for Chinese kale seedling production.

3.2 Materials and Methods

Rhizosphere microorganisms such as bacteria and actinomycetes were obtained from a previously published study (Shutsrirung, 2005). A total number of 25 isolates were selected; 8, 6, and 11 isolates of *Azospirillum*, *Beijerinkia*, and actinomycetes, respectively (Table 3.1). All of the isolates were tested for their potential in nitrogen fixing ability, phosphate solubilization, and IAA production. The method of each determination was described below.

Table 3.1 Code number of selected isolates

Genus	Number of isolates	Code number
Azospirillum spp.	8 isolates	VAs 1, VAs 2, VAs 64, VAs 71, VAs 76, VAs 79, VAs 85, and VAs 87
Beijerinckia spp.	6 isolates	VBe 5, VBe 6, VBe 12, VBe 33, VBe 34, and VBe 75
actinomycetes	11 isolates	VAc 11, VAc 40, VAc 65 VAc 66, VAc 67, VAc 68, VAc 73 VAc 74, VAc 77, and VAc 80

3.2.1 Screening of N_2-fixing ability of BMs isolates under laboratory conditions

Each isolate was assessed for nitrogen fixing ability by acetylene reduction assay (ARA). This method determined the activity of the enzyme nitrogenase using acetylene (C_2H_2) gas obtained from calcium carbide (CaC_2). The BMs were cultured in the semi-solid modified Rennie medium. They were incubated at 30 °C for 7 days. The growth of BMs was observed in the medium. Syringe were used to puncture through the rubber caps over the tubes containing BMs. Approximately 5% of the head space was sucked out and replaced with C_2H_2 in the same volume. A certain volume of the gas was incubated at 30°C with each BMs isolate in a closed system for

24hr. After incubation, the ethylene (C_2H_4) that was produced was measured as the reduction of acetylene (C_2H_2) was measured by 1 ml injected into gas chromatography (GC) (Ota *et al*, 1991). High production of ethylene (C_2H_4) gas by BMs was considered as having a high N_2-fixing ability.

3.2.2 Screening for phosphate solubilizing activity

Each isolate was cultivated in Pikovskaya's (PVK) medium to evaluate their phosphate solubilizing ability. All samples were incubated on a rotary shaker at room temperature for 7 days. The rest of the experiments were performed using phosphate solubilizing bacteria inoculated into PVK medium with 0.5% tricalcium phosphate or 0.5% aluminum phosphate with three replications. Only 25 ml of PVK medium without beneficial microorganisms was used as control (after centrifuging standard phosphorus were 0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 ppm). The supernatant (0.5 ml) was drawn off and mixed with 2ml of color reagent and placed in the dark for 30 min. The absorbance readings were taken after exactly 30 min at 820 nm by using a spectrophotometer.

3.2.3 Screening for plant hormone production in BMs isolates

Determination of plant hormones was done by analysis of indole-3-acetic acid (IAA) produced by pure BMs isolates. In order to examine IAA production, each isolate was inoculated in the appropriate medium supplemented with 1% L-tryptophan, which is the precursor of IAA. The BMs were cultured in the broth medium in a dark room at 30 °C for 7 days. All samples were filtered with Whatman No. 5. The chemical reagent (salkovskii reagent) was obtained from 1 mL of 0.5 M

$FECl_3$ and 50 ml of 35% $HClO_4$. Indole-3-acetic acid (MW = 175.19) was used as standard. The IAA concentration of 1 mM was prepared as stock solution. The standard serie were 0, 10, 20, 50, 100, and 150 µM. One mL of standard serie and each sample were pipetted into test tubes and 2 ml of salkovskii reagent was added to each test tube. Then the solution was mixed and incubated for 30 min in the dark room. After incubation the supernatant obtained from the extracellular suspension of each isolate was measured by a spectrophotometer at wavelength 530 nm to determine IAA levels (Gordon and Weber, 1951).

3.2.4 Evaluation of growth promotion efficiency of all selected isolates

A screenhouse experiment was conducted at the Faculty of Agriculture, Chiang Mai University, Thailand. Coconut husk compost was used to produce the seedling media. From preliminary evaluations, the seedling media contained only a small number of microorganisms. Each of the twenty-five selected isolates was used to inoculate Chinese kale seedlings and were grown in selected seedling media (SSM). Three selected isolates (Vas 2, Vbe 75, and Vac 77) were inoculated as a single isolate into Chinese kale seedlings (10^7 CFU /plant) and raised in SSM for 45 days. SSM without microbial addition was used as control treatment. Randomized complete block design (RCBD) was used in this experiment with three replications. Each replication contained 10 seedlings with the following treatments:

1) Control (selected seedling media, SSM)
2) SSM + *Azospirillum* spp. 8 isolates
3) SSM + *Beijerinckia* spp. 6 isolates

4) SSM + actinomycetes 11 isolates

The growth promotion efficiency of selected isolates were evaluated by measurement of shoot and root dry weight and nutrient uptake (N, P, K Ca and Mg) in Chinese kale 20 days after inoculation (DAI). The three isolates that gave the highest growth parameters were selected for the next experiment.

Table 3.2 Chemical properties of selected seedling media

Properties	Analysing Value
pH (H_2O, 1:10)	5.50
Organic matter (%)	35.27
P_2O_5 (%)	0.81
K_2O (%)	0.56
Ca (%)	0.37
Mg (%)	0.25

3.2.5 Statistical analysis and calculation

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

3.3 Results and discussion

3.3.1 Evaluation of growth promotion potential of selected microorganisms

IAA producing, nitrogen fixing and phosphate solubilizing abilities were studied in *Azospirillum* spp. for eight different VAs (Table 3.3). All isolates of *Azospirillum* spp. displayed the potential to produce IAA with values ranging from 9.89 – 194.97 mg/L. The highest values of IAA producing were observed in VAs 64, VAs 2, and VAs 1 with values of 194.97, 182.76, and 179.52 mg/L, respectively. Nitrogen fixing values in *Azospirillum* spp. ranged from 20.78 – 24.94 nmol

C_2H_4/24hr/tube. VAs 85, VAs 1, and VAs 71 showed the highest nitrogen fixing ability with values of 24.94, 23.68, and 22.62 nmol C_2H_4/24hr/tube, respectively. Calcium phosphate solubilizing values ranged from 102.11 – 212.82 µg/mL. VAs 71, VAs 85, and VAs 87 had the highest calcium phosphate solubilizing ability with values of 212.82, 132.97, and 128.95 µg/mL, respectively. Aluminum phosphate solubilizing values ranged from 2.85 – 13.03 µg/mL. VAs79, VAs 64, and VAs 87 showed the highest values of aluminum phosphate solubilizing ability with the following amounts 13.03, 10.97, and 9.86 µg/mL, respectively.

Table 3.3 Screening of IAA producing, N_2-fixing, and phosphate solubilizing abilities of *Azospirillium* spp. under laboratory conditions

Isolate	IAA producing ability (mg/L)	N_2-fixing ability (nmol C_2H_4/24hr/tube)	Phosphate solubilizing ability (µg/mL)	
			Ca-P	Al-P
VAs 1	179.52	23.68	116.60	7.79
VAs 2	182.76	21.45	104.39	5.26
VAs 64	194.97	20.78	108.81	10.97
VAs 71	126.01	22.62	212.82	3.64
VAs 76	157.13	21.27	119.28	2.85
VAs 79	9.89	21.64	102.11	13.03
VAs 85	94.83	24.94	132.97	9.48
VAs 87	38.09	20.85	128.95	9.86

There were six different VBe in which IAA producing, nitrogen fixing and phosphate solubilizing abilities were measured in *Beijerinckia* spp. (Table 3.4). All isolates of *Beijerinckia* spp. displayed the potential to produce IAA with values ranging from 18.90 – 87.52 mg/L. The highest IAA concentrations were observed for VBe 75 with 87.52 mg/L, VBe 33 with 63.07 mg/L, and VBe 5 with 47.30 mg/L. Nitrogen fixing values ranged from 1.29 – 132.37 nmol C_2H_4/24hr/tube. The highest values were measured in VBe 33, VBe 6, and VBe 5 with values of 132.37, 44.18, and 41.30 nmol C_2H_4/24hr/tube, respectively. Calcium phosphate solubilizing values in *Beijerinckia* spp. ranged from 41.00 – 62.00 µg/mL. VBe 5, VBe 33, VBe 6, and VBe 12 showed the highest ability to solubilize calcium phosphate with values of 62.00, 54.00, 45.00, and 45.00 µg/mL, respectively. Aluminum phosphate solubilizing values ranged from 2.45 – 31.41 µg/mL. VBe 5, VBe 34, and VBe 33 showed the highest ability to solubilize aluminum phosphate with values of 31.41, 21.37, and 15.07 µg/mL, respectively.

Table 3.4 Screening of IAA producing, N_2-fixing, and phosphate solubilizing abilities of *Beijerinkia* spp. under laboratory conditions

Isolate	IAA producing ability(mg/L)	N_2-fixing ability (nmol C_2H_4/24hr/tube)	Phosphate solubilizing ability (µg/mL)	
			Ca-P	Al-P
VBe 5	47.30	41.30	62.00	31.41
VBe 6	35.39	44.18	45.00	14.87
VBe 12	22.07	1.29	45.00	3.62
VBe 33	63.07	132.37	54.00	15.07
VBe 34	18.90	16.70	44.00	21.37
VBe 75	87.52	25.44	41.00	2.45

Eleven different VAc were studied for IAA producing, nitrogen fixing and phosphate solubilizing abilities in actinomycetes (Table 3.5). All isolates of actinomycetes displayed the potential to produce IAA with values ranging from 5.67 – 460.31 mg/L. The highest values were observed in VAc 65, VAc 73, and VAc 77 with values of 460.31, 272.33, and 255.06 mg/L, respectively. Nitrogen fixing ability levels ranged from 9.27 – 21.54 nmol C_2H_4/24hr/tube. The highest levels were measured in VAc 6, VAc 74, and VAc 66 with values of 21.54, 18.59, and 17.92 nmol C_2H_4/24hr/tube, respectively. Calcium phosphate solubilizing values ranged from 128.61 – 260.96 µg/mL. VAc 6, VAc 11, and VAc 80 showed the highest ability to solubilize calcium phosphate with the amounts 260.96, 249.53, and 238.09 µg/mL, respectively. In actinomycetes aluminum phosphate solubilizing values ranged from 1.47 – 14.82 µg/mL. VAc 40, VAc 11, and VAc 74 showed the highest ability to solubilize aluminum phosphate with the amounts 14.82, 5.97, and 4.25 µg/mL, respectively.

Table 3.5 Screening of IAA producing, N_2-fixing, and phosphate solubilizing abilities of actinomycetes under laboratory conditions

Isolate	IAA producing ability (mg/L)	N_2-fixing ability (nmol C_2H_4/24hr/tube)	Phosphate solubilizing ability (µg/mL)	
			Ca-P	Al-P
VAc 6	16.68	21.54	260.96	2.89
VAc 11	5.67	9.27	249.53	5.97
VAc 40	113.49	13.29	224.37	14.82
VAc 65	460.31	15.38	226.65	2.10
VAc 66	152.01	17.92	186.25	1.47
VAc 67	208.69	10.20	181.21	2.40
VAc 68	213.84	16.50	192.50	2.53
VAc 73	272.33	14.67	219.33	3.63
VAc 74	254.18	18.59	128.61	4.25
VAc 77	255.06	15.23	157.78	2.72
VAc 80	189.78	10.83	238.09	3.05

3.3.2 Evaluation of growth promotion efficiency of all selected isolates on Chinese kale seedlings

There were 25 isolates of beneficial microorganisms that were studied. There were 2 types of beneficial microorganisms studied and those were bacteria (*Azospirillum* and *Beijerinckia*) and actinomycetes. *Azospirillum* spp. isolates gave shoot and root dry weights that were significantly increased compared to control. *Azospirillum* 2 (VAs 2) resulted in 3.05 g/10plants of shoot dry weight and 0.44 g/10plants of root dry weight per 10plants (Table 3.6). *Beijerinckia* 75 (VBe 75) gave significantly improved the shoot and root dry weight with 2.80 and 0.26 g/10plants, respectively (Table 3.7). For actinomycetes 77 (VAc 77) the results showed significantly higher shoot and root dry weight than most of the treatments (3.42 and 0.41 g/10plants, respectively) (Table 3.8).

The inoculations with *Azospirillum* isolates, as displayed in Table 3.6, have demonstrated that treatment with VAs 2 showed the highest shoot dry weight with 3.05 g/10plants. The second highest was VAs 87 with 2.87 g/10plants and then VAs 76 with 2.81 g/10plants. Treatment with VAs 2 also gave the highest root dry weight with 0.44 g/10plants. The second highest was VAs 87 with 0.39 g/10plants and that was followed by VAs 85 with 0.35 g/10plants. These values were significantly higher than that of the control. The results indicated that specific isolates of *Azospirillum* were able to enhance plant growth.

Table 3.6 Shoot and root dry weight of Chinese kale seedlings grown in selected seedling media inoculated with *Azospirillum* spp.

Treatment	**Dry weight (g/10plants)**	
	Shoot	**Root**
1. Control	2.47 abc/1	0.24 d
2. VAs 1	2.35 bcd	0.27 d
3. VAs 2	3.05 a	0.44 a
4. VAs 64	1.80 d	0.28 d
5. VAs 71	2.16 cd	0.28 d
6. VAs 76	2.81 ab	0.29 cd
7. VAs 79	2.05 cd	0.24 d
8. VAs 85	2.75 ab	0.35 bc
9. VAs 87	2.87 ab	0.39 ab
F – test	**	**
C.V. (%)	13.54	12.07

** *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*

The inoculations with *Beijerinckia* isolates as shown in Table 3.7 have demonstrated that VBe 75 showed the highest shoot dry weight of 2.80 g/10plants, followed by VBe 34 with 2.60 g/10plants. The other isolates did not surpass the shoot dry weight of the control which was 2.47 g/10plants. Treatment with VBe 5 gave the highest root dry weight of 0.35 g/10plants, followed by VBe 6 with 0.34 g/10plants, and then VBe 34 with 0.29 g/10plants. The results showed that specific isolates of *Beijerinckia* were able to enhance plant growth.

Table 3.7 Shoot and root dry weight of Chinese kale seedlings grown in selected seedling media inoculated with *Beijerinckia* spp.

Treatment	**Dry weight (g/10plants)**	
	Shoot	**Root**
1. Control	2.47 ab[/1]	0.24 bc
2. VBe 5	2.41 ab	0.35 a
3. VBe 6	2.11 bc	0.34 a
4. VBe 12	1.97 cd	0.21 c
5. VBe 33	1.67 d	0.27 b
6. VBe 34	2.59 a	0.29 b
7. VBe 75	2.79 a	0.26 bc
F – test	**	**
C.V. (%)	10.63	9.53

*** indicates the effect is significant at $P < 0.01$*
[/1] Values within each column followed by same letter are not significantly different at $P < 0.01$

The results of the inoculations with actinomycetes isolates are shown in Table 3.8. Treatment with VAc 77 showed the highest shoot dry weight of 3.42 g/10plants, followed by treatment with VAc 40 resulting in 3.06 g/10plants and then VAc 68 with 3.00 g/10plants. Treatment with VAc 68 also gave the highest root dry weight of 0.44 g/10plants, followed by VAc 11 with 0.42 g/10plants and VAc 77 with 0.40 g/10plants. These results indicated that certain isolates of actinomycetes were able to enhance plant growth.

Table 3.8 Shoot and root dry weight of Chinese kale seedlings grown in selected seedling media inoculated with actinomycetes

Treatment	Dry weight (g/10plants)	
	Shoot	Root
1. Control	2.47 cd[/1]	0.24 e
2. VAc 6	2.23 d	0.28 de
3. VAc 11	2.66 bc	0.42 a
4. VAc 40	3.06 ab	0.33 bcd
5. VAc 65	2.25 cd	0.32 cd
6. VAc 66	2.53 cd	0.37 abc
7. VAc 67	2.37 cd	0.27 de
8. VAc 68	3.00 ab	0.44 a
9. VAc 73	2.49 cd	0.28 de
10.VAc 74	2.57 cd	0.37 abc
11.VAc 77	3.42 a	0.40 ab
12.VAc 80	2.35 cd	0.35 abcd
F – test	**	**
C.V. (%)	9.49	13.60

***   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*

3.3.3 Effect of *Azospirillum* spp. on nutrient uptake in Chinese kale seedlings

Inoculation with *Azospirillum* spp. isolates had a significant effect on phosphorus and calcium uptake (Table 3.9). When compared to control, *Azospirillum* 2 (VAs 2) increased the amount of phosphorus uptake the most with a value of 19.7 mg /10plants. The nutrient uptake of calcium was also increased the most in VAs 2 with a value of 19.7 mg /10plants. The analysis of the uptake of nitrogen, potassium, and magnesium showed no statistically significant differences when compared with the control treatment.

Table 3.9 Nutrient uptake by Chinese kale seedlings grown in selected seedling media inoculated with *Azospirillum* spp.

Treatment	Nutrient uptake (mg/10plants)				
	N	P	K	Ca	Mg
1. Control	56.7 c[/1]	17.0 abc	109.3 abc	11.0 c	10.3 b
2. VAs 1	85.7 ab	16.0 abcd	113.7 ab	15.3 abc	14.7 ab
3. VAs 2	82.0 abc	19.7 a	120.0 a	19.7 a	18.0 a
4. VAs 64	66.3 bc	12.7 d	82.0 c	14.3 abc	13.3 ab
5. VAs 71	80.7 abc	14.3 bcd	93.7 abc	15.7 abc	14.3 ab
6. VAs 76	107.3 a	18.3 a	116.3 ab	18.7 ab	18.3 a
7. VAs 79	69.7 bc	14.0 cd	89.0 bc	13.7 bc	14.0 ab
8. VAs 85	93.0 ab	18.0 ab	106.0 abc	18.3 ab	17.7 a
9. VAs 87	85.7 ab	18.3 a	117.7 a	19.3 a	17.3 a
F – test	ns	*	ns	*	ns
C.V (%)	20.63	13.86	15.56	19.19	20.52

* *indicates the effect is significant at $P< 0.05$*
[/1] *Values within each column followed by same letter are not significantly different at $P < 0.05$*
ns indicates the effect is not significant at $P< 0.05$

3.3.4 Effect of *Beijerinckia* spp. on nutrient uptake in Chinese kale seedlings

Inoculation with *Beijerinckia* spp. isolates had a significant effect on the uptake of nitrogen, phosphorus, potassium, calcium, and magnesium (Table 3.10). Beijerinckia 75 (VBe 75) had the highest increases for the following nutrients: nitrogen, phosphorus, potassium, and calcium while magnesium was not different than control. The values of these nutrients were 96.3, 17.0, 121.7, 19.7, and 10.3 mg /10plants, respectively. The highest magnesium uptake was in VBe 34 with16.7 mg /10plants, which was significantly increased when compared to the control.

Table 3.10 Nutrient uptake by Chinese kale seedlings grown in selected seedling media inoculated with *Beijerinckia* spp.

Treatment	Nutrient uptake (mg/10plants)				
	N	**P**	**K**	**Ca**	**Mg**
Control	56.7 c[/1]	17.0 a[/2]	109.3 ab	11.0 c	10.3 c
VBe 5	88.3 ab	16.0 ab	113.3 a	17.3 ab	16.3 ab
VBe 6	74.7 bc	14.3 ab	106.3 ab	14.7 bc	14.0 bc
VBe 12	88.3 ab	13.3 bc	89.3 bc	17.0 ab	14.0 bc
VBe 33	72.0 bc	11.3 c	69.3 c	14.0 bc	13.3 bc
VBe 34	79.7 ab	16.0 ab	114.3 a	17.7 ab	16.7 ab
VBe 75	96.3 a	17.0 a	121.7 a	19.7 a	10.3 c
F – test	*	**	**	**	*
C.V (%)	14.05	10.54	11.01	13.34	17.56

* *indicates the effect is significant at P< 0.05*
** *indicates the effect is significant at P< 0.01*
[/1] *Values within each column followed by same letter are not significantly different at P < 0.05*
[/2] *Values within each column followed by same letter are not significantly different at P < 0.01*

3.3.5 Effect of actinomycetes on nutrient uptake in Chinese kale seedlings

Inoculation with actinomycetes isolates increased the uptake of the following nutrients: nitrogen, phosphorus, calcium, and magnesium. With actinomycetes 77 (VAc 77) the following nutrients were increased significantly: nitrogen, phosphorus, calcium, and magnesium. The values for these nutrients were 105.3, 23.3, 23.3, and 22.3 mg/10plants, respectively. These were the highest values and were significantly different when compared with control treatments. The analysis of the uptake of potassium was not statistically significant when compared with the control treatment (Table 3.11).

In this study treatment of Chinese kale with specific isolates showed improved growth and nutrient uptake. It was found that the addition of beneficial microorganisms to seedling media can increase the shoot and root dry weight. The following 3 isolates, *Azospirillum* 2 (VAs 2), *Beijerinckia* 75 (VBe 75), and actinomycetes 77 (VAc 77), which are representative of each of the three types of beneficial microorganisms tested, showed the highest increase in the amount of dry matter within the plant when compared with control after 30 days. This observed growth increase is a result of inoculation with bacteria at the time of planting. The same 3 above mentioned isolates also had the highest overall nutrient uptakes.

Table 3.11 Nutrient uptake by Chinese kale seedlings grown in selected seedling media inoculated with actinomycetes

Treatment	**Nutrient uptake (mg/10plants)**				
	N	**P**	**K**	**Ca**	**Mg**
1. Control	56.7 d	17.0 c	109.3 bcde	11.0 e	10.3 d
2. VAc 6	87.3 abc	15.7 c	108.3 cde	15.7 cd	16.0 c
3. VAc 11	94.7 abc	16.7 c	123.7 abcd	17.3 bcd	18.3 abc
4. VAc 40	103.0 ab	20.0 b	130.3 ab	19.3 bc	21.0 ab
5. VAc 65	86.0 abc	15.7 c	110.0 bcde	17.0 bcd	15.3 c
6. VAc 66	93.0 abc	17.0 c	106.3 de	18.0 bcd	18.0 bc
7. VAc 67	80.3 c	16.0 c	116.7 abcde	15.3 d	15.7 c
8. VAc 68	100.3 abc	20.7 ab	130.0 abc	20.0 ab	19.3 abc
9. VAc 73	83.7 bc	16.7 c	109.3 bcde	17.3 bcd	18.7 abc
10.VAc 74	95.7 abc	16.7 c	109.3 bcde	18.0 bcd	18.0 bc
11.VAc 77	105.3 a	23.3 a	132.7 a	23.3 a	22.3 a
12.VAc 80	87.3 abc	16.0 c	98.7 e	15.7 cd	17.3 bc
F – test	**	**	ns	**	*
C.V (%)	13.93	9.78	11.25	13.10	14.59

* *indicates the effect is significant at P< 0.05*
** *indicates the effect is significant at P< 0.01*
/1 *Values within each column followed by same letter are not significantly different at P < 0.05*
/2 *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< 0.05, 0.01

Another study showed that the addition of bacteria such as *Azospirillum, Beijerinckia*, and *Azotobacter* to the production of seedlings resulted in increased weight of shoots and roots (Jacoud *et al.*, 1999; Swaminathan and Srinivasan, 2006). In comparison with this experiment treatment with *Azospirillum* spp. had the highest root dry weight when compared with *Beijerinckia* spp. and actinomycetes. The results of another study showed that *Azospirillum* spp. could also produce plant hormones called indole 3-acetic acid (IAA) and effect root growth and elongation

(Zakharova *et al*., 1999). This is consistent with the research of Akbari *et al.* (2007) that found IAA hormone is produced from *Azospirillum* when stimulated with wheat formation and resulted in increased weight and root length. Similarly, the research by Nassar *et al.* (2003) found that actinomycetes can promote the growth, freshness, dry weight, and the root's bean length.

When the potential of microorganisms to increase nutrient uptake was studied, it was found that inoculation of seedling media with beneficial microorganisms increased nutrient uptake. Although for some of the treatments there was no significant difference when compared to the control. This may be due to the time of harvest or the duration of the study may need to be extended. It was impossible to distinguish clearly. Egamberdiyeva and Hoflich (2004) found that when cotton was effectively inoculated with bacteria, it increased the nutrient uptake of nitrogen, phosphorus, and potassium and grew taller with increased dry weight.

3.5 Conclusion

Nutrient uptake as well as shoot and root dry weights were effectively increased with the addition of beneficial microorganisms to the seedling media of Chinese kale. There were 3 isolates that were representative of each type of beneficial microorganism tested which had both the highest dry matter increases and nutrient uptakes. Therefore inoculation with any of these top performing isolates on Chinese kale seedlings has the potential to producing high quality crops and reducing agrochemical contamination.