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Antioxidant Activity, Vitamin C Content and Growth of Chinese Kale in Response to High Humus Seedling Media and Beneficial Microorganisms

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ABSTRACT

Nutritional values (antioxidant activity and vitamin C content), growth and nutrient uptake of Chinese kale seedlings in response to high humus seedling media and beneficial microorganisms were evaluated in the screenhouse. Selected seedling media (SSM) were mixed with each of the three selected isolates (Azospirillum sp., Beijerinckia sp. and actinomycetes) and various percentages of leonardite. The results showed that SSM with actinomycetes plus 15% leonardite produced the highest shoot and root dry weight of Chinese kale seedling and all these values were significantly higher than the control. This treatment also produced the highest nutrient uptake (N, P, K, Ca and Mg). Furthermore, this treatment produced the highest vitamin C content (33.08 and 44.66 µg/ml) and antioxidant activity (1.9285 and 4.733 µmol trolox/g) at both the seedling stage (20 days after inoculation, or 20 DAI) and harvest time (40 days after inoculation, or 40 DAI), respectively. For Chinese kale, application of SSM with actinomycetes plus 15% leonardite not only improved plant growth and nutrient uptake but also increased nutritional values (antioxidant activity and vitamin C content) at both a seedling and at harvest. SSM with Beijerinckia sp. and 10% leonardite provided the second highest values of vitamin C content and antioxidant activity, also exceeding the control. Our results demonstrated that the improvement of vitamin C content and antioxidant activity of Chinese kale by bioorganic inputs in the production system is of interest and could lead to better consumer health.

Keywords: Antioxidant, Beneficial microorganisms, Chinese kale, Leonardite, Vitamin C

INTRODUCTION

Chinese kale (*Brassica oleracea* var. alboglabra), a vegetable, has been grown commercially in northern Thailand for more than two decades. It is grown primarily in the mountains, with production increasingly continuously in response to strong demand from both domestic and export markets. Increasing demand has led to increased use of agrochemicals – fertilizers, pesticides and insecticides – threatening the environment and nearby communities. Use of local materials such as seedling media and compost in the production systems offers the potential for higher profit and a healthier environment for the farmers. Jierwiriyapant et al., (2012) reported that efficiency and low production costs were two of the factors that influenced farmers to adopt organic agricultural methods. The use of compost and beneficial microorganisms as biofertilizer, in preference to agrochemicals, on Chinese kale farms is of interest as well as in line with government policies to reduce the use of agrochemicals, and thus reduce the cost of production (Shutsrirung, 2010). Cooper et al., (1998) found that humic acid extracted from soil, peat or leonardite (high humic acid containing material) increased the phosphorus content of turf grass by 3-5%. Humic acid from leonardite significantly increased root weight of turf grass over the control. Studies have shown that humic substances could enhance seed germination of maize, wheat and barley. Root development of maize was double when humic substances were added to the nutrient solution (Kononova and Pankova, 1950; Dixit and Kishore, 1967; Chen and Avid, 1990).

The current concept of more nutritious foods places the emphasis on foods that contain more protein, fiber and vitamins as well as specific phytochemicals, such as the polyphenolic antioxidants found in fruits and vegetables (Scalbert et al., 2005). Studies have revealed that Chinese kale contains phytochemicals such as glucosinolates, Beta-carotene, flavonoids and vitamin C to fight against cancer (Van Poppel et al., 1999; Talalay et al., 2001; La et al., 2009). A study of the antioxidant activities of 22 common vegetables, green tea, and black tea indicated that kale had the highest antioxidant activity against hydroxyl radicals followed by Brussels sprouts, alfalfa sprouts, beets, spinach, and broccoli (Guohua et al., 1996). Many studies have shown that organic fertilizers and/or beneficial microorganisms improved crop yield and/or nutritional quality. Elazar et al., (1989) found that *Azospirillum* could increase the root surface area of maize. Ravi et al., (2004) concluded that inoculation of *Azospirillum* strain OAD-2 significantly increased plant height, number of leaves per plant, branches per plant and total dry mass accumulation in Gaillardia pulchella compared to other inoculations and/or uninoculated control. The use of microorganisms not only enhances plant growth but also suppresses plant pathogens. Several actinomycetes isolates provided a high inhibitory efficiency against some soil and air borne pathogens and one isolate (HOU1) substantially improved root growth of brassica (Chinese kale) seedlings (Kunasakdakul et al., 2012). Soltoft et al., (2010) concluded that organically grown onions, carrots, and potatoes generally have more health-promoting secondary metabolites in comparison with the conventionally cultivated ones. Kequan and Liangli (2006) studied the total phenolic content (TPC) and antioxidant properties of various vegetables; and suggested that kale, spinach, broccoli and rhubarb are the better dietary sources of natural antioxidant activities and phenolic compounds.

The objective of this work was to determine the effects of high humus seedling media and beneficial microorganisms on antioxidant activity, vitamin C content and growth of Chinese kale at the seedling stage (20 days after inoculation, or 20 DAI) and harvest time (40 days after inoculation, or 40 DAI).

MATERIALS AND METHODS

First screenhouse experiment

Chinese kale (*Brassica oleracea* var. alboglabra) was cultivated in the screenhouse of the Faculty of Agriculture, Chiang Mai University, Thailand. Randomized complete block design (RCBD) was used in this experiment. Selected seedling media (SSM, coconut husk compost) without leonardite and microbial addition was used as control treatment. The media was mixed with leonardite (humic acid = 57.96%) in various percentages: 5, 10 and 15% (w/w). The mixed media (SSM + leonardite) were used to raise the seedlings with and without beneficial microorganisms (16 treatments, 3 replications) (Table 1). In the treatments with beneficial microorganisms, three isolates – *Azospirillum* sp. (VAs 2), *Beijerinckia* sp. (VBe 75) and actinomycetes (VAc 77) selected from the previous study (Shutsrirung, 2010) – were inoculated as a single isolate to Chinese kale seedlings (10^7 CFU plant^{-1}) after one week of germination. Growth parameters; shoot and root dry weight: and nutrient content were measured at 20 DAI.

Second screenhouse experiment

From the first experiment, the two best treatments from the first screenhouse experiment were selected to evaluate the efficiency of high humus seedling media and beneficial microorganisms on antioxidant activity and vitamin C content. The two selected media were coconut husk seedling media + 10% leonardite + *Beijerinckia* sp. (VBe 75) and coconut husk seedling media + 15% leonardite + actinomycetes (VAc 077) (Table 1). Selected seedling media (coconut husk compost) was used as control treatment. Randomized complete block design (RCBD) was applied in this experiment (3 treatments with 3 replications) (Table 2). After one week of seeding, each of the two selected isolates was applied to the seedlings under each treatment. At the seedling stage and harvest time, content of vitamin C and antioxidant activity were evaluated using the HPLC (High Performance Liquid Chromatography) and DPPH (2,2-diphenyl-1-picrylhydrazyl) method, respectively.

Analysis of vitamin C

Fresh Chinese kale was collected for analysis at 20 DAI and harvest time. Vitamin C was immediately analyzed on the same day of sample preparation. Chinese kale samples were weighed (1 g) and mixed with 10 ml of the extracting solution (3% metaphosphoric acid and 8% acetic acid). The mixture was homogenized at high-speed blender for 1 min and then centrifuged at 10000 g (refrigerated at 4°C) for 20 min. The supernatant was poured out and separately collected. The extracting procedure of the same solid sample was repeated twice, then the supernatant was again collected and mixed with the previous supernatant. The amount of vitamin C content was determined in the supernatant samples and in the standard (ascorbic acid; 10, 100, 200 µg/ml) by HPLC (Shimadsu, UV-detector). For HPLC analysis, an Ultra Aqueous C18 column (5 particle size, µm 250 mm × 4.6 mm I.D.) was used. The column flow rate was 1.5 mL/min with the UV detection at 242 nm, where mobile phase A consisted of 100% 10mM potassium phosphate (pH 2.5).

Analysis of antioxidant activity

Chinese kale sample was extracted by mixing the dried samples with 10 ml of ethanol using a magnetic stirrer at room temperature for 24 h. Each extracted solution sample was filtered through Whatman No. 1 filter paper. All filtered sample solution samples were analyzed with DPPH (2,2-diphenyl-1-picrylhydrazyl) method as described by Brand-Williams et al. (1995). In brief, 1.8 ml of the DPPH solution (DPPH: Tris buffer: 85% EtOH (1:1:1)) was mixed with the filtered solution samples or standard (Trolox; 0, 60, 120, 180, 240, 300, 400, 500, 600 µl) to get the final volume of 2.4 ml before placing all samples in the dark for 30 min. The absorbance readings were taken at exactly 30 min at 525 nm by using a UV-visible spectrophotometer (Shimadsu, UV-1601).

Statistical analysis

All data were analyzed by using Statistix 8.0 (Tallahassee, FL, USA). Data was analyzed by one-way ANOVA, followed by the least significant difference (LSD) test at 95%. Significance was accepted at $p < 0.05$.

RESULTS

Plant growth and nutrient content

The results of this study (Table 1) have demonstrated that SSM with actinomycetes + 15% leonardite (Treatment 16) showed the highest shoot dry weight of 1.69 g/plant, followed by SSM with *Beijerinckia* sp. + 10% leonardite (Treatment 12) of 1.65 g/plant and (SSM with *Azospirillum* sp. + 5% leonardite (Treatment 10) of 1.62 g/plant. These values of shoot dry weight were significantly higher than that of the control. SSM with actinomycetes + 15% leonardite (Treatment 16) also gave the highest root dry weight of 0.21 g/plant, followed by SSM with *Azospirillum* sp. + 5% leonardite (Treatment 8) of 0.20 g/plant, and SSM with *Azospirillum* sp. + 15% leonardite (Treatment 14) of 0.18 g/plant.

Table 1. Shoot and root dry weight of Chinese kale seedlings inoculated with microorganisms to selected seedling media.

Treatment No.	Treatment	Dry weight (g/plant)	
		Shoot	Root
1	Control (selected seedling media)	1.4767 bcd	0.1645 abcd
2	SSM + *Azospirillum* sp. (VAs 2)	1.3933 de	0.1492 bcd
3	SSM + *Beijerinckia* sp. (VBe 75)	1.2133 e	0.165 abcd
4	SSM + actinomycetes (VAc 77)	1.5667 abcd	0.122 cd
5	SSM + 5% leonardite	1.55 abcd	0.1676 abcd
6	SSM + 10% leonardite	1.56 abcd	0.157 abcd
7	SSM + 15% leonardite	1.5333 abcd	0.1503 abcd
8	SSM + *Azospirillum* sp. (VAs 2) + 5% leonardite	1.5567 abcd	0.1979 ab
9	SSM + *Beijerinckia* sp. (VBe 75) + 5% leonardite	1.45 cd	0.1579 abcd
10	SSM + actinomycetes (VAc 77) + 5% leonardite	1.6233 abc	0.1732 abcd
11	SSM +*Azospirillum* sp. (VAs 2) + 10% leonardite	1.5067 abcd	0.1144 d
12	SSM + *Beijerinckia* sp. (VBe 75) +10% leonardite	1.65 ab	0.141 bcd
13	SSM + actinomycetes (VAc 77) + 10% leonardite	1.5333 abcd	0.1266 cd
14	SSM + *Azospirillum* sp. (VAs 2) + 15% leonardite	1.6233 abc	0.1833 abc
15	SSM + *Beijerinckia* sp. (VBe 75) +15% leonardite	1.5533 abcd	0.1512 abcd
16	SSM + actinomycetes (VAc 77) + 15% leonardite	1.69 a	0.2123 a
	F – test	*	*
	C.V (%)	7.81	23.64

Note: Means with different letters in the same column differ significantly. * indicates significant at $P < 0.05$.

The results (Table 2) showed that high values of N uptake were obtained in SSM with actinomycetes (Treatment 4), SSM with *Azospirillum* + 10% leonardite (Treatment 11) and SSM with 10% leonardite (Treatment 6) at 60.42, 52.96, and 51.87 mg/plant, respectively. The P uptake values in SSM with actinomycetes + 15% leonardite (Treatment 16) (10.92 mg/plant), SSM + 5% leonardite (Treatment 5) (10.58 mg/plant) and SSM + 10% leonardite (Treatment 6) (10.39 mg/plant) were higher than that of the control (10.09 mg/ plant). SSM with actinomycetes (Treatment 4) produced the highest value of K uptake (132.73 mg/plant). The highest value of Ca uptake (23.85 mg/plant) was obtained in SSM with *Azospirillum* + 15% leonardite (Treatment 14), significantly higher than that of the control (13.57 mg/plant). The maximal level of Mg uptake was achieved in the combined inoculation of SSM with actinomycetes + 15% leonardite (Treatment 16) (6.77 mg/ plant), which was significantly higher than that of the control (5.55 mg/plant).

Table 2. Nutrient uptake by Chinese kale seedlings grown in selected seedling media inoculated with microorganisms.

Treatment No.	N uptake	P uptake	K uptake	Ca uptake	Mg uptake
	(mg/plant)				
1	41.97 efg	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	115.1 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	9.9 abc	90.69 bcd	20.45 b	5.84 abcde
8	41.12 efg	10.23 abc	100.53 bcd	15.95 fg	5.2 ef
9	44.37 cdefg	8.86 c	98.47 cd	16.66 defg	5.4 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 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

Note: Means with different letters in the same column differ significantly. ** indicates significant at $P < 0.01$.

Vitamin C content in Chinese kale

The results in Table 3 indicated that maximum vitamin C content was obtained by SSM with actinomycetes + 15% leonardite (Treatment 3) (33.08 µg/ml), followed by (SSM with *Beijerinckia* + 10% leonardite (Treatment 2) (30.07 µg/ml) and the control (27.43 µg/ml). However, these differences were not significantly different. By 40 DAI, however, the maximum vitamin C content obtained in SSM with actinomycetes + 15% leonardite (Treatment 3) (44.66 µg/ml) was significantly higher ($p<0.01$) than that of the control (28.39 µg/ml). The vitamin C content (30.80 µg/ml) of SSM with *Beijerinckia* + 10% leonardite (Treatment 2) was also higher than that of the control but the values were not significantly between these two treatments (Table 3).

Table 3. Effect of high humus seedling media and beneficial microorganisms on vitamin C content of Chinese kale.

Treatment	% Humic acid	Vitamin C content (µg/ml)	
		20 DAI	40 DAI
1. Control (selected seedling media)	26.06	27.43	28.39 b
2. SSM + VBe 75 + 10% leonardite	30.75	30.07	30.80 ab
3. SSM + VAc 077 + 15% leonardite	35.49	33.08	44.66 a
	F – test	ns	*
	C.V. (%)	23.56	32.59

Note: DAI = Days after inoculation. Means with different letters in the same column differ significantly. ns = non-significant. * indicates significant at P < 0.05.

Antioxidant activity of Chinese kale

The highest antioxidant activity at the seedling stage (20 DAI) was obtained in SSM with actinomycetes + 15% leonardite (Treatment 3) (1.9285 µmol trolox/g sample), followed by SSM with *Beijerinckia* + 10% leonardite (Treatment 2) (0.9978 µmol trolox/g sample) and the control (0.9509 µmol trolox/g sample) (Table 4). The value of antioxidant activity obtained by SSM with actinomycetes + 15% leonardite (Treatment 3) was significantly ($p<0.01$) higher than that of other treatments. At harvest (40 DAI), the values of antioxidant activity did not differ significantly between treatments. However at this stage, the maximum antioxidant activity was recorded with SSM with actinomycetes + 15% leonardite (Treatment 3) (4.733 µmol trolox/g sample), followed by SSM with *Beijerinckia* + 10% leonardite (Treatment 2) (4.352 µmol trolox/g sample) and the control (4.337 µmol trolox/g sample). Our results indicated that antioxidant activity (DPPH assay) of Chinese kale obtained by SSM with actinomycetes + 15% leonardite (Treatment 3) was highly significant ($p<0.01$) at 20 DAI when compare with that of the control.

Table 4. Effect of high humus seedling media and beneficial microorganisms on antioxidant activity.

Treatment	% Humic acid	Antioxidant activity (µmol trolox/g sample)	
		20 DAI	40 DAI
1. Control (selected seedling media)	26.06	0.9509 b	4.337
2. SSM + VBe 75 + 10% leonardite	30.75	0.9978 b	4.352
3. SSM + VAc 077 + 15% leonardite	35.49	1.9285 a	4.733
	F – test	**	ns
	C.V. (%)	31.64	19.95

Note: DAI = Days after inoculation. Means with different letters in the same column differ significantly. ns = non significant. ** indicates significant at P < 0.01.

DISCUSSION

In this study, high humus materials together with beneficial microorganisms enhanced plant growth, particularly SSM with actinomycetes + 15% leonardite that produced 14.9% and 29.0% higher shoot and root dry weight over the control, respectively. The additions of *Azospirillum* sp. + 5% leonardite and *Azospirillum* sp. + 15% leonardite into SSM produced 20.3 and 11.4% higher root dry weight over the control, respectively (Table 1). Our results were in accordance with other studies. Bashan et al., (1990) reported that inoculation of wheat with *Azospirillum brasilense* significantly increased plant dry weight. Khalid et al., (2004) found that inoculation of high IAA producing rhizobacteria produced up to a 13.5% increase in root elongation in wheat and a 37.7% increase in shoot elongation of wheat. Furthermore, Jacoud et al., (1999) suggested that root surface areas of maize could be increased by inoculating *Azospirillum lipoferum*. Diaz-Zorita and Fernandez-Canigia (2009) inoculated wheat (*Triticum aestivum* L.) seed with a liquid formulation containing *Azospirillum brasilense* INTA Az-39 strain. The crops exhibited more vigorous vegetative growth, including a 12.9% greater shoot and 22.0% dry matter accumulation.

On the average, our results indicated that, application of high humus material together with beneficial microorganisms not only enhance plant growth but also increased plant nutrient uptake. The inoculation of actinomycetes VAc 077 in Treatment 4 resulted in higher N (43.9%), K (22.5%), Ca (27.2%) and Mg (18.2%) uptake than those of the control. In addition, SSM with actinomycetes VAc 077 + 15% leonardite also produced higher N (10.2%), P (8.2%), K (2.2%), Ca (57.0%) and Mg (21.9%) uptake than those of the control (Table 2). Other studies also showed similar results. Akbar et al., (2009) found that inoculating *Azospirillum brasilense* (native or Sp7) on *Triticum aestivum* L. (cv. Baccros and Mahdavi) produced significantly higher grain yield by 29% over the control. Additionally, the grains contained more N (22.8%), P (59.5%) and K (34%) when compared to the control plants. Fernandez-Escobar et al., (1996) reported that foliar application of leonardite extracts to young olive plants stimulated shoot growth when they were grown without the addition of mineral elements to the irrigation water. Under field conditions, application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, B, Mg, Ca and Fe in leaves.

Application of beneficial microorganisms together with leonardite showed a tendency to improve vitamin C content in Chinese kale particularly SSM with actinomycetes + 15% leonardite that gave 20.6 and 57.3% higher vitamin C content over the control at 20 and 40 DAI, respectively. Furthermore this treatment also resulted in approximately twice the value of antioxidant compared to the control at 20 DAI (Table 3 and 4). Other studies have also shown the positive effect of organic inputs on nutritional values of vegetables. Soltoft et al., (2010) concluded that organically grown onions, carrots, and potatoes generally have higher contents of health-promoting secondary metabolites in comparison with the conventionally cultivated ones. Reviewing 41 published studies comparing the nutritional value of organically grown and conventionally grown fruits, vegetables and grains, certified nutrition specialist Virginia Worthington concluded that there were significantly more of several nutrients in organic crops. These included 27.0% more vitamin C, 21.1% more iron, 29.3% more magnesium, and 13.6% more phosphorus (Worthington, 2001).

CONCLUSION

Growth parameters and nutrients content of Chinese Kale seedling were improved by microbial and leonardite application. The best mixed media was SSM with actinomycetes plus 15% leonardite that showed the highest ability to promote good plant growth and gave the highest shoot and root dry weight. The highest nutrients uptake (P, Ca and Mg) of Chinese kale seedling was also obtained by this treatment. The second best media was SSM with *Beijerinckia* sp. plus 10% leonardite. These best two bioorganic mixed media show promise for improving the growth parameters and nutrient content of Chinese kale seedlings. Application of high humus materials (leonardite) and beneficial microorganisms in the two best mixes of SSM media not only improved plant growth and nutrient uptake of Chinese kale but also increased the nutritional values (antioxidant activity and vitamin C content) of Chinese kale. The highest values of vitamin C content and antioxidant activity of Chinese kale at 20 DAI was obtained by the best mixes of media and the values were significantly higher than that of the control. This indicated that when selected seedling media was mixed with actinomycetes (VAc 077) plus 15% leonardite, the mixed media was able to promote the vitamin C content and antioxidant acitvity. Our results suggested that high humus materials and beneficial microorganisms would ensure good performance and high quality vegetables after transplanting.

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