
Effects of cultivated practices on the growth, phenolic content, antioxidant activity and Ca content of Chinese kale (*Brassica oleracea* L. var. *alboglabra*)

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Abstract Various cultivation practices did not significantly increase the total phenol content (TPC), antioxidant activity (AA), and calcium (Ca) content in Chinese kale (*Brassica oleracea*). The results indicated that conventional cultivation (CC), good agricultural practice (GAP), and local plant bioextract (LPB) significantly gave the highest leaves chlorophyll content at 23 and 48 days after seed sowing (DASS), fresh weight/m², and leaves width compared to the controlled treatment. The highest leaves length was affected by GAP and LPB, where the highest petiole length was affected by GAP compared to other treatments. The TPC, AA and Ca content were not affected by any cultivation practices. These results implied that cultivation practices influenced some growth traits of *B. loeracea* but did not influence the TPC, AA and Ca content.

Keywords: Cultivation, Phenol, Conventional practice, Bioextract

Introduction

Chinese kale (*Brassica oleracea* L. var. *alboglabra*) belongs to the *Brassica* family. It is widespread in the People's Republic of China and Southeast Asia with a large growing area and a marketable supply in these regions (Sun *et al.*, 2011). For Thailand, it is a popular vegetable for year-round consumption since it is easily grown in every region of the country. Kale is a great source of beta-carotene regardless of how it is washed or cooked, as it retains this value, but consumed fresh provides a high amount of vitamin C and minerals (Toyota Thailand Foundation and Mahidol University, 1997). It is also a widely consumed vegetable, which is rich in antioxidants and anticarcinogenic compounds (Wang *et al.*, 2017) and several phenolic compounds (Sun *et al.*, 2012). In addition, the total polyphenol content is high

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at 5.29-5.61 mg GE/g dry weight and performs an antioxidant capacity between 27.46-31.36% (Prasoprattanachai, 2009). Factors affecting the antioxidant index of the same vegetable are genetic, the environment and cultivation practice (Bravo, 1998). It has been revealed that the antioxidant activity and amount of phenolic compounds of organically grown Chinese kale was higher than that of the conventionally grown species (Sakunasing, 2009). Even though there is little information on the real influence of the cultivation on the levels of some important phytochemicals in Chinese kale. This study aimed to examine its effect on some growth characteristics, the TPC, AA and Ca content.

Materials and methods

Soil sample and liquid bioextract preparation

The experiment was conducted in greenhouses at the Faculty of Agriculture and Agricultural Industry, Surindra Rajabhat University, Surin Province, Thailand (latitude 16.8191844 and longitude 100.2569015). It was carried out from January to March, 2020. Both before and after Chinese kale cultivation, soil samples were collected at a depth of 0-20 cm, air-dried, and passed through a 2-mm sieve before some analyses of the soil properties were undertaken. The daily day temperature and relative humidity were recorded from the first DASS to harvesting day. Liquid bioextract from the native plant species was prepared from the fresh components of 3 kg of *Leucaena leucocephala* shoot, 20 kg of *Azolla microphylla*, 3 kg of chopped *Pennisetum purpureum* X *P. americanum*, 3 kg of male inflorescence (bell) of *Musa* (ABB group), 3 kg of ripe *Carica papaya* L. pericarp, 3 kg of ripe *Musa* (ABB group) pericarp, 2l of molasses, 2l of effective microorganisms, and 4l of plain water. All components were thoroughly mixed and stirred in a fermentation container, which was placed under room temperature conditions for 60 days before straining only the liquid portion to analyze the content of the plant nutrients and phytochemical substances.

The experiment was conducted using a randomized complete block design (RCBD) with five replications (seven plants per replication). Four treatments are shown in Table 1.

Commercial Chinese kale seeds were sown in a 1 x 4 meter plot under greenhouse conditions on January 10, 2020, and harvested on February 28, 2020. The chlorophyll content at 23 and 48 days after seed sowing (DASS) was measured on the three fully expanded uppermost leaves using a chlorophyll meter (SPAD502). The stem fresh weight per m², leaves width and length, and petiole length were recorded on the harvested day (48 DASS). The TPC, AA and Ca contents of the whole stem were quantified after the plant samples were dried at a temperature of 70 °C for 48 hours using a hot air oven.

Table 1. Components of the various treatments

Treatments	Fertilization		Quantity of Seeds Used /4m ²	Spacing of Seedlings	Spraying of <i>Trichoderma harzianum</i>	Spraying of LPB	Watering	Pest Management
	Chemical	Manure						
Without practical cultivation (control)	None.	None.	4 g, mulching with rice straw after sowing.	<u>1st practice</u> Removal by hand at 20 days after germination to provide spacing of 10 cm. <u>2nd practice</u> Removal by hand at 30 days after germination to provide spacing of 20 cm.	None.	None.	Twice a day at 8-9 a.m. and 3-4 p.m.	None.
Conventional cultivation (CC)	<u>1st application</u> 113 g of 46-0-0 ratio/4 m ² at 14 days after germination. <u>2nd application</u> 113 g of 15-15-15 NPK ratio/4 m ² . at 28 days after germination	Broadcasting of 5 kg/4m ² as basal fertilizer before seeds sowing.	4 g, mulching with rice straw after sowing.	<u>1st practice</u> Removal by hand at 20 days after germination to provide spacing of 10 cm. <u>2nd practice</u> Removal by hand at 30 days after germination to provide spacing of 20 cm.	None.	None.	Twice a day at 8-9 a.m. and 3-4 p.m.	Following the recommendation of the Department of Agricultural Extension (DOAE).

Table 1. (Continued)

Treatments	Fertilization		Quantity of Seeds Used/4m ²	Spacing of Seedlings	Spraying of <i>Trichoderma harzianum</i>	Spraying of LPB	Watering	Pest Management
	Chemical	Manure						
Good Agricultural Practice (GAP) cultivation	<u>1st application</u> 75 g of 15-15-15 NPK ratio/4m ² at 20 days after seeds sowing. <u>2nd application</u> 50 g of 46-0-0 NPK ratio/4m ² at 35 days after seeds sowing.	Broadcasting of 10 kg/4m ² as basal fertilizer before seeds sowing.	5g, mulching with rice straw after sowing.	Removal by hand at 20 days after seeds sowing to provide spacing of 15 cm.	Spraying of <i>Trichoderma harzianum</i> to the soil at the rate of 600 cc/m ² before seeds sowing.	None	Twice a day at 8-9 a.m. and 3-4 p.m. using the certified source of water by the Surin Agricultural Research and Development Center (SARDC), Department of Agriculture (DOA).	Following the recommendation of SARDC, DOA.
Local Plant Bioextract (LPB) cultivation	None.	Broadcasting of 30kg/4m ² as basal fertilizer before seeds sowing.	5g, mulching with rice straw after sowing.	Removal by hand at 20 days after sowing the seeds to provide spacing of 15 cm.	None.	4 times at 7-day intervals. <u>1st practice</u> 15 days after germination. <u>2nd practice</u> 22 days after germination. <u>3rd practice</u> 29 days after germination. <u>4th practice</u> 36 days after germination.	Twice a day at 8-9 a.m. and 3-4 p.m.	In the case of the incidence of pests, bioproducts like wood vinegar (pyroligneous acid) and/or <i>Trichoderma harzianum</i> were allowed to be used.

Chemical properties analysis of the soil samples

The soil pH was determined at a 1:2 ratio of soil and deionized water, and the electrical conductivity (EC) was determined at a 1:5 ratio of soil and deionized water. The soil pH and electrical conductivity were measured using a pH meter and an EC meter, respectively (Land Development Department, 2010). Organic matter was measured by using the Walkley and Black method (Walkley and Black, 1947). The total N was analyzed using a Leco FP 828 nitrogen analyser at the Faculty of Agriculture, Chiang Mai University, Thailand. The available P was extracted using Bray II measured by the ammonium molybdate method and determined using a spectrophotometer at a wavelength of 882 nm (Bray and Kurtz, 1945). Exchangeable K, Ca, Mg and Na were extracted using the ammonium acetate method. Exchangeable K and Na were measured by using an atomic emission spectrophotometer (AES) where exchangeable Ca and Mg were measured by using an atomic absorption spectrophotometer (AAS) (Land Development Department, 2010). Extractable S and B were extracted using the barium chloride method and calcium chloride-manital extraction, respectively (Theerachindakhajorn, 2011) The available Fe, Cu, Zn and Mn were extracted, using the DTPA method (Lindsay and Norvell, 1978), and the soil texture was classified as loam using a hydrometer method (Land Development Department, 2004).

Chemical properties analysis of the liquid bioextract

Liquid bioextract (LB) pH and electrical conductivity were measured using a pH meter and EC meter, respectively (Land Development Department, 2010). The total N was determined using dry combustion (LECO Corporation, 2018), whereas the total P was measured by using the vanadomolybdate method (Land Development Department, 2010). The elements K and Na were measured by AES, whereas Ca, Mg, Fe, Mn, Zn and Cu were measured by AAS (Hanlon, 1998). B was determined using a colorimetry method (Suwanawong, 2001), and an analysis of the phytochemical substances of the liquid bioextract was conducted.

The free IAA content was determined using the HPLC/RF method, whereas the free GA₃ content and free cytokinin were determined using the HPLC/PDA method (Naprom and Sringam, 2009). The TPC was measured by the Folin-Ciocalteu method (Folin and Ciocalteu, 1927), and the DPPH radical scavenging activity was determined using a free radical method (Brand-Williams *et al.*, 1995).

Data of the growth parameters and phytochemical substances were analysed by analysis of variance (ANOVA) employing a statistical software package. A comparison of the means was carried out by Duncan's multiple range test (DMRT) at $p \leq 0.05$.

Results

Chemical properties of the soil before and after Chinese kale cultivation

To evaluate the soil chemical properties before and after Chinese kale cultivation, soil samples from the plots at 3 days before introducing the treatments and at the harvested day were collected. The soil pH, total nitrogen (N), phosphorus (P), potassium (k), calcium (Ca), sodium (Na), sulphur (S), manganese (Mn) and boron (B) after cultivations were greater than before cultivations. However, electrical conductivity (EC), organic matter (OM), cation exchange capacity (CEC), magnesium (Mg), iron (Fe) and zinc (Zn) after cultivations were lower than before cultivations (Table 2). Copper (Cu) content was a nutrient recorded less than 1 ppm both before and after cultivations.

Table 2. Soil chemical properties before and after Chinese kale cultivation

Chemical Properties	Before Cultivation	After Cultivation
pH	7.39	8.49
EC (mS/cm)	152.50	105.80
OM (%)	2.29	2.21
Total N (%)	0.10	0.21
CEC (meq/100g)	13.80	10.22
Avilable P (ppm)	265.27	348.28
Exchangeable K (ppm)	381.27	786.88
Exchangeable Ca (ppm)	3,922.80	4,300.00
Exchangeable Mg (ppm)	182.20	146.98
Exchangeable Na (ppm)	33.92	59.60
Extractable S (ppm)	30.31	48.04
Available Fe (ppm)	13.11	8.82
Available Cu (ppm)	< 1.00	< 1.00
Available Zn (ppm)	13.87	0.95
Available Mn (ppm)	17.90	34.98
Extractable B (ppm)	0.67	1.10

Chemical properties of LB

The quantity of some chemical properties of the liquid bioextract were examined. The liquid bioextract pH was 3.72, with electrical conductivity 10.28 mS/cm. Total nitrogen of 0.33%, potassium and calcium content of 0.74 and 0.15%, respectively. The content of phosphorus, magnesium, iron, manganese, zinc, copper and boron were 114.48, 802.00, 41.06, 66.13, 23.39, 1.42 and 10.75 ppm, respectively. The free GA₃, free IAA and free cytokinin content of 8.18, 0.062 and 0.607 ppm, respectively (Table 3).

Table 3. Chemical properties of liquid bioextract

Chemical Properties	Detectable Amount
pH	3.72
EC (mS/cm)	10.28
Total N (%)	0.33
P (ppm)	114.48
K (%)	0.74
Ca (%)	0.15
Mg (ppm)	802.00
Fe (ppm)	41.06
Mn (ppm)	66.13
Zn (ppm)	23.39
Cu (ppm)	1.42
B (ppm)	10.75
Free GA3 (ppm)	8.18
Free IAA (ppm)	0.062
Free Cytokinin (ppm)	0.607

Chinese kale growth characteristics

Results showed that the leaves chlorophyll content at 23 DASS had a highly significant difference (Table 4). CC, GAP and LPB significantly showed higher means of 48.76, 50.14 and 49.44 SPADunit, respectively when compared to the control. Also, the leaves chlorophyll content at 48 DASS from CC, GAP and LPB significantly showed the higher means of 46.56, 46.96 and 46.62 SPADunit, respectively when compared to the control. The fresh weight/m² of the controlled treatment significantly showed the lowest fresh weight/m² compared to CC, GAP and LPB with a score of 1.60, 2.22, 2.26 and 2.18 kg, respectively. The leaves width from CC, GAP and LPB gave higher means with a dimension of 10.21, 10.23 and 10.51 cm, respectively when compared to the controlled treatment. The LPB and GAP cultivation significantly performed the highest leaves length with 13.16 and 13.00 cm when compared to the CC and controlled treatment. Furthermore, the GAP cultivation statistically gave the highest petiole length of 10.24 cm followed by the LPB cultivation, CC and controlled treatment, respectively.

Total phenol content, antioxidant activity and calcium content

The results showed that the TPC, AA and Ca contents in all treatments were not significantly different (Table 5).

Table 4. Growth characteristics of Chinese kale as affected by various types of cultivations

Treatment	Leaves Chlorophyll Content SPAD		Fresh Weight /m ² (kg)	Leaves Width (cm)	Leaves Length (cm)	Petiole Length (cm)
	Unit					
	23DASS	48DASS				
Control	46.78b	43.03b	1.60b	8.53b	11.72c	8.67c
Conventional Cultivation	48.76a	46.56a	2.22a	10.21a	12.56b	8.54c
Good Agricultural Practice	50.14a	46.96a	2.26a	10.23a	13.00a	10.24a
Local Plant Bioextract	49.44a	46.62a	2.18a	10.51a	13.16a	9.74b
F-test	**	**	**	**	**	**
CV (%)	2.42	1.85	5.29	2.90	1.57	2.50

** = significantly different at $p \leq 0.01$.

Table 5. The TPC, AA and Ca contents as affected by various types of cultivations

Treatment	TPC (mg GE/g)	AA (umol TE/g)	Ca Content (%)
Control	1.59	3.14	3.32
Conventional Cultivation	1.53	2.81	3.46
Good Agricultural Practice	1.74	3.20	3.25
Local Plant Bioextract	1.78	3.06	3.06
F-test	ns	ns	ns
CV (%)	15.94	8.23	10.10

ns = not significantly different at $p \leq 0.05$.

Discussion

The leaves chlorophyll content at 23 and 48 DASS in all cultivation practices were significantly the highest in the conventional cultivation, good agricultural practice and local plant bioextract, which was possibly due to the nutrient content in the fertilizers used in each treatment. Nitrogen is a structural element of chlorophyll and protein molecules, and it thereby affects the formation of chloroplasts and accumulation of chlorophyll in them (Daughtry, 2000). Moreover, phosphorus affects the stability of chlorophyll in plant (Bojovic and Stojanovic, 2003). Hence, the increased chlorophyll content of this study might be the possible result of phosphorus. Furthermore, potassium content in chemical fertilizer and cow manure of all tested treatments, causing adequate potassium for chlorophyll synthesis. Thus, our result was conformed to the recent work of Zhao *et al.* (2016) who reported that Chlorophyll a, b and (a + b) of a maize inbred line, which

was sensitive to potassium deficiency was significantly decreased under different K deficiency treatments. The results from the controlled treatment of the present study was similar to those reported by Lu *et al.* (2016) who found that the total concentration of chlorophyll decreased under the K deficiency.

The fresh weight/m² and leaves width of the controlled treatment significantly showed the lowest value compared to the three treatments. This indicated that without supplying any macronutrients, N, P and K, affected such traits of the tested plant. As N, P and K were supplied to the soil, the N and P function was mainly as constituents of proteins and nucleic acids where the K function was mainly in osmoregulation, the electrochemical equilibria in cells and their compartments and the regulation of enzyme activities (Marschner, 1995). Such the functions of N, P and K caused a significantly increased fresh weight/m² and leaves width of the tested plant.

The leaves length of the tested plant was significantly the highest in the GAP and LPB cultivation. This proved that the nutrient content in the CC did not significantly affect the leaves length as presented in the GAP and LPB. This was interesting that the LPB gave the highest leaves length as well as the GAP, which was possibly due to the macronutrients, micronutrients and hormones content found in the liquid bioextract causing an extension of the leaves length. Even though liquid bioextract contains low nutrient content, it still contains macronutrients, micronutrients and natural hormones. The present study was conformed to Tripathi *et al.* (2015) who reported that micronutrients were required in minute amounts by plants, but inexorably played an eminent role in plant growth and development. Plant hormones found in liquid bioextract were free IAA, free GA₃ and free cytokinin. Additionally, auxins were involved in cellular elongation as well as the effect of gibberellins on stimulating cell division and/or cell elongation, and cytokinins promoted cell division (Arteca, 1996).

Good agricultural practice gave the highest petiole length, which was possibly due to the spacing among the tested plants being 15 cm which was closer than the CC, causing them to compete against each other. In addition, 10 kg of manure applied to the soil in the GAP was a higher rate than the CC that may promote the length of the petiole, which was possibly due to the macronutrients and micronutrients contained in the manure. Our results were in agreement with Tripathi *et al.* (2015) who reported that micronutrients play an eminent role in the growth and development in plant species.

The total phenol content and antioxidant activity were not significantly different. It was indicated that different cultivation practices did not affect the TPC and AA. Furthermore, the results of the present study were similar to those reported by Kesarvani *et al.* (2014) who found that

phenolic compounds did not change under different farming systems. Moreover, phenolic compounds and antioxidants rely highly on genotypic as well as environmental factors (Adom and Liu, 2002) with minimal changes due to agronomic practices (Kesarvani *et al.*, 2014). Antioxidant activity showed insignificant differences among the cultivation practices, which was possibly due to adequate soil nutrients and favourable environmental conditions during the growing period, as tested plants may not provide the different antioxidants content. This conformed with Ahmed *et al.* (2010) who reported that in adverse environmental conditions; such as, low availability of the nutrients to the plants under organic farming, plants become highly tolerant while providing higher antioxidant enzymes. This was in agreement with Kesarvani *et al.* (2014). Any alteration of the agronomic practices from conventional to organic farming could bring minimal or no changes to secondary metabolites. However, this present study did not conform to several findings, which were focused on differentiating only organic and conventional practices; such as, *Brassica oleraceae* (Sakunasing, 2009), *Impomoea aquatica* (Prasopratnachai, 2009 and Ren *et al.* 2017).

The Ca content of the tested plants showed no significant differences among the treatments. Chinese kale is found to be a highly calcium-accumulated crop compared to other *Brassicaceae* vegetables (Thailand Toyota Foundation and Mahidol University, 1997). Our result revealed that the exchangeable Ca of the soil of the present study, both before and after cultivation, showed the highest contents were 3,922.80 ppm (0.39%) and 4,300.00 ppm (0.43%), respectively. Likewise, the Ca content of the tested plants from all treatments ranged from 3.06-3.46% of dry weight. Marschner (1995) reported that the Ca content of plants varied between 0.1 and > 5.0% of dry weight depending on the growing conditions. These results proved that the Ca content of the studied soil was at an adequate level for the growth of the tested plant.

In conclusion, *B. oleracea* grown under CC, GAP and LPB significantly performed the leaves chlorophyll content, fresh weight/m² and leaves width that higher than the control. However, the GAP significantly increased the leaves and petiole length. Interestingly, the GAP and LPB significantly increased the leaves length even though chemical fertilizers were not supplied to the soil. The TPC, AA and Ca content were not significantly changed by any type of cultivation. It was proved that some changes of such traits depended on the cultivation compared to the control.

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