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The potential of cabbage waste extract as a bio-stimulant for enhancing growth, biochemical constituents, and oil quality of thyme (*Thymus vulgaris*)

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Thyme (*Thymus vulgaris L.*) is an important cultural aromatic plant, whose herb and essential oils (EOs) have been used in many industrial and medicinal applications. Herb and EO yields are often negatively influenced by various factors, so it is important to keep finding new growing procedures that increase the quantitative content of herbs and EOs. Therefore, this paper is focused on the effects of applying cabbage extract (CE) at different concentrations on morphological parameters, mineral contents, total carbohydrates, and the quantitative and qualitative content of EOs in *T. vulgaris* plants. Two trials were conducted during two successive seasons 2017/2018 and 2018/2019 to assess the growth and essential oil response of thyme plants to different concentrations of natural plant extract. Cabbage extract (1, 2, 4, 6, 8 %) was sprayed on thyme plants four times during each season. Besides, spraying cabbage extract had a noticeable positive effect on vegetative growth parameters and the oil percent of thyme plants. All concentrations caused increases in N%, P%, K%, and carbohydrates % and caused variable effects between all oil compositions as compared to control plants. Moreover, foliar spray with 2 and 4% of CE caused an increment in the values of morphological parameters, carbohydrates, minerals, and all oil components as compared with control plants. The major compounds of thyme oil (thymol, p –Cymene, and γ- terpinene) showed the highest percentage in oil of herb harvested at the first and second harvests (H1 and H2) after foliar spray with 2 and 4% CE. In conclusion, the leaves waste of cabbage plants can be used as a bio-stimulant.

1. Introduction

The decrease in natural resources and the environmental disparity induced by current agricultural practices presented a serious challenge to food and nutritional drift sustainability. The overall population has gradually risen (1.13 percent annually) and the food demand has been steady. A significant issue is the adverse effect of ecological threats arising from the non-judicial use of chemical fertilizers and pesticides and from sustainable management of soil fertility (Wezel *et al.*, 2014). Unwanted soil biological and chemical changes have not only called into question sustainable food production but have also called for troubling malnutrition. In addition, the changing climate scenario has added enormous unforeseen farming costs. The economic and environmental cost of food rising today is much costlier than in the last decades. Cost-efficient and environmentally sustainable farming practices are necessary in order to combat such situations, and bio-stimulants are a feasible option in this context (Rathore *et al.*, 2009; El-Serafy and

El-Sheshtawy 2020). There is a lot of interest in natural products in agriculture (horticulture), which will at the same time boost the yield and biological value of the plants grown without adverse effects on the natural environment. These groundbreaking products are plant-growth bio-stimulants that can be applied effectively to sustainable farming. This behavior leads to the effective use of environmental limited resources (e.g., water) by plants and protects them from harmful agents caused by stressful conditions and pathogens (El-Serafy, 2018; Stamford *et al.*, 2019; Sofy *et al.*, 2020). Bio-stimulants consist of different substances or microorganisms that have been found to boost plant growth and development, improve nutrition uptake, abiotic stress tolerance, and crop quality characteristics (Shubha *et al.*, 2017; Caradonia *et al.*, 2018; Rezaei-Chiyaneh *et al.*, 2019). Plant growth bio-stimulants don't provide plants with sufficient quantities of essential nutrients but boost rooted system uptake and promote plant growth under stress because of increased antioxidant activity (Du Jardin 2015). Several metabolic processes such as photosynthesis, respiration, absorption of ions, and nucleic acid synthesis have been impaired by it. Bio-stimulants improve the availability of food, increase antioxidants, improve the capacity of water retention metabolism, and increase the development of chlorophyll. In addition to several benefits in agricultural practice, the use of bio-stimulants is suggested as a safe method for enhancing food crop nutrition (Latif and Mohamed 2016; Prakash and Verma 2016; Colla *et al.*, 2017; Akladious and Mohamed 2018).

There are different classes of bio-stimulants including seaweed and botanicals extracts, humic and fulvic acids, proteins hydrolysates, other nitrogen compounds, beneficial bacteria, fungal products, chitosan, and other biopolymers, and inorganic compounds (Mohamed and Gomaa 2012; Calvo *et al.*, 2014; Du Jardin 2015). Some research focused on the production of organic stimulants and selected plant biomass from higher plants, which are readily available and recognized for their specific properties, i.e., mugwort (*Artemisia vulgaris L.*), calendula (*Calendula offcinalis*), purple coneflower (*Echinacea purpurea*), chamomile (*Matricaria chamomilla*) and basil (*Ocimum basilicum L.*) (Godlewska *et al.*, 2019). The obtained plant extracts contain plant growth-promoting substances, such as amino acids, vitamins (Paradikovic *et al.*, 2011), nutrients, micro and macro elements, betaines, mannitol (Saa *et al.*, 2015), polyphenols and phytohormones (Ertani *et al.*, 2016). In addition, the extracts from plants promote the absorption and transportation of micro and macro elements of the soil in the plant (Paradikovic *et al.*, 2011).

This study has focused on the production of bio-stimulants from white cabbage (*Brassica oleraceae*) which is one of the most widely grown plants in the world. It belongs to the genus Brassica and the mustard family, Brassicaceae (Cruciferae). Brassicaceae is a monophyletic group of 338 familiar genera and about 3700 species worldwide, except in Antarctica (Al-Shehbaz *et al.*, 2006). White cabbage plays an important role in many countries cultures and traditional cooking and is commonly used in traditional medicine. White cabbage is an important source of phytonutrients in the human diet due to its inexpensive prices and availability in local markets. White cabbage is a cheap, but nutritious food source, which offers nutrients and phytochemicals to promote health. Phytochemicals have been very recent in scientific study, and white cabbage as a major source of glucosinolates, phenolic substances, carotenoids, and vitamins, is well known (Kapusta-Duch *et al.*, 2012; Avato and Argentieri, 2015).

Moreover, white cabbage was used as the raw material for processing bio-extract (the outside cabbage leaves that are peeling off until the cabbage is sold on the market). A source of inorganic nutrients and amino acids was shown to be bio extract from cabbage leaves waste (Grubb and Abel, 2006) and also includes minerals such as phosphorus, potassium, calcium, and iron. Cabbage is one of the most common vegetable plants in the world, besides tomatoes and onions (Nyatuame *et al.*, 2013).

Thyme is one of the most significant species in the family of the Lamiaceae and is used in the food, cosmetic and pharmaceutical industries. The key components of volatile thymus oils are thymol and carvacrol (Nickavar *et al.*, 2005; Trindade *et al.*, 2018). Essential oils play a key role as antiviral, antibacterial, antimycotic, insecticidal, and herbivore defensive in plants in nature (Bakkali *et al.*, 2008). It includes a large range of active phytochemicals (for example, flavonoids, terpenes, polyphenols, and coumarins). It is a supplement for the cancer-prevention agent that is late recommended and has a variety of useful effects such as anti-spasmodic, antioxidants, and anthelmintic (Sale-

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hi *et al*., 2018). Also, because of its bactericidal and fungicidal effects, it is important as a crude medicine in the production of plant drugs. The alcoholic extract is antiseptic and expectorant and is an essential component of cough medicines (Nikolić *et al*., 2014). In addition, *T. vulgaris* and thymol are very promising agents to protect agricultural plants and stored products (Matusinsky *et al*., 2015; Park *et al*., 2017).

Little research used the cabbage leaves extract as a bio-stimulant, therefore, the objective of this study was to evaluate the effect of different concentrations of cabbage waste leaves extract on the morphological, biochemical, and oil composition of *T. vulgaris,* and to study the effect of the extract on thyme under harvesting frequency.

2. Materials and Methods

2.1. Cabbage extracts preparation and chemical composition

Fresh leaves of cabbage were cut into small pieces, dried, ground, and extracted. For extraction, ethyl alcohol 80% was added to the powder and shacked on a shaker at room temperature for 48 hrs. Extracts were purified by filtering twice through Whatman filter paper no.1. After purification, the crude ethanolic extracts were concentrated using a rotary evaporator at 45 °C under reduced pressure. Each extract was diluted to the required dose (0, 1, 2, 4, 6, and 8%).

Folin reagent was used to determine the total soluble protein content according to the method of Lowry *et al*., (1951). The phenol-sulphuric acid method was used to determine the total carbohydrates in cabbage extract according to Dubois *et al*. (1956). The absorbance read was at 490 nm using a spectrophotometer. The amounts of total phenols in the plant extract were measured using the folin reagent at 725 nm UV-Vis spectrophotometer according to Dihazi *et al*., (2003) method and the gallic acid curve (99.5 percent) used as a standard. Total nitrogen was determined using a modified Kjeldahl procedure (Bradstreet, 1965). Phosphorus was measured as molybdovanadophosphoric acid and was read at 470 nm on a visible light spectrophotometer (Franson, 1975).

Calcium, magnesium, and potassium were determined by atomic absorption or emission spectrophotometry (Hanlon, 1998). Sulphur was measured using a gravimetric method (Hanlon, 1998). Fe, Zn, Cu, and Mn were determined by atomic absorption spectrophotometer (Marguí *et al*., 2022). The ascorbic acid (vitamin c) concentration of the cabbage extract was analyzed using the method described by Mukherjee and Choudhuri (1983). The tocopherol content was measured at wavelength 520 nm using a 2.2-dipyridyl reagent according to the method of Philip *et al*. (1954). Thiamine, cellulose, and hemicellulose were determined according to Bishop *et al*. (1958) with modification by Dever *et al*. (1968). The amino acid analyser was used to determine amino acid fractions in the leaves of cabbage plants according to El-Gala and Amberger (1988). Essential and non-essential amino acids were injected as standard.

2.2. Experimental layout and design

Seeds of thyme (*T. vulgaris*) were obtained from the Enza Zaden, Assem Doss Co. Egypt. Waste leaves of cabbage were obtained from markets. The seeds were sown in October during the two successive seasons of 2017/2018 and 2018/2019 in the nursery and germinated after about two weeks. In the last week of November, uniform and healthy seedlings of about 10 cm in height were transplanted into 35 cm diameter plastic pots filled with a prepared growing medium composed of sandy loam soil at the Experimental Farm of the Faculty of Agriculture, Cairo University, Egypt. Before sowing, the physical and chemical properties of the soil of the experiment were determined by standard methods according to Jackson (1973). The soil texture during the two growing seasons was consisting of 31.7 and 33.1% sand, 39.6 and 40.2% silt, and 27.3 and 25.1% clay respectively. Chemical analysis of the soil during the two growing seasons showed that pH = 7.5 and 7.3 respectively and available N, P, and K were 45.6 and 47.9, 7.8 and 7.4, and 415 and 434 ppm respectively (Table 1).

The experimental layout for each season was a Randomized complete Blocks Design, with three replications. Thyme plants were sprayed with cabbage extract (1, 2, 3, 6, 9 %) while control plants were sprayed with water by hand sprayer four times along each season starting from six weeks after transplanting and every month thereafter. Thyme plants were harvested twice (first and second harvest at 3 and 6 months after transplantation) manually 10 cm above the soil

Table 1. Physical and chemical properties of the experimental soil in the two growing seasons.

Soil properties	2017	2018
Coarse sand %	1.4	1.6
Fine sand %	31.7	33.1
Silt %	39.6	40.2
Clay %	27.3	25.1
Soil texture	Clay Loam	Clay Loam
pH	7.5	7.3
Organic matter %	1.93	1.85
Available N ppm	45.6	47.9
Available P ppm	7.8	7.4
Available K ppm	415.0	434.0

ppm: part per million

surface. At harvest, the following parameters were estimated: plant height, fresh and dry weights of the plant (dry weight was carried out by drying at 40°C for 72 hours in an electric oven), essential oil percentage, and composition. Some chemical parameters are measured in the dried leaves as nitrogen, phosphorus, potassium, and carbohydrates. Essential oil yield was also calculated. The extracted essential oil was dehydrated over anhydrous sodium sulphate and stored in a refrigerator until Gas Chromatography Mass Spectrometry (GC/MS) analysis.

2.3. Determination of essential oil content

Volatile oil percentage was determined in dry herbs according to the method described in the Egyptian Pharmacopoeia (1984) using Clevenger's apparatus for the determination of essential oil according to Guenther (1961).

2.4. Determination of carbohydrates

Total carbohydrates in dry herbs of thyme plants were determined based on the method of phenol sulfuric as described by Dubois *et al.* (1956). Pure glucose was used as the standard.

2.5. Determination of minerals

For the determination of minerals in dry herb of thyme plants, approximately 1.0 g of the dried leaves were put in a micro-Kjeldahl flask and pure HNO_3 (5 mL) was added. The samples were left to stand overnight at room temperature (25 ± 2°C). Then, the flask contents were heated to 120°C for 3 h in a Kjeldatherm block digestion system. After cooling at room temperature, H_2O_2 (2 mL) was added, and the samples were heated again to 120°C until digestion was completed (approximately 2 h). Finally, colourless solutions were filtered with a Whatman no. 42 filter paper into 100 mL volumetric flasks and diluted (Baker and Smith, 1974). The N concentration was determined using a micro-Kjeldahl apparatus as per Horwitz (1956). The blue colour method was followed to assess the P concentration by reducing molybdenum to molybdophosphoric acid in sulfuric acid to exclude arsenate (Franson, 1975). Standard reagents, such as sulfomolybdic acid (H_2MoO_7S), molybdenum blue, diluted H_2MoO_7S, and 8% (w/v) $NaHSO_3$-H_2SO_4, were used. The K concentration of the herb was assessed using a flame photometer as outlined by Page *et al.* (1982).

2.6. Determination of oil composition using GC-MS analysis

A GC-MS instrument with the following specifications was used for qualitative and quantitative analyses: TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA) coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole

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Mass Spectrometer). The GC/MS system was equipped with a TG-WAX MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness.(The carrier gas was helium at a flow rate of 1.0 mL min^{-1} and a split ratio of 1:10 using the following temperature program: 40°C for 1 min; rising at 4.0°C min^{-1} to 160°C and held for 6 min; rising at 6°C min^{-1} to 210°C and held for 1 min. The injector and detector temperatures were held at 210°C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection, and NIST library) and the remained compounds were identified by comparing their mass spectra and relative retention indices of the peaks with those of standard compounds under the same conditions (Vimolmangkang *et al*. 2010).

2.7. Statistical Analysis

In each growing season, normality distributions of data for different traits were checked out by the Shapiro and Wilk technique (Shapiro and Wilk 1965). Data for the studied traits were analysed as a factorial experiment arranged in randomized complete blocks with three replications. A combined analysis of variance was conducted for the two seasons according to Gomez and Gomez (1984). A homogeneity test of error variances was performed by using the Levene test (Levene 1961). Thus, if the hypothesis that the two error variances are homogeneous cannot be rejected, the combined analysis of variance was computed. Tukey's HSD (honestly significant difference) test at the 5% level of significance was used to examine differences among treatment means. The estimates of simple correlation coefficients (r) were calculated between all possible pairs of the studied traits according to the method described by Steel *et al*. (1997) and the statistical significance of correlations was calculated according to Gomez and Gomez (1984).

3. Results

3.1. Chemical composition of cabbage extract

The chemical composition of cabbage leaf extract is shown in Table 2, the cabbage extract is rich in some macro and microelements and vitamins, i.e. thiamine, ascorbic acid, and tocopherols, as well as some osmoprotectants, i.e. amino acids. Also, it contains carbohydrates, protein, fibre, cellulose, and hemicellulose. The extract has high concentrations of zinc, sodium, and manganese (170, 130, and 120 mg $100g^{-1}$DW) respectively, and also high concentrations of potassium, sulphur, phosphorus, calcium, and nitrogen (1.2, 1.1, 0.79, 0.68, and 0.42 g $100g^{-1}$DW) respectively. The extract has high concentrations of vitamin C and vitamin E about 36.6 and 15.0 mg $100g^{-1}$DW respectively. In addition, the extract has high amounts of amino acids such as aspartic acid, glutamic acid, alanine, leucine, proline, threonine, and glycine (70.1, 53.2, 40.2, 39.6, 36.3, 35.2 and 30.1 mg $100g^{-1}$ DW) respectively.

3.2. Effect of cabbage extract on morphological parameters

Foliar application of cabbage leaf extract (CE) had a positive effect on studied vegetative growth parameters of thyme plants compared to control (spraying with water) (Table 3). The results show significant differences among treatments for all studied traits (plant height, plant fresh weight, plant dry weight, and oil percent). Foliar spraying with CE at 4 % concentration gave the highest value of plant height (29.3 cm) and the highest values of plant fresh and dry weights as well as oil percent (25.0, 6.63 g, and 0.15%) respectively. The results in Table 4 showed that the second harvest H2 gives the high pronounced increases in all growth parameters like plant height (29.5 cm), fresh weight (26.1 g), plant dry weight (6.85 g), and oil percent (0.14%). The data also in table 4 showed that the CE at 4% concentration gave the highest values in all growth parameters when the plants were harvested after 6 months of transplantation (H2).

3.3. Effect of cabbage extract on mineral contents and total carbohydrate

Herein, spraying thyme plants with different concentrations of CE (1, 2, 4, 6, and 8%) caused a significant increase in N, P, K, and total carbohydrates as compared to control plants (water spray) (Table 4). The most pronounced increases were recorded in plants sprayed with 2 and 4% of cabbage extract about (3.49 and 3.50%) in N content, (0.48 and 0.48%) in P con-

Table 2. Chemical analysis of cabbage powder per 100 g dry weight

Component	Concentration	Component	Concentration (mg)
Protein (%)	13.2	Aspartic acid	70.1
Fat (%)	1.6	Threonine	35.2
Carbohydrate	68.7	Serine	25.0
Fiber (%)	5.48	Glutamic acid	53.2
Nitrogen (g)	0.42	Proline	36.3
Phenolic compounds (mg)	90.5	Glycine	30.1
Calcium (g)	0.68	Alanine	40.2
Magnesium (g)	0.11	cysteine	2.27
Phosphorus (g)	0.79	Valine	28.5
Potassium (g)	1.2	Isoleucine	21.0
Copper (mg)	0.4	Leucine	39.6
Iron (mg)	4.5	Tyrosine	22.5
Sulfur (g)	1.1	Phenylalanine	24.1
Sodium (mg)	130	Histidine	10.5
Zn (mg)	170	Lysine	30.2
Manganese (mg)	120	Arginine	22.0
Cellulose (%)	13.7	Tryptophan	4.90
Hemicellulose (%)	11.1		
Vitamin B1 (Thiamine)(mg)	0.124		
Vitamin C (ascorbic acid) (mg)	36.6		
Vitamin E (tocopherols) (mg)	15.0		

tent, (4.10 and 4.18%) in K and (21.2 and 22.3%) in total carbohydrate respectively as compared with control plant. In addition, the plants harvested at the first harvest time after 3 months of transplantation gave the highest value of all the above contents compared with the plants harvested at the second harvest time.

3.4. Effect of cabbage extract on oil composition by GC/MS analysis

The chemical composition of the essential oil of *T. vulgaris* was determined on sample sets from the second (2019) season, thirteen compounds were identified. The identified compounds were grouped into three categories: (i) major compounds (more than 10%), (ii) minor compounds same as (1% and 10%), and (iii) trace compounds (less than 1%) (data are not shown). In addition, thymol (35.9- 56.7%), p -cymene (4.70-18.3%), and γ- terpinene (4.10-17.4%) were the major compounds in *T. vulgaris* (Table 5). The minor compounds were β -myrcene, α -terpinene, cis- sabinene hydrate, linalool, borneol, thymyl methyl ether, carvacrol methyl ether, and caryophellene, and the trace compounds were α-thujene and β-pinene.

Foliar spray with different concentrations of CE showed variable effects between all oil compositions. Foliar spray with 2 and 4% of CE caused an increment in the values of all oil components as compared

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Table 3. Effect of foliar spray with cabbage extract on plant height, plant fresh weight, plant dry weight, and oil percent of thyme under different harvesting times

Treatments		Plant H (cm)	FW/P (g)	DW/p (g)	Oil (%)
CE%					
0		19.7±1.5 d	13.7±1.6 d	3.01±0.50 d	0.06±0.010 d
1		24.7±2.2 b	19.2±2.9 bc	4.40±0.82 c	0.09±0.009 c
2		25.9±2.2 b	21.0±3.1 b	5.26±1.05 b	0.12±0.011 b
4		29.3±2.2 a	25.0±3.2 a	6.63±1.23 a	0.15±0.019 a
6		25.2±0.9 b	18.4±2.3 c	4.23±0.68 c	0.13±0.015 b
8		22.5±1.1 c	14.6±1.3 d	3.31±0.38 d	0.08±0.009 c
Harvest:					
H1		19.6±0.5 b	11.2±0.4 b	2.09±0.10 b	0.07±0.004 b
H2		29.5±0.8 a	26.1±1.2 a	6.85±0.43 a	0.14±0.007 a
CE% x Harvest					
0%	H1	15.0±0.7 g	8.6±0.3 i	1.38±0.09 j	0.03±0.005 i
0%	H2	24.4±0.8 de	18.8±0.8 e	4.64±0.21 e	0.10±0.006 d-f
1%	H1	18.1±0.7 f	9.9±0.6 hi	1.74±0.12 i	0.07±0.007 gh
1%	H2	31.4±1.6 b	28.5±1.2 c	7.05±0.32 c	0.12±0.009 d
2%	H1	19.6±0.7 f	10.6±0.6 g-i	1.91±0.13 hi	0.09±0.008 e-g
2%	H2	32.2±1.9 b	31.3±0.7 b	8.61±0.58 b	0.15±0.009 c
4%	H1	22.5±1.2 e	14.7±0.7 f	2.98±0.13 f	0.09±0.009 e-g
4%	H2	36.1±0.6 a	35.2±1.6 a	10.28±1.17 a	0.21±0.009 a
6%	H1	22.9±0.5 e	12.7±0.4 fg	2.42±0.14 g	0.08±0.006 f-h
6%	H2	27.4±1.2 c	24.1±3.2 d	6.05±0.84 d	0.17±0.009 b
8%	H1	19.3±0.7 f	10.7±0.5 gh	2.13±0.14 h	0.06±0.005 h
8%	H2	25.6±1.0 cd	18.5±1.1 e	4.48±0.25 e	0.11±0.006 de

CE = Ethanolic extract of Cabbage leaves (Cabbage Extract).
Values are means ±SD. Different letters next to the mean values in each column indicate significant difference according to Tukey's test

with control plants. The major compounds of thyme oil (thymol, p –cymene, and γ- terpinene %) showed the highest percentages in oil of the herb harvested at the first and second harvests (H1 and H2) after foliar spray with 2 and 4% CE. These compounds represented about 80 % of the oil constituents of thyme. In addition, the highest percentages of the major compounds in *T. vulgaris* oil were recorded in the plants harvested at the second harvest time H2 than the first harvest time H1.

Table 4. Effect of foliar spray with cabbage extract on mineral contents (N, P, K) and total carbohydrate of thyme under different harvesting time

Treatment	Harvest	N%	P%	K%	TC
CE%					
Control		3.20±0.35 b	0.38±0.02 b	3.14±0.15 b	16.6±0.8 c
1%		3.42±0.29 a	0.46±0.03 ab	3.94±0.13 a	19.4±0.5 b
2%		3.49±0.27 a	0.48±0.04 a	4.10±0.14 a	21.2±0.5 ab
4%		3.50±0.32 a	0.48±0.04 a	4.18±0.12 a	22.3±0.8 a
6%		3.35±0.34 ab	0.50±0.05 a	3.82±0.26 a	19.8±1.0 b
8%		3.32±0.35 ab	0.45±0.05 ab	3.68±0.19 ab	19.4±0.4 b
Harvest					
H1		4.09±0.03 a	0.52±0.02 a	4.10±0.09 a	19.6±0.7 a
H2		2.67±0.04 b	0.39±0.01 b	3.52±0.11 b	20.0±0.4 a
CE% x Harvest					
Control	H1	3.98±0.07 a	0.41±0.03 c	3.38±0.12 b-d	15.1±0.6 e
Control	H2	2.41±0.06 c	0.35±0.04 c	2.90±0.21 d	18.1±0.6 de
1%	H1	4.06±0.07 a	0.51±0.03 b	4.15±0.08 a-c	19.6±0.9 b-d
1%	H2	2.78±0.03 b	0.41±0.03 c	3.72±0.17 a-d	19.1±0.9 b-d
2%	H1	4.09±0.09 a	0.55±0.03 ab	4.34±0.16 a	22.0±0.6 ab
2%	H2	2.89±0.03 b	0.41±0.02 c	3.86±0.13 a-c	20.4±0.3 b-d
4%	H1	4.21±0.05 a	0.54±0.04 ab	4.42±0.09 a	24.0±0.6 a
4%	H2	2.79±0.09 b	0.42±0.06 c	3.94±0.09 a-c	20.58±0.6 b-d
6%	H1	4.11±0.08 a	0.61±0.03 a	4.23±0.15 ab	17.9±0.7 de
6%	H2	2.60±0.06 bc	0.39±0.02 c	3.40±0.37 b-d	21.7±0.9 a-c
8%	H1	4.09±0.06 a	0.52±0.04 b	4.06±0.12 a-c	18.8±0.6 cd
8%	H2	2.56±0.09 bc	0.38±0.03 c	3.30±0.12 cd	20.0±0.6 b-d

CE = Ethanolic extract of Cabbage leaves (Cabbage Extract).
Values are means ±SD. Different letters next to the mean values in each column indicate significant difference according to Tukey's test

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Table 5. Effect of foliar spray with cabbage extract on oil composition (%) of thyme under different harvesting times during the second season

Treatment / Component		β -Myrcene	α -Terpinene	ρ -Cymene	γ-terpinene	Cis- sabinene hydrate	Linalool	Borneol	Thymyl methyl ether	Carvacrol methyl ether	Thymol	Caryophellene
CE%												
Control		1.27±0.06 d	1.47±0.03 a	14.5±0.3 ab	9.0±1.4 b	1.57±0.12 a	3.19±0.13 a	4.90±0.39 a	2.21±0.36 b	1.79±0.10 bc	49.7±3.1 a	2.83±0.15 b
1%		1.76±0.04 c	1.49±0.05 a	14.6±1.7 a	10.5±3.1 a	1.53±0.04 ab	2.83±0.10 b	3.65±0.64 bc	2.15±0.27 b	2.02±0.05 a	51.9±6.2 a	3.54±0.42 a
2%		1.97±0.04 bc	1.60±0.09 a	13.4±2.7 b	10.3±2.8 ab	1.44±0.03 b	2.81±0.15 b	4.04±0.25 b	2.48±0.36	1.53±0.09 d	50.1±6.4 a	2.52±0.26 c
4%		2.10±0.03 ab	1.64±0.03 a	10.6±1.7 c	11.2±2.1 a	1.12±0.08 c	2.72±0.09 b	3.34±0.34 c	2.51±0.06 b	1.56±0.05 d	55.3±4.6 a	2.91±0.22 b
6%		1.95±0.04 bc	1.69±0.27 a	9.6±2.2 c	10.2±1.4 ab	1.11±0.04 c	2.69±0.22 b	3.50±0.34 c	3.11±0.46 a	1.84±0.07 b	55.0±0.16 a	3.42±0.16 a
8%		2.27±0.05 a	1.64±0.11 a	9.8±0.2 c	10.1±2.9 ab	1.18±0.19 c	2.14±0.34 c	3.75±0.58 bc	2.21±0.14	1.74±0.09 c	51.6±6.4 a	3.36±0.51 a
Harvest												
H1		1.84±0.08 b	1.42±0.03 b	9.0±0.8 b	5.1±0.4 b	1.40±0.05 a	2.69±0.08 b	4.33±0.15 a	2.67±0.11 a	1.71±0.07 b	65.2±1.5 a	3.46±0.21 a
H2		1.94±0.08 a	1.75±0.08 a	15.1±0.8 a	15.3±0.5 a	1.25±0.09 b	2.77±0.16 a	3.39±0.32 b	2.21±0.23 b	1.79±0.05 a	39.40±1.0 b	2.73±0.13 b
CE% x Harvest												
Control	H1	1.24±0.12 f	1.43±0.02 b	14.6±0.6 b	6.0±0.3 c	1.32±0.06 cd	3.00±0.17 cd	4.03±0.10 cd	3.00±0.02 bc	1.60±0.12 c-e	56.2±2.3 c	2.50±0.06 d
Control	H2	1.30±0.03 f	1.51±0.05 ab	14.4±0.2 b	12.0±0.6 b	1.82±0.06 a	3.37±0.12 a	5.77±0.12 a	1.41±0.06 g	1.98±0.06 ab	43.3±1.2 de	3.15±0.09 c
1%	H1	1.72±0.05 e	1.40±0.03 b	10.8±0.6 c	3.6±0.3 d	1.61±0.03 b	3.03±0.09 b-d	5.04±0.29 ab	2.69±0.12 b-d	1.94±0.06 a-c	65.7±1.7 ab	4.48±0.06 a
1%	H2	1.81±0.06 de	1.58±0.05 ab	18.3±0.6 a	17.4±0.6 a	1.45±0.03 bc	2.63±0.06 e	2.26±0.02 e	1.60±0.23 fg	2.10±0.03 a	38.1±1.2 de	2.60±0.06 d
2%	H1	1.92±0.07 b-e	1.40±0.06 b	7.4±0.2 d	4.1±0.1 d	1.48±0.04 bc	2.48±0.06 e	3.54±0.23 d	3.28±0.10 b	1.32±0.03 e	64.3±1.7 bc	3.09±0.06 c
2%	H2	2.02±0.05 b-e	1.79±0.06 ab	19.4±0.6 a	16.5±0.3 a	1.39±0.03 c	3.14±0.08 bc	4.53±0.06 bc	1.68±0.06 fg	1.74±0.03 bd	35.9±1.7 e	1.95±0.06 e
4%	H1	2.04±0.02 a-d	1.64±0.06 ab	6.8±0.1 d	6.5±0.1 c	1.30±0.01 cd	2.54±0.05 e	4.09±0.06 cd	2.63±0.02 c-e	1.45±0.01 de	65.0±2.9 a-c	2.43±0.06 d
4%	H2	2.15±0.03 a-c	1.65±0.03 ab	14.5±0.3 b	15.9±0.6 a	0.95±0.03 ef	2.89±0.06 d	2.58±0.04 e	2.39±0.04 de	1.67±0.02 b-e	45.7±1.7 d	3.38±0.06 bc
6%	H1	1.90±0.06 c-e	1.27±0.04 b	4.7±0.2 e	7.0±0.1 c	1.08±0.06 e	2.21±0.06 f	4.23±0.12 cd	2.08±0.06 ef	1.99±0.06 ab	74.0±2.3 a	3.76±0.12 b
6%	H2	2.00±0.06 b-e	2.12±0.44 a	14.6±0.2 b	13.3±0.1 b	1.13±0.06 de	3.17±0.10 b	2.77±0.12 e	4.13±0.06 a	1.69±0.03 b-d	36.0±1.2 e	3.07±0.04 c
8%	H1	2.21±0.06 ab	1.40±0.06 b	10.0±0.3 c	3.6±0.1 d	1.61±0.06 b	2.88±0.12 d	5.04±0.12 ab	2.36±0.29 de	1.94±0.03 a-c	65.7±1.9 ab	4.48±0.12 a
8%	H2	2.33±0.06 a	1.87±0.04 ab	9.6±0.3 c	16.6±0.4 a	0.75±0.03 f	1.40±0.06 g	2.45±0.06 e	2.06±0.02 ef	1.54±0.02 de	37.4±0.6 de	2.23±0.06 de
T		**	ns	**	**	**	**	**	**	**	*	**
H		**	**	**	**	**	**	**	**	*	**	**
T x H		ns	*	**	**	**	**	**	**	**	**	**

CE = Ethanolic extract of Cabbage leaves (Cabbage Extract).

Values are means ±SD. Different letters next to the mean values in each column indicate significant difference according to Tukey's test

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3.5. Response curve of dry weight and oil% to different levels of cabbage extract (CE %)

Linear and quadratic regression models were used to examine the response of dry weight (g plant^{-1}) and oil% for thyme plants to different levels of cabbage extract (Figure 1). For dry weight (Figure 1 a), the results of regressing coefficient indicated that cabbage extract (CE %) at 1.0 % decreased the dry weight by 0.014. The R^2 was higher for quadratic response (83.0%) than linear (0.1%). These results indicated that 83.0% of the variation in dry weight could be explained by the quadratic regression model following the equation: $y= 3.14 + 1.42 x – 0.18 x^2$ (i.e. the quadratic model was a more important and better fit than the linear model). Also Figure 1 (a), for the quadratic curve, dry weight = 6.63 g plant^{-1} was the maximum when CE % (x) = 4%. For oil% (Figure 1 (b) results of regressing coefficient indicated that an increase of 1.0 % CE, caused an increase of 0.003 %. Also, Figure 1 (b) showed that R2 was increased from 7.7% (linear) to 99.2% (quadratic). These results exhibited that 99.2 % of the variation in oil % could be explained by the quadratic regression model following the equation: $y= 0.057 + 0.042 x – 0.005 x^2$. Meanwhile, oil% scored the maximum value (0.15%) at 4% CE.

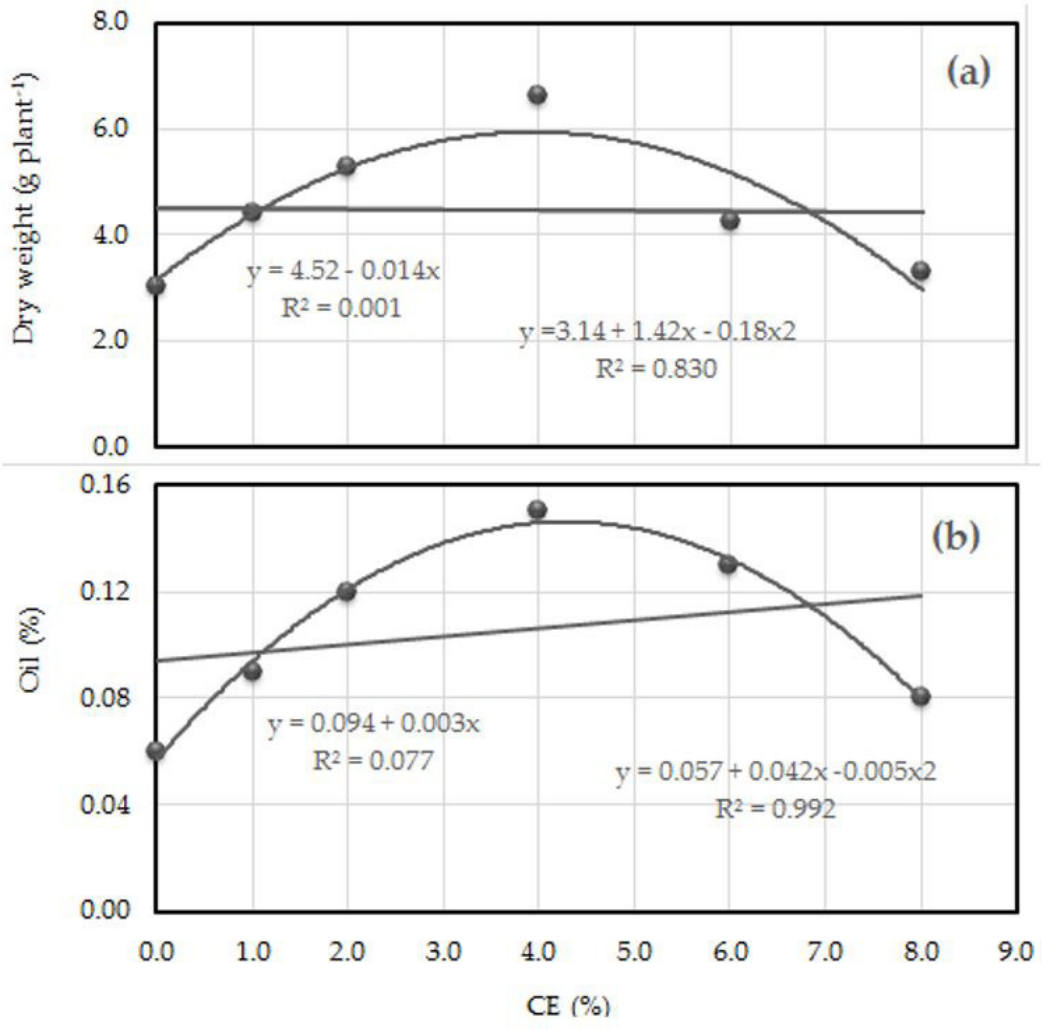

Figure 1. Linear and quadratic response of dry weight (a) and oil (b) to the level of cabbage extract

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Table 6. Pearson correlation coefficient of evaluated traits of thyme

	N	P	K	TC	Oil	PH	FW
N	1						
P	0.856**	1					
K	0.744**	0.887**	1				
TC	-0.012ns	0.227ns	0.464 ns	1			
Oil	-0.676**	-0.392ns	-0.109 ns	0.471ns	1		
PH	-0.724**	-0.413ns	-0.132 ns	0.309 ns	0.900**	1	
FW	-0.754**	-0.524ns	-0.214 ns	0.249 ns	0.897**	0.981**	1
DW	-0.751**	-0.535ns	-0.222 ns	0.231 ns	0.905**	0.973**	0.996**

ns: non-significant. ** Significant at $p < 0.001$.
PH; plant height, FW, fresh weight, DW; dry weight, TC; total carbohydrate

3.6. Correlation matrix

The matrix in Table (6) shows the simple correlation between each pair of variables. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables. Dry and fresh weight (g plant^{-1}) was positively and significantly correlated with oil% (r = 0.905** and r = 0.897**, respectively), plant height (r = 0.973** and r = 0.897**, respectively). However, the correlation coefficients between dry and fresh weight with N% content were significant and negative (-0.751** and -0.754**, respectively). Plant height (cm) was positively and significantly correlated with oil% (r = 0.900**). However, plant height and oil% showed negatively and significantly correlated with N% content (-0.724** and -0.676**, respectively). N, P, and K % contents were positively and significantly correlated with each other (Table 6). These results indicated that plant height and fresh and dry weights contribute to increasing in oil %. These relationships need to be considered by the thyme breeder when searching for superior genotypes that have desirable traits. However, the matrix of correlation showed positive and highly significant approximately full correlation coefficients (≈ 1.00) for many relations in Table 6, indicating that these traits are identical and we can use any one of them to identify the other.

4. Discussion

The cabbage extract is rich in macro and microelements, carbohydrates, protein, fiber, phenolic compounds, cellulose, hemicellulose, and vitamins, i.e. thiamine, ascorbic acid, and tocopherols, as well as some amino acids (Table 2). In the human diet, white cabbage is commonly used. It is mainly common because of the growth in local farmers' practices, customer preferences, affordable prices, and local availability. Moreover, this plant is most famous because of the abundance of antioxidants and anti-cancer agents such as polyphenolic contents, glucosinolates, carotenoids, and vitamin C (Park *et al*., 2014a, b) and potential anti-obesity properties (Williams *et al*., 2013). White cabbage is an essential source of dietary fiber, phytochemicals, and vitamins. Up to 40% of the white cabbage leaves, commonly used as fertilizer or animal feed, have been reported to be lost after processing (Nilnakara *et al*., 2009).
An idea was therefore suggested of using outside cab-

bage leaves which are normally discarded for the production of added-value items. Of particular concern are many products that contain the most abundant food fibre powder and phytochemical substances, including glucosinolates (Tanongkankit *et al.*, 2012). In addition, white cabbage has been also reported as a good source of phenolic compounds (Park *et al.*, 2014a). Also, tocopherols (vitamin E analogues) together with vitamin C are compounds with proven antioxidant activity and contribute to white cabbage health benefits (Podsedek, 2007).

Spraying CE extract at 2 and 4 % concentrations significantly increased plant height, plant fresh and dry weights, and oil % compared to the control. Maximum plant height, plant fresh and dry weight, and oil % were obtained in thyme herbs foliar sprayed with CE at 4% (Table 4). The positive effects of CE on the growth of thyme plants might result from the presence of an appropriate mix of inorganic nutrients and organic nutrients, such as amino acids in cabbage extract. Using the cabbage bio-extract resulted in high levels of leaf biomass and volatile oil with the highest content of carvone and menthol which was noted also in *Mentha spicata* and *Mentha arvensis* (Sitthithaworn *et al.* 2011). Cabbage is a sulfur-rich plant since the glucosinolate accumulating in the cabbage can be broken up to generate primary sulphur. For the synthesis of monoterpenoid compounds in volatile oil, sulfur is a donor of the methyl group and a source of methylene groups (Avato and Argentieri 2015). The increment in fresh and dry weights of thyme herb may be due to the effect of cabbage extract which enhanced the growth of the herb resulting from cell division and elongation in the meristematic zones and due to the rich of cabbage extract in macro and micronutrients and amino acids (Park *et al.*, 2014a). In addition, cabbage extract contains minerals that produced increases in plant growth and oil content. NP treatments produced the highest growth and essential oil of garden thyme (*Thymus vulgaris L.*) compared with the control treatment (Sharafzadeh, 2011).

Maximum plant height, plant fresh and dry weights, and oil % were obtained in plants harvested at the second harvest time H2 (Table 3). These findings were in line with Said-Al Ahl *et al.* (2018), who found that at the second (April) crop and afterward the third (August), the fourth (October), the first (February), and finally the sixth (December) harvests, the highest essential oil yield of thyme was obtained.

This may be due to that the second and the third harvests happened at more suitable growth stages for herbage and oil production. Also, Zhekova *et al.* (2011) also reported an increase from 0.3% to 0.41% in the essential oil content of fresh thyme when the blossoming stage changed from the beginning of blossom to full blossom. In addition, Khazaie *et al.* (2008) reported an increasing trend in the essential oil content of *T. vulgaris* as the irrigation interval increases and the harvest time increases. Significant differences in herb fresh weight and essential oil content, when harvested on different dates, were also reported previously by Ezz (2009) and Hegazy *et al.* (2016). Plant growth and yield are affected by the surrounding environment including temperature, humidity, and light intensity, which influence the various physiological parameters and the photosynthesis process (Zhou *et al.* 2022).

The most pronounced increases in mineral contents and total carbohydrate % were recorded in plants sprayed with 2 and 4% of cabbage extract as compared with control plants and plants harvested at the first harvest time after 3 months of transplantation (Table 4). The cabbage extract contains elements (N, P, and K) that caused an increase in the mineral and carbohydrates in thyme herbs. N plays an important role in the synthesis of plant constituents through the action of different enzyme activity and protein synthesis (Jones *et al.*, 1991) reflected in the increase in growth parameters. The presence of N in cabbage extract increased the vegetative growth, essential oil, fixed oil, total carbohydrates, soluble sugars, and NPK content of *Nigella sativa L.* plants (Khalid, 2001). Also, the high concentrations of P in cabbage extract had a stimulating effect on the growth parameters, total carbohydrates, soluble sugars, mineral contents, and the percentage of essential oil production from chamomile flowers compared with the control (Nassar *et al.*, 2004).

The major compounds of thyme oil (thymol, p –cymene, and γ- terpinene %) showed the highest percentage in oil harvested from herbs at the first and second harvests (H1 and H2) after foliar spray with 2 and 4% (Table 5). Variation in the composition of

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essential oils is influenced by different plant parts and their different stages of development and modifications due to the environment (Pirbalouti *et al*., 2013). These factors influence the plant's biosynthetic pathways and, consequently, the relative proportion of the main constituents. Harvesting time and environmental conditions are very important to obtain higher and better-quality essential oil content (Pirbalouti *et al*., 2013). Optimizing the harvesting time is vital for maximizing the quality of essential oil. Several studies have demonstrated that essential oil accumulation and its composition were affected by water stress and harvest dates (Said-Al Ahl and Omer, 2016). Essential oils (EOs) are synthesized through secondary metabolic pathways of plants as communication and defense molecules. In addition to their important roles in direct and indirect plant defenses against herbivores and pathogens, reproduction (through the attraction of pollinators and seed disseminators), and plant thermotolerance, EOs are responsible for the specific taste and aroma of plants. These characteristics, together with their diverse biological activities, have made them highly attractive for industrial purposes, food processing, perfumery, and medicine, including the development of plant protection products (Pavela and Benelli, 2016).

5. Conclusion

The present study indicated that different concentrations of cabbage waste leaf extract affected plant growth, oil percentage, nutrient uptake, and total carbohydrate in *T. vulgaris*. Thus, the utilization of cabbage waste leaves extract could be pondered as a strong biotechnological approach for plant growth development in sustainable agriculture systems. In addition, cabbage extract is constructed to positively change biological activities, essential oil content, and its major constituents in *T. vulgaris*. It concluded that the foliar spray with 2 and 4% cabbage extract gave the highest values of oil components under the two harvest times, so that, the cabbage extract can be used as a bio-stimulant.

Conflict of Interest

The authors declare no conflict of interest. Besides, the funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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