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Increasing of bioactive compounds in *Mentha cordifolia* Opiz., kitchen mint via $ZnSO_4$ biofortification during plantation

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ABSTRACT

Background: Plant growth generally requires both macronutrients and micronutrients. One of the most important micronutrients for plants is zinc. Zinc is an essential nutrient for every life form, including plants. In particular, zinc aids enzymatic processes and many biochemical reactions. When plants receive an inadequate amount of zinc, it leads to the loss of imperative biochemical reactions, which is also related to inhibition of plant growth. Therefore, the yield of the plant is relatively low. Interestingly, some scientific evidence have demonstrated a positive relation between zinc intake and the amount of essential oil and yield of plants. *Mentha cordifolia* Opiz., kitchen mint, is one of the top seven vegetables consumed in Thailand. The increase of some essential minerals in plants or biofortification during plantation has resulted in the increase of essential oils and chlorophyll. The bioactive compounds of essential oil provide antibacterial benefits. Additionally, chlorophyll can be utilized for against sinusitis, purifying the blood, and cleansing of toxins from intestines.

Objective: Our study aimed to evaluate the effects of fortifying zinc into plantation soil on growth characteristics, essential oil droplets, and overall quality of *Mentha cordifolia* Opiz.

Methods: *Mentha cordifolia* Opiz were planted in soil fortified with $ZnSO_4$ at 0 (control), 100, and 200 ppm and grown for 3 months. During the growth period, physical characteristics were observed. After harvesting, proximate analyses were conducted as well as determination of minerals, and chlorophyll content, were conducted. Microbiological and sensory tests were also performed.

Results: The increase of growth characteristics correlated with the increase of zinc concentration. The sizes of mint leaves were larger, the stalks were plumper, and the length of the roots were longer—although not significantly different—and the production of essential oil significantly increased. The approximate composition contents including protein, fat, ash, and fiber of plants grown in fortified $ZnSO_4$ soil, increased when compared to the

control. The leaves grown from the soil containing the 100 ppm $ZnSO_4$ treatment possessed the highest chlorophyll content, related to lowest in a* value. Overall, the kitchen mint fortified with 100 ppm $ZnSO_4$ seemed to be the most tolerable sample when analyzed for color and sensory attributes. Furthermore, it was discovered that 200 ppm $ZnSO_4$ treatment demonstrated the highest production of essential oil and lowest number of microorganisms.

Conclusion: In conclusion, soil fortified with $ZnSO_4$ at 100 ppm during seeding increased greenness, chlorophyll content, and consumer acceptability of the mint leaves. Darker and larger oil droplets were found in the mint leaves obtained from plants grown in soil fortified with 200 ppm $ZnSO_4$.

Keywords: Fortification, Growth characteristics, Zinc deficiency, kitchen mint, oil drop

INTRODUCTION

All living things including plants, animals, and humans require zinc (Zn) for their growth and reproduction. Zinc deficiency in humans inhibits physical growth and compromises the immune system. In fact, zinc deficiency is among the top five causes of death and disease in many developing countries [1]. Furthermore, the human body needs zinc for normal growth and development from the fetal stage throughout adolescence [2]. For example, DNA synthesis, neurosensory functions, and cell-mediated immunity all require zinc [3]. Manufacturers of oysters and other meat products, as well as a fortified cereal, claim their products have a high zinc content [4]. From our review of scientific studies, it was found that many plants may lack zinc when low zinc soil or alkaline soil was used to plant. Plants with zinc deficiency exhibited poor integrity, growth performance, and were low in yield and quality [5]. On the other hand, the growth, dry matter yield, and essential oil production of plants increased significantly when mint was grown in soil supplemented with zinc [6]. *Mentha cordifolia* Opiz. (Family Lamiaceae), commonly known as kitchen or marsh mint, is among the top seven vegetables consumed in Thailand. Mint is consumed in multiple forms—raw, in mildly cooked food, or in herbal tea. Mint is easily grown not only in Thailand but also in many Southeast Asian countries [7]. People in Thailand use kitchen mint as a traditional medicine to relieve gastrointestinal problems, asthma, muscle spasms, and inflammation. Some scientific data and evidence have demonstrated the biological effects of *M. cordifolia*, including antimutagenicity [8], analgesic quality, anti-nociceptive [9], anti-inflammatory, antioxidant activities [10], cold relief, flu, fever, motion sickness, and aiding of digestion problems [11]. Ferruzzi and Blakeslee [12] reported that the therapeutic properties of chlorophyll, which is usually found in green vegetables, can be summarized as being beneficial against sinusitis and skin rashes, purifying the blood and body overall, cleansing of toxins and the intestines, and liver detoxification.

Although Thailand is a tropical country with a wide variety of vegetables or plants, the World Health Organization reported that Thai people have moderate levels of zinc deficiency [13]. It is hypothesized that the zinc content in the plantation soil is not high enough. Therefore, this present work aimed to fortify zinc in soil and monitor growth characteristics, proximate compositions, and sensory tolerability of *Mentha cordifolia* Opiz.

MATERIALS AND METHODS

Chemicals and media

Our study used analytical grade chemicals. Zinc sulfate, potassium sulfate, copper sulfate, purified acid sand, calcium carbonate, and boric acid were purchased from Ajax Finechem, Apdivision of Nuplex Industries (Aust) Pty Ltd, New Zealand. Sulfuric acid, sodium hydroxide, and hydrochloric acid were obtained from Merck KGaA, Thailand. Methyl red, bromocresol green, ethanol, acetone, and petroleum ether were procured from LAB-SCAN, Ireland. Anhydrous sodium sulfate was purchased from QReC, New Zealand. All media for microbiological analysis was analytical grade including plate count agar, potato dextrose agar, EC broth, lauryl sulfate broth, and triple sugar iron agar purchased from Merck KGaA. Buffered peptone water, eosin methylene blue agar, selenite cysteine broth, lactose broth, brilliant green agar, tetrathionate broth base, XLD agar, and hektoen enteric agar were purchased from Difco Michigan, U.S.A.

Soil preparation for planting of kitchen mint

A field experiment was conducted at the Faculty of Agro-Industry, Prince of Songkla University, May 2014. Containers with a diameter of 19 cm and a depth of 13 cm were used for planting. Samples of soil mixed with 0, 100, and 200 ppm $ZnSO_4$ were put separately into the containers. All soil samples were subjected to analysis for mineral contents and physicochemical characteristics, such as organic matter, pH, cation exchange capacity, electrical conductivity, and available nitrogen—phosphorus and potassium.

Seeding preparation

The kitchen mint seeds were soaked in tap water overnight. The floating seeds were discarded, and the same amount of soaked vital seeds were planted separately into each prepared soil sample. Thereafter, the same amount of water was sprayed onto each planted pot twice a day for 3 months. During the growth process, the growth of the tagged leaf and plant height were measured.

Harvesting and analysis

Plants aged 3 months were harvested, and their leaves, stalks and roots were cut and separated. Each plant part was washed with tap water and drained before being weighed and counted. The leaves were then subjected to qualitative analyses.

Approximate composition

The leaf samples were analyzed for the contents of moisture, ash, fat, and protein according to the method of AOAC and fiber according to AOCS [14, 15]. The analyses were run in triplicate and standard deviations were calculated for differences among each trial.

Determination of macro and micro mineral

Samples were digested with nitric acid and hydrogen peroxide and filtered through Whatman No.1 filter paper. The final volume was adjusted to 100 mL using distilled water. Afterwards, the samples were analyzed using Inductively Couple Plasma-Optical Emission Spectrometer (ICP-OES) method [16].

pH and titratable acidity

Ten grams of each sample was homogenized in 40 mL distilled water for 2 minutes with a homogenizer and the pH was measured at room temperature by pH meter (Docu-pH Meter,

Satorius, Germany. Titratable acidity was carried out using AOAC method [14]. The homogenized sample was briefly prepared using a similar method to those used for pH measurement, before being filtered by Whatman No.4 filter paper. Thereafter, 5 mL of supernatant was added with 25 mL of distilled water, followed by 3 drops of phenolphthalein indicator then titrated with 0.1 M NaOH to reach the endpoint at pH 8.1, with the solution turning a pale pink. The results were expressed as L-ascorbic acid content.

Color value

The tagged leaf was measured for color value as *L*, a** and *b** using color meter (Color Flex, Hunter lab, U.S.A.). The color value was expressed as Commission International de I' Eclairage (CIE) Lab coordinates where *L** represents the luminosity (0 = black, 100 = white), *a** the redness (*a**> 0) or greenness (*a**< 0), and *b** the blueness (*b**> 0) or yellowness (*b**< 0).

Determination of chlorophyll (a,b) and total chlorophyll

The sample was analyzed using the AOAC method [17]. 3 grams of the sample were briefly ground with calcium carbonate and purified acid sand, which helped increase the extraction of chlorophyll and mixed with 10 mL of 80% acetone. The sample was then vacuum filtered through Whatman No.1 filter paper using a porcelain Buchner funnel. The residue material was re-extracted under the same conditions, until the green color of the residue on filter paper disappeared. All of the extracts were then pooled, with excess anhydrous sodium sulfate being added in order to absorb water before reparation of the chlorophyll by filtering. The final volume was adjusted to 100 mL with absolute acetone. Chlorophyll a and b were measured with a spectrophotometric absorbance at 665 and 649 nm, respectively. The concentration of both chlorophyll forms were calculated in units of mg/L according to the following equations:

$$\text{Chlorophyll a} = 11.63\text{Abs.}_{665} - 2.39\text{Abs.}_{649}$$
$$\text{Chlorophyll b} = 20.11\text{Abs.}_{649} - 5.18\text{Abs.}_{665}$$
$$\text{Total chlorophyll} = 17.72\text{Abs.}_{649} + 6.45\text{Abs.}_{665}$$

Determination of oil droplet

A fresh leaf sample from each treatment was cut cross-sectionally to view the oil droplet via digital photography using a Nikon Alphaphot 2 YS2 microscope.

Microbiological quality

Ten grams of each sample was blended with 90 ml of 0.1% sterilized peptone water to obtain 10^{-1} dilution before being diluted to appropriate dilution (10^{-2} -10^{-6}), in order to determine total viable count, *Escherichia coil*, *Staphylococcus aureus*, as well as yeast and mold. For *Salmonella* spp. count, 10 grams of each sample was blended with 90 mL of tryptic soy broth (TSB) followed by analysis using the method of Bachmann and Earles [18].

Sensory acceptability

Thirty blinded volunteers comprising of graduate students and technicians from the Department of Food Technology, Prince of Songkla University, who were familiar with consumption of fresh vegetables, recruited for sensory evaluation. The panelists were asked

to evaluate the samples for preferences in color, odor, taste, flavor and overall acceptability of each sample using a nine-point hedonic scale, from "1 - dislike very much" to "9 - like very much".

Statistical analysis

This experiment was run in a completely randomized design (CRD). Data was analyzed as mean ± standard deviation (SD) using one-way analysis of variance (ANOVA), then mean values were compared by Duncan's multiple range test (DMRT) at a significance level of 0.05.

RESULTS AND DISCUSSION:

Chemical composition and element in the soil sample

Initial values of moisture content, total nitrogen, organic matter, available phosphorus, cation exchange capacity (CEC), pH, electric conductivity (EC), aluminum, zinc, potassium, calcium, magnesium, and sulfur in the soil are shown in Table 1.

Table 1. Chemical composition and element of the soil

Sample	Parameters	Quantity	Unit
Soil (Initial)	Moisture Content	21.07	Percent
	Total nitrogen	0.47	Percent
	Organic matter	11.11	Percent
	Available P	146.65	mg/kg
	Cation exchange capacity (CEC)	31.96	Meq/100g
	pH	7.3	-
	Electric conductivity (EC)	355	µS/cm
	Al	0.06	Meq/100g
	Zn	31.429	mg/kg
	K	1,142.22	mg/kg
	Ca	11,739.68	mg/kg
	Mg	1,859.05	mg/kg
	S	682.124	mg/kg
Fortified with 100 ppm $ZnSO_4$	Zn	46.071	mg/kg
Fortified with 100 ppm $ZnSO_4$	Zn	50.840	mg/kg

Effect of fortified $ZnSO_4$ into the soil on growth characteristic and yield of kitchen mint

According to the results shown in Table 2, with increased concentrations of $ZnSO_4$, the kitchen mint leaves were bigger, the stalks were plumper, and the roots were longer; however, the last result was not significant. In fact, zinc is required for the synthesis of tryptophan which is a precursor of Indole-3-3 acetic acid (IAA), a plant hormone that stimulates cell division and stretching of the cells [19]. This may explain the growth of the

leaves, stalk, and roots when mint was grown in fortified $ZnSO_4$ soil. However, mint grown in fortified $ZnSO_4$ soil produced less volume compared with the control sample. Therefore, the yield of leaves, stalks, and roots (on a fresh basis) of the mint planted in fortified soil did not increase significantly. These results indicate a certain amount of zinc increased division and elongation of plant cells, leading to an increase of plant part size but not an increase in plant propagation. However, during plantation it was noted that mint grown in the soil fortified with 100 ppm $ZnSO_4$ experienced highest degree of worm infestation, despite the fact that all plant samples were grown in the same nursery. Hassan reported that some plants produced specific secondary metabolites including phenolic compounds, which act as insect attractants [20]. This may explain why there was no significant increase in leaf yield in mint grown in soil fortified with 100 ppm $ZnSO_4$, although photosynthesis determined by chlorophyll content was the highest for this soil sample. These results demonstrated 200 ppm of $ZnSO_4$ fortification increased zinc content in plants up to 47.44% as shown in Table 2. According to $ZnSO_4$ content in the plant leaves, it was discovered that the percentage increase of zinc tended to be lower when $ZnSO_4$ at 200 ppm was applied. It indicates that the plant started to saturate with zinc at this level and may have given rise to toxicity.

Table 2 Effect of $ZnSO_4$ fortification on growth characteristic and yield of kitchen mint parts and zinc level in kitchen mint leaves

Sample	Leaves			Stalks			Roots		Zn in Kitchen mint (ppm)	Zn increase (%)
	Width (cm)	Length (cm)	Yield (%)	Height (cm)	Diam-eter (cm)	Yield (%)	Length (cm)	Yield (%)		
Control	4.41±0.39^{c}	3.14±0.25c	42.89	40.43±6.29^{b}	0.60±0.01^{c}	39.25	43.12±4.45^{ns}	17.86	32.390	0
100 mg/ml	5.31±0.27^{b}	4.19±0.27^{b}	43.07	57.76±5.80^{a}	0.69±0.01^{a}	38.12	49.40±6.89^{ns}	18.81	44.821	38.38
200 mg/ml	6.46±0.21^{a}	4.47±0.24^{a}	44.31	63.77±7.10^{a}	0.65±0.01^{b}	37.27	50.10±7.03^{ns}	18.42	47.755	47.437

A-C means columns with different letters are significantly different ($p<0.05$)

Effect of fortified $ZnSO_4$ on chemical composition of the kitchen mint leaves

Moisture content of the mint leave samples was between 86.40 and 86.66 %, as shown in Table 3. The leaves obtained from 100 ppm $ZnSO_4$ fortification had the highest protein content followed by 200 ppm $ZnSO_4$ and the control group respectively. Additionally, when the soil was fortified with more $ZnSO_4$, the fat, ash, and fiber content contained in the leaves was higher, with the highest in the leaves from 200 ppm fortification. Kalaikandhan *et al*. reported that synthesis of protein, nucleic acid, growth substances, chlorophyll, lipid, and secondary metabolites were increased from assistance of zinc [21]. Additionally, it was noted that mint grown in soil fortified with 200 ppm $ZnSO_4$ had a different mint odor that was strongly pungent and not the appealing smell typical of kitchen mint. Unsurprisingly, the total carbohydrates were highest in the leaves of the control group, which was a typical characteristic of normal vegetables which mainly contain carbohydrates [22].

Table 3 Effect of $ZnSO_4$ fortification on chemical composition, pH and titratable acidity (TA) of the fresh kitchen mint leaves

Parameter	Control	100 ppm	200 ppm
Moisture (%)	86.66±0.03[a]	86.40±0.07[b]	86.66±0.05[a]
Protein (%)	3.34±0.09[c]	5.05±0.04[a]	4.03±0.05[b]
Fat (%)	2.11±0.04[c]	2.86±0.04[b]	3.00±0.02[a]
Ash (%)	2.19±0.03[c]	2.33±0.10[b]	2.47±0.02[a]
Fiber (%)	1.43±0.01[c]	1.44±0.06[b]	1.52±0.02[a]
Carbohydrates (%)	4.30±0.08[a]	1.95±0.03[c]	2.33±0.04[b]
pH	6.56±0.02[c]	6.75±0.02[a]	6.64±0.01[b]
TA (g of L-ascorbic/L)	1.07±0.08[a]	0.75±0.04[b]	0.80±0.08[ab]

A-C means row with different letters are significantly different ($p<0.05$)

The pH of a plant reflects the acidic and basic components found in the plant, particularly ascorbic acid, phenolic compounds, and essential oil content. In fact, acidity of plant materials reflects the amounts of weak organic acid, mainly ascorbic acid and other components, such as citric acid and phenolic compounds [23]. The higher the acidity, the lower the pH value of sample, as discovered in the low buffering capacity of the material—a leafy vegetable [24]. Mint leaves from the fortified 100 ppm $ZnSO_4$ group showed the highest pH and the lowest titratable acidity (Table 3). This may due to two possible reasons; (a) D-glucose, a form of carbohydrate produced in the mint was decreased and/or (b) ascorbic acid content was highly used for controlling normal functions in the mint grown in fortified soil with 100 ppm $ZnSO_4$. Rai *et al* [25] reported that plants exposed to polluted air possessed higher vitamin C compared with those in non-polluted air, due to a stress-response mechanism [25]. Moreover, Burt stated that plants having a high content of essential oils were classified as a low acid material because the pH of the oils was about 6.7-7.3 [26].

Table 4 Effect of $ZnSO_4$ fortification on color value of the kitchen mint leaves

		Lower Side			Upper Side		
Sample	**Position**	**L***	**a***	**b***	**L***	**a***	**b***
Control	leaf base	67.52+0.85[b]	-5.72±0.14[b]	16.32±0.44[a]	71.62±0.44[b]	-5.10±0.06[b]	16.28±0.14[a]
	leaf mid	67.65±0.91[b]	-5.38±0.53[a]	15.83±0.20[b]	69.30±0.56[b]	-5.04±0.35[a]	16.50±0.37[a]
	leaf apex	64.85±0.89[b]	-5.20±0.01[a]	13.40±0.50[b]	68.87±0.05[a]	-4.83±0.92[b]	14.87±0.31[b]
100 ppm	leaf base	63.61±0.63[c]	-6.63±0.13[c]	16.95±0.46[a]	66.21±0.63[c]	-5.91±0.40[c]	15.08±0.75[b]
	leaf mid	64.70±0.42[c]	-6.57±0.05[b]	16.37±0.07[a]	63.16±0.16[b]	-5.31±0.07[b]	14.42±0.15[b]
	leaf apex	64.13±0.60[b]	-6.30±0.10[b]	17.08±0.43[a]	66.02±0.64[b]	-5.23±0.22[c]	17.87±0.98[a]
200 ppm	leaf base	68.92±0.40[a]	-5.39±0.16[a]	14.67±0.10[b]	75.30±0.65[a]	-4.94±0.06[a]	15.76±0.19[ab]
	leaf mid	73.15±0.42[a]	-5.31±0.03[a]	14.68±0.10[c]	73.11±0.47[a]	--4.94±0.09[a]	14.72±0.20[b]
	leaf apex	71.21±0.27[a]	-5.21±0.06[a]	17.07±0.14[a]	68.87±0.05[a]	-4.46±0.19[a]	16.80±0.36[a]

A-C means columns with different letters are significantly different ($p<0.05$)

Effect of $ZnSO_4$ on color values and chlorophyll content in kitchen mint

The color value in the CIE system as L*, a* and b* of the leaves at the base, middle, and the apex of both sides, upper and lower sourced from different $ZnSO_4$ fortification concentrations are shown in Table 4. Results indicated that the upper side of the leaf at base and apex

position from the sample fortified with 100 ppm $ZnSO_4$ had the highest green (a* value) followed by the control and 200 ppm $ZnSO_4$ fortification samples respectively. This may be related to the proper concentration of zinc aiding the synthesis of chlorophyll as shown in Table 5. Fortification of 200 ppm $ZnSO_4$ in the soil resulted in decreased greenness for the plant, possibly due to the overload of $ZnSO_4$ fortification, which induces toxicity. However, the symptoms of toxicity in the plant tested is still unclear. Fontes and Cox [27] reported that in common plants the leaves were brown or had brown spots when soil contained more than 80 ppm zinc. Nevertheless, the level of zinc deficiency or toxicity in plants still needs further investigation, as each plant may respond to zinc content differently.

Table 5 Effect of $ZnSO_4$ fortification on chlorophyll of the kitchen mint leaves

Sample	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Control	1083.11 ± 0.36^{b}	419.43 ± 0.31^{b}	1502.55 ± 0.49^{b}
100 ppm	1195.78 ± 0.36^{a}	506.36 ± 0.30^{a}	1700.91 ± 0.66^{a}
200 ppm	1004.14 ± 0.04^{c}	413.40 ± 0.32^{c}	1417.30 ± 0.08^{c}

A-C means columns with different letters are significantly different ($p<0.05$)

Effect of $ZnSO_4$ on hoard essential oil of kitchen mint leaves

The essential oil determination of the plant leaves from digitalized photography using the Nikon Alphaphot2 microscope at 40x10, 100-120V, 50/60 Hz, 0.8 A is shown in Figure 1. The results demonstrated that the essential oil drop of the leaves of the control plant was the smallest, while the largest size and darker color of the essential oil droplet was found in the leaves of mint grown in soil fortified with 200 ppm $ZnSO_4$. The larger size and darker color of the essential oil droplet from the leaves obtained from fortified 200 ppm $ZnSO_4$ may be a good explanation of antimicrobial activity to be discussed later. Additionally, the participants noticed that the mint grown in the 200 ppm fortified $ZnSO_4$ had a strange flavor, a strong burning sensation, had a pungent smell, and gave a less cool feeling probably due to the quality (darker color) and quantity (bigger size) of the oil drop as shown in the Figure 1 (c).

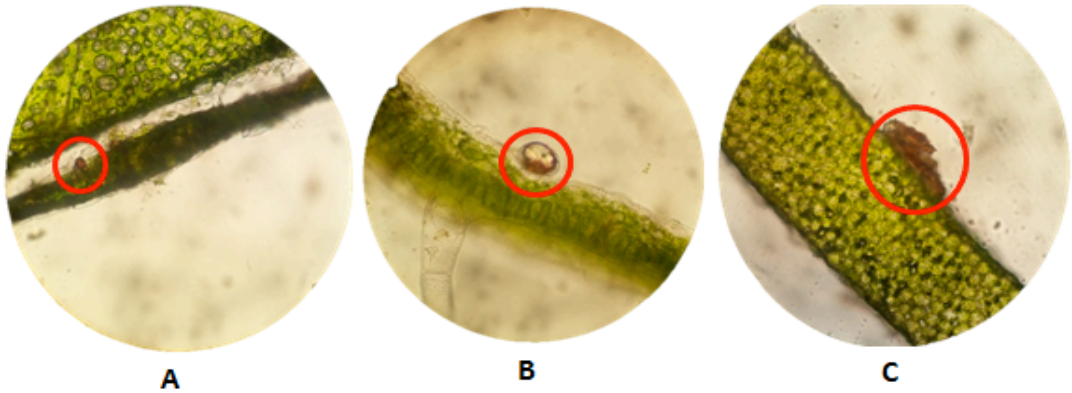

Figure 1 The essential oil drop of kitchen mint fortified with Zn at different concentration (A) Control (B) 100 ppm Zn fortification and (C) 200 ppm Zn fortification

Effect of $ZnSO_4$ on microbiological test in kitchen mint

Generally, major groups of microorganisms found in common fresh kitchen mint are bacteria, yeasts, and molds. However, species and a number of microorganisms depend on many factors, such as raw material, farm management, season, climate, harvesting techniques, and

management after harvesting, which includes washing, cutting, and storage [18]. It was discovered that the control sample contained the highest number of total viable count of 7.63×10^4 CFU/g, yeast and mold of 4.9×10^2 CFU/g, followed by the sample obtained from 100 ppm and 200 ppm $ZnSO_4$ fortification, respectively, although the differences were not significant (Table 6). The possible reason for the leaves obtained from 200 ppm $ZnSO_4$ fortified kitchen mint containing fewer microorganisms than others was that the zinc induced a higher essential oil content which exhibited an antimicrobial property [28]. However, the number of *Staphylococcus aureus,* which was found in small numbers and was not significantly different in each sample while the number of *Escherichia coli* in the sample obtained from 200 ppm $ZnSO_4$ fortification was smallest. There was no *Salmonella* detected in any sample. The *E.coli* and *S.aureus* contamination indicated the poor microbiological quality of water supply during planting, harvesting, and post harvesting [29]. The results highlight that for mint growth with $ZnSO_4$ at 200 ppm there is a significant effect on *E.coli* count. Many researchers reported that *E.coli* was highly sensitive to essential oils such as menthol [30], cineole [31], limonene, carvone, and pulegone [32]. Generally, total viable count, yeast and mold, *S.aureus*, *E.coli,* and *Salmonella* of all treatments in this experiment followed the fresh vegetables for exporting standard which did not exceed 1×10^6 CFU/g, 1×10^4 CFU/g, 100 CFU/g, 100 MPN/g and not detected, respectively [33].

Table 6 Effect of $ZnSO_4$ fortification on microbiological test of the kitchen mint leaves

Sample	Yeast and Mold CFU/g	TPC CFU/g	*Salmonella* CFU/g	*Staphylococcus* MPN/g	E.coli MPN/g
Control	4.9×10^2	7.63×10^4	ND	9.2±0.02	9.2±0.02
100 ppm	4.76×10^2	6.57×10^4	ND	9.2±0.01	9.2±0.02
200 ppm	3.2×10^2	5.17×10^4	ND	9.2±0.02	3.6±0.01

ND: Not detected

Effect of $ZnSO_4$ on sensory acceptability in kitchen mint

Sensory acceptability of kitchen mint grown in the soil fortified with zinc sulfate at different concentration evaluated by 30 panelists using the 9-point hedonic scale is illustrated in Table 7.

Table 7 Effect of $ZnSO_4$ fortification on sensory acceptability of the kitchen mint leaves

Sample	Color	Odor	Taste	Flavor	Overall
Control	7.58 ± 0.86^{ns}	6.77 ± 1.14^{b}	6.46 ± 0.95^{b}	6.81 ± 0.75^{ns}	6.69 ± 0.97^{b}
100 ppm	7.77 ± 0.71^{ns}	7.38 ± 0.98^{a}	7.31 ± 0.68^{a}	7.19 ± 0.75^{ns}	7.27 ± 0.87^{a}
200 ppm	7.73 ± 0.83^{ns}	7.15 ± 0.97^{ab}	6.96 ± 0.96^{a}	7.08 ± 0.98^{ns}	7.27 ± 1.04^{a}

A-C means columns with different letters are significantly different ($p<0.05$)

The results demonstrate that overall and taste attributes of the leaves fortified with 100 ppm $ZnSO_4$ and 200 ppm $ZnSO_4$ were significantly higher than those of controls, with a P-value less than 0.05. However, no significant differences in color and flavor characteristics were detected from any sample. The odor score of the sample with 100 ppm $ZnSO_4$ fortification was significantly higher than the control but not significantly higher for the 200 ppm $ZnSO_4$ fortification. However, some panelists suggested that the kitchen mint grown in the soil fortified with 100 ppm $ZnSO_4$ had a stronger and clearer natural odor when compared to the

control sample. Additionally, the panelists also commented on the sharp taste and burning sensation of leaves obtained from 200 ppm $ZnSO_4$ fortification while the leaves at 100 ppm $ZnSO_4$ fortification possessed a significant cool taste, similar to the characteristic taste of menthol [34]. It is emphasized that the sharp taste and burning sensation may relate to some the appearance of different compounds in the mint when the plant was grown in the soil fortified with 200 ppm $ZnSO_4$; this needs to be further investigated

CONCLUSION

In conclusion, $ZnSO_4$ fortification in soil can increase the level of zinc content in kitchen mint leaves significantly, in addition to improving growth characteristics, yield, protein, and fat content, as well as consumer acceptability scores. The fortification with $ZnSO_4$ 100 ppm of the mint leaves demonstrated the highest chlorophyll content. However, darker and larger size of the oil droplet was found in the mint leaves obtained from plants grown in soil fortified with 200 ppm $ZnSO_4$. The plant grown in soil fortified with 100 ppm $ZnSO_4$ was the optimum choice based on sensory score and suitable for consumption— whereas plants grown in soil fortified with 200 ppm $ZnSO_4$ may be suitable for essential oil production.

Competing interest: None to declare

Author's contribution: Timaporn Srirattanakul, BSc, MSc is a Nutraceutical and Functional Food and performed all of the laboratory work for the study and provided statistical analysis and assisted in writing the manuscript.

Sunisa Siripongvutikorn, PhD is an Assistant Professor of Food Technology. She is principal investigator for this study providing oversight and contributing fundamental conceptualization for the research, writing the grant proposal and manuscript.

Chutha Sae-Wong, PhD is a Pharmaceutical Sciences. She coordinated the initiatives to accelerate the development and subsequent production of the intervention meal. She also contributed in the study design and assisted in writing the manuscript.

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