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Full Length Research Paper

The effect of bio-extract from cabbage waste on growth, yield and quality of volatile oil extracted from *Mentha spicata* and *Mentha arvensis* var. *piperascens*

Worapan Sitthithaworn[1*], Theerasak Yeepoosri[2], Wullapa Yeepoosri[1], Kamol Mundee[1] and Danai Vannavanich[2]

[1]Faculty of Pharmacy, Srinakharinwirot University, Ongkarak, Nakonnayok 26120, Thailand.
[2]Rajamangala University of Technology, Thanyaburi, Pathumthani 12130, Thailand.

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The purpose of this study was to determine the effect of bio-extract produced from cabbage waste on the leaf biomass, yield and chemical compositions of volatile oils from spearmint (*Mentha spicata* L.) and Japanese mint (*Mentha arvensis* L. var. *piperascens* Malinv.). The spearmint and Japanese mint were grown in an open field and supplemented with three different fertilizers: bio-extract from cabbage waste, sulphur fertilizer, and a combination of sulphur fertilizer and bio-extract from cabbage waste. The plants were harvested during flowering and were analysed to determine the biomass and oil productivity. We determined that the bio-extract from cabbage waste was an effective nutrient supplement for the cultivation of spearmint and Japanese mint. For spearmint, use of the bio-extract yielded the greatest productivity of volatile oil since it resulted in the highest quantity of leaf biomass and in a high quantity of volatile oil with the greatest carvone content. For Japanese mint, the bio-extract yielded volatile oil with a menthol content equivalent to that of other supplements and we determined that application of bio-extract along with sulphur fertilizer was appropriate to enhance the biomass for Japanese mint.

Key words: *Mentha spicata* L., *M. arvensis* L. var. *piperascens* Malinv., bio-extract, volatile oil.

INTRODUCTION

Mentha spicata L. and *Mentha arvensis* L. var. *piperascens* Malinv. (Lamiaceae) have been cultivated and grow quite well in several areas of Thailand. *Mentha spicata* L., commonly known as spearmint (SM), produces carvone as the major oil component, while *M. arvensis* L. var. *piperascens* Malinv., commonly known as Japanese mint (JM), is a source of menthol (Dewick,1997). The oil of spearmint and the oil of Japanese mint are widely used for flavouring pharmaceuticals and oral preparations, such as toothpastes and mouthwashes. As the demand for the essential oils of SM and JM becomes greater, large scale cultivation for extraction of the essential oils should be performed. To achieve desirable products of high yield, a high-yielding clone has to be accompanied with proper soil preparation, fertilizer application, plant spacing, weed control as well as proper time and technique of harvesting (Canter et al., 2005). Sulphur fertilization is reported to be necessary for the synthesis of primary and secondary metabolites in plants (for a review, see Lewandowska and Sirko A, 2008). Sulphur is utilised in *S*-adenosyl-methionine (SAM) formation. SAM is a key substrate in numerous enzymes. It also serves as a donor of methyl group and a source of methylene groups for the synthesis of monoterpenoid compounds in volatile oil. Sulphur fertilization has also been reported to have a significant effect on the yield of volatile oil in sweet basil(*Ocimum basilicum*) (Zheljazkov et al., 2008).

During the past few decades, the use of non-chemical fertilizer has become a common practice. The use of non-chemical fertilizers was introduced with alternative

*Corresponding author: E-mail: worapan@swu.ac.th, Tel.: 663-739-5094, Fax: 663-739-5096.

Table 1. Physico-chemical properties and amino acid composition of bio-extract from cabbage waste tr[a] indicated that < 0.1 mg/100 mg

Parameter	Value
pH	3.6
Specific gravity	1.05
Macronutrients (%)	
N	0.38
P	0.02
K	0.73
Ca	0.29
Mg	0.10
S	0.17
Amino acid composition (mg/100 g)	
Aspartic acid	77.4
Threonine	31.2
Serine	29
Glutamic acid	63.2
Pralines	21.1
Glycine	34.3
Alanine	43.9
cysteine	3.37
Valine	31.7
Methionine	tr[a]
Isoleucine	23
Leucine	41.6
Tyrosine	20.3
Phenylalanine	26.6
Histidine	11.8
Lysine	34.4
Arginine	23.2
Tryptophan	5.16

agriculture system, which involves the use of bio-extracts. A bio-extract is an organic liquid made from vegetables, fruits, and animal substance fermented with sugar or molasses. It contains minerals, vitamins and amino acids. It is also rich in microorganisms that benefit the soil, and it provides nutrients to the plants. Bio-extract has become an alternative to chemical substances for improving crop production while maintaining and improving soil quality for long term use without toxic residue. The quality of bio-extract varies depending on the source of raw materials used to produce it. In this study, cabbage (*Brassica oleracea*, Brassicaceae) waste, the outer leaves of cabbage that are peeled off before cabbages are distributed in the market, was used as the raw material to produce bio-extract. Cabbage is a sulphur-rich plant because the glucosinolate accumulated in cabbage is able to breakdown to produce elemental sulphur (Grubb and Abel, 2006).

Our previous study revealed that, in addition to sulphur compound, an amino acid mixture should be used to supplement SM and JM growth to enhance the production of volatile oil with optimum carvone content for SM and high menthol content in JM (Vimolmangkang et al., 2010). As bio-extract contains both sulphur compound and amino acids, it is attractive to use as a supplement to boost growth and volatile oil production in these plants. In addition, cabbage waste is available all year, so it can provide sufficient material to produce bio-extract.

This study was carried out to determine whether or not bio-extract from cabbage waste is able to promote growth and high-quality volatile oil production of SM and JM.

MATERIALS AND METHODS

Preparation of bio-extract from cabbage waste

Bio-extract was made by mixing cabbage and molasses in a 4:1 ratio and water was added to the mixture to make 10 L. The fermentation was allowed for 20 days. 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 (AOAC. 1990). The amino acid composition was determined by ion-exchange chromatography with post-column derivatisation using ninhydrin. The post-column reaction between ninhydrin and amino acid eluted from column was monitored at 440 and 570 nm, and the chromatogram was used for determination of amino acid composition. Quantification of the amino acids was performed by comparison of the integrated area under the peaks against those from a separation of a standard calibration mixture (Fitzpatrick, 1949; Moore et al., 1958). The physico-chemical properties and the amino acid composition of the bio-extract are given in Table 1.

Nutrient supplements

The nutrient powder (Accent hydroponics, Thailand) was composed of 14.3% ammonium nitrate, 2.3% potassium dihydrogen phosphate, 10.0% potassium nitrate, 2.8% potassium phosphate, 7.8% magnesium sulphate, 0.10% manganese sulphate, 0.006% copper sulphate, 0.005% zinc sulphate and 0.003% ammonium molybdate in stock A and 8.6% calcium nitrate and 0.19% chelated iron in stock B. Stock solution A was made by dissolving 20 kg of stock A in 44 L of water. Likewise, stock solution B was made by dissolving 20 kg of stock B in 44 L of water. Plant standard nutrient was made by mixing stock solution A and stock solution B in a 1:1 ratio and water was added to the solution mixture to make a nutrient concentration of 2 to 3 mS/cm, as determined by the conductivity factor (CF). Plant standard nutrient supplemented with sulphur fertilizer was made by adding 500 g of magnesium sulphate to 10 L of stock solution A prior to combining with stock solution B (Vimolmungkung et al., 2010). The plant standard nutrient supplemented with bio-extract was made by adding 100 g of the concentrated bio-extract to 10 L of stock solution B prior to mixing with stock solution A.

To make standard nutrient supplemented with bio-extract and sulphur fertilizer, 100 g of the concentrated bio-extract was added to 10 L of stock solution B and 500 g of magnesium sulphate was added to 10 L of stock solution A prior to combining the stock solution. The nutrient solution was optimized at pH 6 to 7.

Cultivation

M. spicata L. and *M. arvensis* L. var. *piperascens* Malinv. were propagated by cutting shoots (10 cm long) from the mother plant and removing the lower leaves. The shoots were planted in soil free of weeds. When the plants were rooted (4 weeks), they were ready for transfer. The experiment was set up in a randomised complete block design, with three treatments including sulphur fertilizer, the bio-extract, and a combination of the bio-extract and sulphur fertilizer; three replicates were completed for each treatment. Fifty plantlets were used in each replicate of a single treatment. The plot size for a single replicate was 1 x 2 m. There were five rows in each plot, and the distance between the rows was 10 cm. Within the rows, the space between plants was 10 cm. The experiments were conducted in 2008 at the Faculty of Pharmacy, Srinakharinwirot University, Ongkarak, Nakonnayok, which is located in central Thailand. The plants were raised under black nylon netting that gave 50% shade protection. The photo period was natural day length (12 to 14 h per day). The average temperature was 30°C and varied from 26 to 33°C.

The annual rainfall for the region is about 1300 mm. Before planting, basal fertilizers containing nitrogen, potassium and phosphorus were applied and mixed well with the top soil of plot area to provide optimum nutrient supply for plant growth. The mixed soil had 300 ppm of nitrogen, 250 ppm of potassium and 50 ppm of phosphorus. The soil was well drained with a sandy-loam texture and pH 6.6. The plants were irrigated by sprinkler twice daily and the nutrient solutions were supplied at a 4 L per plot to the plants weekly.

Harvesting and analysis of volatile oil

At full bloom stage, leaf biomass and size of leaves were measured and recorded. The aerial portion of 30 plants was randomly selected. The oil content was determined from fresh leaves following hydrodistillation in a Clevenger-type apparatus for 2 h. Refractive index of volatile oil was determined at 25°C using a RX-500-Atago refractometer and volatile oil composition was analysed using an Agilent GC/MS (Model 7685 Series Injector) as described previously (Vimolmangkang et al., 2010).

Statistical analyses

Data are presented as mean values ± standard error calculated from triplicate determinations. Analyses of variance were performed at a level of $P < 0.05$ to evaluate the significance of differences between mean values.

RESULTS AND DISCUSSION

It has been observed in the mint species that harvesting the plants during full bloom yielded the greatest quantity of biomass, the greatest oil yield and the optimum oil quality (Rajeswara et al., 1999). Thus, in this study, the biomass, volatile oil content and oil compositions of SM and JM were determined during the full-bloomc period. The volatile oil obtained from fresh leaves exhibited the characteristics of SM and JM oils (Dewick, 1997). The volatile oils were pale yellow in colour with refractive indices of 1.477 to 1.482 for SM and 1.458 to 1.459 for JM. When comparing SM raised with sulphur fertilizer with the bio-extract from cabbage waste and with a combination of bio-extract plus sulphur fertilizer, it was clear that the bio-extract from cabbage waste provided the appropriate nutrients to promote the growth of SM because SM treated with the bio-extract yielded the greatest quantity of leaf biomass (Figure 1a) as determined by the ratio of leaf weight based on aerial portion. For JM, the bio-extract gave rise to the greatest quantity of leaf biomass when it was applied together with sulphur fertilizer (Figure 1a).

However, the bio-extract alone could promote growth equivalent to that of the sulphur fertilizer alone, the leaf biomass obtained from JM supplemented with the bio-extract alone was equivalent to that obtained from JM supplemented with sulphur fertilizer. Therefore, the bio-extract did not limit the growth of JM. Thus, it is worth applying the bio-extract as an alternative to inorganic sulphur fertilizer to increase the biomass of both SM and JM. An important concern in the farming of volatile oil-producing plants is the quantity of oil that accumulates in the plants. In this study, when comparing the bio-extract with sulphur fertilizer, it was clear that the bio-extract alone significantly increased the production of volatile oil in SM (Figure 1b). The volatile oil content observed in SM supplemented with the bio-extract was comparable with that observed in SM supplemented with the combination of sulphur fertilizer and the bio-extract. The volatile oil constituents observed in SM supplemented with the bio-extract were similar to those observed in SM cultivated with other types of supplementation. The major component was carvone. Other constituents were limonene, 1,8-cineole, menthone, α-pinene, β-pinene and sabinene. SM is characterised by the content of the monoterpenoid carvone, which accounts for 60 to 70% of the total oil composition in SM (Kokkini et al., 1995).

We found that all types of treatment could result in high levels of carvone at the harvesting period; SM supplemented with the bio-extract exhibited the highest carvone level (Figure 2a). Amino acid and inorganic supplements were reported to serve as raw materials for volatile oil biosynthesis, and oil compositions depend primarily on plant maturity (Duriyaprapan and Britten, 1982). It was clear that the bio-extract from cabbage waste contained an appropriate mix of inorganic nutrients and organic nutrients, such as amino acids, and could be applied to SM not only to support growth and plant maturity but also to serve as raw materials for volatile oil production. In contrast to SM, different treatments did not appear togreatly affect the volatile oil production in JM or the percentage of menthol in the oil (Figure 2b). The monoterpenoid constituents presented in JM raised with the bio-extract were similar to those observed in JM raised with other supplementations. The predominant compounds were menthol, limonene, menthone and neomenthol. Therefore, supplementation of JM with the bio-extract yielded the volatile oil without significant alterations of the quality.

The bio-extract from cabbage waste was demonstrated to be a source of inorganic nutrients and amino acids

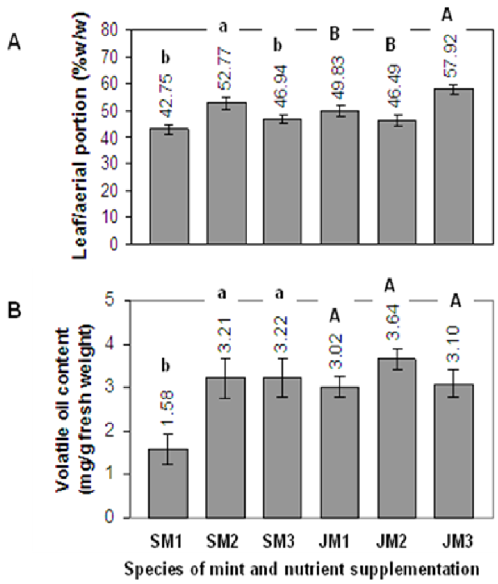

Figure 1. Differences in leaf biomass (A) and volatile oil content (B) of spearmint (SM) and Japanese mint (JM) supplemented with sulphur fertilizer (SM1 and JM1), bio-extract (SM2 and JM2), or bio-extract plus sulphur fertilizer (SM3 and JM3). Values represent mean ± S.E. Different letters within each species of mint indicate significant differences at p = 0.05.

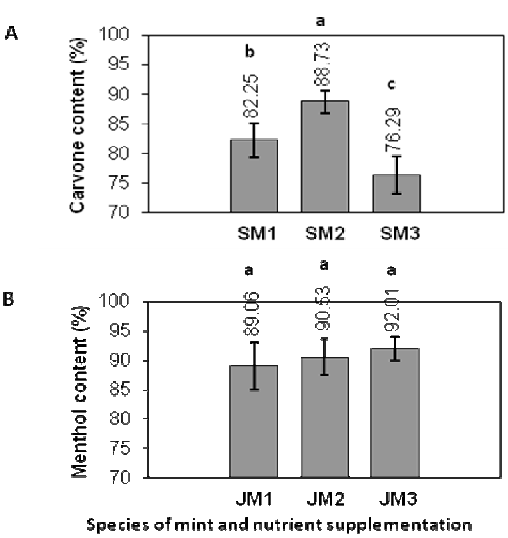

Figure 2. Carvone content (A) in spearmint (SM) and menthol content (B) in Japanese mint (JM) supplemented with sulphur fertilizer (SM1 and JM1), bio-extract (SM2 and JM2), or bio-extract plus sulphur fertilizer (SM3 and JM3). Values represent mean ± S.E. Different letters indicate significant differences at p = 0.05.

(Table 1) and it was supplied to both SM and JM to support the production of volatile oil in both plants. The concentration of bio-extract used in this study was suitable for growth and volatile oil production in SM. In JM, however, although the bio-extract yielded volatile oil without significant alterations in the quality as compared with sulphur fertilizer and with the bio-extract plus sulphur fertilizer, bio-extract should be applied to JM together with sulphur fertilizer to yield the greatest biomass.

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