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Yield performance and chemical constituents of selected local lines of *Ocimum* accessions

(Prestasi penghasilan dan kandungan kimia baka titisan terpilih aksesi *Ocimum* tempatan)

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Key words: local *Ocimum* lines, evaluation, herb and essential oil yield, chemical constituents

Abstrak
Enam titisan selasih tempatan telah dinilaikan di petak kecil bagi menentukan potensi hasil serta kandungan kimia. Berdasarkan pemetikan hasil empat kali setahun, empat titisan (BM 02, BM 05, BM 14 dan BM 16), yang tidak berbeza secara ketara antara satu dengan yang lain, memberikan hasil herba segar yang tertinggi (49.68–62.33 t/ha). Pengeluaran minyak pati yang tertinggi diperoleh daripada BM 05 (178.7 kg/ha), BM 14 (182.3 kg/ha) dan BM 16 (161.6 kg/ha). Titisan BM 14 dengan pulangan minyak pati yang tertinggi (0.35%) dan keseragaman pengeluaran minyak pati dari keempat-empat pemetikan hasil, adalah yang paling berpotensi. Kandungan jenis kimia yang terdapat dalam setiap aksesi turut dibincangkan.

Abstract
Six selected lines of local *Ocimum* accessions were evaluated in small plots for the determination of yield potential and chemical constituents. Based on four harvests per year, four lines (BM 02, BM 05, BM 14 and BM 16) gave the highest fresh herb yield between 49.68–62.33 t/ha. They were not significantly different from another. The highest essential oil production were obtained from BM 05 (178.7 kg/ha), BM 14 (182.3 kg/ha) and BM 16 (161.6 kg/ha). Line BM 14 which gave the highest average oil recovery percentage of 0.35% and consistency in essential oil production from the four harvests, seems to be the most promising. The chemical constituents of each of the accessions were also discussed.

Introduction

Ocimum spp., collectively known as basil, are native of tropical and subtropical regions of Asia, Africa, and South and Central America. Basil (locally called selasih and kemangi) has long been recognized as a rich and diverse source of essential oils. It is one of the most popular culinary herbs in North America, sold as fresh-cut, dried products, and as ornamental plants (Simon et al. 1999). Extracted essential oils from the leaves and flowering tops are used extensively by the perfume, pharmaceutical and food industries (Simon et al. 1984) and

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in aromatherapy (Chaplin 1999). These plants as well as oils obtained from them have received a lot of attention for their potential medicinal properties (Ketzis 1999). Extracts from the plants are used in traditional medicine and have been shown to contain biologically-active constituents that have insecticidal, nematicidal, fungistatic and antimicrobial functions (Simon et al. 1990).

According to Simon et al. (1999), based on geographical origins, several types of essential oils from basil are traded in the international market. They are the European type sweet basil which is considered having the highest quality aroma, containing linalool and methyl chavicol as the major constituents; the Egyptian type, very similar to the European which contains a higher percentage of methyl chavicol; the Reunion type, from the Comoro Islands, Madagascar, Thailand and Vietnam which is characterized by high concentration of methyl chavicol. Methyl cinnamate-rich basil is commercially produced in Bulgaria, India, Guatemala and Pakistan, while eugenol-rich basil comes from Java, Russia and North Africa.

In Malaysia, in spite of its long existence, basil is still being planted in household gardens. It is used to flavour foods, as medicine and in rituals (Burkhill 1966). Very limited documented information is available about its yield performance and the chemical constituents of its essential oils. This evaluation is to determine the fresh herb and essential oil yield production as well as the chemical constituents of selected lines of local basil, and to identify potential lines for commercial planting, if the need arises.

Materials and methods

Six lines selected based on potential yields and chemical constituents were used as treatments in this evaluation. They were BM 02, BM 05, BM 10, BM 13, BM 14, and BM 16. A vegetatively propagated clone, BM 14C, which gave the highest essential oil yield from a previous evaluation (Muhamad Ghawas and Ahmad 2002) was used as a check. Seedlings of about a month old were field planted in early May 2000 on Rengam Series soil at MARDI Research Station in Kluang, Johor. The experimental site, previously left fallow to grasses, is about 100 m above sea level and has a mean rainfall of about 2 300 mm with no regular dry season (Nieuwolt 1982).

The experimental design was a randomized complete block with three replications. Each treatment plot consisted of six-row plots with 26 plants per row. The planting distance was 30 cm within row and 60 cm between rows, giving a density of about 55 556 plants/ha. Large producers plant 70 000 to 84 000 plants/ha (Fletcher 1996). The plants were grown in the open, irrigated when required during the early stage of growth, and at later stages they were grown under rainfed conditions. No nipping or pinching of flowering tops was carried out during the evaluation.

Fertilizer application consisted of 30 g/plant of NPK compound (15:15:15) applied at two equal splits on the first and second month after field planting, respectively, amounting to 250 kg/ha of each N, P_2O_5 and K_2O, or an equivalent to 1.67 t/ha of the NPK compound. Side dressing with N in the form of urea, at a rate of 60 kg/ha, was applied within a week following each harvest. Simon (1995) suggested applying 50–70 kg of actual N/ha as a side dressing following each harvest. $N:P_2O_5:K_2O$ at a ratio of 1:1:1 with N rate of 230–300 kg of actual N/ha be broadcast and ploughed in before field planting.

Harvesting was done at about full bloom stage. Individual plant was cut manually at 15–20 cm from the ground level. Harvesting intervals of four crops/year (main and three ratoons) ranged between 85 days and 95 days, giving a total growth period of 362 days for each treatment, except for BM 16 for which a longer growth period of 101–107 days was required between harvests. In this line, only the main and two ratoon crops were obtained within the evaluated year. The biomass from each treatment plot was weighed fresh for herb yield.

For determination of essential oil content, random samples weighing 20 kg from each treatment plot were distilled for 2 hours, within 3 hours of harvesting using a portable water-steam distillation unit. The recovery percentages of each treatment were obtained by calculating the volume of essential oil obtained from every unit weight of fresh herb distilled. The oil yield was the product of fresh herb yield and the recovery percentage. Analyses of variance and Duncan Multiple Range Test (DMRT) were carried out for the parameters evaluated. Significant differences among the evaluated lines in yield production in small plots on a per hectare basis were used to determine their performance.

Selected chemical constituents of the essential oils were determined using a Gas Chromatograph-Mass Selective Detector (GCMSD). The chemical components present in the basil oil were separated using Gas Chromatograph (GC) utilizing a temperature programming technique, starting at 60 °C, increasing the temperature at a rate of 5 °C/minute to a final temperature of 260 °C. The separated components were identified by the electron impact ionization (EI) mass spectroscopy technique. The yield potential and chemical contents were used as the basis for identifying promising local basil lines.

Results and discussion

In this evaluation, four harvests (main and three ratoon crops) were obtained within a year of field planting, except for BM 16 which produced only three harvests.Yield production and recovery percentages of the treatments vary in each harvest. The fresh herb yield ranged from 6.70–15.92 t/ha, 7.77–22.34 t/ha, 8.48–28.29 t/ha and 8.86–12.49 t/ha for the main, 1st, 2nd and 3rd ratoon crops respectively *(Table 1)*. An evaluation on fertilizer and irrigation

Table 1. Fresh herb and essential oil yield, oil recovery percentages (Rec) and growth period (Gp) of each harvest of the main crop, 1st, 2nd, and 3rd ratoons of selected *Ocimum* lines at Kluang, Johor

Line	Main crop				1st ratoon				2nd ratoon				3rd ratoon				Total Gp
no.	Herb (t/ha)	Oil (kg/ha)	Rec* (%)	Gp (days)	Herb (t/ha)	Oil (kg/ha)	Rec (%)	Gp (days)	Herb (t/ha)	Oil (kg/ha)	Rec (%)	Gp (days)	Herb (t/ha)	Oil (kg/ha)	Rec (%)	Gp (days)	(days)
BM 02	15.92	45.99	0.29	86	9.25	19.05	0.21	89	15.82	53.56	0.34	95	11.53	33.90	0.29	92	362
BM 05	15.65	33.12	0.21	91	14.74	43.43	0.30	85	15.37	54.41	0.35	92	11.07	47.70	0.32	94	362
BM 10	10.83	23.02	0.22	87	4.88	10.28	0.21	88	13.83	29.10	0.21	93	8.93	14.47	0.12	94	362
BM 13	11.48	21.27	0.20	87	10.61	13.12	0.13	95	8.48	23.76	0.28	87	9.71	23.06	0.23	93	362
BM 14	14.23	44.30	0.31	95	7.77	47.12	0.41	90	15.19	51.37	0.35	90	12.49	39.51	0.33	87	362
BM 16**	11.70	19.07	0.16	101	22.34	64.28	0.29	106	28.29	78.22	0.27	107	–	–	–	–	314
BM 14C	6.70	16.37	0.26	92	7.77	28.89	0.38	90	11.11	41.65	0.38	90	8.86	29.00	0.32	90	362
Mean	12.36	30.68	0.25	89.7	9.17	26.98	0.27	89.8	13.30	42.31	0.31	91.2	10.43	31.27	0.27	91.7	
Percent	27.4	23.4			20.3	20.6			29.4	32.2			23.0	23.8			

*Fresh basis

**Not included in the mean and percentage calculation requirements of local basil on bris soil in Kelantan (Engku Ismail 2001) reported that with an application of 2.5 t/ha NPK compound fertilizer (12:12:17:2 +TE), fresh herb yield of 7.3 t/ha and 13.0 t/ha were obtained for the main and ratoon crops, respectively.

The overall percentages of fresh herb yield for the treatments, not including BM 16, were 27.3, 20.3, 29.3 and 23.0% for the main, 1st, 2nd, and 3rd ratoon crops, respectively. Their corresponding essential oil production were 23.4, 20.6, 32.2 and 23.8%. In this evaluation, the highest yield production was obtained from the 2nd ratoon. The reduction in yield was quite drastic at the fourth harvest (3rd ratoon). This may be due to the short-lived perennial nature of the lines, except BM 16 which is a perennial. Factors such as physiological decline, senescence and mechanical damage probably contributed to the reduction in yield. For maximization of yield production, replanting is suggested after four rounds of harvesting. In the Mediterranean areas, and other countries with a similar climate, basil is grown as a short-lived perennial, with 3–5 harvests per year, while in temperate zones basil is harvested only 1–3 times per year (Simon 1999).

In general, large variations in growth and yield performance could be expected in any crop production due to differences in climatic conditions, plant type, cultural and management practices and other related factors. In essential oil production, intrinsic factors such as genotype, state of maturity and part of plant harvested, and extrinsic factors such as light, temperature, water and nutrients, will strongly influence the oil yield and composition (Ahmad et al. 1999). Based on the data from one year production, significant differences in the parameters evaluated were obtained among the six lines as compared to the check, BM 14C *(Table 2)*. Four lines, BM 02, BM 05, BM 14 and BM 16, which were not significantly different from each other, gave the highest fresh herb yields ranging from 49.68–62.33 t/ha. Anguiler et al. (1999), reported yields of fresh herb and essential oils of sweet basil of 5–20 t/ha and 40 kg/ha, respectively. In California, United States, fresh herb yield ranged from 15–25 t/ha (Fletcher 1996).

In this evaluation, three lines (BM 05, BM 14 and BM 16) which were not significantly different between one another, gave the highest essential oil production. Their respective oil yields were 178.7, 182.3 and 161.6 kg/ha *(Table 2)*. These yields were 39.4–57.3% higher than the check (115.9 kg/ha). Compared to the average yield of essential oil and recovery percentages of selected and improved strains of seven *Ocimum* spp. in India (Puspangadan et al. 1995), these oil yields and recovery rates were lower. The respective average fresh herb yield, essential oil production and recovery percentages in India ranged from 40–60 t/ha, 160–300 kg/ha and 0.40–0.50%.

The average oil recovery percentages in this evaluation ranged from 0.19–0.35%. Line BM 14 gave the highest average recovery percentage (0.35%) and was significantly higher compared to the other lines. Its highest oil recovery percentage of 0.41% was obtained from the first ratoon crop. In this evaluation, this factor probably contributed to the consistency in essential oil production of BM 14, ranging from

Table 2. Fresh herb and essential oil yield and oil recovery (fresh basis) of selected *Ocimum* lines at Kluang, Johor (Main crop + 3 ratoons)

Line no.	Yield		Oil recovery (%)
	Fresh herb (t/ha)	Oil (kg/ha)	
BM 02	52.51ab*	152.5 b	0.28b
BM 05	56.82a	178.7ab	0.30b
BM 10	38.47cd	76.9d	0.19e
BM 13	40.13bcd	81.2d	0.21d
BM 14	49.68abc	182.3a	0.35a
BM 16	62.33a	161.6ab	0.24c
BM 14C	33.92d	115.9c	0.33a

*Mean values in the same column with different letters are significantly different ($p < 0.05$) according to DMRT

39.51–51.37 kg/ha over the four harvests. In the United States, an analysis on oil recovery (fresh basis) of more than 60 accessions of *Ocimum* spp. harvested during full bloom gave a range of 0.04–0.70% (Simon et al. 1990).

Oil constituents partly determine the quality of the essential oil and the products that could be developed by end-users. The profile of the top five chemical constituents of the six lines and the check clone is in *Table 3*. BM 02, BM 05, BM 10, BM 14, and BM 14C were high in methyl chavicol content, ranging from 69.4–94.7%. BM 13 was high in geranial (28.5%) and neral (22.9%), while BM 16 gave high percentages of eugenol (43.7%) and trans beta ocimene (27.6%). The highest linalool content was obtained from BM 14 (10.8%) followed by BM 13 (6.4%).

For essential oil production of these local basil, three lines, i.e. BM 05, BM 14 and BM 16, gave the highest yield. Among them, line BM 14, with an annual yield of 182.3 kg/ha seems most promising. Factors such as high oil recovery percentage and consistency in yield production from harvest to harvest contribute to its identification and selection. Based on principal constituents of exotic basil which contained mainly methyl chavicol (70–88%), with small amount of linalool, cineole, camphor, eugenol, limonene and citinellol (Lawless 1992), the oil from line BM 14 may possibly be used as an ingredient in the manufacture of fragrances, soaps, dental products and in major food categories especially meat products and savouries.

Conclusion

Line BM 14 with essential oil yield of 182.3 kg/ha, highest in oil recovery percentage, consistency in the production in the four harvests and containing the chemical constituents mentioned earlier was identified and selected among the local basil lines. Higher yield production may be expected with better management and cultural practices which need to be evaluated in the future to establish local agronomic requirements. In addition, a more efficient distillation unit for the extraction of essential oil needs to be developed in the future to improve oil recovery.

Table 3. Percentages of top five chemical constituents of basil lines at Kluang, Johor

Line no.	Chemical constituents	%	Line no.	Chemical constituents	%
BM 02	Methyl chavicol	84.2	BM 14	Methyl chavicol	69.9
	Trans beta ocimene	4.4		Linalool	10.8
	Camphor	1.3		Geranial	2.1
	Eugenol	1.3		Trans beta ocimene	2.1
	Linalool	1.2		Neral	1.6
BM 05	Methyl chavicol	94.7	BM 16	Eugenol	43.7
	Trans beta ocimene	2.4		Trans beta ocimene	27.6
	1,8 - cineole	1.7		Germacrene	10.4
	Linalool	1.2		Alpha bergamotene	5.7
				Beta caryophyllene	2.7
BM 10	Methyl chavicol	69.4	BM 14C	Methyl chavicol	86.7
	Linalool	5.7		Linalool	2.4
	Trans beta ocimene	3.7		Trans beta ocimene	2.4
	Alpha - cadinol	3.4		1,8 - cineole	1.3
	1,8 - cineole	3.0		Beta caryophyllene	1.3
BM 13	Geranial	28.5			
	Neral	22.9			
	Methyl chavicol	11.0			
	Cis alpha bisbolene	7.8			
	Linalool	6.4			

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