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Factors Affecting Biomass Growth and Production of Essential Oil from Leaf and Flower of *Salvia leucantha* Cav.

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Abstract: This study was conducted to investigate factors affecting biomass growth and production of essential oils from leaf and flower of *Salvia leucantha* Cav. (Mexican Sage). The light intensity (37-151 $\mu mol.m^{-2}s^{-1}$), water input (76-152 ml/day), and concentration of salicylic acid (0-0.5 mM) were varied and the biomass growth was recorded. The essential oils contained in the leaf and flower were extracted using a steam-distillation technique. The yield of essential oil both from flower and leaf varied from 1-2.76 % and 0.36-0.8 % on a dry weight basis, respectively. The productivity of essential oil was estimated in the range of 0.57-3.96 kg/ha.year and 2.26-4.36 kg/ha.year for both leaf and flower. The highest estimated productivity of essential oil (4.36 kg/ha.yr) was obtained when *Salvia leucantha* was cultivated without any shade net (receiving a light intensity of 301 µmol.m-2s-1) with a water input of 152 ml/day (100 % water field capacity) and sprayed with 0.25 mM salicylic acid on the surface of the flower once a week. The essential oil from both leaf and flower contain aristolane (7.31-16.33 %) which may have a potential application as anti-malaria substance.

Key words: *Salvia leucantha* Cav; essential oils; light intensity; salicylic acid; aristolane.

Introduction

Mexican Bush Sage (*Salvia leucantha*) is categorized as a Lamiaceae perennial plant which is indigenous of East Mexican and Tropical America with the distribution area spreading from Middle America plateau, European Mediterranean, Northern African to Continental of Asian [1]. *Salvia leucantha* plant has a relatively wide pharmacological and medicinal spectrum that encompass antioxidant, antimicrobial, and antispasmodic activity, neuroprotective agent, menstrual and digestive disorder medication, and blood circulatory regulator [2,3]. In Indonesia, *Salvia leucantha* can withstand being grown on plateau region as an introductory plant at an altitude of 500-1300 meter above sea level.

The leaf and flower of *S. leucantha* contain 0.6 wt% and 0.07 wt% essential oil, respectively [4,5]. The essential oil mainly comprises of bornyl acetate (11.4-23.9 %), β-caryophyllene (6.5-13.9 %), caryophyllene oxide (13.5 %), germacrene-D (13.8 %) and spathulenol (7-12.1 %) [4,5,6].

The essential oil is located around fine hair attached on flower calyx surface, flower, stem, leaf, pedicel, corollary flower, and petiole. The oil can be isolated through hydrodistillation and steam distillation of the *S. leucantha* flower [7]. The accumulation of volatile constituent in the essential oil depends primarily on growing conditions which can be modified physically or biologically

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throughout the period of cultivation. The yield of essential oil from *S. leucantha* can be improved by several conditions particularly elicitation process through the application of salicylic acid foliar spray at different concentration, irradiating light at reduced intensity using shade net, and regulation of water field capacity during the cultivation phase.

Foliar application of salicylic acid (at a concentration of 3 M) as an elicitor applied on *Thymus daenensis* had been proven to be the most effective method to enhance the yield of essential (extracted from the leaf) from 2.5 to 3.5 wt%, d.b [8]. In another study, aromatic herbs like *Ocimum basilicum* and *O. americanum* that were subjected to water deficiency during the cultivation phase experienced an increase in the essential oil yield extracted from the flower particularly from 0.24 to 0.38 wt%, d.b. and from 0.23 to 0.30 wt%, d.b., for both species, respectively [9]. Previous study [10] reported that the essential oil yield of *Salvia sclarea* extracted from its flower were of 0.1 wt%, d.b. and 8.7 wt%, d.b. when subjected to a reduction of light intensity using a shade net of 55 % and 75 %, respectively. Therefore, this study aimed to investigate the response of *Salvia leucantha* when subjected to a different foliar application of salicylic acid, water input, and light intensity towards biomass growth and its production of essential oil.

Material and method

Materials

One hundred and twenty plants of *S. leucantha* were obtained from Cihideung, Cigugur, Bandung Barat Regency, West Java Province, Indonesia. The plants were identified at the Indonesian Life Sciences Institute (LIPI), Bogor as *Salvia leucantha* Cav. Salicylic acid, ethanol, and hexane were obtained from School of Life Sciences and Technology, Institut Teknologi Bandung. Plastic shade net (55% and 75%) were purchased from JM Tani in Bandung, West Java Province, Indonesia.

Methods

Acclimatization and cultivation of *Salvia leucantha*

Acclimatization of *S. leucantha* at Kebun Percobaan Manoko, Lembang, Indonesia was carried out for one week. During the acclimatization period, the medium used was soil: compost: husk with a composition ratio of (1:1:1). The plant was watered at a full water capacity of the medium on a daily basis. After one week of acclimatization, the plants were subjected to different treatments particularly foliar spray of salicylic acid (0-0.50 mM), water input (50-100 % of water field capacity), and light intensity (0-75 % shade rate). The foliar application of salicylic acid was carried once a week by spraying the aerial part of the plant including leaf, flower, and axillary branch. Water input to the plants was varied by watering the plants once in three days at different percentage of water field capacity which was equivalent to 76 ml/day (50%), 114 ml/day (75 %), and 152 ml/day (100 %). The variation of light intensity was carried out using plastic with different shade rate which has an equivalent light intensity of 37, 151 and 301 $\mu mol.m^{-2}s^{-1}$. Growth parameters including plant height, number of leaf, axillary branch, and flower were determined on a regular basis during the cultivation period (52 days). At the end of the cultivation period, the plants were harvested and leaf were maintained at turgor conditions.

Extraction of essential oil

The essential oil contained in the *Salvia leucantha* leaf and flower was extracted using a steam distillation technique. Prior to that, the leaf and flower were shade-dried at room temperature and relative humidity at 70 % for 3 days. The steam distillation was carried out at 96°C (boiling point of water at 1.050 meter above sea level) for 4 hours. Following the condensation process, the oil and water phase were separated using a Dean-Stark receiver. The extracted oil was stored at the refrigerator to prevent it from being oxidized.

Determination of moisture content

The total moisture content of the sample was determined by heating the sample in the oven at 103°C until constant weight was obtained [11]. The moisture content of the sample after shade-dried for 3 days was also determined by determining the difference in weight before and after the shade drying process. The relative water content of the leaf was measured based on Wetherley method [12].

Estimation of yield and productivity of essential oil

The yield and productivity of essential oil were estimated using Eq. (1) to Eq. (3). The oil yield was calculated by dividing the amount of extracted oil to the dry weight of the sample. The productivity of biomass and essential oil was estimated for a cultivation area of 10.000 m^2 (1 ha) at Kebun Percobaan Manoko, Lembang at similar conditions performed in this study. It was assumed that the distance between each plant in the cultivation area was 1 m (10.000 plant/ha). The annual productivity of the biomass (kg/ha.yr) was estimated based on the amount of fresh weight obtained after the biomass was harvested at Kebun Percobaan Manoko, Lembang. It was also assumed that the leaf and flower of *Salvia leucantha* can be harvested twice a year.

$$\text{Oil yield (wt\%)} = \frac{\text{weight of extracted oil (g)}}{\text{Dry weight of sample (g)}} \times 100 \quad (1)$$

Biomass productivity (kg / ha.year) = Fresh weight of sample (g) x harvesting period (1/year) / cultivation area (ha) (2)

Oil productivity (kg / ha.year) = biomass productivity (kg / ha.year) x oil yield (wt%) (3)

Composition analysis of essential oil from *Salvia leucantha* using Gas chromatography-Mass spectrometry

The composition of essential oil from *Salvia leucantha* oil were analyzed by gas chromatography-mass spectrometry (Shimadzu's operation system GC-2010-GCMS-QP2010) using fused-silica capillary column HP-5 MS (30 m x 0,25 mm, film thickness of 0,25 µm). The injector was set at 250°C with a separation ratio of 1/30. The oil sample was dissolved in hexane at a volume ratio of sample to solvent (1:10, v/v %). The temperature was programmed at 50°C for the first 5 minutes and then being increased gradually to a final temperature of 250°C with a temperature increasing rate of 3°C/minute. Helium gas was used as mobile phase carrier at a flow rate of 1 ml/minute. Injection volume of oil sample to the column was 1 µL. Retention indexes for each detected compound was determined based on the approach of Van den Dool and Kratz [13] using an n-alkane as a standard.

Results and discussion

The effect of foliar application of salicylic acid, water input, and light intensity towards biomass growth and its yield and productivity of essential oil was investigated and the results are shown in Table 1. The moisture content of the sample after shade-dried for 3 days varied from 12.6 to 27.6 %. This variation might possibly due to the differences of air temperature and air circulation on a daily basis. The differences in those factors might cause a low energy efficiency which would lead to longer time for drying [14].

Effect of salicylic acid on biomass growth, essential oil yield and productivity

Foliar application of salicylic acid on the leaf of *S. leucantha* successfully increased the estimated biomass productivity from 282 to 586 kg/ha.yr. Salicylic acid has been reported to act as a regulator for plant flowering, retard senescence, and prerequisite for the biosynthesis of auxin or cytokinin [15]. The latter might have caused the plant to produce shadier leaf. In contrast, foliar application of salicylic acid on the flower of *Salvia leucantha* did not increase the productivity of the biomass. When the flower was not sprayed with salicylic acid, the estimated biomass productivity was 233 kg/ha.yr. Foliar application of salicylic acid (0.25-0.5 mM) on the flower decreased the estimated biomass productivity to 158 to 170 kg/ha.year. According to the study reported by Senaratna [16], although salicylic acid may induce flowering, it may also inhibits flowering and growth depending on its concentration and also influenced by the plant species and developmental stage.

Unlike the estimated productivity of the biomass, the yield of essential oil extracted from the flower increased from 1 to 2.8 wt%, d.b. when the concentration was increased from 0 to 0.25 mM. The oil yield slightly decreased to 1.8 %

Table 1. Biomass productivity as well as essential oil yield and productivity from *Salvia leucantha* at different cultivation conditions

Condition*	Sample origin	Moisture content after shade-drying (%)	Biomass productivity (kg/ha.year)	Oil yield (%)	Oil productivity (kg oil/ha.year)
Control	Flower	23.50	233.0	1.00	2.33
SA 0,25 mM		12.62	158.4	2.76	4.36
SA 0,50 mM		26.57	169.6	1.83	3.09
SN 55 %		25.60	108.0	2.10	2.26
SN 75 %		n/a	1.8	n/a	n/a
WI 50 %		18.60	153.2	1.57	2.40
WI 75 %		19.40	111.6	2.28	2.54
Control	Leaf	15.40	282.0	0.70	2.17
SA 0,25 mM		17.60	488.6	0.53	3.11
SA 0,50 mM		19.85	586.1	0.81	3.96
SN 55 %		18.20	244.0	0.67	1.63
SN 75 %		15.40	183.0	0.62	1.13
WI 50 %		15.10	160.1	0.36	0.57
WI 75 %		17.40	174.4	0.44	0.76

SA: concentration of salicylic acid; SN: shade net; WI: water input

(dry weight) when the concentration of salicylic acid was further increased to 0.5 mM. The observable increased of oil yield derived from *S. leucantha* flower was also reported within the same *Lamiaceae* family by Haiati and Rowshan [17]. They reported a 16 wt% increased relative to the control plant when *Satureja hortensis* was subjected to a foliar application of salicylic acid (0.14 mM) on the leaf. A different profile was observed that when salicylic was sprayed on the leaf of *S. leucantha*. The oil yield decreased from 0.7 to 0.5 wt%, d.b. when the concentration of salicylic acid was increased from 0 to 0.25 mM. However, the oil yield slightly increased to 0.8 wt%, d.b. when the concentration of salicylic acid was further increased to 0.5 mM. This slight decrease might be caused by the differences in the level of moisture content which would lead to a lower distillation efficiency.

The productivity of the essential oil was estimated using Eq. (3) which is a function of the oil yield and biomass productivity. Hence, the profile of estimated productivity of the essential oil resembles the profile of the oil yield. Highest estimated productivity of essential oil (4.4 kg/ha.yr) was obtained when the flower of *S. leucantha* was sprayed with 0.25 mM. Approximately 4 kg/ha.yr of essential could also be obtained when the leaf was sprayed with 0.5 mM salicylic acid. This result was higher compared to previous study by Lawrance [18] whom reported a 1.4 kg/ha. yr essential oil productivity of *S. sclary* planted at North Carolina, United States of America.

Effect of water input on biomass growth, essential oil yield and productivity

Water input to the plants was varied by watering the plants once in three days at different percentage of water field capacity which was equivalent to 76 ml/day (50 %), 114 ml/day (75 %), and 152 ml/day (100 % control). From the table, it can be seen that reducing the water input decreased the estimated biomass productivity of the flower from 233 to 112 kg/ha.yr. Similarly, the estimated biomass productivity of the leaf decreased from 283 to 174 kg/ha.yr. As such highlights the importance of sufficient water input for the plant growth. This is in line with Farooq [19] whom stated that the loss of turgidity might inhibits cell elongation which also limits its growth.

The essential oil yield extracted from the flower increased from 1 wt% to 2.3 wt% when the amount of water input was decreased from 152 ml/day (100 % water field capacity) to 114 ml/day (75 % water field capacity). However, further decreased in the amount of water input to 76 ml/day (50 % water field capacity) resulted in lower oil yield (1.6 wt%). However, the water input treatment strongly reduced the essential oil yield extracted from the leaf. These differences might have caused by the effect of water input toward oil gland structure. Water stress could affect oil gland cell integrity and cellular structure of the leaf which will possibly accumulate lesser oil [20]. In contrast, Werker [21] reported that the flowers' calyx of *Salvia sclarea* and *Salvia fructicosa* had denser structure which will accumulate more oil. Similar to the oil yield, the flower's essential oil productivity subjected to both water input variations increased from 2.3 to 2.5 kg/ha.year. The productivity of leaf's essential oil from both water input variations decreased from 2.2 to 0.6 kg/ha.yr.

Effect of light intensity on biomass growth, essential oil yield and productivity

The plants were also subjected to different light intensity by using different shade net particularly 55 % and 75 % shade net. Plants that were cultivated under a shade net of 75 % and 50 % received a light intensity of 151 and 37 μmol.$m^{-2}s^{-1}$ whereas plants that were not cultivated using a shade received full sunlight with an intensity of 301 μmol.$m^{-2}s^{-1}$. From Table 1, it can be observed that reducing the light intensity decreased the estimated biomass productivity of the flower from 233 to 1.8 kg/ha.year whereas the estimated biomass productivity of the leaves decreased from 282 to 183 kg/ha.year. These results clearly showed that lower light intensity provided lower signal to induce flowering as well as lower energy to accumulate more leaves' biomass.

The flower's essential oil yield from the plants that were cultivated under the shade net of 55 % increased from 1 to 2.1 wt%. In contrast, the essential oil extracted from the leaf slightly decreased from 0.7 to 0.67wt%. Unlike the shade net of 55 % treatment, plants that were cultivated under the shade net of 75 % producing scant flowers for the distillation process which cannot be quantified. Most possibly, the energy obtained through photosynthesis was allocated only for the cell maintenance. Similar to the treatment under the shade net of 55 %, the essential oil obtained from the leaf of the plants under the shade net of 75 % were slightly reduced from 0.7 to 0.62 wt%. The plants' response toward light intensity could vary amongst species or developmental stage. Li [22] reported an increase on *Salvia officinalis* oil yield from 0.34 to 0.38 wt% which was cultivated under a shade net of 45 %.

The flowers' essential oil productivity from the plants cultivated under the shade net of 55 % was slightly decrease from 2.33 kg/ha.year to 2.26 kg/ha. year. In line with the essential oil extracted from flowers, leaves' essential oil productivity strongly decreased from 2.17 kg/ha. year to 1.63 kg/ha. year. This result similarly occurred on the plants cultivated under the shade net of 75 %, decreasing the productivity from 2.17 kg/ha. year to 1.13 kg/ha. year. When cultivated under the shade net, plants produced lower biomass and consequently giving lower essential oils productivity.

Effect of water input and light intensity on the composition of *Salvia leucantha* oil

The composition of essential oil extracted from both leaf and flower of *S. leucantha* was analyzed using GC-MS and the results are shown in Table 2 and Table 3. The essential oil extracted from the leaf of controlled plants (water input of 152 ml/day, light intensity of 301 μmol.$m^{-2}s^{-1}$ and 0 M salicylic acid) mainly consists of δ-cadinene (9.69 %), β-cububene (13.93 %), aristolane (11.2 %) and β-farnesene (1.89 %). The essential oil extracted from the flower of controlled plant contains a higher amount of β-farnesene (22.6 %) followed by δ-cadinene (13.9 %), germacrene-D (13.8 %), and aristolane (13.7 %). In contrast, β-farnesene was not detected in the essential oil extracted from the flower of *S. leucantha*, both when the plants were given different amount of water input particularly 76 ml/day (50 % water field capacity), 114 ml/day (75 % water field capacity). Instead, the essential oil from both con-

Table 2. Composition of essential oil extracted from the flower of *Salvia leucantha* at different cultivation conditions

Compound	Composition (%)				
	Control	WI 75 %	WI 50 %	SN 55 %	Reference [4]
Bornyl acetate	-	-	-	-	23.9
β-Farnesene	22.59	-	-	10.6	-
Farnesol	0.95	18.95	20.61	-	-
Aristolane	13.72	13.33	16.33	7.31	-
trans-Caryophyllene	11.53	12.7	16.20		-
β-Caryophyllene	-	-	-	5.31	13.9
δ-Cadinene	13.86	11.35	13.38		-
Germacrene-D	13.80	10.94	12.67	3.46	13.8
Germacrene-B	4.86	3.74	-		0.3
γ-Cadinene	3.07	2.74	3.44		1.3
α-Humulene	0.44	-	-		0.8
Calamenene	4.29	3.99	4.42		1
Farnesene	0.46	0.60	0.95		-
cis-Muurola-3,5-diene	-	-	-		10.8
Bicyclogermacrene	-	-	4.88		8.7
Eicosane	1.09	-	-		-
Hexadecane	-	-	2.42		-
Hexadecanoic acid	2.82	-	-	10.36	-
Methyl-cyclopentane	-	18.85	-	13.36	-

SN: shade net; WI: water input

ditions mainly consist of farnesol (18.9-20.6 %), aristolane (13.3-16.3 %), *trans*-caryophyllene (12.7-16.2 %) δ-cadinene (11.4-13.4 %). The essential oil extracted from the flower of plants that were subjected to a light intensity of 37 ìmol.m^{-2} s^{-1} (shade net of 55 %) has rather a different composition compared the controlled plants and primarily consists of methyl-cyclopentane (13.4 %) â-farnesene (10.6 %), hexadecanoic acid (10.4 %) and aristolane (7.3 %).

According to the study carried out by Negi [4] the essential oil extracted from the flower of *S. leucantha* contains up to 23.9 % bornyl acetate. Previous studies also reported that the essential oil from *S. leucantha* contains bornyl acetate in the range of 11.4 to 27.8 % [5,6]. However, all the authors did not further characterize whether the detected bornyl acatete is (+)-bornyl acetate or (-)-bornyl acetate.

In contrast, bornyl acetate was not detected in all the samples investigated in this study. As such may be due to plant ontogeny in which could lead the biosynthesis pathway to other constituents. Chalcat [23] reported that Artemisia ketone was at the highest during the peak of flowering stage. In contrast, Japanese Mint contains highest menthol during the first stage of flowering and decreased toward the plant's age [24]. In addition, the difference in composition of the essential oil obtained in this study could be caused by growing condition applied during the cultivation period. Aside from those factors, the stage development of plant and harvesting time may create a significant different proportion of any constituent which should be present in the oil.

Based on the study conducted by Rupashinge [25] farnesene isomer is synthesized from farnesyl pyrophosphate through farnesol dehydration. Water deficiency triggered the inhibition of farnesene isomer formation so that the accumulation of farnesol as a preceding precursor was observed. Farnesene itself has been commonly used as a

Table 3. Composition of essential oil extracted from the leaf of *Salvia leucantha* (water input of 152 ml/day. light intensity of 301 mmol.m^{-2}s^{-1} and 0 M salicylic acid)

Compound	Composition (%)	
	Control	Reference [5]
β-Elemene	0.6	-
Germacrene A	6.16	2.8
β-Carryophyllene	7.87	10.7
Aristolane	11.24	-
Alloaromadendrene	3.29	-
β-Farnesene	1.89	-
β-Cadinene	9.69	-
β-Cubebene	13.93	0.9
Bicyclogermecrene	5.13	0.5
Farnesene	1.16	-
γ-Cadinene	3.59	6.5
Calamanene	5.71	0.5
α-Amorphene	0.75	-
Germacrene B	0.45	0.9
Caryophyllene oxide	1.39	0.4
Hexahydrofarnesyl acetone	2.16	-
Abietatriene	0.66	-
Phytol	7.37	-
Bornyl Acetate	-	27.8
α-Himachalene	-	10.5
Germacrene D	0.79	4.5
Spathulenol	-	12.1

pheromone for insects and has a potential usage as carbon building block [26]. Aristolane was also found in the oil derived upon water deficiency treatment as a sesquiterpenoid which has antimicrobial and antioxidant effects [27]. Furthermore, this substance is classified as a rare terpenoid which has a potential as anti-malaria activity [28]. Aristolane is synthesized from mevalonic acid pathway with the primary precursor of farnesyl diphosphate transforming to bicyclogermacrene [29].

Conclusions

This study had investigated the effect of foliar application of salicylic acid, water input and light intensity on the growth biomass growth and production of essential oils from leaf and flower of *Salvia leucantha*. The biomass productivity of the flower varied from 1.8 to 233 kg/ha.year whereas the biomass productivity of the leaf varied from 160 to 586 kg/ha.year. The essential oils contained in the leaf and flower were extracted using a steam-distillation technique. The yield of essential oil both from flower and leaf varied from 1-2.76 % and 0.36-0.8 % on a dry weight basis, respectively. The productivity of essential oil was estimated in the range of 0.57-3.96 kg/ha.year and 2.26-4.36 kg/ha.year for both leaf and flower. The highest estimated productivity of essential oil (4.36 kg/ha. year) was obtained when *S. leucantha* was cultivated without any shade net (receiving a light intensity of 301 μmol.m^{-2}s^{-1}) with a water input of 152 ml/day (100 % water field capacity) and sprayed with 0.25 mM salicylic acid on the surface of the flower once a week. The essential oil from both leaf and flower contain aristolane (7.31-16.33 %) which may have a potential application as anti-malaria substance.

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