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MORPHOLOGICAL CHARACTERS AND ESSENTIAL OIL CONSTITUENTS EXTRACTED OF TWO CLOVE VARIETIES (*SYZYGIUM AROMATICUM* (L.) MERR. & L. M. PERRY.) FROM AMBON ISLAND, INDONESIA

Hariyadi[1], Asri Subkhan Mahulette[2*], Sudirman Yahya[1] and Ade Wachjar[1]

[1]Department of Agronomy and Horticulture, IPB University (Bogor Agricultural University), Bogor, Indonesia.
[2*]Agrotechnology Study Program, Faculty of Agriculture, Pattimura University, Ambon, Indonesia.

Abstract

The aim of this study was to determine the morphological characters and essential components of clove oil extract from buds, stalks, and leaves of *Syzygium aromaticum* var. Tuni and *S. aromaticum* var. Zanzibar. The results showed that the Tuni clove varieties had a larger size of the leaves, fruit and seed morphology than the Zanzibar variety. The results of the essential component characterization are obtained information that bud oil of Tuni variety was composed of eugenol (67.9%), the stem oil contained eugenol (80.6%), the leaf oil contained eugenol (60.5%). The bud oil of Zanzibar variety was constituted of eugenol (47.4%), the stem oil contained eugenol (67.5%), the leaf oil contained eugenol (63.5%). The main components of both varieties of clove oil were eugenol, followed by caryophyllene, eugenyl acetate, and other minor components. Climate factors such as rainfall, humidity and air temperature on Ambon Island did not significantly affect the eugenol content of both varieties.

***Key words** :* Aromatic clove, Maluku, *Syzygium aromaticum*, Tuni, volatile oil, Zanzibar.

Introduction

Syzygium aromaticum (L.) Merr. & L. M. Perr. (Myrtaceae), known as clove, is an Indonesian native plant especially from the Maluku islands (Milind and Deepa, 2011; Rathinam and Viswanathan, 2018; Alfian *et al.* 2019; Mahulette *et al.* 2019a). As part of the center of clove origin in the Maluku Islands, Indonesia, Ambon Island has many typical clove germplasm including aromatic cloves varieties (Milind and Deepa, 2011). *S. aromaticum* var. Tuni is typically from Maluku which is widely cultivated compare to *S. aromaticum* var. Zanzibar. The two types of clove varieties were morphologically different but still in the aromatic clove group. To date, dried clove buds are the main portion of the plant to be utilized, but the extraction of essential oils from these two varieties from Ambon island is still limited.

Clove oil is produced by plants via secondary metabolic pathways (Atanasova-pancevska *et al.,* 2017), can be extracted from buds (bud oil), flower stalks (stem oil), and leaves (leaf oil) (Riyanto *et al.,* 2016; Uddin *et al.*, 2017) through hydrodistillation, steam distillation or dry distillation (Sahraoui and Boutekedjiret, 2015; Rassem *et al.,* 2016; Nam *et al.,* 2017). essential oil and its derivative products such as oleoresin (Kamatou *et al.,* 2012; Gaspar *et al.,* 2018) are commercially advantage and widely used in the health, food, cosmetics industries (Chóez-guaranda *et al.,* 2017; Nejad *et al.,* 2017), food preservatives, pesticides, and fumigants (Kamatou *et al.,* 2012; Xu *et al.,* 2016). The main component of clove oil is eugenol which has antiseptic and analgesic activities (Riyanto *et al.,* 2016), anti-inflammatory (Chicca *et al.,* 2014), anti-oxidants (Hamed *et al.,* 2012; Kasai *et al.,* 2016), anti-fungal (Pinto *et al.,* 2009), anti-bacterial (Mohamed and Badri 2017), anti-viral (Rathinam dan Viswanathan 2018), anti-cancer (Rahman *et al.,* 2018), and widely used in the treatment of teeth and gums (Pulikottil and Nath, 2015).

Previous studies on clove essential oil originated from

***Author for correspondence :** E-mail : mahulette_07@yahoo.co.id

Ambon Island found that the main components were eugenol, caryophyllene, and eugenyl acetate, where eugenol was the highest and most abundant constituent in stem oil (97.2–98.8%) (Sohilait, 2015). The results of the study informed the volatile components of cloves on Ambon Island but were not specific to certain varieties. Maluku Province as the center of origin of clove plants has a high diversity of clove germplasm. The cloves of Tuni and Zanzibar varieties are part of the clove germplasm in Maluku and are widely cultivated by farmers. Until now, information about the content of essential oils from flower buds, flower stalks, and leaves of both varieties is still limited. Both clove varieties are classified as cultivated cloves from the aromatic group. Other studies of aromatic cloves from different parts of the morphology have also been reported. *S. aromaticum* from Bangladesh found that bud oil was composed of eugenol (60–90%), eugenyl acetate, caryophyllene, and other minor components, and leaf oil contained eugenol (82–88%), small amounts of eugenyl acetate, and other minor components, while stem oil was composed of eugenol (90–95%) and several other minor components (Uddin *et al.,* 2017). Previous research also reported that clove buds contained 21.3% essential oil with eugenol content of around 78–95%, flower stalks contained 6% essential oil with eugenol content 89–95%, while leaves contained 2–3% essential oil with eugenol content about 80–85% (Hadi, 2012). The difference in the composition of essential oils in plants other than being influenced by the parts of plant organs analyzed is also influenced by other factors such as climate. Ambon Island, where the cloves of Tuni and Zanzibar varieties have grown, have climatic conditions that can affect the content of essential oils produced. Ambon Island, where the cloves of Tuni and Zanzibar varieties have grown, have climatic conditions that can affect the content of essential oils produced (Amini *et al.,* 2016; Da Silva *et al.,* 2016). This paper reported the morphological characters and composition and concentration of the components of clove essential oil extracted from buds, flower stalks, and leaves of *S. aromaticum* var. Tuni and Zanzibar originated from Ambon Island. The knowledge of the clove essential oil components is important for identifying its potential uses, as such information about the composition and concentration of essential oil of both Ambon clove varieties is still limited.

Materials and Methods

Plant material

Plant material for morphological characterization and volatile components are taken from productive plants> 15 years old. Samples were collected from Lilibooy Village, Ambon Island, Maluku (03°44’46.8"E–128°01’10.1"S at an elevation of 103.8 m) on September 2018 during the harvest time. The number of plants taken consisted of 10 Tuni and Zanzibar varieties, respectively. Measurement of plant morphology refers to modified IPGRI (1980). The measurement of length using a ruler, diameter using a caliper, weighing the weight using digital scales, while the determination of color using the RHS color chart (2015). 10 samples of leaves, flowers, fruits, and seeds were taken for morphological measurements. The measured leaves are the fourth leaf from the shoot (Ruhnayat 2007), while the fruits and seeds taken are physiologically mature. Flower bud, flower stalks and leaf samples for distillation are carried out after plant morphology measurements.

Buds and stalks were dried for 3–4 days before being distilled while the leaves were dried on ventilated drying oven at 30°C until the water content dropped to 10–15%. To see the relationship between the main components of clove oil of both varieties with climate factors, climate data from Pattimura Meteorological Station (2018) were used which included rainfall, air temperature, and air humidity data during the period of flower initiation to flowering bud harvesting.

Essential oil extraction

The dried clove buds were grinded before it was distilled, while the dried flower stalks and leaves were distilled directly. The process of distillation used hydrodistillation method for 6 hours at 100°C until there was no more oil coming out. The extracted oil was then added to anhydrous sodium sulfate (Na_2SO_4) and was inserted inside a dark bottle. The oil was stored in a tight container with a temperature of 2°C. The component of clove oil was analyzed using GC/MS. The volume of essential oil (v/w) obtained from bud, stalk, and leaf were 1.3%, 1.0%, 0.4% for Tuni variety, and 1.3%, 1.2%, 0.35% for Zanzibar variety, respectively.

GC/MS analysis

The component of essential oils was analyzed by Agilent Technologies 7890 Gas Chromatograph system, which is equipped with HP INNOWAX capillary column (30 m × 0.25 i.d., film thickness 0.25 ìm) and 5975 mass selective detector and chemistation data system. Clove oil was injected into the column using the GC/MS syringe as much as 1 µL and carried by helium gas. The column temperature was increased from 60°C (held 0 minutes) to up to 150°C (held for 1 minute) and then the temperature was increased 20°C/minute until 210°C (held for 10 minutes). The mobile phase flow rate was set to

0.6 mL/minute, the injector temperature was 250°C, 12 kPa pressures, and the injector split ratio was set to 250:1.

Data Analysis

Analysis of morphological characters and essential components of GC/MS results is presented descriptively. The chromatogram peaks that arose from the GC/MS results were compared with the mass spectrum of the Wiley 7 library 2003 and NIST 2005 v.2.0 on chemistation data system (Wenqiang *et al., 2007;* Hossain *et al., 2012)*. The acquired volatile components were grouped in a heatmap using the R Stat 3.1.0 program (package: metabolomics, shiny, heatmap, shinyheatmaply). Principal Component Analysis (PCA) to see the relationship between the main components of clove oil and climate factors used by XLSTAT software version 2019.1.

Results and Discussion

Morphological characters

The morphology of the cloves of Tuni and Zanzibar varieties is shown in Fig. 1. The measurement results of the morphological characters of the two varieties were found to be informed that the cloves of the Tuni variety had elliptical leaves with a length of 9.14±0.22 cm, the width of 4.18±0.29 cm, leaf area of 28.50±2.43 cm. Old leaf color Deep yellowish green/green group (141B), shoot color moderate red/grayed red group (179D). Flower buds are funnel-shaped, length 2.06±0.12 cm, diameter 0.49±0.01cm, pale color greenish yellow/yellow group (2D). Fruits and seeds are conical. Physiological ripe fruit has a length of 2.62±0.18 cm, a diameter of 1.30±0.10 cm, the color of the dark purple/purple group (79A). Seed length 1.98±0.08 cm, diameter 0.95±0.05 cm, light purple/purple group (75B).

Zanzibar varieties have elliptical leaves with a length of 8.18±0.78 cm, the width of 3.38±0.52, leaf area of 23.30±4.30 cm^2. Old leaf color Deep yellowish green/ green group (141B), shoot color moderate red/grayed red group (179D). Flower bud is round-shaped funnel, length 1.87±0.11 cm, width 0.50±0.01, color strong pink/ red group (49A). Fruit and seeds are conical. The fruit has a length of 2.24±0.09 cm, the width of 1.36±0.05 cm, the color of the dark purple/purple group (79A). Seeds have a length of 1.55±0.05 cm, a diameter of 0.81±0.02 cm, a light purple/purple group (75B).

Climatic conditions

In Maluku province-Indonesia, the Tuni variety cloves have a broad distribution in almost all Maluku regions compared to Zanzibar varieties. The two clove varieties grew from coastal areas to mountain forests in Maluku and were cultivated in the form of agroforestry systems

(A) (B)

Fig. 1: Morphology of clove Tuni (A) variety, Zanzibar variety (B).

along with other plantation crops. On Ambon Island, Maluku Province, the clove is distributed at an altitude of 0-700 m. According to Pattimura Meteorology Station data (2018), in the clove harvest year 2018, the average rainfall in the distribution area on Ambon Island is 5 435 mm^{-1}, the average temperature is 24.9 ^{o}C, average humidity is 85.6%. The distribution of rainfall, air temperature, and humidity during the flower filling period until the flower bud harvest of both varieties are presented in Fig. 2.

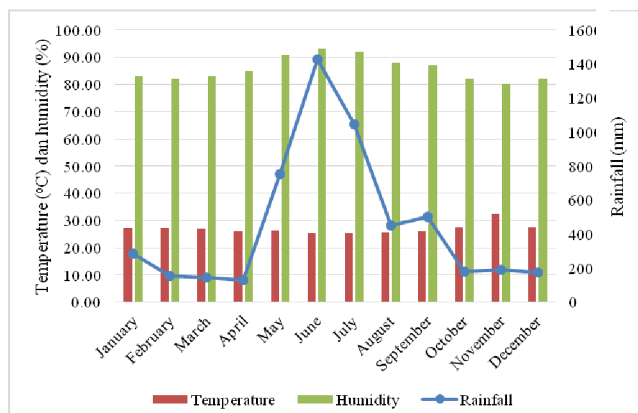

Fig. 2: Climate conditions in the locations of the clove varieties of Tuni and Zanzibar varieties on Ambon Island.

The cloves of Tuni and Zanzibar varieties on Ambon Island enter a period of flower initiation in June and flower bud harvest at the beginning of September. During the harvest season in 2018, peak rainfall coincides with the flower initiation period, ie in June (1430 mm) with relatively high humidity (93%) and low air temperature (25.40 ^{o}C). The rainfall decreases to 501 mm in September, along with the flower bud harvest. At that time the air humidity on Ambon Island ranged 87% with an air temperature of 26 ^{o}C.

The components of clove essential oils from bud, stalk, and leaf

The GC/MS analysis of clove oil (*S. aromaticum*) of Tuni and Zanzibar varieties from Ambon Island showed various essential oil components were listed in Table 1. The main components of buds, flower stalks, and leaves from the two varieties have various compositions and concentrations. The GC/MS chromatogram results of Tuni variety showed five volatile oil components were identified from buds, flower stalks, and leaves. While, in Zanzibar, variety six volatile oil components were identified from bud and flower stalk, and five volatile oil components were identified from leaf. The bud oil of Tuni varieties was composed of volatile components of which eugenol (67.9%), caryophyllene (21.1%), eugenyl acetate (6.2%), α-humulene (1.3%), patchouli alcohol (1.2%); stem oil consisted of eugenol (80.60%), caryophyllene (13.0%), eugenyl acetate (2.5%), α-humulene (1.6%), caryophyllene oxide (1.2%); and leaf oil contained eugenol (60.5%), caryophyllene (32.2%), α-humulene (3.5%), eugenyl acetate (2.1%), and caryophyllene oxide (1.0%). Bud oil of Zanzibar varieties are composed of eugenol (47.4%), caryophyllene (25.3%), eugenyl acetate (19.1%), α-humulene (3.3%), δ-cadinene (1.9%), copaene (1.5%); stem oil in the form of eugenol (67.5%), caryophyllene (24.8%), α-humulene (2.7%), eugenyl acetate (1.8%), patchouli alcohol (1.5%), cryophyllene oxide (1.0%); leaf oil in the form of eugenol (63.5%), caryophyllene (29.1%), α-humulene (3.2%), eugenyl acetate (2.8%), caryophyllene oxide (1.0%).

The results of this study revealed that the highest values of eugenol were found in flower stalks in compare with the extracted oils from the buds and leaves. The Tuni variety had the highest concentration values of eugenol in the flower stalk (80.6%) but contained low levels of caryophyllene (13.0%). In this variety, caryophyllene levels were the highest in the extracted leaves oil (32.2%), while the highest levels of eugenyl acetate were found in the extracted oil from buds (6.2%) but found in low levels in the extracted oil from flower stalks and leaves (2.2% and 3.5%). The same condition was determined in Zanzibar variety, in which eugenol was determined as the main component and was found in high levels in the extracted oil from flower stalks (67.5%) then followed by the leaves (63.5%) and was the lowest in buds (47.4%). In this variety, caryophyllene was found in almost the same concentration in the extracted oil from buds, flower stalks, and leaves (25.3%, 24.9%, 29.1%, respectively). While the highest levels of eugenyl acetate were found in bud oil (19.1%) and in the lowest level in both stem and leaf oil (1.8% and 2.8%, respectively).

The main components of the extracted oil from bud, stalk, and leaf

Essential oils of cloves (*S. aromaticum*) were distilled from three parts of the plant; buds, flower stalks, and leaves. Apart from having different compositions, essential oils contain specific components of each distilled part of the plant (Fig. 3). Analysis results have shown that the components of the extracted essential oil from *S. aromaticum* can be divided into two groups. Eugenol, caryophyllene, eugenyl acetate, and α–humulene as the first group and caryophyllene oxide, patchouli alcohol, copaene, δ-cadinane as the second group. The first group is considered as the main components of clove oil (*S. aromaticum*) because it was found in high concentration while the second group is considered as minor as its presence in clove oil was in low concentration. According to the analyzed plant parts, two groups were found; the main group included the extracted clove oil from buds of

Table 1 : The chemical composition of essential oils (*S. aromaticum*) of Tuni and Zanzibar varieties originated from Ambon Island.

S. aromaticum varities	Buds				Stalks				Leaves			
	RT	Q	Components	Conc. (%)	RT	Q	Components	Conc. (%)	RT	Q	Components	Conc.(%)
Tuni	50.0	98	Eugenol	67.9	50.0	97	Eugenol	80.6	50.0	98	Eugenol	60.5
	26.9	99	Caryophyllene	21.1	26.9	99	Caryophyllene	13.0	26.9	99	Caryophyllene	32.2
	51.3	99	Eugenyl acetate	6.2	51.3	99	Eugenyl acetate	2.5	30.7	99	α-humulene	3.5
	30.7	98	α-humulene	1.3	30.7	99	α-humulene	1.6	51.3	99	Eugenyl acetate	2.1
	50.2	99	Patchouli alcohol	1.2	46.2	91	Cryophyllene oxide	1.2	46.2	94	Cryophyllene oxide	1.0
Zanzibar	50.0	98	Eugenol	47.4	50.0	98	Eugenol	67.5	50.0	98	Eugenol	63.5
	26.9	99	Caryophyllene	25.3	26.9	99	Caryophyllene	24.8	26.9	99	Caryophyllene	29.1
	51.3	99	Eugenyl acetate	19.1	30.7	98	α-humulene	2.7	30.7	98	α-humulene	3.2
	30.7	98	α-humulene	3.3	51.3	99	Eugenyl acetate	1.8	51.3	99	Eugenyl acetate	2.8
	35.6	99	δ-cadinene	1.9	50.2	99	Pathchouli alcohol	1.5	46.2	95	Caryophyllene oxide	1.0
	21.6	99	Copaene	1.5	46.2	94	Cryophyllene oxide	1.0				

RT=retention time. Q=quality. Data were obtained from six essential oils from Tuni and Zanzibar varieties from June to September 2018.

Zanzibar variety and the rest of the extracted oil from other plant parts as a second group. Although bud oil of Zanzibar (first group) did not contain caryophyllene oxide and patchouli alcohol, it contained copaene and δ-cadinane. In contrast to the second group that contained all the volatile components of essential oil but lacked both copaene and δ-cadinane. Heatmap results showed that the main components of the extracted clove oil (*S. aromaticum*) of Tuni and Zanzibar varieties from all the analyzed plant parts was eugenol followed by caryophyllene, eugenyl acetate, and other minor components.

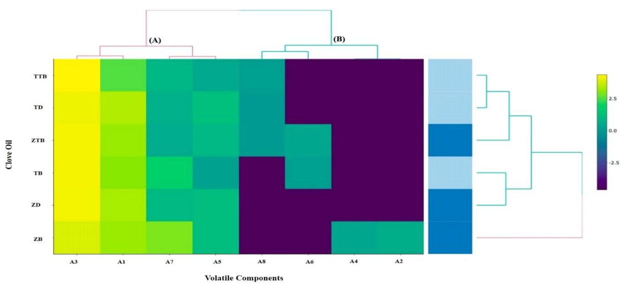

Fig. 3: Heatmap for each group of the essential oil of *S. aromaticum*. Main components (A), minor components (B). Components: Caryophyllene (A1), δ-cadinane (A2), eugenol (A3), copaene (A4), α-humulene (A5), patchouli alcohol (A6), eugenyl acetate (A7), caryophyllene oxide (A8). Clove oil: bud oil-Tuni (TB), stalk oil-Tuni (TTB), leaf oil-Tuni (TD), bud oil-Zanzibar (ZB), stalk oil-Zanzibar (ZTB), leaf oil-Zanzibar (ZD).

Principle Component Analysis (PCA)

The cloves of Tuni and Zanzibar varieties have the main components, eugenol, caryophyllene, eugenyl acetate, and α-humulene. The main components are influenced by climate factors such as rainfall, air temperature, and humidity. Analysis of the relationship of the main components with climate factors was analyzed using the Principle Component Analysis (PCA) (Figure 4). The results of PCA analysis obtained two eigenvalues which are 4.20 with the variability of 60.05% and 2.39 with the variability of 34.16%. Both eigenvalues have a variety of data is 94.21 (% cumulative/total variant). The PCA results in this study showed that eugenol and eugenyl acetate did not correlate with environmental factors while caryophyllene and α-humulene were negatively correlated with rainfall and humidity. Higher rainfall and air humidity will reduce the content of caryophyllene and α - humulene. Furthermore, caryophyllene and α-humulene also have a positive correlation with air temperature. The higher the air temperature will increase the content of caryophyllene and α-humulene.

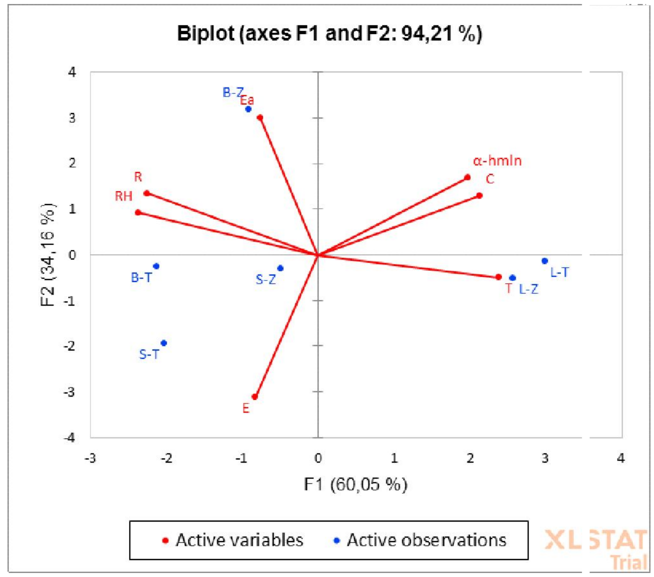

Fig. 4: *Principle Component Analysis* (PCA) the relationship between the main components of clove oil and climate factors. Main component: eugenol (E), caryophyllene (C), eugenyl acetate (Ea), á–humulene (á-hmln); Climate factors: rainfall (R), air temperature (T), air humidity (RH).

The cloves of Tuni and Zanzibar varieties have almost the same morphology but the difference is prominent in leaf size and shoots color. Zanzibar varieties have slightly smaller leaf sizes and reddish shoots than Tuni varieties. Both varieties are classified as cultivated cloves from the aromatic group which can produce essential oils. GC/MS analysis results indicated that Tuni and Zanzibar varieties were composed of almost the same main components of essential oil in the extracted oil from buds, flower stalks, and leaves. Both clove oil varieties show the main components, eugenol, caryophyllene, eugenyl acetate, and α-humulene. Such results were in accordance with the previous research results that reported that the main components of clove essential oil (*S. aromaticum*) are eugenol followed by caryophyllene, and acetoeugenol (Atanasova-pancevska *et al.,* 2017; Uddin *et al.,* 2017; Gaspar *et al.,* 2018).

Eugenol as the main component is at the highest concentration then followed by caryophyllene in the whole bud oil, stem oil, and leaf oil. Eugenol and caryophyllene as the main component were found at the highest concentration compared to other components from all the part of the plant of both varieties. Such results are in accordance with other reports that confirm the main component of *S. aromaticum* is eugenol then caryophyllene and other minor components (Gaspar *et al.,* 2018). The relatively high concentration values of eugenol and caryophyllene in the extracted clove oil (*S. aromaticum*) determines its quality. Previous reports confirmed that levels of eugenol and caryophyllene were crucial in high-quality clove oil (Riyanto *et al.,* 2016; Gaspar *et al.,* 2018). Both eugenol and caryophyllene are responsible for the distinctive aroma of clove oil. Eugenol is a phenylpropanoid with a molecular weight of 164.2 g/mol, soluble in organic solvents, has a pale yellow color, and with distinctive aroma (Kurniawan *et al.,* 2017; Nejad *et al.,* 2017). Caryophyllene is a sesquiterpene that found in high levels in clove oil which is used in natural remedies and also as a fragrance (Rios *et al.,* 2014; Vijayalaxmi *et al.,* 2015).

In this study, eugenol was found to be highest in the stem oils of both varieties. This obtained results were in accordance with the previously reported results that revealed the clove oil can be obtained from buds, flower stalks, and leaves (Sohilait 2015; Riyanto *et al.,* 2016), and eugenol concentration level reached 95% in the distillate extracted oil from the flower stalks (Nejad *et al.,* 2017; Uddin *et al.,* 2017). Although the extracted oil from stalks has the highest levels of eugenol, it was reported that it was contained lower levels of caryophyllene and eugenyl acetate in compare with the oil extracted from both buds and leaves (Jentzsch *et al.,* 2017).

The production of essential oils of a plant is affected by several factors including plant parts, physiological variations, environmental conditions, geographical areas, genetic factors and evolution (Dolveni *et al.,* 2016; Amelia *et al.,* 2017; (Dolveni *et al.* 2016; Amelia *et al.* 2017; Mahulette *et al.* 2019b). In this study, the production of volatile components is influenced by the part analyzed, also influenced by the environment in which it grows. During the flower initiation period of both varieties on Ambon Island, rainfall and humidity were very high while the air temperature was low. The climatic conditions have more influence on caryophyllene and α-humulene while eugenol and eugenyl acetate are not significantly affected by these climate factors. These results indicate that climate conditions affect the production of plant secondary metabolites. Production of plant secondary metabolites is strongly influenced by environmental conditions which have a strong influence on the chemical composition of plants (Maksimovic´ *et al.,* 2007; Amini *et al.,* 2016; Da silva *et al.,* 2016).

Conclusion

Tuni clove varieties have a slightly larger size of leaves, fruit flowers and seeds morphology than Zanzibar varieties. The whole bud oil, stem oil, leaf oil of both varieties have the main components namely eugenol then followed by caryophyllene, eugenyl acetate, and α-humulene. Rainfall, air temperature, air humidity at the growth of the two varieties in Ambon Island did not affect the content of eugenol and eugenyl acetate clove oil but it affected the content of caryophyllene and α-humulene.

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References

Alfian, A., A.S. Mahulette, M. Zainal Hardin and A. Bahrun (2019). Morphological character of raja clove (*Syzygium aromaticum* L. Merr & Perry.) native from Ambon Island. IOP Conference Series: *Earth and Environmental Science*, **343(012150)**:1–4. doi:10.1088/1755-1315/343/1/012150

Amelia, B., E. Saepudin. A.H. Cahyana, D.U. Rahayu, A.S Sulistyoningrum and J. Haib (2017). GC-MS analysis of clove (*Syzygium aromaticum*) bud essential oil from Java and Manado. *Aip conf. Pro,.* 1862.doi:10.1063/1.4991186.

Amini, F., G.R. Asghari, S.M. Talebi, M. Askary and M. Shahbazi (2016). Effect of environmental factors on the compounds of the essential oil of *Lippia citriodora*. *Biologija.,* **62(3)**:194–201.doi:10.6001/biologija.v62i3.3372.

Atanasova-pancevska, N., J. Bogdanov and D. Kungulovski (2017). Antimicrobial activity and chemical composition of two essential oils and eugenol from flower buds of *Eugenia caryophyllata*. *Open biol. Sci. J.*, **3(1)**:16–25.doi:10.2174/2352633501703010016.

Chicca, A., D. Caprioglio, A. Minassi, V. Petrucci, G. Appendino, O. Taglialatela-scafati and J. Gertsch (2014). Functionalization of â-caryophyllene generates novel polypharmacology in the endocannabinoid system. *Acs. Chem. Biol.,* **9(7)**:1499–1507.doi:10.1021/cb500177c.

Dolveni, M.F., S. Bladimiro, H. Vanessa, R.L. Beltran and C. Juan (2016). Chemical composition of the essential oil of *Morella parvifolia* (Benth.) Parra-O. from the Venezuelan Andes. *Emirates J. Food Agric.*, **28(4)**:288–290.doi:10.9755/ejfa.2015-11-977.

Gaspar, E.M., B.J. Sci, T. Res, E.M. Gaspar, R. Duarte and J.C. Santana (2018). Volatile composition and antioxidant properties of clove products. *Biomedical.*, **5(4)**:1–7.

Gupta, M., D. Singh, P. Gularia and S. Gupta (2015). GC-MS analysis and identifications of chemical constituents of *Syzygium aromaticum*, *Brassica compestris* and *Cow ghee*. *J. Chem. Pharm. Res.,* **7(1)**:568–572.

Hadi, S. (2012). Pengambilan minyak atsiri bunga cengkeh (clove oil) menggunakan pelarut n-heksana dan benzena. *J. Bahan Alam Terbarukan.,* **1(2)**:25–30.(in Indonesian)

Hamed, S.F., Z. Sadek and A. Edris (2012). Antioxidant and antimicrobial activities of clove bud essential oil and eugenol nanoparticles in alcohol-free microemulsion. *J. Oleo Sci.*, **61(11)**:641–648.doi:10.5650/jos.61.641.

Hossain, M.A., R.A. Al-Hashmi, A.M. Weli, Q. Al-riyami and Al-J.N (2012). Constituents of the essential oil from different brands of *Syzigium caryophyllatum* L. by gas chromatography-mass spectrometry. *Asian Pac. J. Trop. Biomed.,* **2(3)**:1446–1449.

IPGRI (1980). *Tropical fruit descriptor*. Thailand: ipgri-southeast asia regional committee.

Jentzsch, P.V, F. Gualpa, L.A. Ramos and V. Ciobotã (2017). Adulteration of clove essential oil: detection using a handheld raman spectrometer. *Flavour Fragr. J.,* **33(2)**:184–190.

Kamatou, G.P., I. Vermaak and A.M. Viljoen (2012). Eugenol - from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. *Molecules.,* **17(6)**:6953–6981.

Kasai, H., M. Shirao and M. Ikegami-kawai (2016). Analysis of volatile compounds of clove (*Syzygium aromaticum*) buds as influenced by growth phase and investigation of antioxidant activity of clove extracts. *Flavour Fragr. J.,* **31(2)**:178–184.

Kurniawan, M.A., S. Matsjeh and S. Triono (2017). Conversion of eugenol to methyleugenol: computational study and experimental. *Aip. Conf. Proc*., **1823**:1–9.

Mahulette, A.S., Hariyadi, S. Yahya and A. Wachjar Alfian (2019)a. Morphological traits of Maluku native forest clove (*Syzygium aromaticum* L. Merr & Perry.). *J. Trop. Crop Sci.,* **6(2)**:105–111.

Mahulette, A.S., Hariyadi, S. Yahya and A. Wachjar (2019)b. The physicochemical components and characteristic from essential oils of forest cloves *Syzygium aromaticum* (Myrtaceae) in Maluku Province, Indonesia. *Plant Arch.,* **19(2)**:466–472.

Maksimovic, M., D. Vidic, M. Milos¡, M.E. S¡olic´, S. Abadzic´ and S. Siljak-yakovlev (2007). Effect of the environmental conditions on essential oil profile in two dinaric effect of the environmental conditions on essential oil profile in two dinaric salvia species/ : S. *Brachyodon vandas* and S. *Officinalis l. Biochem. Syst. Ecol.*, **35**:473–478.

Milind, P. and K. Deepa (2011). Clove: a champion spice. *Int. J. Res. Ayurveda Pharm.,* **2(21)**:47–54.

Mohamed, S.G. and A.M. Badri (2017). Antimicrobial activity of *syzygium aromaticum* and *citrus aurantifolia e*ssential oils against some microbes in Khartoum, Sudan. *Ec. Microbiol.,* **6**:253–259.

Nam, P.N., P. Thi, K. Lien,T. T Hoa, C.M. Huong and D. Thien (2017). Microwave assisted soxhlet extraction of essential oil from vietnamese star anise fruits (*Illicium verum hook.* F.) and their chemical composition. *Emirates. J. Food Agric.*, **29(2)**:131–137.

Nejad, S.M., H. Özgüneº and N. Baºaran (2017). Pharmacological and toxicological properties of eugenol. *Turk J. Pharm. Sci.,* **14(2)**:201–206.

Pinto, E., L. Vale-silva, C. Cavaleiro and L. Salgueiro (2009). Antifungal activity of the clove essential oil from *Syzygium aromaticum* on candida, aspergillus and dermatophyte species. *J. Med. Microbiol.,* **58(11)**:1454–1462.

Pulikottil S. and S. Nath (2015). Potential of clove of *Syzygium aromaticum* in development of a therapeutic agent for periodontal disease: a review. *South African Dent. J.,* **70(3)**:108–115.

Rahman, M.F., M.N. Haykal, N.A. Siagian, P.M. Sriepindonnta and N.A. Tampubolon (2018). Synthesis and proapoptotic activity on cervical cancer cell of ester eugenol 1-(3-methoxy-4-hydroxy)phenyl-2-propylmethanoate. *Iop Conf. Ser. Mater. Sci. Eng*., **299(1)**:1–7.

Rassem, H.H.A, A.H. Nour and R.M. Yunus (2016). Techniques for extraction of essential oils from plants: a review. *Aust. J. Basic Appl. Sci.,* **10(1016)**:117–127.

Rathinam, P. and P. Viswanathan (2018). Anti-virulence potential of eugenol-rich fraction of *Syzygium aromaticum* against multidrug resistant uropathogens isolated from catheterized patients. *Avicenna J. Phytomed.,* **8(5)**:416–431.

Rios, N., C. Y*α*nez, L. Rojas, F. Mora, A. Usubillaga and P. Vit (2014). Chemical composition of essential oil of apis mellifera propolis from falcó n state, venezuela. *Emirates J. Food Agric.,* **26(7)**:639–642.

Riyanto., H. Sastrohamidjojo and E. Fariyatun (2016). Synthesis of methyl eugenol from crude cloves leaf oil using acid and based chemicals reactions. *Iosr J. Appl. Chem.,* **9*(*10)**:105–112.

Ruhnayat, A. (2007). Aplikasi model pemupukan berimbang pada tanaman cengkeh (*Syzigium aromaticum*). *Bul. Littro*., **28(2)**:149–158.

Safrudin, I., A. Maimuly and A.R. Prihadi (2015). Effect of crushing of clove bud (Syzygium aromaticum) and distillation rate on main constituents of the essential oil. *Am. J. Essent. Oils nat. Prod.,* **2(3)**:12–15.

Sahraoui N. and C. Boutekedjiret (2015). Innovative process of essential oil extraction: steam distillation assisted by microwave. *Prog. Clean Energyean Energy*, **1**:831–841.

Da silva, E.A.J.D.A., V.P.D. A da Silva, C.C.F. Alvesi, J.M. Alves, E. L. Souchie and L.C.D.E.A. Barbosa (2016). Harvest time on the content and chemical composition of essential oil from leaves of guava. *Ciência Rura.*, **46(10)**:1771–1776.

Sohilait, H.J. (2015). Chemical composition of the essential oils in *Eugenia Caryophylata*, Thunb from Amboina Island. *Sci. J. Chem*,. **3(6)**:95–99.

Uddin, M.A., M. Shahinuzzaman, M. Sohel Rana and Z. Yaakob (2017). Study of chemical composition and medicinal properties of volatile oil from clove buds (*Eugenia caryophyllus*). *Int. J. Pharm. Sci. Res*., **8(2)**:895–899.

Vijayalaxmi, A., V. Bakshi and N. Begum (2015). Anti-arthritic and anti inflammatory activity of beta caryophyllene against freund's complete adjuvant induced arthritis in wistar rats. *J. Bone Reports Recomm,.* **1(2)**:1–10.

Villa-ruano, N., Y. Pacheco-hern*α*ndez, E. Rubio-rosas, J.A. Zarate-reyes, E. Lozoya-gloria and R. Cruz-duran (2017). Chemical profile, nutraceutical and anti-phytobacterial properties of the essential oil from *Dalea Foliolosa* (fabaceae). *Emirates J. Food Agric.*, **29(9)**:724–728.

Wenqiang, G., L. Shufen, Y. Ruixiang, T. Shaokun and Q. Can (2007). Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods. *Food chem.*, **101**:1558–1564.

Xu, J.G., T. Liu, G.P. Hu and X.M. Cao (2016). Chemical composition, antibacterial properties and mechanism of action of essential oil from clove buds against Staphylococcus aureus. *Molecules.,* **21(9)**:1–13.