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Diversity characterization of three varieties of *Cymbopogon nardus* under different shade conditions

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Abstract. *Danata NH, Aini N, Udayana C, Setiawan A, Yamika WSD, Prambudi R. 2023. Diversity characterization of three varieties of* Cymbopogon nardus *under different shade conditions. Biodiversitas 24: 3574-3582. Cymbopogon nardus* L. is a perennial aromatic plant distinguished for producing one of the world's most vital essential oils, citronella. This plant requires abounding sunlight to maximize the growth and quality of essential oils. The development of citronella can be implemented with an agroforestry system as an intercrop. However, the deterring factor restricting the propagation of citronella as an intercrop in agroforestry systems is the unavailability of adequate sunlight, consequently deteriorating the growth and quality of essential oils. This study investigated three varieties of C. nardus, i.e., Seraiwangi 1, Sitrona 1 Agribun, and Sitrona 2 Agribun, at distinctive shade proportions, i.e., 0%, 25%, 50%, and 75% shade. The examined parameters included the growth, physiological responses, yield, and quality of essential oils, i.e., citronellal, citronellol, and geraniol. Results demonstrate that the various types and shade percentages affect most examined parameters. The Seraiwangi 1 variety grown under 0% and 25% shade had nearly the same value, while the Sitrona 1 Agribun and Sitrona 2 Agribun varieties continued to experience declines ranging from 0%, 25%, 50%, to 75%. Nevertheless, shade extensively influences physiological responses such as photosynthetic rate and stomata width, and contrastingly there was a cipher effect on the stomata length. In the yield parameters, the oil concentration declined with the increase in shading percentage. The Seraiwangi 1 delivered superior quality of citronellal, citronellol, and geraniol compared to Sitrona 1 Agribun and Sitrona 2 Agribun varieties.

Keywords: Agroforestry, *Cymbopogon*, essential oils, shade, varieties

INTRODUCTION

Citronella (*Cymbopogon nardus* L.) is an aromatic plant distinguished for producing a vital essential oil known as citronella. This plant belongs to the Poaceae family. It is a perennial plant from South India and Sri Lanka (Hamzah et al. 2014). The development of citronella plants is widespread in tropical and subtropical regions such as America, Africa, and Asia. It is cultivated commercially in several countries, including Burma, Sri Lanka, India, Taiwan, and Indonesia (Kaur et al. 2021). The main ingredients of citronella, i.e., citronellal, citronellol, and geraniol, make this plant an aromatic. Citronella oil is recognized worldwide for its aromatherapeutic properties and various applications. The raw material is utilized to manufacture soap, perfume, cosmetics, flavoring, and insect repellent (Silva et al. 2011). Functioning as a repellent, it deters mosquitoes, thereby playing a vital role in mitigating the detrimental effects of malaria and dengue fever. Furthermore, it is readily available at a cost-effective price, is environmentally friendly, and has been endorsed by the community for its distinctive aroma (Zulfikar et al. 2019). Besides being used as an essential oil, citronella is also used as a herbal medicine beneficial for the body to maintain glucose, reduce hypertension, anticancer, flatulence, digestive disorders, cramps and intestinal irritation, and gastritis (Widiputri et al. 2019).

Citronella oil is one of 12 essential oils exported by Indonesia to various destination countries. Citronella oil contributes to the third largest essential oil export revenue after patchouli and vetiver oil (Sulaswatty et al. 2019). Citronella oil is a volatile compound in the terpenoid groups of secondary metabolites. Terpenoids are composed of five-carbon (C5) chains that are synthesized via the mevalonate (MVA) pathway and the 2-methylerythritol 4-phosphate pathway with acetyl CoA as the precursor (Mahajan et al. 2020). With the exponential increase in public health awareness, the urgency for domestic essential oils is imperative, attributed to their aromatherapeutic applications, including reed, electric, candle diffusers, and aerosol kind. When added to candles for aromatherapy purposes with a concentration of 37.5%, Citronella oil can relax blood vessels and reduce blood pressure in hypertensive patients (Yelfi et al. 2019).

The expansion of citronella plant development is urgently required to meet the accelerating and ceaseless market demands. However, the available land has been retained; it cannot be reallocated for medicinal and aromatic plant development to prioritize food crop commodities and attain the primary human necessities. Agroforestry is a competent alternative that can be implemented for the efficacious propagation of citronella plants. Citronella plants require abounding irrigation and light intensity to produce successful yields and quality essential oils. The limiting factor in developing citronella as an intercrop in agroforestry systems is the unavailability of optimum sunlight, which can deteriorate essential oils' growth and quality. The shade negatively affects light accessibility and other agro-climatic conditions, e.g., air temperature, soil temperature, humidity, and CO_2 concentration (Song et al. 2012). The shade also leads to diminished stomatal conductivity, inhibited CO_2 transport, and low transpiration, decreasing nutrient distribution and assimilated yields (Shafiq et al. 2021). Reducing the intensity of solar radiation in shaded conditions pronouncedly reduced the content of essential oils (Degani et al. 2016).

The planting material used is also fundamental in determining cultivation success. Planting materials can be in accessions, lines, or varieties that certain institutions have officially issued. Numerous superior varieties of citronella are procreated in Indonesia, i.e., Seraiwangi 1, Sitrona 2 Agribun, and Sitrona 2. Each variety has its characteristics and uniqueness following the growing conditions, quality, and quantity of the expected crop yields. The plant's type, spacing, and age also characterize the agroforestry system. Therefore, it is pivotal to establish the varieties suitable for growth in shaded conditions and the percentage tolerance of shade.

MATERIALS AND METHODS

The study was conducted from June to October 2022. It was located in the experimental garden of the Faculty of Agriculture Universitas Brawijaya, in Jatimulyo Village, Lowokwaru Sub-district, Malang, East Java, Indonesia. The experimental site is situated at an altitude of approximately 445 meters above sea level (masl). The air temperature ranges from 22-26℃ with air humidity ranging from 74-82%. The tools used in the experiment included the leaf area meter (LAM), lux meter, digital scale, oven, measuring cup, meter, ruler, bucket, hoe, scissors, stationery and treatment board, distillation tools, and a 20 mL glass bottle. The planting materials used were Seraiwangi 1, Sitrona 1 Agribun, and Sitrona 2 Agribun varieties, bamboo shade buffer, shading net, and manure, including N, P, and K fertilizers.

The experiment incorporated a split-plot design with three replicates with two primary factors. The first factor included the main plot with four distinct percentage shading: N0 (no shade), N25 (25% shading), N50 (50% shading), and N75 (75% shading). The second factor as subplots consisted of three varieties, namely V1 (Seraiwangi 1), V2 (Sitrona 1 Agribun), and V3 (Sitrona 2 Agribun).

Moreover, four parameters were observed, i.e., growth, physiology, yield, and oil quality. Growth parameters were further categorized as plant length, number of leaves and tillers, leaf area, fresh weight, and dry weight. Physiological parameters included chlorophyll index, photosynthetic rate, and stomata characteristics. Yield parameters comprised fresh herbs' weight and the essential oil yield per plant. Oil quality parameters chiefly consisted of essential oil content and quality of citronellal, citronellol, and geraniol concentrations. Observations were made when the plants reached 13-15 WAP (weeks after plant). Plant length was measured from the base of the plant to the top of the petal. The number of leaves was measured by counting open and healthy leaves, i.e., leaves that are not attacked by pests or diseases and are not withered. The number of tillers was observed by counting the number of tillers formed. Leaf area was measured using the ALA (Average Leaf Area) method. The ALA method measures one plant as a leaf sample using a leaf area meter/LAM (x), then dividing by the plant's number of leaves (Widaryanto et al. 2019).

$$\text{Average leaf area}^{-1}(y) = \frac{X}{\text{Number of leaf samples}}$$

Where:
x : leaf area of all leaf samples using LAM
Leaf area : $\sum$ leaf x (*y*)

Fresh weight was measured by weighing the sample using an analytical balance. The dry weight of the plant was carried out by oven-drying the plant sample for 2×24 h at a temperature of 80°C until a constant dry weight was obtained, and then the plant sample was weighed. The chlorophyll index measurement was carried out using the Minolta Chlorophyll meter SPAD-502. The rate of photosynthesis was measured using a Li-cor LI-6400XT Portable Photosynthesis System. The stomatal characteristics observed included stomatal density, length, and width, accessed using an Olympus BX51 microscope with an Olympus DP 24 microscope camera with 40x magnification. The fresh weight of the herb was observed by weighing the economic part of the *C. nardus* plant, which produces oil, namely the leaf part.

The oil content of the crop was carried out by multiplying the weight of fresh herbs by the essential oil content (%). Therefore, the steam distillation method was carried out to obtain the oil content (%)derived from 1,000 g of fresh herb and put in a distillation kettle for 4 h. The citronellal, citronellol, and geraniol content was determined using gas chromatography-mass spectroscopy (GC-MS) analysis.

Data analysis

Observational data were analyzed using analysis of variance (F test) at 5%. If there was a significant effect between treatments, further tests were carried out using Duncan Multiple Range Test at 5%.

RESULTS AND DISCUSSION

Plant length

Different varieties and shade percentages affect the length of *C. nardus* plants (Figure 1). The Seraiwangi 1 variety had a higher plant length than the Sitrona 1 Agribun and Sitrona 2 Agribun varieties. Sitrona 1 Agribun had a lower average plant length than other varieties. The higher the shade percentage was, the longer the *C. nardus* plant was. The 25% shade treatment had plant length that was not significantly different from 0% or no shade treatment. When shade increased by 50%, the plants lasted 75% longer due to inadequate sunlight.

Number of leaves

Different varieties and shade percentages influence the number of leaves of *C. nardus* (Figure 2). In observing the number of leaves, there was no interaction between *C. nardus* varieties and the level of shade percentage. The variety with the highest number of leaves was the Seraiwangi 1 variety. This value was not significantly different from the Sitrona 1 Agribun variety. Sitrona 2 Agribun had the least number of leaves among other varieties. Increasing the percentage of shading could significantly reduce the number of leaves. Giving 25% shading and no shading had the same value. An increase in 50% shading reduced the number of leaves by 72.57% compared to no shading and then declined drastically at 75% shading, with a diminishing percentage reaching 86.32%.

Number of tillers

In observing the number of tillers, each factor, i.e., the different varieties and the shade percentage, affects the number of *C. nardus*. However, there was no interaction between the two factors, as depicted in Figure 3. The Sitrona 1 Agribun variety had more tillers than the Seraiwangi 1 and Sitrona 2 Agribun. The Agribun Seraiwangi 1 and Sitrona 2 varieties tended to have the same number of tillers. Increasing the percentage of shading was significantly reduce the number of tillers formed. The highest number of tillers was in the unshaded treatment, followed by 25% shaded treatment, then significantly declined in 50% and 75% shaded treatments, with a decreasing percentage of 72.46% and 87.35%, respectively.

Leaf area

The observation of leaf area includes an interaction between the two factors: different varieties and shade percentage. The Seraiwangi 1 variety had relatively the same leaf area at 0% and 25% shade, then decreased significantly at 50% and 75%. The Sitrona 1 Agribun variety had the highest leaf area at 0% shade, decreased at 25% shade, and equivalently diminished at 50% and 75% shade with almost the same leaf area values. Sitrona 2 Agribun variety had leaf area, which continued to decline sequentially at 0%, 25%, and 50% shade, and the lowest leaf area was at 75% shade. Leaf area parameters are exhibited in Figure 4.

Fresh weight

The use of varieties and the different shade percentages play a pivotal role in measuring the fresh weight of *C. nardus* produced. There is an interaction for each different *C. nardus* variety and shade percentage levels, as presented in Figure 5. Overall, an increase in the shade percentage inhibited the fresh plant weight. The fresh weight of the Agribun Seraiwangi 1 and Sitrona 1 varieties had almost identical patterns, namely a decrease at 25% shade level and a drastic decrease at 50% and 75% shade levels, which were insignificant. The average fresh weight produced by the Sitrona 2 Agribun variety continued to experience a significant decrease with an increase in the percentage of shade of 25%, 50%, and 75%, which had the lowest fresh weight value.

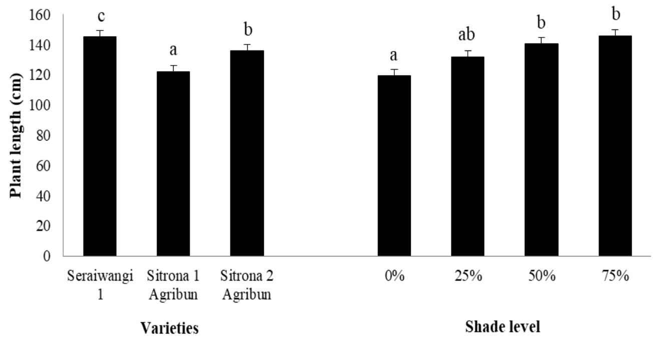

Figure 1. The effect of different varieties and the percentage of shade on the plant length *of Cymbopogon nardus*

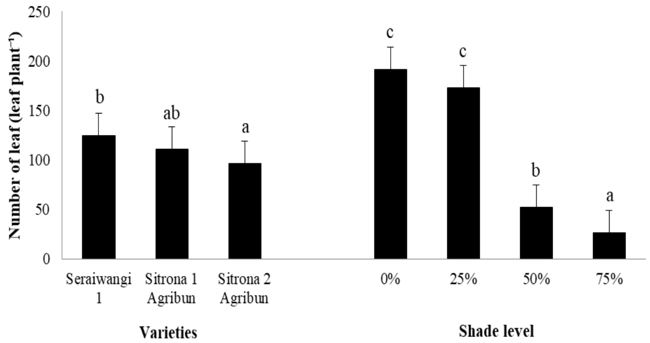

Figure 2. The effect of different varieties and the percentage of shade on the number of the leaf *of Cymbopogon nardus*

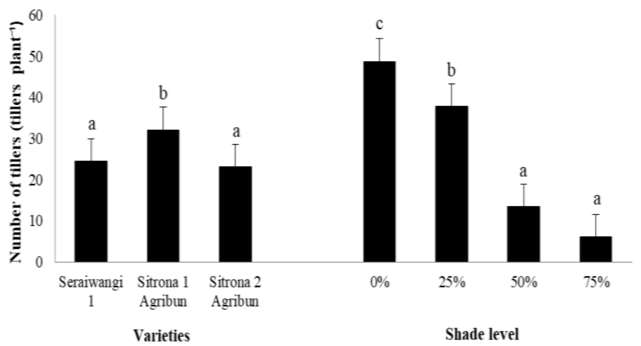

Figure 3. The effect of different varieties and the percentage of shade on the number of tillers *of Cymbopogon nardus*

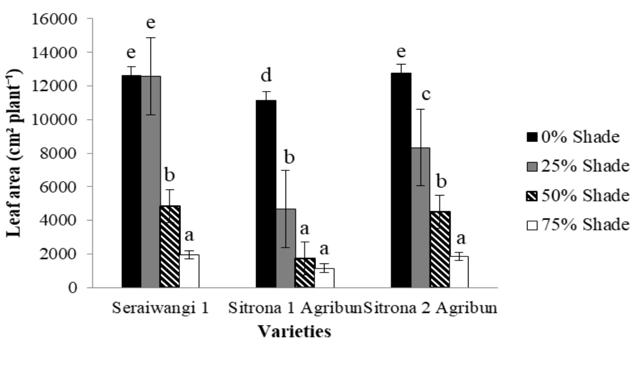

Figure 4. Characteristics of leaf area in the three varieties of *Cymbopogon nardus* at different shade levels

Dry weight

The observed pattern of plant dry weight is almost the same as that of the fresh weight of *C. nardus*. The highest biomass was produced in the no-shade treatment (Figure 6). The Seraiwangi 1 and Sitrona 2 Agribun varieties had nearly identical patterns; an increase in the shade percentage significantly reduced the dry weight of the plants gradually with the treatment of 0%, 25%, 50%, and 75% shade. The average dry weight of the Sitrona 1 Agribun variety under 25% shade was lower than the no-shade treatment. Then it decreased drastically at 50% and 75% shade with values that were not significantly different.

Chlorophyll index

Different shade percentages affect the chlorophyll index, whereas varieties do not (Figure 7). The three varieties, which included Seraiwangi 1, Sitrona 1 Agribun, and Sitrona 2 Agribun, did not affect the chlorophyll index contained in the leaves. On the other hand, the different percentages of shade affected the chlorophyll index. Citronella plants grown in shaded conditions had a higher chlorophyll index. The lowest chlorophyll index was found in the no-shade treatment, then it increased in the 25% shade treatment and significantly increased in the 50% and 75% shade treatment. The chlorophyll index at 50% and 75% shade were not significantly different.

Photosynthetic rate

Observing the photosynthetic rate of *C. nardus* plants demonstrates that although the different shade percentage has an effect, the use of distinct varieties does not affect the photosynthetic rate. The parameters of the photosynthetic rate are presented in Figure 8. The varietal treatment showed no effect on the photosynthetic rate of *C. nardus*. The Seraiwangi 1, Sitrona 1 Agribun, and Sitrona 2 Agribun varieties had nearly the same photosynthetic rate. In contrast to the shade treatment, the shade percentage difference indicated a contrasting photosynthetic rate. The higher the percentage of shade, the lower the rate of photosynthesis that runs. The highest photosynthetic rate was found in the no-shade and 25% shade treatments, which had insignificant values. The rate of photosynthesis continued to decrease at 50% and 75% shade treatment.

Stomatal characteristics

The stomatal characteristics observed were density, length, and width, as presented in Figure 9. The stomatal density of *C. nardus* is presented in Figure 10. Based on the observations, there is an interaction between the use of *C. nardus* varieties and the percentage of shade treatment. The Seraiwangi 1 and Sitrona 1 Agribun varieties had nearly identical patterns. Stomatal density continued to increase until 50% shading and then decreased to 75% shading. Meanwhile, in the Sitrona 2 Agribun variety, stomata density increased at 25% treatment and then decreased at 50% and 75% shade treatment.

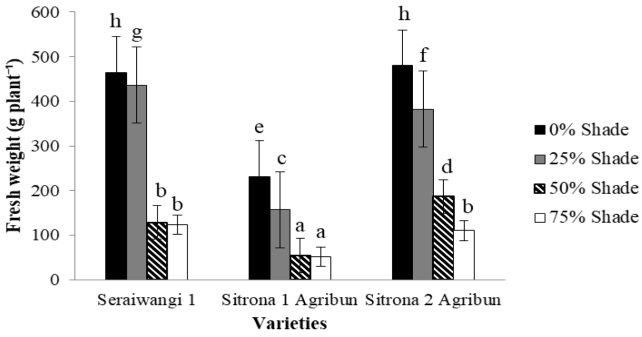

Figure 5. Characteristics of fresh weight of the three varieties of *Cymbopogon nardus* at distinct shade levels

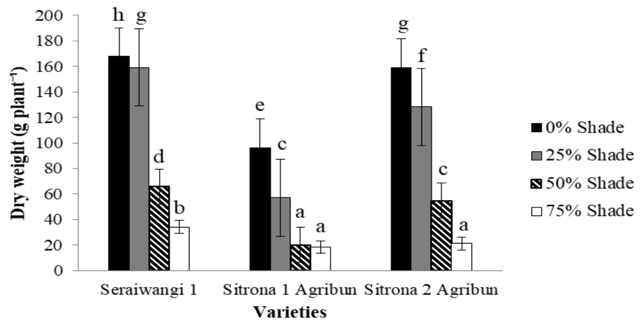

Figure 6. Characteristics of the dry weight of the three varieties of *Cymbopogon nardus* at various shade levels

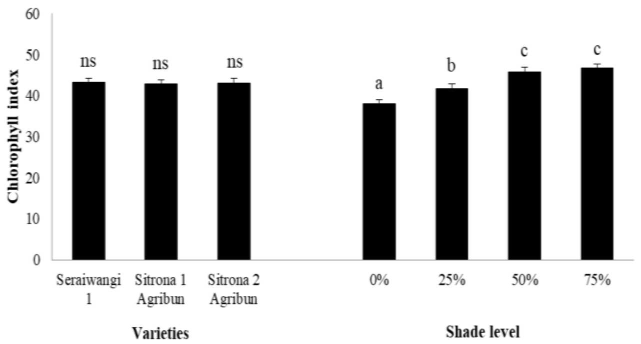

Figure 7. The effect of different varieties and the percentage of shade on the chlorophyll index of *Cymbopogon nardus*

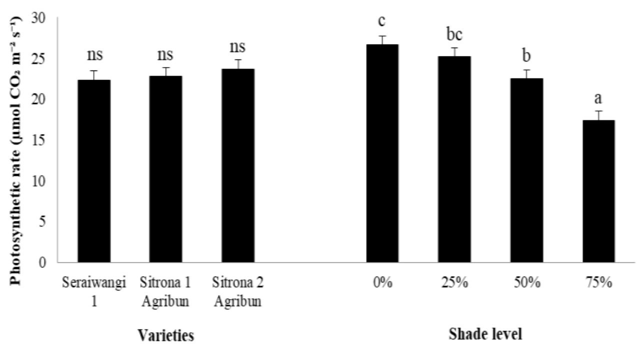

Figure 8. The effect of different varieties and the percentage of shade on the photosynthetic rate of *Cymbopogon nardus*

In addition to stomatal density, other characteristics observed were the length and width of the stomata. Based on the observations, there was no interaction between the use of varieties and the shade percentage on the length and width of stomata, as depicted in Table 1. Regarding stomata length, varieties, and shade percentage do not affect stomata length. In contrast, differences in varieties and shade levels affect the stomata's width. Treatment without shade tended to have a wider stoma than *C. nardus* under shaded conditions. Shading 25%, 50%, and 75% tended to have the same width of stomata. The Sitrona 2 Agribun variety had the smallest stomata width, while Seraiwangi 1 and Sitrona 1 Agribun had the same width.

The weight of fresh herbs

The weight of fresh herbs parameters was observed with the leaf biomass of *C. nardus* because the part of the plant that is distilled and contains essential oils is the leaves. There is an interaction between the use of *C. nardus* varieties with different shade percentages, as depicted in Figure 11. The Agribun Seraiwangi 1 and Sitrona 1 varieties shared nearly identical patterns, and fresh herb weight decreased at 25% shade and then declined drastically at 50% shade and 75% with the same weight value of fresh herbs. The Sitrona 2 Agribun variety experienced a significant decrease from 0%, 25%, and 50% to 75% shade treatment. The Seraiwangi 1 variety had a higher fresh herb weight than other varieties.

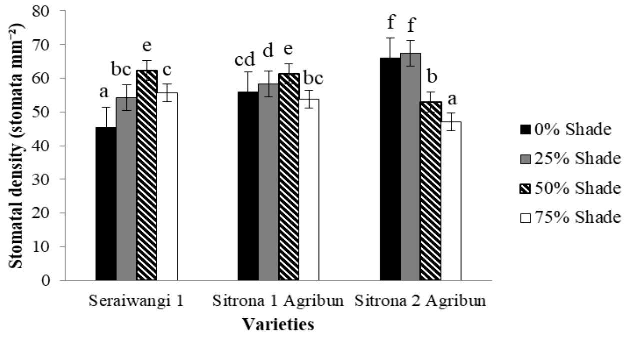

Figure 9. Characteristics of the stomatal density of the three varieties of *Cymbopogon nardus* at different shade levels

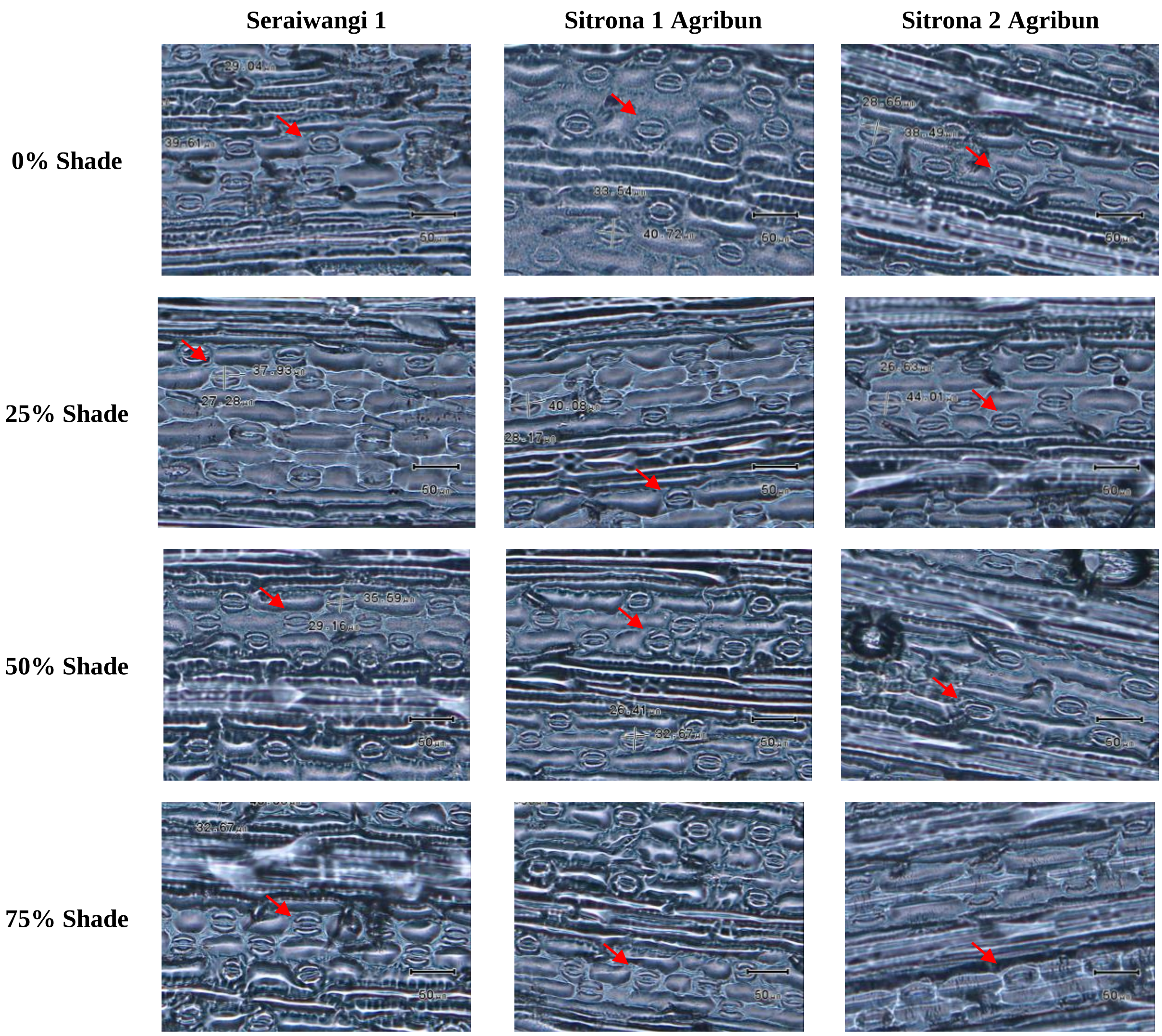

Figure 10. The effect of varieties of *Cymbopogon nardus* and shade levels on stomatal characteristics. Red arrows indicate each variety's length, width, and stomatal density at different shade conditions

Essential oil concentration

Different varieties release contrasting concentrations of essential oils. Increasing the shade percentage could significantly decrease the concentration of the essential oil produced. The Agribun Seraiwangi 1 and Sitrona 2 varieties had essential oil concentration values that reduced as the shade percentage increased. In contrast, the concentration of essential oil of the Sitrona 1 Agribun variety decreased to 50% shade and then slightly increased to 75%. Parameters of essential oil concentrations are presented in Table 2.

Essential oil yield

The essential oil yield was the observed yield of planting oil. It was calculated by multiplying the essential oil concentration and the weight of fresh herbs. This observation was crucial because it demonstrated the essential oil yield obtained in a unit area. Observations of essential oil yields show an interaction between the varieties used and the different shade percentages. The parameters of essential oil yield are presented in Figure 12.

The highest oil yields were obtained in the no-shade treatment. Seraiwangi 1 and Sitrona 1 Agribun varieties decreased by 25% and then decreased drastically at 50% and 75% shade with the same value. The Sitrona 2 Agribun essential oil yield decreased significantly at 0%, 25%, 50% shade, and 75% shade.

Essential oil composition

The main essential oils produced by *C. nardus* are citronellal, citronellol, and geraniol. The content is established through GC-MS observations separated by retention time. The various shade percentages and varieties used significantly affect the oil quality. The Seraiwangi 1 variety tended to have higher citronellal, citronellol, and geraniol concentrations than other varieties, followed by Sitrona 2 Agribun and Sitrona 1 Agribun varieties with the lowest compound content. The results of the essential oil composition of *C. nardus* are presented in Table 3.

Discussion

Following several examined parameters, it demonstrated that citronella is a plant that requires abounding sunlight to support the growth and yield of essential oils. Increasing shade percentage inhibited growth and biomass in all the observed varieties. Light is the indispensable factor affecting plant morphology, productivity, and photosynthesis and is essential for regulating plant growth (Hou et al. 2018); cultivating citronella in the shade when it triggers longer plant growth is termed etiolation. Many plant species respond to shade by accelerating stem and leaf elongation, repressing branching, increasing leaf area (specific leaf area), and decreasing photosynthate allocation to roots (Semchenko et al. 2012). At a certain percentage, shade in agroforestry systems induces intercropping to develop longer, broader leaves and more branches (Mohanty et al. 2019). The response of leaf elongation and increasing specific leaf area is an adaptation of plants to retrieve more sunlight.

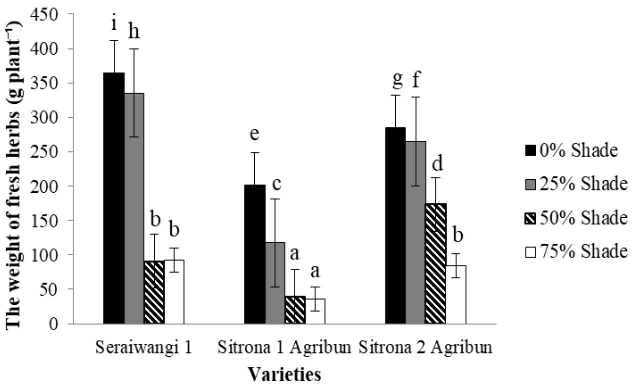

Figure 11. Characteristics of the weight of fresh herbs in the three varieties of *Cymbopogon nardus* at distinct shade levels

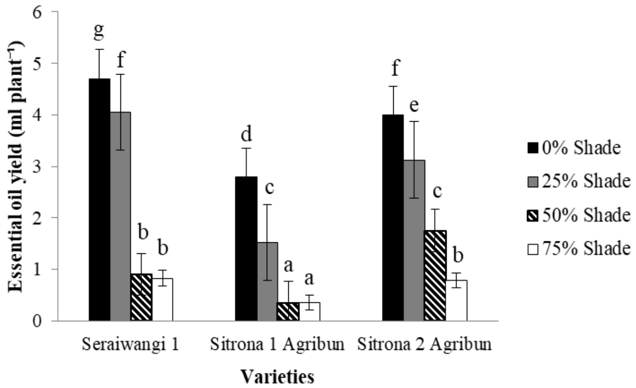

Figure 12. Characteristics of essential oil yield of the three varieties of *Cymbopogon nardus* at distinct shade levels

Table 1. Effect of varieties of *Cymbopogon nardus* and shade levels on the stomatal length and stomatal width

Treatment	Stomata	
	Length (µm)	Width (µm)
Shade level (%)		
0	39.89 ns	30.17 b
25	41.13 ns	27.37 a
50	38.31 ns	26.90 a
75	38.07 ns	27.57 a
CV (%)	7.3	4.91
Varieties		
Seraiwangi 1	38.77 ns	29.52 b
Sitrona 1 Agribun	38.19 ns	29.36 b
Sitrona 2 Agribun	41.09 ns	25.13 a
CV (%)	6.59	9.1

Table 2. Essential oil concentration of the three varieties of *Cymbopogon nardus* at different shade levels

Shade level (%)	Varieties		
	Seraiwangi 1	Sitrona 1 Agribun	Sitrona 2 Agribun
0	12.9	13.9	14
25	12.1	12.9	11.8
50	9.9	8.9	10.1
75	8.9	9.9	9.3

Table 3. Characteristics of the three varieties of *Cymbopogon nardus* on essential oil composition at different shade levels

Shade level (%)	Citronellal (%)			Citronellol (%)			Geraniol (%)		
	Seraiwangi 1	Sitrona 1 Agribun	Sitrona 2 Agribun	Seraiwangi 1	Sitrona 1 Agribun	Sitrona 2 Agribun	Seraiwangi 1	Sitrona 1 Agribun	Sitrona 2 Agribun
0	32.8	30.08	22.58	10.1	7.42	5.71	23.73	24.11	20.68
25	31.65	30.83	20.47	9.37	7.46	4.93	23.81	24.18	18.73
50	34.29	21.47	23.42	9.5	7.77	7.01	22.11	21.07	22.56
75	32.15	25.19	25.61	8.96	7.44	6.4	20.77	20.68	19.17

Plants undergo the process of photosynthesis, where light is the energy that catalyzes the conversion of atmospheric carbon dioxide (CO_2) and water (H_2O) into glucose ($C_6H_{12}O_6$) and oxygen (O_2). The light absorbed by the leaf surface area and light distribution affects growths and yields. The essential oil contained in the citronella is a product of secondary metabolism, found in all parts of the plant, occurring in the roots, stems, and leaves; however, the best yield and quality are derived from the leaves (Saputra et al. 2020). Therefore, leaf observation parameters are critical in citronella plants. The number of leaves, the number of tillers, and the leaf area of the plants in the shaded conditions had fewer numbers than the treatment without shade. The impact of the reduction in light intensity can be observed in the leaf phenotype due to the larger density of the leaves caused by solar radiation. Under shaded conditions with low light intensity, it induces generative organs, namely flowering, and accumulates less leaf biomass (Inderaja et al. 2018). Plants in low light conditions show wider leaf morphology than plants in full light conditions, allowing plants to absorb more light (Wan et al. 2020).

Light sources and light absorption are vital to produce plant biomass. In extremely shaded conditions, the unavailability of light negatively influences the photosynthetic system, thereby affecting the assimilation produced. The assimilated results obtained from photosynthesis can be discovered from the dry weight parameters of the plant. The dry weight of citronella plants at 0% shade conditions was higher than the treatment with shade. Increasing the shading percentage of 50% and 75% could significantly reduce the dry weight produced. In shaded conditions, the assimilated product is allocated to form chlorophyll to optimize light absorption. Research conducted on *Cymbopogon flexuosus* (Nees ex Steud.) W.Watson plants illustrated that the chlorophyll content in shaded conditions was substantially higher than in full sunlight. Minimal light intensity might have allocated primary metabolic assimilates such as total protein, amino acids, and carbohydrates to synthesize chlorophyll as an adaptation of plants to increase light absorption (Thakur et al. 2019). The response of plants to shade conditions is to increase the chlorophyll content, especially chlorophyll b. It is associated with light harvesting and electron transport so plants can maximize photosynthesis in low-light conditions (Dai et al. 2009).

The different shade percentages demonstrate the difference in the physiological response of *C. nardus*. An increase in the shade percentage indicates that the rate of photosynthesis is declining. The 25% shade had almost the same photosynthetic rate as the no-shade treatment; however, at 50% and 75% shade, the rate of photosynthesis decreased drastically. The low rate of photosynthesis is affected by chlorophyll content, nutrient content such as nitrogen, and stomatal density (Suryanto et al. 2014). In shaded conditions, more chlorophyll is formed as a plant adaptation process to increase the light absorption and transfer of PSII. However, the light energy obtained is not entirely converted into photosynthetic products; hence the biomass produced is comparatively less (Fan et al. 2019). The characteristics of stomata illustrated that the higher the shade is, the more stomata are in a unit area; however, this does not guarantee an increase in the rate of photosynthesis. Closer stomata exhibit a greater number of smaller sizes. Investigating the stomata width parameter demonstrates that stomata open wider under no-shade conditions than in shaded conditions. The results concur with the previous studies utilizing the electron micrographs that stomata open wide at 100% light conditions, then shrink slightly at 40% light and almost close at 10% light intensity (Yang et al. 2020). *C. nardus* is a C4 plant where ambient CO_2 is initially fixed as a four-carbon acid (malate) in mesophyll cells through PEPCase, and then, malate is transferred into the bundle sheath cell. Malate undergoes decarboxylation to produce CO_2 with a high concentration around rubisco in the Calvin cycle. Shade conditions in C4 plants induce the release of CO_2 into the bundle sheath cells through malate decarboxylation to slow down, consequently leading to reduced CO_2 absorption and transport in photosynthesis (Li et al. 2014). C4 plants are more adaptive to intense light and high temperatures than C3 plants (Satrapová et al. 2013). An increase in temperature above 32°C can reduce the activation of rubisco as a CO_2 binding enzyme which then affects the decrease in photosynthetic activity. In C4 plants, the rate of photosynthesis can increase up to 40°C. This is due to the high levels of CO_2 in mesophyll chloroplasts (Bejia and Semahegn 2022). The majority of plants with the C4 type are monocots, such as grasses and sedges.

The shade conditions affect the growth, yield, physiological response, and quality of *C. nardus* plant essential oil. The higher the shade percentage, the lower the essential oil content of *C. nardus*. Previous studies illustrate that shading treatment affects the content of essential oils such as linalool and eugenol as the characteristic taste of basil increases in total irradiation and decreases in shaded conditions (Chang et al. 2008). Another study on *Eucalyptus citriodora* Hook. plants revealed that a higher percentage of shade led to the growth of thicker stems, broader petals, and more branches;

however, the content of citronellal and β-Citronellol was not significantly different at 0%, 50%, and 75% shade (Degani et al. 2016). In observing *C. nardus* plants, the most influential factor in the quality of essential oils was the use of varieties, whereas the shade tended to fluctuate. Sequentially, the varieties containing the highest citronellal, citronellol, and geraniol are Seraiwangi 1, Sitrona 2 Agribun, and Sitrona 1 Agribun. Changes in leaf morphology can affect the micromorphology, consequently causing an impact on the essential oil produced. Leaf micromorphology is required to identify the secretory structures responsible for the biosynthesis and storage of bioactive compounds, including essential oils (Fernandes et al. 2013).

Based on this experimental result, it can be established that citronella can theoretically be used as an intercrop in agroforestry systems with shading conditions ranging up to 25%. An increase in the percentage of shade above 25% resulted in reduced biomass and essential oil yields. The growth and yield of intercrops can decrease under tree plantations during the subsequent growth of trees each year (Kaur et al. 2017). Seraiwangi 1 variety grown under 0% and 25% shade shared nearly the identical value, while the Sitrona 1 Agribun and Sitrona 2 Agribun varieties continued to experience declines ranging from 0%, 25%, and 50% to 75%. On the observation of essential oil quality, the Seraiwangi 1 variety had superior quality than the Sitrona 1 Agribun and Sitrona 2 Agribun varieties corresponding to citronellal, citronellol, and geraniol observations. Previous research on soybean plants demonstrated that at 25% shade, the plants could still maintain optimum growth (Khalid et al. 2019). Many commercial crops have been grown in agroforestry systems and declared economically profitable (Thakur et al. 2019). Citronella oil production proliferated significantly by 3%-4% per year, with favorable selling prices starting from Rp.120,000 to Rp.150,000 and increasing to Rp.215,000 to 350,000 (Wahyudi 2021). The advantages of the agroforestry system are not only from the economic aspect of land use but also from the ecological and social aspects. Therefore, to avail the ecological advantages, citronella plants have the potential to augment biodiversity in ecosystems, canopy the soil surface to reduce evaporation in the dry season as well as mitigate surface runoff during the rainy season to repress erosion.

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REFERENCES

Bejia T, Semahegn Z. 2022. Effects of increased iemperature on photosynthesis of C3 and C4 plants. J Nat Sci Res 13 (11): 19-25. DOI: 10.7176/jnsr/13-11-03.

Chang X, Alderson PG, Wright CJ. 2008. Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. Environ Exp Bot 63: 216-223. DOI: 10.1016/j.envexpbot.2007.10.017.

Dai Y, Shen Z, Liu Y, Wang L, Hannaway D, Lu H. 2009. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. Environ Exp Bot 65: 177-182. DOI: 10.1016/j.envexpbot.2008.12.008.

Degani AV, Dudai N, Bechar A, Vaknin Y. 2016. Shade effects on leaf production and essential oil content and composition of the novel herb *Eucalyptus citriodora* Hook. J Essent Oil Bear Plants 19 (2): 410-420. DOI: 10.1080/0972060X.2014.890080.

Fan Y, Chen J, Wang Z, Tan T, Li S, Li J, Wang B, Zhang J, Cheng Y, Wu X, Yang W, Yang F. 2019. Soybean (*Glycine max* L. Merr.) seedlings response to shading: leaf structure, photosynthesis and proteomic analysis. BMC Plant Biol 19 (34): 1-12. DOI: 10.1186/s12870-019-1633-1.

Fernandes VF, de Almeida LB, Feijó EVdRS, Silva DdC, de Oliveira RA, Mielke MS, Costa LCdB. 2013. Light intensity on growth, leaf micromorphology and essential oil production of *Ocimum gratissimum*. Rev Bras Farmacog 23 (3): 419-424. DOI: 10.1590/S0102-695X2013005000041.

Hamzah MH, Che MH, Abidin ZZ, Jamaludin H. 2014. Comparison of citronella oil extraction methods from *Cymbopogon nardus* grass by ohmic-heated hydro-distillation, hydro-distillation, and steam distillation. BioResources 9 (1): 256-272. DOI: 10.15376/biores.9.1.256-272.

Hou W, Luo Y, Wang X, Chen Q, Sun B, et al. 2018. Effects of shading on plant growth, flower quality and photosynthetic capacity of Rosa hybrida. AIP Conf Proc Biotechnol Bioeng. DOI: 10.1063/1.5034257.

Inderaja BM, Pradhita O, Hanifah R, Manurung R, Abduh MY. 2018. Factors affecting biomass growth and production of essential oil from leaf and flower of *Salvia leucantha* Cav. J Essent Oil Bear Plants 21 (4): 1021-1029. DOI: 10.1080/0972060X.2018.1506711.

Kaur H, Bhardwaj U, Kaur R. 2021. *Cymbopogon nardus* essential oil: a comprehensive review on its chemistry and bioactivity. J Essent Oil Res 33 (3): 205-220. DOI: 10.1080/10412905.2021.1871976.

Kaur N, Singh B, Gill RIS. 2017. Productivity and profitability of intercrops under four tree species throughout their rotation in North-Western India. Indian J Agron 62 (2): 160-169.

Khalid MHB, Raza MA, Yu HQ, Sun FA, Zhang YY, Lu FZ, Si LL, Iqbal N, Khan I, Fu FL, Li WC. 2019. Effect of shade treatments on morphology, photosynthetic and chlorophyll flourescence characteristics of soybean (*Glycine max* L. Merr). Appl Ecol Environ Res 17 (2): 2551-2569. DOI: 10.15666/aeer/1702_25512569.

Li T, Liu LN, Jiang CD, Liu YJ, Shi L. 2014. Effects of mutual shading on the regulation of photosynthesis in field-grown sorghum. J Photochem Photobiol B Biol 137: 31-38. DOI: 10.1016/j.jphotobiol.2014.04.022.

Mahajan M, Kuiry R, Pal PK. 2020. Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants. J Appl Res Med Aromat Plants 18: 1-10. DOI: 10.1016/j.jarmap.2020.100255.

Mohanty S, Thakur NS, Gunaga RP, Gajbhiye N. 2019. Influence of *Melia dubia* Cav. spatial geometries on growth, herbage yield and essential oil constituents of *Cymbopogon martinii* (Roxb.) Wats. J Essent Oil Bear Plants 22 (3): 630-648. DOI: 10.1080/0972060X.2019.1642144.

Saputra NA, Wibisono HS, Darmawan S, Pari G. 2020. Chemical composition of *Cymbopogon nardus* essential oil and its broad spectrum benefit. IOP Conf Ser: Earth Environ Sci 415 (1): 6-13. DOI: 10.1088/1755-1315/415/1/012017.

Satrapová J, Hyvönen T, Venclová V, Soukup J. 2013. Growth and reproductive characteristics of C4 weeds under climatic conditions of the Czech Republic. Plant Soil Environ 59 (7): 309-315. DOI: 10.17221/77/2013-pse.

Semchenko M, Lepik M, Götzenberger L, Zobel K. 2012. Positive effect of shade on plant growth: amelioration of stress or active regulation of growth rate? J Ecol 100 (2): 459-466. DOI: 10.1111/j.1365-2745.2011.01936.x.

Shafiq I, Hussain S, Raza MA, Iqbal N, Asghar MA, Raza A, Fan Y-f, Mumtaz M, Shoaib M, Ansar M, Manaf A, Yang W-y, Feng F. 2021. Crop photosynthetic response to light quality and light intensity. J Integr Agric 20 (1): 4-23. DOI: 10.1016/S2095-3119(20)63227-0.

Silva CF, Moura FC, Mendes MF, Pessoa FLP. 2011. Extraction of citronella (*Cymbopogon nardus*) essential oil using supercritical CO_2: Experimental data and mathematical modeling. Braz J Chem Eng 28 (2): 343-350. DOI: 10.1590/S0104-66322011000200019.

Song R, Kelman D, Johns KL, Wright AD. 2012. Correlation between leaf age, shade levels, and characteristic beneficial natural constituents of tea (*Camellia sinensis*) grown in Hawaii. Food Chem 133 (3): 707-714. DOI: 10.1016/j.foodchem.2012.01.078.

Sulaswatty A, Rusli MS, Abimanyu H, Tursiloadi S. 2019. Quo Vadis Minyak Serai Wangi dan Produk Turunannya. Lembaga Ilmu Pengetahuan Indonesia Press, Jakarta. [Indonesian]

Suryanto P, Putra ETS, Kurniawan S, Suwignyo B, Sukirno DAP. 2014. Maize response at three levels of shade and its improvement with intensive agro forestry regimes in Gunung Kidul, Java, Indonesia. Procedia Environ Sci 20: 370-376. DOI: 10.1016/j.proenv.2014.03.047.

Thakur NS, Mohanty S, Gunaga RP, Gajbhiye NA. 2019. *Melia dubia* Cav. spatial geometries influence the growth, yield and essential oil principles content of *Cymbopogon flexuosus* (Nees Ex Steud.) W.Watson. Agrofor Syst 94: 985-995. DOI: 10.1007/s10457-019-00465-6.

Wahyudi A. 2021. Sistem produksi minyak serai wangi berkelanjutan. Perspektif Review Penelitian Tanaman Industri 20 (2): 94-105. DOI: 10.21082/psp.v20n2.2021.94-105. [Indonesian]

Wan Y, Zhang Y, Zhang M, Hong A, Yang HY, Liu Y. 2020. Shade effects on growth, photosynthesis and chlorophyll fluorescence parameters of three *Paeonia* species. PeerJ 8: e9316). DOI: 10.7717/peerj.9316.

Widaryanto E, Roviq M, Saitama A. 2019. An effective method of leaf area measurement of sweet potatoes. Biosci Res 16 (2): 1423-1431. https://www.isisn.org/BR16(2)2019/1423-1431-16(2)2019BR19-214.pdf.

Widiputri DI, Gunawan-Puteri MD, Kartawiria IS. 2019. Benchmarking study of *Cymbopogon citratus* and *C. nardus* for its development of functional food ingredient for anti-diabetic treatment. Iconiet Proc 2 (2): 109-114. DOI: 10.33555/iconiet.v2i2.20.

Yang H, Dong B, Wang Y, Qiao Y, Shi C, Jin L, Liu M. 2020. Photosynthetic base of reduced grain yield by shading stress during the early reproductive stage of two wheat cultivars. Sci Rep 10 (14353): 1-15. DOI: 10.1038/s41598-020-71268-4.

Yelfi A, Napitupulu TP, Meliana DU. 2019. Antihypertension activity of *Cymbopogon nardus* (L.) Rendle as an aromatherapy candle material at community health Center Kapuk II West Jakarta, Indonesia. IOP Conf Ser Earth Environ Sci 308: 1-7. DOI: 10.1088/1755-1315/308/1/012037.

Zulfikar W, Aditama, Sitepu FY. 2019. The effect of lemongrass (*Cymbopogon nardus*) extract as insecticide against *Aedes aegypti*. Intl J Mosq Res 6 (1): 101-103.