J. Hort. Indonesia, Agustus 2024, 15(2): 112-118
DOI : http://doi.org/10.29244/jhi.15.2.112-118
Tersedia online di http://journal.ipb.ac.id/index.php/jhi

p-ISSN 2087-4855 e-ISSN 2614-2872
Terakreditasi Sinta 2 No. 200/M/KPT/2020

The Effect of Drought Stress on Phyllanthin and Quercetin Contents of Green Meniran Plant (*Phyllanthus niruri* L.)

Pengaruh Cekaman Kekeringan terhadap Kandungan Filantin dan Kuersetin Tanaman Meniran Hijau (*Phyllanthus niruri* L.)

Nailulkamal Djamas[1,2*], Adi Pancoro[1], Lukita Devy[2]

Diterima 25 Oktober 2023/ Disetujui 23 Juli 2024

ABSTRAK

Penelitian tentang pengaruh cekaman kekeringan terhadap produksi filantin dan kuersetin pada meniran hijau (*Phyllanthus niruri* L.) telah dilakukan. Tujuan penelitian yaitu: Menentukan dampak cekaman kekeringan terhadap pertumbuhan vegetatif tanaman meniran hijau dan menentukan taraf kapasitas lapang serta waktu panen yang optimal untuk memperoleh konsentrasi optimal filantin dan kuersetin. Penelitian dilakukan dari September 2021 hingga September 2022 di Laptiab BRIN menggunakan tiga taraf Kapasitas Lapang (KL) (30, 60 dan 100%) dan dua waktu panen (2 dan 4 minggu). Parameter yang diamati adalah parameter morfologi serta kandungan filantin dan kuersetin. Hasil penelitian menunjukkan bahwa cekaman kekeringan dan waktu panen memberikan perbedaan yang nyata pada kandungan filantin dan tidak berbeda nyata pada kandungan kuersetin. Kandungan filantin tertinggi diperoleh pada 100% KL dan waktu panen 4 minggu setelah tanam (MST). Kesimpulan dari penelitian ini adalah: Cekaman kekeringan dapat mengurangi pertumbuhan vegetatif dari tanaman meniran hijau dan untuk memperoleh pertumbuhan vegetatif yang optimal, meniran hijau sebaiknya tidak diberikan cekaman kekeringan; Untuk memperoleh kandungan filantin dari tanaman meniran hijau dengan tingkat kandungan yang paling tinggi, taraf kapasitas lapang dan waktu panen yang optimal adalah pada 100% KL dan waktu panen empat minggu; Kandungan kuersetin dari meniran hijau tidak dipengaruhi secara signifikan oleh perbedaan taraf cekaman kekeringan dan waktu panen.

Kata kunci : rekayasa irigasi, pertumbuhan vegetatif tanaman, rekayasa metabolit sekunder, teknik budidaya tanaman obat, waktu panen

ABSTRACT

Research on the effect of drought stress on the production of phyllanthin and quercetin in green meniran (Phyllanthus niruri L.) has been carried out. The research objectives are to determine the impact of drought stress on the vegetative growth of green meniran plants and determine the optimal level of field capacity and harvest time to obtain optimal concentrations of phyllanthin and quercetin. The research was conducted from September 2021 to September 2022 at the BRIN Laboratory using three levels of Field Capacity (KL) (30, 60, and 100%) and two harvest times (2 and 4 weeks). The observed parameters were morphologic parameters and the content of phyllanthin and quercetin. The results showed that drought stress and harvest time made a significant difference in the phyllanthin content and not a significant difference in the quercetin content. The highest phyllanthin content was obtained at 100% KL and harvest time 4 weeks after planting (WAP). The conclusions of this research are: Drought stress can reduce the vegetative growth of green meniran plants and to obtain optimal vegetative growth, green meniran should not be subjected to drought stress; To obtain the highest level of phyllanthin content from green meniran plants, the optimal level of field capacity and harvest time is 100% KL and a harvest time of four weeks; The quercetin content of green meniran is not significantly influenced by differences in the level of drought stress and harvest time.

Keywords: irrigation engineering, harvest time, medicinal plant cultivation techniques, plant vegetative growth, secondary metabolite engineering

[1)]Sekolah Ilmu dan Teknologi Hayati, Institut Teknologi Bandung (ITB), Jl. Ganeca No. 10, Bandung, 40132, Jawa Barat, Indonesia
[2)]Pusat Riset Hortikultura, Badan Riset dan Inovasi Nasional (BRIN), Cibinong Science Center,
Jl. Raya Jakarta – Bogor, Cibinong, Kabupaten Bogor, 16915, Jawa Barat, Indonesia
E-mail: nail004@brin.go.id (*penulis korespondensi)

INTRODUCTION

Indonesia has good prospects for the development of medicinal plant agro-industry. More than 9609 species of Indonesian plants are known to have medicinal properties. 74% of medicinal plants grow wild in forests and the remaining 26% have been cultivated. Of those that have been cultivated, more than 940 types of plants are used as traditional medicine (Yassir and Asnah, 2018).

One of the plants that is usually used as an ingredient for traditional medicine is green meniran. Green meniran (*Phyllanthus niruri* L.) is a plant from the Phyllanthaceae family with medicinal benefits. *P. niruri* has been scientifically evaluated in various clinical trials and has been shown to activate the immune system in infectious diseases, such as chronic hepatitis B, tuberculosis, pneumonia, vaginitis, and varicella infections (Melyani and Sujarwati, 2021).

The chemical compounds contained in meniran herb include saponins, flavonoids, polyphenols, phyllanthin, hypophyllanthin, and potassium salts. These compounds interact with each other to increase their antioxidant activity. Clinically, meniran extract has been shown to act as an immunomodulator or able to stimulate a person's immune system so that it is immune to disease attacks (Tambunan et al., 2019).

Phyllanthin and quercetin are secondary metabolites in meniran plants. Secondary metabolites are compounds resulting from the biogenesis of primary metabolites. In plants, secondary metabolites function as chemical compounds that generally have the ability for bioactivity and as plant protectors from disease and environmental disturbances, and are generally produced in higher plants (Purwati, 2017).

One way to increase the production of secondary metabolites in meniran plants is to provide drought stress to meniran plants. Plants that are stressed by drought close their stomata to stop water loss. The rate of transpiration and CO_2 uptake significantly decreases as a result of stomata closing, but the supply of reducing equivalents (NADPH and H^+) increases, which raises the ratio of NADPH to $NADP^+$. All metabolic activities are accelerated by the enormous excess of NADPH + H^+, which consumes reducing equivalents and leads to the creation of highly reduced natural chemicals such glucosinolates, phenols, terpenoids, alkaloids, and cyanogenic glycosides. (Mahajan et al., 2020). Therefore, in this study drought stress will be carried out on meniran plants using two factors. The first factor uses three types of water content or field capacities, namely 30, 60 and 100%. The second factor uses two harvest times, the first harvest at 2 weeks after planting and the second at 4 weeks after planting. The aims of this study are: 1) To determine the effect of drought stress on the morphology of green meniran plants, 2) To obtain the optimal level of drought stress and harvest time to produce phyllantine and quercetin in green meniran with the highest content.

MATERIALS AND METHODS

The research was carried out at the Agricultural Production Laboratory, LAPTIAB (Agro-Industrial Technology and Biotechnology Development Laboratory) National Research and Innovation Agency (BRIN), B.J. Habibie Science and Technology Area and in the PT Nano Herbaltama Internasional laboratory in Serpong, South Tangerang during September 2021 to September 2022. Randomized Complete Block Design (RCBD) is a limited random design that first groups experimental units into homogeneous groups, called blocks, and then randomly determines treatment within these groups. So, the main objective of grouping experimental units is to make them as homogeneous as possible within the group relative to the dependent variable being studied and to try to make different groups as heterogeneous as possible relative to the dependent variable (Susilawati, 2015). An RCBD was created with 3 replications of each treatment with four individual plants per replication. Meniran seeds come from Jember, East Java. There are two treatment factors, namely field capacity and harvest time so the total sample is 108. There are three levels of drought stress treatment, namely 30, 60, and 100% Field Capacity. There are two harvest times, namely 2 weeks and 4 weeks. Two treatment factors are combined into one treatment package, for example, 30% field capacity combined with a two-week harvest time and 60% field capacity combined with a four-week harvest time.

Meniran plants are planted from sown meniran seeds and then transferred to planting media in the form of soil in polybags (20 x 20 x 20 cm^3) mixed with manure in a ratio of 1:1 until the total mass reaches around 5 kg. The field capacity (KL) given for drought stress treatment of planting media is determined and refers to what was done by Khaerana et al. (2008). Water is provided based on a treatment code that indicates field capacity. The three field capacities shown are 30%, 60%, and 100%. The soil moisture sensor with the Arduino 2560 + microcontroller is optimized to indicate water availability in the planting medium based on soil moisture levels. The microcontroller is programmed to read soil moisture in percentage terms. Irrigation or watering is applied to maintain field capacity based on microcontroller calibration data (Radi et al., 2018). Stress was given 7 days after planting (DAP). Watering is done manually using a water sprinkler. The volume of water is then given until the sensor shows the expected field capacity figure. Treatment was given for 2 weeks for the first harvest and 4 weeks for the second harvest. An illustration of water pouring into each container can be seen in Figure 1 (No measurements were made of light intensity at the location). The water in the measured water container is given based on the field capacity value on the field capacity detector connected to the soil in the meniran pot. For 100% field capacity, water is provided at the maximum volume. For field capacities of 30% and 60%, the water volume is given with a tolerance limit of 10% below or above field capacity.

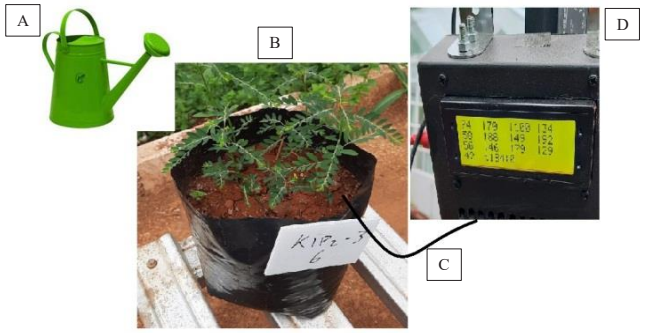

Figure 1. Illustration of water pouring into each container: (A) water container, (B) pot of meniran, (C) cable that is connecting the field capacity detector to the soil, (D) field capacity detector.

The volume of water that must be provided is calculated from the difference between the current field capacity and the supposed field capacity, and the calculations are based on the ratio of water volume to the initial field capacity that was calculated previously.

Morphological observations were carried out every week from the beginning of the drought stress treatment until harvest time arrived. Morphological observations were made on plant height (cm), leaf number, and the presence of flowers and fruit from each meniran in polybags. Meniran harvest was carried out in two different harvesting treatments with different plants, namely the first harvest at 2 weeks and the second harvest at 4 weeks. Harvesting is carried out on all parts of the meniran plant, then the biomass of the meniran plant is weighed and the dry and wet weight of the meniran roots is weighed.

Phyllantin levels were measured using the HPLC (High-Performance Liquid Chromatography) brand Waters e2695 Separations Module based on the method used by Murugaiyah and Chan (2007). Measurement of quercetin levels was carried out using UV-Vis spectrophotometry, Labtron Equipment Ltd., UK model LUS-B13 with a Double Beam UV-Vis Spectrophotometer type based on the method carried out by Krisyanella et al. (2013). Data analysis was carried out using the following methods: a) Analysis of variance (ANOVA) and b) Duncan's Multiple Range Test. Measurement of quercetin levels was not carried out by HPLC because there was a stock of quercetin standards in our laboratory by using UV-Vis spectrophotometry.

RESULTS AND DISCUSSION

Analysis of Variance in Morphology

Data recapitulation of variance analysis in morphology can be seen in Table 1, analyzed by SAS Statistic Software. The statistical analysis was conducted based on Gomez and Gomez (1995).

The table above showed that plant height, number of leaves, crown wet weight, root wet weight, and root dry weight were significantly different in drought stress. Leaf number and root wet weight were significantly different at harvest time. The existence of flowers and fruits was not significantly different in drought stress and harvest time. All the parameters were not significantly different in the interaction between drought stress and harvest time. Drought stress seems to have reduced the productivity of meniran in the form of plant height, leaf number, and biomass. Trisilawati and Pitono (2012) stated that the low productivity of plants due to water deficit stress is caused by decreased nutrient uptake, photosynthesis and respiration processes.

Distribution of Plant Height in Drought

According to the statistical analysis, plant height were very significantly different in drought stress. Furthermore, Duncan's Multiple Range tests were carried out to see further the difference between the given drought stress and the height of the meniran plants. The distribution of plant height during drought can be seen in Figure 2.

The mean of plant height between the three given drought stresses showed significant differences. Plant height growth decreased in line with the increasing drought stress given.

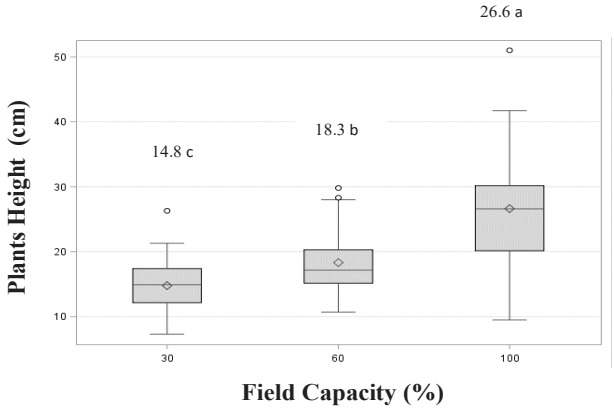

Figure 2. Distribution of Plant Height in Drought

This was also reported by Manurung et al. (2019) on tabat barito plants (*Ficus deltoidea* Jack) where increasing drought stress, namely 40% field capacity, can reduce plant height by 10.3% compared to 100% field capacity. From Figure 2, we can see that drought stress can inhibit the vegetative growth process in the form of plant height in meniran plants. The greater the drought stress, the higher the inhibition of vegetative growth. Drought stress causes a lack of water intake in plants to carry out the process of photosynthesis, resulting in a decrease in plant growth. The decreased rate of photosynthesis reduces the energy sources needed for cell division and enlargement, so the plant is stunted. Drought stress causes inhibition of cell division activity which causes no enlargement of cell mass or content so cell enlargement is inhibited (Buchory et al., 2020).

Distribution of Leaf Number in Drought

According to the statistical analysis, leaf numbers were very significantly different in drought stress. Furthermore, Duncan's Multiple Range tests were carried out to see further the difference between the given drought stress and the number of leaves of the meniran plants. The distribution of the leaf number in drought can be seen in Figure 3.

The leaf number between the two drought stress conditions showed a significant difference. The average number of leaves decreased in line with increasing drought stress. From Figure 3, we can see that drought stress can inhibit the vegetative growth process in the form of several leaves in meniran plants. Plant cells will experience cycles to be able to grow and develop. During the cell cycle, certain materials control it so that the cell cycle process runs normally, namely enzymes, proteins, and hormones. In drought conditions, the activity of cytokinin hormones which function in cell division and maintaining cell turgor becomes inhibited so that shoots cannot be initiated and leaves fall off easily. Conversely, drought conditions will increase the production of the hormone ethylene. Ethylene is a hormone that plays a role in leaf aging so that the leaves fall (Yusuf, 2019).

Analysis of Variance in Phyllanthin's and Quercetin's Content

Data recapitulation of analysis of variance in phyllanthin's and quercetin's content can be seen in Table 2, analyzed by SAS Statistic Software. The statistical analysis was conducted based on Gomez and Gomez (1995).

The table above showed that phyllanthin's content was significantly different in drought stress and harvest time. Quercetin's content was not significantly different in drought stress and harvest time. All the parameters were not significantly different in the interaction between drought stress and harvest time. From the table above, it can be seen that drought stress and harvest time have an effect on the phyllantin content in meniran plants but have no effect on the quercetin content. Drought stress affects the secondary metabolite content of a plant (Utami et al., 2020). Harvest time affects the secondary metabolite content of a plant (Buchory et al., 2020).

Table 1. Data Recapitulation of Analysis of Variance in Morphology

No.	Parameters	Drought	Harvest Time	Drought x Harvest Time
1	Plant Height	**	ND	ND
2	Leaf Number	**	**	ND
3	Existence of Flowers	ND	ND	ND
4	Existence of Fruits	ND	ND	ND
5	Crown Wet Weight	**	ND	ND
6	Root Wet Weight	**	*	ND
7	Root Dry Weight	*	ND	ND

Description: * = significantly different ($0.01<\alpha\leq0.05$), ** = very significantly different ($\alpha\leq0.01$), ND = not significantly different ($\alpha>0.05$)

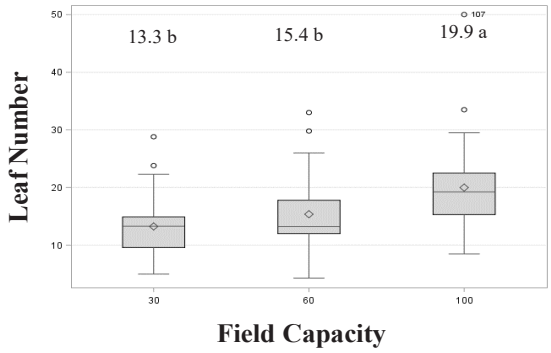

Figure 3. Distribution of Leaf Number in Drought

Table 2. Data Recapitulation of Analysis of Variance in Phyllanthin's and Quercetin's Content

No.	Parameters	Drought	Harvest Time	Drought x Harvest Time
1	Phyllanthin's content	*	**	ND
2	Quercetin's content	ND	ND	ND

Description: * = significantly different (0.01<α≤0.05), ** = very significantly different (α≤0.01), ND = not significantly different (α>0.05)

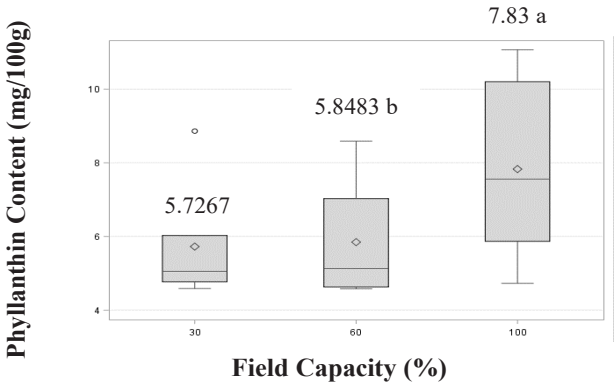

Figure 4. Distribution of Phyllanthin Content in Drought

Distribution of Phyllanthin Content in Drought

According to the statistical analysis, phyllanthin content was significantly different in drought stress. Furthermore, Duncan's Multiple Range test was carried out to see further the difference between the given drought stress and the phyllanthin content. The distribution of phyllanthin content in drought can be seen in Figure 4.

The mean of phyllanthin content (in mg/100 g) between the two drought stress conditions showed a significant difference but not with the other two drought stress. Field capacity (KL) of 100% resulted in an average phyllanthin content of 7.83 mg/100 g. 60% KL produced an average phyllanthin content of 5.8483 mg/100 g and 30% KL produced an average phyllanthin content of 5.7267 mg/100 g. The phyllanthin content decreased in line with the decrease of field capacity or water content that is given to the meniran plant.

We can see from Figure 4 that the decrease in drought stress can cause a decrease in phyllanthin content. That is the opposite of the theory that concludes the increase of drought stress could increase the secondary metabolite content of the plant. Water stress treatment does not always increase the active ingredient content of the plants, the increased drought stress can decrease the synthesis of secondary metabolite in turmeric (Suciastuti and Sudjino, 2019). Of all the drought stress treatments, the highest phyllanthin content was obtained by 100% field capacity.

Distribution of Phyllanthin Content at Harvest Time

According to the statistical analysis, phyllanthin content was very significantly different at harvest time. Furthermore, Duncan's Multiple Range test was carried out to see further the difference between the harvest time and the phyllanthin content. The distribution of phyllanthin content at harvest time can be seen in Figure 5.

The mean of phyllanthin content (in mg/100 g) between the two harvest times showed a significant difference. Two weeks of harvest time resulted in an average phyllanthin content of 5.0278 mg/100 g meanwhile four weeks of harvest time produced an average phyllanthin content of 7.9089 mg/100 g. The phyllanthin content decreased in line with the acceleration of harvest time that is given to the meniran plant.

We can see from Figure 5 that the decrease in harvest time can cause a decrease in phyllanthin content. This can happen because plants can respond differently to short-term and long-term stress. Plants with long-term stress will reduce certain elements in secondary metabolism so that plants can reduce energy expenditure until the end of the stress which is one of the plant strategies to survive in unfavorable environmental conditions (Krol, 2014). However, as the harvest time increases, the secondary metabolites contained by plants will increase. Of all the harvest time treatments, the highest phyllanthin content was obtained within four weeks of harvest time.

Distribution of Quercetin Content during Drought and Harvest Time

According to the statistical analysis, quercetin content was not significantly different in drought stress and harvest time. Furthermore, Duncan's Multiple Range test was carried out to see further the difference between the given drought stress and the quercetin content and between the harvest time and the quercetin content. The distribution of quercetin content during drought and harvest time can be seen in Figures 6 and 7.

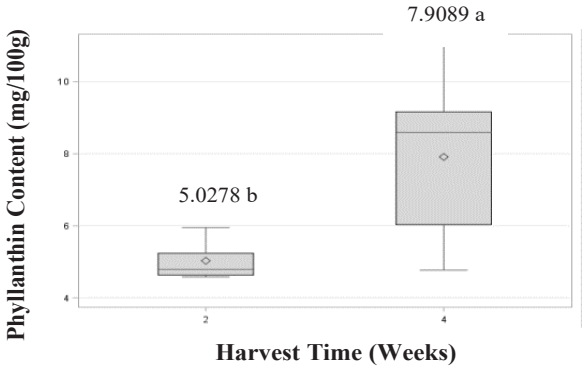

Figure 5. Distribution of Phyllanthin Content at Harvest Time

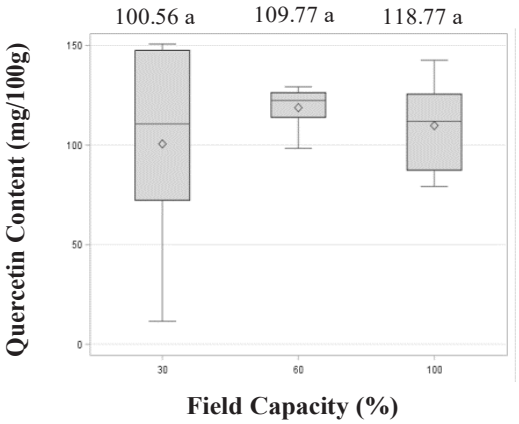

Figure 6. Distribution of Quercetin Content in Drought

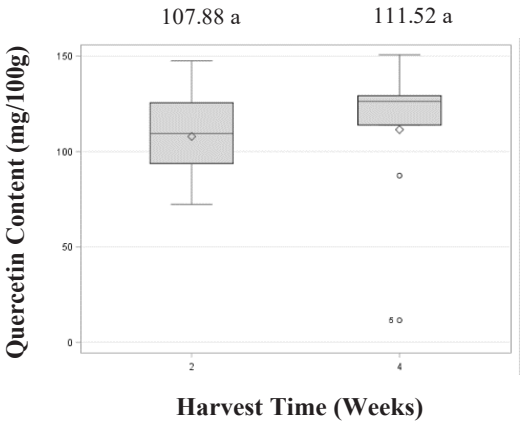

Figure 7. Distribution of Quercetin Content at Harvest Time

We can see from Figures 6 and 7 that there were no significant differences in quercetin content during drought and harvest time. The average quercetin content (in mg/100 g) was not significantly different in different drought or harvest times. This could be caused by the plant acclimatization process in green meniran. Plants of course undergo an acclimatization process to drought stress and harvest time so that they can avoid the metabolic imbalance experienced by plants when drought stress occurs during the two harvest periods (Kleinwachter et al., 2014).

CONCLUSION

The conclusions of this research are: 1) Drought stress can reduce the vegetative growth of green meniran plants and to obtain optimal vegetative growth, green meniran should not be subjected to drought stress, 2) To obtain the highest level of phyllanthin content from green meniran plants, the optimal level of field capacity and harvest time is 100% KL and a harvest time of four weeks. 3) The quercetin content of green meniran is not significantly influenced by differences in the level of drought stress and harvest time.

ACKNOWLEDGEMENT

Hereby we thank Lembaga Pengelola Dana Pendidikan (LPDP) RI for the grant for research and scholarship to study in Biotechnology of Institut Teknologi Bandung for the Magistrate Program.

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