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Effect of Water Availability on the Growth and Secondary Metabolites of Zodia (*Evodia suaveolens)*

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ABSTRACT

Zodia are included in medicinal plants because it contains secondary metabolites such as flavonoids, tannins, alkaloids, steroids/triterpenoids that have many benefit and also as mosquito repellents. The growth and content of secondary metabolites in plants can be affected by environmental factors such as water availability. Research on the cultivation of this crop is still limited. Environmental modification in cultivation is needed to increase its secondary metabolites, and the study of the potential of this plant as medicine are necessary. This study aimed to determine the effect of water availability on the growth and secondary metabolites of zodia. The research was carried out in July-November 2021 in Greenhouse, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta. The method used was a completely randomized design, one treatment factor with 4 levels namely, 100, 80, 60 and 40% of field capacity. Each treatment was repeated 6 times so that there were 24 experimental polybags. The data analysis used is Analysis of Variance, and the Duncan's Multiple Range Test at the 5% level. The results showed that the availability of water at a level of 60% field capacity gave the best average growth parameters (plant height and number of leaves) among other treatments. The flavonoid content in each treatment showed the same average. The results of the GCMS (Gas Cromatography and Mass Spectroscopy) test of zodia leaves showed that there were dominant bioactive compounds, namely ethylbenzene, octadecamethyl cyclononasiloxane, pentadecanoid acid and heptadecanoid acid. It is suspected that the content of these compounds has the potential as antifungal, antibacterial, antioxidant and anticancer.

Keywords: Antioxidant; Cow manure; Medicinal plants; Mosquito repellent; Phytochemical

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INTRODUCTION

Zodia (*Evodia suaveolens*) is a plant that classified as an orange tribe (Rutacea), this plant is native to Indonesia precisely in Papua Region. Zodia is included in ornamental plants and medicinal plants that have many benefits and also known as mosquito repellent. Rahayu et al. (2008) stated that not only as an ornamental plant, the zodia is also in demand by people living in mosquito-prone areas, because this plant is known to be effective in repelling Aedes aegypti mosquitoes. Zodia leaves can be distilled to produce essential oils (Kardinan 2007). In addition, zodia has the potential for other medications such as the effectiveness of the zodia against headache healers, rubbing drugs, cancer cell killers, as well as antifungals and antibacterials. Ngibad (2019), revelead that chemicals contained in zodia leaves after phytochemical screening are flavonoid compounds, tannins, steroid alkaloids/triterpenoids, and saponins, when studied further, will be very useful to find out which compounds are included in drugs. The cultivation of this plant is still not carried out yet, environmental modifications in its cultivation to increase secondary metabolites and studies on the potential of this plant as a medicine are needed. The cultivation of plants is influenced by various factors, especially environmental factors both biotic and abiotic. One of the important abiotic factors is water.

SPlants need mineral nutrients to grow and complete their life cycle, water is necessary for nutrient absorption in plants (Belliturk et al., 2019). Water availability is one of the main limiting factors for crop production. Too much water will cause aeration stress and a small amount of water will cause drought stress, therefore the availability of water must be sufficient according to plant needs. Generally, biotic and abiotic stress in plants tends to increase the production of secondary metabolite compounds. Secondary metabolite compounds are produced through pathways outside the biosynthesis of carbohydrates and proteins. Tatang (2019) stated that there are three main pathways for the formation of secondary metabolites, namely the malonic acid pathways of the compounds produced including fatty acids such as myristic, oleic, lauric, stearic, palmitic, linolenic, linoleic, glycerides, polyacetylene, phospholipids, and glycolipids. Second, the mevalonate acid pathway is terpenoid, essential oil, menthol, squalent, streoid, monoterpenoid, saponin, and the third factor the shikimic acid pathway includes tannins, phenol, benzoic acid, lignin, coumarin, benzoic amino acids, cinnamic acid and quinones.

The limited availability of nutrients causes the use of carbon in cellular metabolism not to be used for cell growth, but rather to produce secondary metabolites (Nofiani 2008). The content of secondary metabolites in plants can increase because of water stress, In addition to reducing growth and productivity plant responses to water stress can increase levels of the amino acid proline and secondary metabolites (Trisilawati and Pitono 2012). The purpose of this study was to determine the growth and content of secondary metabolites of zodia at various levels of water availability

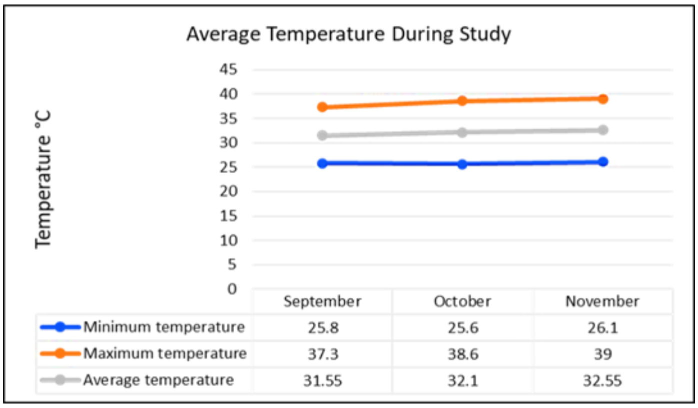


Figure 1. Average temperature during study

MATERIAL AND METHOD

Place and Time Of Research

The research was conducted from September to November 2021 at Greenhouse, Faculty of Agriculture, Sebelas Maret University (UNS), Surakarta. This location is located at coordinates 7°33'41.7" LS and 110°51'32.6" E and is at an altitude of 96 meters above sea level. Based on temperature observations in the environment during the study, the average minimum and maximum temperatures in each month were found to range from 25-39°C (Celcius).

Materials and Tools

This research was carried out starting from the preparation of tools and materials, planting, treatment by watering the zodia according to the field capacity for once every 5 days, treatments and observations carried out once a week, maintenance to harvest and post-harvest. The research materials used in this study are three-month old zodia plant, soil, cow manure, and water. The necessary tools are polybags, bucket, hose, label paper, stationery, analytical scales, stationery, writing books, envelopes, clear insulation, plastic, camera, and oven. The planting medium used was a mixture of inceptisol soil and cow manure in a ratio of 2:1.

Based on the laboratory analysis, the soil pH was 6.57 (neutral), total N 0.16% and available P was 6.48 ppm (low), while the available K was 0.54 cmol.kg^{-1} (moderate). Based on the description of the content listed on the cow manure packaging, it is known that the pH is 7.12 (neutral), total N 1.58%, available P was 1.48 ppm, available K was 1.33 cmol.kg^{-1}, and material organic was 38,66%.

Table 1. Chemical analysis of soil and cow manure

Growing media	Organic matter (%)	pH	N (%)	P (ppm)	K (cmol.kg^{-1})
Soil	12.20	6.57	0.16	6.48	0.54
Cow Manure	38.66	7.12	1.58	1.48	1.33

Research Methods

This study used a Complete Randomized Design, with 1 treatment factor, namely water availability treatment consisting of 4 levels, namely, 100% (C0), 80% (C1), 60% (C2), and 40% (C3) of field capacity (FC). The observation parameters in this study were the growth and yield components of zodia plants, namely plant height, number of leaves, root length, fresh and dry weight of plants, antioxidant content, flavonoids, and GC-MS (Gas Cromatography and Mass Spectroscopy) analysis.

Antioxidant Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) method is a simple, rapid, sensitive, and reproducible method for testing antioxidant activity, DPPH is a free radical compound that is often used to assess the antioxidant activity of several compounds or extracts, so the method is suitable for assessing the antioxidant activity of ethanol extracts. (Puspita, 2020). Fresh zodia leaves were extracted and then dissolved using methanol. The extract was then added to DPPH solution and incubated at 37°C. Then the sample was inserted into the cuvette until it was full to measure the absorbance. The data obtained is then calculated to determine the percentage of antioxidant activity with the equation (Eq. 1) (Kamoda et al., 2021):

$$\%\ \text{antioxidant activity} = \frac{A0}{A1}\ X\ 100\% \quad \text{(Eq. 1)}$$

Where A0 is the absorbance at DPPH without sample, A1 = Absorbance on DPPH after adding sample.

Flavonoid Content

Quantitative analysis of flavonoids can be done using a UV-Vis spectrophotometer by entering the absorbance value of the sample into the equation of the quersetin standard curve at 350-450 nm wave length. The sample tested was zodia leaf simplisia weighing 1 gram.

Gas Cromatography and Mass Spectroscopy (GC-MS) Analysis

The mechanism of GCMS is to separate mixed compounds into single compounds based on chemical properties and the analysis time required. The test was carried out on a sample of 1 g of dry simplisia from zodia leaves that had been extracted with methanol. Gas chromatography is used to detect volatile compounds under high vacuum and low pressure when heated. Meanwhile, mass spectrometry plays a role in determining molecular weights, molecular formulas, and producing charged molecules**.**

Data analysis

The observational data was analyzed using Analysis of Variance (ANOVA). If it has a real difference, it will be further tested using DMRT (Duncan's Multiple Range Test) level of 5%.

RESULT AND DISCUSSION

Parameters of Growth

Water availability affects plant height and the number of leaves *(Table 2)*. Availability of water levels of 60% FC and 40% FC gave a fairly high average plant height. The number of leaves at 100% FC water availability (107 leaves) was significantly different from the 60% FC treatment (124 leaves), and at 40% field capacity produces the longest roots (11.5 cm). Water availability treatment with a level of 60% field capacity gives the highest average plant height (18 cm). Manan et al. (2015) stated that the availability of water is related to the process of nutrient absorption by plants in metabolic processes. Plants respond to water availability by increasing vegetative growth such as plant height, number of branches, number of leaves, and stem diameter. Table 2 also shows that the 100% FC level treatment is significantly different from 60% FC and produces the lowest crop height compared to other treatments. because the 100% field capacity makes the soil excess water so that it becomes saturated. The availability of water, both deficient and excess will be able to interfere with plant growth (Suwati 2019). The results of research by Marsha et al. (2014) showed that the average plant height due to the time of water administration every day with a water volume of 100% FC was lower (14.83 cm) than 80% FC (16.05 cm) caused by water saturation conditions. All soil pores are filled with water which causes the washing of nutrients so that they cannot be absorbed optimally by plants' roots.

Table 2. Effect of water availability on parameter of growth of zodia

Water Availability	Plant Height (cm)	Number of leaves	Root Length (cm)
C0	12.58 ± 3.63 a	107 ± 16.00 a	10.25 ± 2.30 a
C1	14.75 ± 2.86 a	113 ± 23.12 a	11.00 ± 0.66 a
C2	18.00 ± 4.90 b	143 ± 26.94 b	10.75 ± 1.44 a
C3	16.83 ± 4.11 b	124 ± 20.91 b	11.50 ± 1.55 a

Note: Numbers followed by the same letter in the same column show that there is no significant difference in DMRT at the 5% level. C0 (availability of water 100% field capacity); C1 (availability of water 80% field capacity); C2 (availability of water 60% field capacity); C3 (availability of water 40% field capacity).

Based on the final observations *(Table 2)* it showed that the 60% FC (C2) treatment produced the highest average number of leaves (143 leaves). This is presumably because the availability of water with a level of 60% FC is optimal so that the plants grow well. Plants need mineral nutrients to grow and complete their life cycle, water is necessary for nutrient absorption because it can help plants distribute nutrients as food through the roots (Belliturk et al., 2019). Paramatha's research (2019) on *Paspalum conjugatum* grass showed that the application of water content of 75% FC gave the highest yield on almost all variables compared to other water content treatments.

The availability of water for plants must be sufficient, neither excess nor deficiency. Root length describes the plant's ability to obtain a supply of water including nutrients in the deeper soil layers. The increase in root length and volume is an important morphological response in the adaptation process of plants to water shortages (Abidin 2021). This is in accordance with Sawitri's research (2018) that rice plants in conditions lack of water for rice roots try to spread widely in dry and dense soil by increasing the number and length of roots to be able to absorb water and nutrients more efficiently.

Parameters of Yield

Plants with water availability at 60% field capacity (Table 3) produced the highest dry and fresh weights than the other treatments. Meanwhile. the highest chlorophyll content was found at the 40% field capacity (FC) level. Table 2 also shows that the treatment level of 100% field capacity water availability produces dry weight. fresh weight and low chlorophyll content. because the plants experience excess water.

Based on Table 3. it can be seen that availability of water has no significant effect on plant fresh weight. The highest fresh weight was found at 60% FC (C2). which was 11.5 g. The difference in fresh weight of treatment 100% FC (C0) and 60% FC (C2) was 4.66 g. The availability of water for plants is particularly important because water can dissolve nutrients in the soil that are absorbed by the roots so that they are channeled to the leaves where the photosynthesis process takes place. This is in line with (Eka 2016) which states that water absorption through roots affects plant fresh weight so root also plays an important role for plants. The more leaves, the fresh weight and dry weight of the plant will also increase. This is in accordance with the statement of Luthfiana (2019) which states that the fresh weight of plants is in line with the number of leaves per plant. because plants can optimally absorb sunlight along with Mg and water nutrients through the roots needed in the photosynthesis process.

Table 3. Effect of water availability on yield variables of zodia

Water Availability	Fresh Weight (g)	Dry Weight (g)	Chlorophyll (mg.L^{-1})
C0	6.84 ± 3.49 a	1.47 ± 0.75 a	0.38 ± 0.24 a
C1	7.59 ± 3.39 a	1.56 ± 0.73 a	0.46 ± 0.31 a
C2	11.50 ± 5.52 a	2.45 ± 1.14 a	0.54 ± 0.30 a
C3	8.40 ± 3.87 a	2.07 ± 0.93 a	0.74 ± 1.02 a

Note: Numbers followed by the same letter in the same column show that there is no significant difference in DMRT at the 5% level. C0 (availability of water 100% field capacity); C1 (availability of water 80% field capacity); C2 (availability of water 60% field capacity); C3 (availability of water 40% field capacity)

Based on the results of the analysis of variance *(Table 3)* it is known that the water availability treatment has no significant effect on the dry weight of the zodia. This is presumably because zodia plants are able to adapt to various conditions of water availability so that water can be absorbed and used efficiently by plants. Giving water to plants should be in accordance with the actual water needs of plants, because the lack or excess of giving water has an adverse effect on plants. The opinion of Aritonang and Surtinah (2018) explains that water and the availability of sufficient nutrients in plants will make cell organelles that play a role in producing food for plants to be maximally formed and can function optimally. Treatment of water availability with a level of 60% FC resulted in the highest average dry weight of 2.45 grams. The number of leaves affects the dry weight of the plant because the leaves are the storage site for the results of photosynthesis of the plant. Yuniarti et al. (2017) stated that plant biomass includes the results of photosynthesis, absorption of nutrients and water.

DMRT results *(Table 3)* also showed that the treatment of water availability was not significantly different to the total chlorophyll of the zodia, this was because the zodia plants were able to absorb the elements of N, Mg, and Fe well so there was no significant difference. Posumah (2017) stated that there are several factors that affect the formation of chlorophyll, one of which is nutrients, including N, Mg and Fe. Table 3 shows that the chlorophyll content in the water availability treatment at 40% field capacity was higher than the chlorophyll content in other treatments. Generally, giving less water will reduce chlorophyll levels because the growth on the leaves will also decrease but there are some plants when in stressed the chlorophyll content will increase. Plants that are tolerant to little water supply or are stressed will adapt and synthesize chlorophyll. This is in accordance with research by Ashri (2006) which shows that drought stress treatment causes an increase in chlorophyll levels in soybean leaves, the highest yield is found in soybean leaves which are more stressed. Bahar et al. (2017) research also showed that there was an increase in pH, K, total acidity, tartaric acid, anthocyanin and TPI quantities depending on periods and stress levels.

Chemical Content Analysis

Based on Table 4 shows that the 100% field capacity (C0) treatment had higher antioxidants than other treatments. The results of the flavonoid test *(Table 4)* show that all treatments have the same average flavonoid content of 0.15% except in the treatment of 40% FC water availability which is 0.13%.

The antioxidant test in this study used 0.03 mm DPPH with methanol as a solvent. Based on Table 4 shows that the treatment 100% FC (C0) antioxidant content was higher than other level of treatment. This is presumably because plants treated with 100% FC can adapt to a lot of water conditions. Sairam et al (2008) stated that plants that can adapt to the availability of excess water have the characteristics of the ability to cope with stress by forming aerenchyma, increasing soluble sugars, increasing glycolytic activity and fermentation enzymes as well as antioxidant resistance mechanisms to overcome conditions after hypoxia and anoxia. Sayuti and Yenrina (2015) stated that antioxidants are sensitive to external environmental conditions such as temperature, pH, oxygen, and salt and are easily damaged by high temperatures.

Table 4. Effect of water availability on the content of antioxidants and flavonoids zodia.

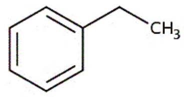
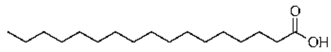
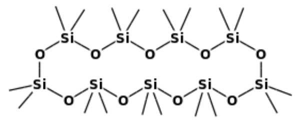
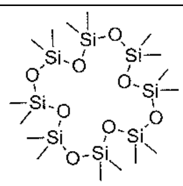
Water Availability	Antioxcidant (%)	Flavonoid (%)
C0	6.87	0.15
C1	3.91	0.15
C2	5.91	0.15
C3	5.45	0.13

Note: C0 (availability of water 100% field capacity); C1 (availability of water 80% field capacity); C2 (availability of water 60% field capacity); C3 (availability of water 40% field capacity)

Generally, stressed plants will produce higher secondary metabolites as a form of defense from an unfavorable environment, but in this study the treatment of 40% FC water availability with less water volume resulted in slightly lower flavonoid content than other level of treatments, because not all plants under water stress are able to produce high secondary metabolites. The results of Naiola's (1996) study on bitter plants with water availability of 40% field capacity showed the lowest andrographolide content compared to other treatments at 100%, 80%, and 60% field capacity. This shows that excessive water stress reduces the quality of the active components of the plant. The synthesis of flavonoids starts from the product of glycolysis, namely phosphoenol pyruvate then the product will enter the shikimate pathway to produce phenylalanine as the starting material for the phenylpropanoid pathway. This pathway produces 4-coumaroyl-CoA which combines with malonyl-CoA and then via chalcone as the first common intermediate to produce flavonoid structures (Nabavi et al. 2020).

Based on the GC-MS test that has been carried out on samples. there are five main compounds or dominant compounds (*Table 5*) namely ethylbenzene. octadecamethyl cyclononasiloxane. hexadecamethyl cyclooctasiloxane, pentadecanoic acid, and heptadecanoid acid. The following are the dominant compounds at each treatment level.

Table 5. Dominant compounds result of GC-MS analysis

No	Chemical Compunds	Area %				Chemical Structure
		C0	C1	C2	C3	
1	Ethyl benzene	10.87	13.12	14.55	11.47	
2	Pentadenoic acid	7.55	15.30	-	8.96	
3.	Heptadecanoid acid	-	-	10.12	-	
4.	Octadecamethyl cyclononasiloxane	16.64	9.37	26.15	29.93	
5.	Hexadecamethyl Cyclooctasiloxane	9.55	13.50	-	9.97	

Note: C0 (availability of water 100% field capacity); C1 (availability of water 80% field capacity); C2 (availability of water 60% field capacity); C3 (availability of water 40% field capacity)

The compounds octadecamethyl cyclononacyloxane ($C_{19}H_{54}O_9Si_9$) and hexadecamethyl cyclooctasiloxane ($C_{16}H_{48}Si_8$) are derivatives of siloxane compounds. Siloxane compounds and their derivatives are generally found in plants. especially the leaves and have various activities (Varothai et al. 2013). Bratty et al. (2020) in his research found that the extract of the siwak tree fruit tested by GCMS detected bioactive compounds. some of which were hexadecamethyl-cyclooctasiloxane and octadecamethyl-Cyclononasiloxane which had shown antibacterial and antifungal properties. Other dominant compounds are pentadenoic acid or pentadecanoic acid and heptadecanoic acid. Pentadecanoic acid is a fatty acid commonly found in nuts. The content of pentadecanoic acid is also found in plants that function as antifungals and antibacterials. the results of Nurmilantina's research (2017) show that in the analysis of the chemical composition of the leaves of sage (*Stenochlaena palustris* Bedd.) which is a plant originating from India. several compounds are obtained. some of which are terpenoids. In addition, pentadecanoic acid compounds ($C_{15}H_{30}O_2$), hexadecanoic acid ($C_{16}H_{32}O_2$), heptadecane cyclo heptatriene, and octadecanoic acids were also found. These compounds are also known as fatty acids which function as a source of calories, antibacterial, antifungal, and anticancer agents (Sjafaraenan and Johannes 2016). Heptadecanoic acid or margaric acid in Changzhi Xu's (2019) study showed that this fatty acid could significantly inhibit the growth of lung cancer cells.

Zodia leaf simplicia was extracted using ethanol solvent. The extract then continued with GC-MS analysis. Based on Figures 2 it can be seen that zodia leaves contain bioactive compounds, C0 (100 % FC) treatment there are 16 compounds, C1 (80% FC) 17 compounds, C2 (60% FC) 12 compounds and C3 (40%) 15 compounds ranging from high to low percent area.

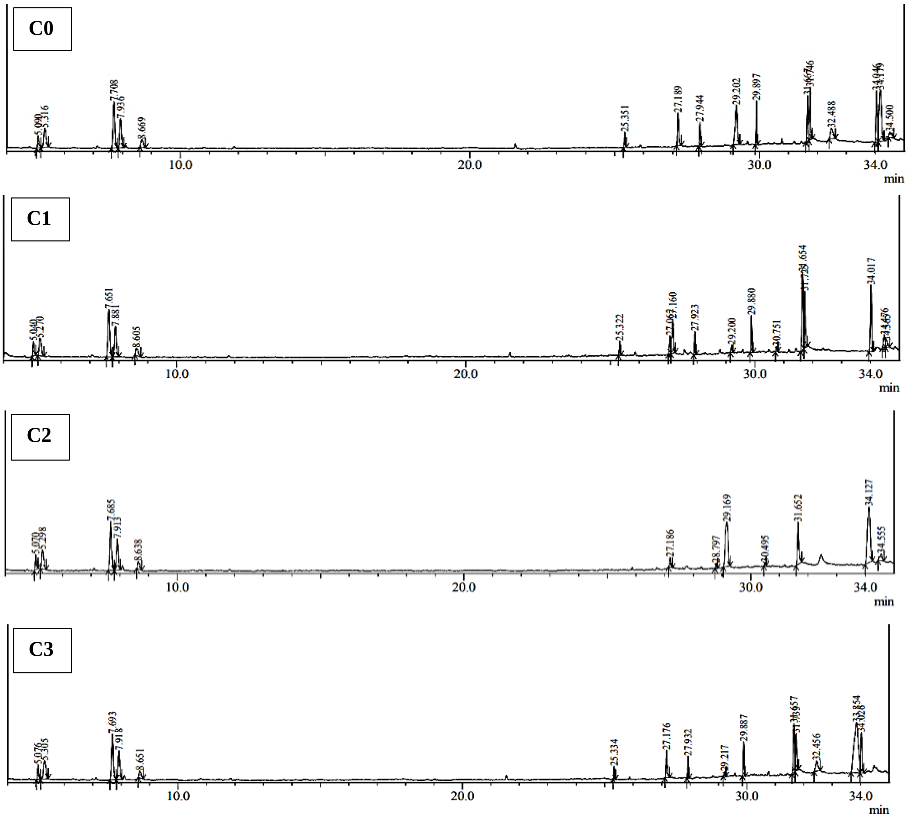


Figure 2. Chromatogram of GCMS analysis of zodia leaves at different levels of treatment (Peak 1: Ethyl benzene; 2: pentadecanoic acid; 3: hepatdecanoic acid; 4: octadecamethyl cyclononasiloxane; 5: hexadecamethyl cyclooctasiloxane)

CONCLUSION

Availability of water affects plant height as well as number of zodia leaves. Availability of water with a level of 60% field capacity is the best treatment for plant height and number of leaves. availability of water does not affect the content of secondary metabolites (flavonoids) zodia

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