

**INTERFERENCE EFFECT OF BABANDOTAN WEED (*Ageratum conyzoides* L.)
ON ANTIOXIDANT AND PATCHOULI OIL YIELD OF *Pogostemon cablin* (Blanco)
Benth cv. Sidikalang**

Anggoro Priyatmoko¹, Sri Darmanti, Lilih Khotimperwati¹

¹ Department of Biology, The Faculty of Science and Mathematics, Diponegoro University

Email: darmantisri@yahoo.co.id

Received: 17 October 2022 | Accepted: 3 July 2023

ABSTRACT

Weed interference results in increased synthesis of reactive oxygen species (ROS). High accumulation of ROS causes oxidative stress and stimulates the activation of the antioxidant defense system in plants. Weed interference causes biotic stress in the form of allelochemicals and abiotic stress in the form of competition for plants which results in the inhibition of growth and development in plants. *Ageratum conyzoides* is an annual weed in plantations and can cause biotic and abiotic stress on surrounding plants. This study aims to determine the effect of *A. conyzoides* interference on antioxidant content in the form of total phenol and total proline in patchouli leaves [*Pogostemon cablin* (Blanco) Benth cv. Sidikalang] as well as the percentage of essential oil yield from *P. cablin*. The study was conducted with a Complete Randomized Design (CRD) with a single factor of *A. conyzoides* interference levels in as many as four treatments (0, 2, 4, 6 individuals of *A. conyzoides* per treatment). The results concluded that higher levels of *A. conyzoides* interference led to an increase in total phenol and total proline in *P. cablin* leaves, but decreased the percentage yield of *P. cablin* essential oil.

Keywords: Interference, antioxidant, ROS.

INTRODUCTION

P. cablin can control 90% of the world market with total exports of patchouli oil around 35%-40% (Badan Pusat Statistik, 2013). Direktorat Jenderal Perkebunan (2017) reported that patchouli plants production in Indonesia is currently declining due to reduced planting land, poor soil conditions, and also the presence of other external disturbances such as weed interference. Weed interference in the environment greatly affects the productivity of cultivated plants. According to Patel *et al.* (2017), weed interference leads to a decrease in crop production by up to 31%. One of the weeds that are often found in patchouli plantations is *A. conyzoides* which is classified as an annual weed (Rosmanah *et al.*, 2017).

The decrease in crop production is due to a decrease in the speed of physiological processes due to weed interference. According to Yang *et al.* (2021) plants that get stressed can decrease the speed of physiological processes such as plant respiration, photosynthesis, water and nutrient absorption, and another physiological metabolism. Increased stress on cultivated plants will cause the synthesis of *reactive oxygen species* (ROS) in cultivated plants will also increase. The accumulation of ROS due to high stress causes damage to cell components, macromolecules, plasma membranes, nucleic acids, and proteins in cultivated plants, thereby reducing their productivity (Kasmiyati *et al.*, 2014). The increase in ROS was responded to by cultivated plants by synthesizing secondary metabolites for defense against

stress (Ejaz *et al.*, 2011). According to Zhang *et al.* (2015), the synthesis of secondary metabolites is due to the enzyme *phenylalanine ammonia-lyase* (PAL) which synthesizes primary metabolites into secondary metabolites.

Allelochemicals are secondary metabolite that plays a role in interactions between plants and as an intermediary in the mechanism of allelopathy and is useful for plant adaptation to environmental changes. According to Schandry *et al.* (2020), allelochemicals are released into the environment through root exudate, evaporation, washing, and also the decay of dead plant parts. The most widely produced allelochemicals produced by plants are phenolic compounds synthesized through the shikimic acid pathway (Rodriguez *et al.*, 2014). Phenols produced by *A. conyzoides* are allelochemicals and have a negative impact on cultivated plants because they increase the activity of peroxidase and superoxide dismutase enzymes which causes the rate of transpiration and photosynthesis rate to decrease (Kilkoda *et al.*, 2015). Allelochemicals from weeds can increase the phenolic content in plants that function as antioxidants. According to Naikoo *et al.* (2019), phenolic compounds are secondary metabolites that will increase if plants get stressed.

According to Moelyaandani *et al.* (2020), the presence of weeds also causes competition for water absorption for plants. Impaired water absorption causes drought stress in cultivated plants and is responded to by the production of proline. Nasrin *et al.* (2020) stated that with the increasing intensity of

drought stress, the proline content in rice plants increases. The stress of weeds also affects the condition of the patchouli oil content produced by *P. cablin*. Patchouli oil is a form of secondary metabolite derived from the terpenoid group and synthesized through the mevalonic acid metabolic pathway (Beek *et al.*, 2018). According to García-Caparrós *et al.* (2019), the high and low levels of plant essential oils depend on the intensity of stress, the length of the stress, and the selection of superior plant seeds.

According to the results of research by Damalas *et al.* (2022) weed interference in the form of *Chenopodium album* and *Sorghum halepense* against *Lupinus albus* L. causes a decrease in fresh weight, dry weight, seed yield, and productivity. The results of research by Kilkoda *et al.* (2015) the presence of the weed *Ageratum conyzoides* caused a decrease in growth in three varieties of *Glycine max* L. Merr. According to the results of research by Mahanta *et al.* (2007), the presence of several weed species around patchouli plants can reduce the quality of essential oils.

Based on literature search, it shows that weed interference can affect the growth of cultivated plants, but more information is still needed about the impact of *A. conyzoides* interference which is patchouli plantation weed on *P. cablin* growth and productivity. Based on this background, it is necessary to conduct research on the physiological response of *P. cablin* due to the interference of *A. conyzoides* to the content of antioxidants, osmoregulators, and essential oil yield from *P. cablin*.

RESEARCH METHOD

Time and Place

This research was conducted from July 2021 to May 2022. Planting is carried out in Perum Jatisari BSB, Mijen District, Semarang City. Analysis of the proline, total phenol, and patchouli oil yield was carried out at the Plant Structure and Function Biology Laboratory, Department of Biology, FSM, Diponegoro University.

Tools and Materials

The main tools needed are a spectrophotometer UV-VIS (Shimadzu UVMMini-1240), analytical balance (Ohaus AR2140), vortex (Thermo Scientific 88882010), water bath (Polyscience WBE10), Whatman Filter Paper No. 42, and steam distillation circuit. The materials used in this research are shoot cuttings of two-month-old *Pogostemon cablin* cv. Sidikalang, seeds of *Ageratum conyzoides*, planting medium with composition soil:manure: husks (2:1:1).

Experimental Design

The experimental design used a Complete Randomized Design (CRD) with a single factor of *A. conyzoides* interference levels with four treatments (0, 2, 4, 6 individual *A. conyzoides* per treatment). Each level of treatment was carried out five repetitions.

Planting and Treatment

The research was conducted in a place shaded by rain. *A. conyzoides* seeds are selected from broodstock on plantations that has already undergone seed ripening characterized by seeds of black color. Seeds

are seeded in prepared fields and selected uniform seedlings are for treatment. Selection of *A. conyzoides* seedlings based on the number of leaves as well as the uniform length of the stem. *P. cablin* seedlings are cuttings of shoots from 1-year-old broodstock with eight leaves and a stem length of 30 cm.

Planting is carried out in polybags of 30 x 30 cm with a planting media weight of 3.5 kg. Planting treatment begins when the age of *P. cablin* is already two months. Planting is adjusted to the level of *A. conyzoides* interference in each polybag by providing a planting distance of 10 cm between *A. conyzoides* and *P. cablin* in the middle of the polybag. Watering is carried out as much as 1 liter in the morning per polybag. Weeding is carried out when non-target weeds are growing on the treatment polybags. *P. cablin* harvesting is carried out at 60 day after planting (DAP) which is characterized by the yellowing of the underside of *P. cablin* leaves. Leaf samples were used for phenol content analysis, and proline was taken at 60 DAP at harvest time. Manguntungi *et al.* (2017) stated that leaves numbers 4 to 6 undergo physiological maturation and also contain maximum secondary metabolites.

Total Phenol

Measurement of total phenols based on the method of Singleton *et al.*, (1999). A total of 0.5 g of leaves were mashed and homogenized with 5 ml of methanol and then filtered using filter paper. A total of 0.5 ml of solution was taken then added with 2.5 ml of 10% Folin Ciocalteu reagent and 10% Na₂CO₃ was

added and homogenized using a vortex for 15 second. The solution was incubated at 40°C for an hour, then the absorbance was read with a spectrophotometer at a wavelength of 760 nm. The total concentration of phenol is based on a standard curve.

Total Proline

Measurement of proline content based on the method of Bates *et al.* (1973). A total of 0.5 gr of leaves were ground and extracted in 10 ml of 3% sulphosalic acid (w/v) then filtered with filter paper. A total of 2 ml of filtrate was reacted with 2 ml of ninhydrin acid solution and 2 ml of glacial acetic acid in a test tube for one hour at 100°C and the reaction process ended with an ice bath. The solution is further extracted with 4 ml of toluene and shaken vigorously with a stirrer test tube for 15 to 20 seconds. The absorbance is read with a spectrophotometer at a wavelength of 520 nm. The concentration of proline is determined by a standard curve of pure proline.

Patchouli Oil Yield

Measurement of the yield of *P. cablin* patchouli oil based on the method of Abdjul *et al.*, (2013) with modifications. Using one whole plant treatment with fresh conditions harvested in the morning. Then it is put into a steam distillation device and heated at a temperature of 100°C for 5 hours until a distillate is obtained. Distillates containing oil and water are put into a split funnel to separate the oil and water, and then MgSO₄ is added to drain the oil from the water. The percentage yield of essential oils is calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Volume of oil produced (ml)}}{\text{Ingredients before processing (G)}}$$

Data Analysis

The quantitative data obtained from this research were analyzed using variance analysis (ANOVA) to determine the effect of treatment on treatment as well as regression analysis. Using Duncan's Multiple Range Test (DMRT) to determine the significant difference between treatments at a confidence level of 95%.

RESULT AND DISCUSSION

The results of the analysis of variance (ANOVA) showed that the interference of *A. conyzoides* weeds affected the total phenol and total proline in *P. cablin* leaves. The results of observations and regression analysis of the total content of phenol and proline can be seen in Table 1, Figure 1, and Figure 2.

Based on the results in Table 1, it shows that the highest total phenol in *P. cablin* leaves in the interference treatment of six *A. conyzoides* individuals (4.000 mg/g) and the lowest total phenol in the treatment without *A. conyzoides* individuals (1.614 mg/g). Figure 1 shows the regression between *A. conyzoides* and *P. cablin* interference is positive with $R^2 = 0.834$, the more individuals *A. conyzoides* causes the higher of total phenol content in *P. cablin* leaves.

The results of research by Singh (2004) on chickpeas, mustard, and rice plant planted on soil containing *A. conyzoides* and residues from *A. conyzoides* plants can increase total phenol in plants. Darmanti (2015) the greater the amount of interference weed with soybean plants, the total phenol content in the leaves of soybean plants increases.

Table 1 Antioxidant Content In The Form of Total Phenol and Proline *P. cablin* (60 DAP)

Individual Count of <i>A. conyzoides</i>	Total Phenol (mg/g)	Total Proline (µg/ml)
0	1.614 ± 0.385 ^a	0.086 ± 0.077 ^a
2	2.288 ± 0.015 ^b	0.150 ± 0.049 ^{ab}
4	2.321 ± 0.012 ^b	0.201 ± 0.011 ^{ab}
6	4.000 ± 0.486 ^c	0.264 ± 0.091 ^b

Numbers followed by different letters on the same parameter showed a marked difference with Duncan's Double Test at a rate of 95%. The data shown is an average ± standard deviation.

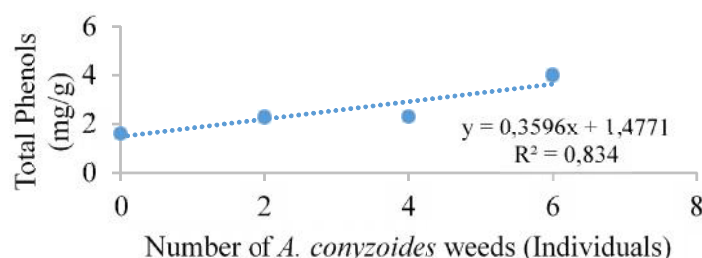


Figure 1 Regression analysis of total phenol *P. cablin* due to *A. conyzoides* weed interference

Naikoo *et al.* (2019) state that phenolic compounds are secondary metabolites that will increase if plants get stressed. Phenolic compounds according to Rodriguez *et al.* (2014) are one of the largest groups of compounds that have a role as natural antioxidants in plants and are synthesized through the shikimic acid pathway. The shikimic acid pathway in plants occurs in chloroplasts and produces L-phenylalanine (L-Phe) which is used for the biosynthesis of various secondary metabolites (Santos-Sánchez *et al.*, 2019).

Phenolics as natural antioxidants in plants have a very important role when plants experience depression. In these conditions, phenol plays a role in neutralizing the occurrence of oxidation reactions caused by excessive ROS due to the high intensity of stress experienced by plants (Parwata, 2015). According to Sharma *et al.* (2012), the way phenolic compounds neutralize ROS in plants is by donating electrons or hydrogen atoms to bind ROS. Phenolic compounds in plants accumulate in the central vacuoles of the cover cells of the stoma and epidermal cells as well as in the subepidermal tissues located in the leaves (Pratyusha, 2022).

Figure 2 shows the regression between *A. conyzoides* and *P. cablin* interference is positive with $R^2 = 0.998$, the more individuals *A. conyzoides* causes the higher of total proline content in *P. cablin* leaves. The results of research by Kumar *et al.* (2018) alleochemicals derived from leaching of *A. conyzoides* cause increased proline content in *Pisum sativum*. The results of the observation of total proline in this research can be seen in Table 1.

Proline is one of the amino acids produced by plants and accumulates in chloroplasts in both choked and non-choked conditions (Kishor *et al.*, 2015). Proline synthesis in plants through two pathways, namely the glutamic acid pathway as the main pathway and also through the ornithine pathway (Liang, 2013). According to Hayat *et al.* (2012) proline in plants in addition to being a good osmoregulator also plays three main roles during plant contamination, namely metal thickening, as an antioxidant, and signal molecules.

According to Dar *et al.* (2015) proline acts as an antioxidant, namely by accumulating proline in large quantities which serves to stabilize ROS when plants are exposed to depression. This is to Rana (2017) opinion

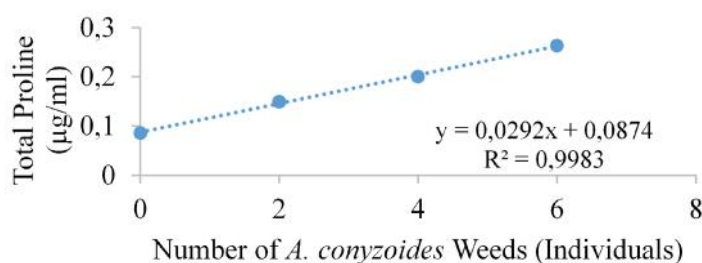


Figure 2 Regression analysis and total regression of *P. cablin* proline due to *A. conyzoides* weed interference

that there is a positive correlation between the accumulation of proline in plants and plant stress or stress in plants. *A. conyzoides* interference causes high total phenol and proline in *P. cablin* leaves which indicates that *P. cablin* is in a choked state so that it can cause inhibition of *P. cablin* growth.

According to Czarnocka *et al.* (2018), phenol and proline are non-enzymatic antioxidants that function to prevent, reduce, or eliminate damage due to high concentrations of ROS in various tissues and plant cells due to stress. Superoxide (O_2^-), hydroxyl radical (OH), hydrogen peroxide (H_2O_2), and singlet oxygen (1O_2) is ROS commonly found when plants are in a choked condition (Mittler, 2017).

The results of research by Afifi *et al.* (2012) showed that rye grass interference caused a high accumulation of H_2O_2 in corn seeds compared to corn seeds that did not experience rye grass interference. The results of research by Kilkoda *et al.* (2015) interference of *A. conyzoides* and *Borerria alata* caused a decrease in growth in three soybean varieties. According to Mukti *et al.* (2020) and Yamawo (2015) weed interference causes competition for resources, namely nutrients, light, water, and growing space as well as interference from weed allelochemicals

so as to increase antioxidants and inhibit the growth of other plants. This is in accordance with the results of research by Fernandez *et al.* (2016) *Quercus pubescens* that experience competition and are exposed to allelochemicals derived from pine leaf juice will experience an increase in phenol and terpene content and growth inhibition including height and biomass of *Q. pubescens*.

The allelochemical *A. conyzoides* causes oxidative stress which can increase the amount of ROS production and increase the concentration of phenol and proline as antioxidants. According to Negi *et al.* (2020), the allelochemical *A. conyzoides* can increase ROS production in the form of superoxide compounds in some rice varieties. High accumulation of ROS in the form of superoxide can increase the activity of the PAL enzyme (Darmanti *et al.*, 2021). The PAL enzyme serves to deflect primary metabolic pathways to secondary metabolic pathways (Zhang *et al.*, 2015).

Based on the ANOVA results in Table 2 showed that *A. conyzoides* weed interference affected the percentage of *P. cablin* patchouli oil yield. The highest percentage of patchouli oil yield *P. cablin* in the interference treatment without *A. conyzoides* individuals

Table 2 Percentage of Patchouli Oil Yield *P. cablin* (60 DAP)

Individual Count Of <i>A. conyzoides</i>	Patchouli Oil Yield (%)
0	1.614 ± 0.111 ^d
2	1.430 ± 0.061 ^c
4	1.247 ± 0.026 ^b
6	0.879 ± 0.086 ^a

Numbers followed by different letters on the same parameter showed a marked difference with Duncan's Double Test at a rate of 95%. The data shown is an average ± standard deviation.

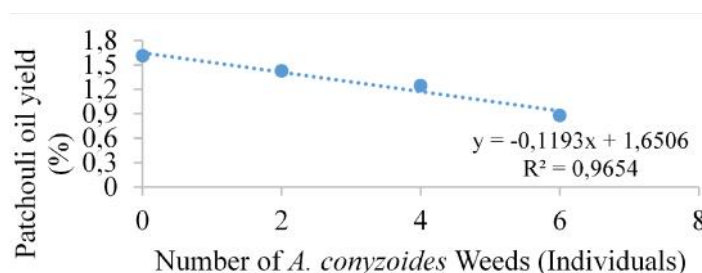


Figure 3 Regression analysis of patchouli cultivar Sidikalang yield due to *A. conyzoides* weed interference

(1.614%) and the lowest percentage of patchouli oil yield in the treatment of six *A. conyzoides* individuals (0.879%). Figure 3 shows the regression between *A. conyzoides* and *P. cablin* interference in negative with $R^2 = 0.965$, the more individuals *A. conyzoides* causes the smaller percentage of *P. cablin* patchouli oil yield. This is by the results of research by Mahanta *et al.* (2007), namely the tighter some types of weeds in patchouli plantations will reduce the yield of essential oil and also the quality of *P. cablin* essential oil. In *P. cablin* patchouli oil, many sesquiterpenes are found which are derivatives of the terpene group with the main compound constituting patchouli oil being patchouli alcohol (Beutel *et al.*, 2020).

According to Aisyah *et al.* (2010) the composition of essential oils in *P. cablin* leaves is patchouli alcohol (32.60%), δ -guaiene (23.07%), α -guaiene (32.60%), seychellene (6.95%), α -patchoulene (5.47%). Souhoka *et al.* (2020) identified the composition of essential oils in *P. cablin* leaves, namely patchouli alcohol (22.7%), δ -guaiene (18.61%), α -guaiene (18.90%), seychellene (5.70%), α -patchoulene (2.88%). According to Lee *et al.* (2020) patchouli alcohol is a sesquiterpene the main component

of patchouli essential oil and is an antioxidant. The α -guaiene compound is one of the constituent compounds of patchouli oil whose percentage is almost the same as patchouli alcohol. The compound α -guaiene is an antioxidant for plants affected by the stress and is derived from the biosynthesis of the mevalonate acid pathway (Kurosaki *et al.*, 2016; Widyasanti *et al.*, 2021). This is in line with Swamy *et al.* (2015) that the phytochemical content of *P. cablin* including patchouli alcohol, patchoulene, guaene, seychellene, eugenol, and pogostol is an antioxidant in *P. cablin*.

According to Beek *et al.* (2018) patchouli oil is the main compound of patchouli oil from the results of secondary metabolites and comes from the terpenoid group and is synthesized through the mevalonate acid metabolism pathway. The synthesis of patchouli oil is strongly influenced by the stress experienced by plants (Setiawan *et al.*, 2021).

Patchouli oil is a secondary metabolite derived from the deflection of the primary metabolite by the enzyme phenylalanine ammonia-lyase (PAL) caused by an increase in ROS along with an increase in stress that spurs a plant to produce more secondary metabolites (Zhang *et al.*, 2015).

According to Agami (2016), a drastic decrease in fresh weight in plants can cause the accumulation of patchouli oil to also be low. This is to the results of research by Abdoune *et al.* (2013) there is a correlation between the decrease in the weight of some types of essential oil-producing plants and the decrease in the content of essential oils.

The results of research by Abdi *et al.* (2019) on *Ocimum basilicum* and *Ocimum americanum* which experienced a decrease in vegetative growth due to the decreased chlorophyll content caused the content of essential oils to also decrease. The results of research by Gorgini *et al.* (2021) on *Lavandula angustifolia* cv. Hidecot and *Lavandula angustifolia* cv. Muneasted which experienced a decrease in plant biomass also saw a decrease in the percentage of essential oils followed by an increase in the content of phenol and proline in *L. angustifolia* cv. Hidecot and *L. angustifolia* cv. Muneasted.

According to Rao *et al.* (2007), weed interference can decrease the essential oil content in citronella cv. Java-II and essential oils on a lemon. According to Moelyaandani *et al.* (2020), weed interference can disrupt water supply levels. Disruption of water supply levels caused *P. cablin* to experience drought stress characterized by high proline content. According to Khalid (2006), the low level of water availability causes disruption of the water absorption process by plants, causing smaller and fewer leaves and can reduce the capacity of essential oils in plants.

According to Nasruddin *et al.* (2019), the decrease in essential oil content can be

caused by disturbances in the photosynthesis process due to lower CO₂ assimilation levels and affects terpene production. Photosynthesis is disturbed due to weed allelopathy which inhibits the absorption of nutrients, especially N elements (Puspitasari *et al.*, 2013).

The release of allelochemicals from the body of *A. conyzoides* can be done using root exudate, evaporation, washing, and also the decay of dead parts of the body of plants (Schandry *et al.*, 2020). According to Kumar *et al.* (2018) *A. conyzoides* releases various types of allelochemicals harmful to other plants such as flavonoids, keloidids, saponins, tannins, anthraquinones, gallic acid, ferulic acid, quamaric acid, hydroxy-benzoic acid, anisic acid, and syringic acid. Research conducted by Isda (2016) allelochemical compounds in the form of flavonoids in *A. conyzoides* can interfere with the growth of other plants by damaging spindle threads during metaphase and also interfere with the activity of cytokinin hormones that play a role in cell division.

The allelochemical of *A. conyzoides* causes inhibition of *P. cablin* growth due to the degree of *A. conyzoides* interference, the length of interference, and also the characteristics of *A. conyzoides* itself. This is to the results of research by Budi (2012) which states that the size of the interference or stress will depend on the type of weed, the intensity of the weed, and the length of competition time between weeds and other plants.

CONCLUSIONS

The interference of several *A. conyzoides* has an impact on the total phenol and total proline content in *P. cablin* leaves as well as the percentage of *P. cablin* patchouli oil yield. The increasing individuals of *A. conyzoides* per treatment causes an increase in the total content of phenol and proline, this is because phenol and proline are antioxidants in *P. cablin* that function to survive the stress of *A. conyzoides*. The increasing individuals of *A. conyzoides* per treatment causes a decrease in the percentage of *P. cablin* essential oil, this is because the increase in antioxidants which is a secondary metabolism causes a decrease in the growth of *P. cablin* so that the content of essential oils also decrease.

ACKNOWLEDGEMENT

The authors of this research would like to thank the Blado District Agricultural Extension Center for helping with the availability of seeds *P. cablin* cv. Sidikalang, and Plant Structure and Function Biology Laboratory of the Department of Biology, FSM, Diponegoro University, which has allowed it to conduct plant analysis.

REFERENCES

- Abdi, G., Shokrpour, M., & Salami, S. A. 2019. *Essential Oil Composition at Different Plant Growth Development of Peppermint (Mentha x piperita L.) Under Water Deficit Stress*. Journal of Essential Oil-Bearing Plants. <https://doi.org/10.1080/0972060X.2019.1581095>
- Abdjul, N., Paputungan, M., Duengo, S. 2013. *Analisis Komponen Kimia Minyak Atsiri Pada Tanaman Nilam Hasil Distilasi Uap Air Dengan Menggunakan Kg-Sm*. MIPAJurusan Pendidikan Kimia Universitas Negeri Gorontalo.
- Abdoune, M. A., Khadir, A., Bendahou, M., Belkaid, A. B. 2013. *Drying Effect on Yield and Antimicrobial Activity of Essential Oils*. International Journal Medicine Aromatic Plants. 3 (1): 93–101. <https://www.researchgate.net/publication/235986594>
- Afifi, M., & Swanton, C. 2012. *Early Physiological Mechanisms of Weed Competition*. Weed Science, 60(4), 542–551. <https://doi.org/10.1614/ws-d-12-00013.1>
- Agami, R. A., Medani, R. A., El-Mola, I. A. Abd., Taha, R. S. 2016. *Exogenous Application With Plant Growth Promoting Rhizobacteria (PGPR) Or Proline Induces Stress Tolerance In Basil Plants (Ocimum basilicum L.) Exposed To Water Stress*. International Journal of Enviromental & Agriculture Research (IJOEAR). 2 (5): 78–92.
- Aisyah, Y., Hastuti, P., Sastrohamidjojo, H., Hidayat, C. 2010. *Increase of the Content of Patchouli Alcohol in Patchouli Oil (Pogostemon cablin Benth) Using Cellulose Acetate Membrane*. AGRITECH. 30 (3): 184-191.
- Badan Pusat Statistik. 2013. *Statistik Indonesia 2013*.
- Bates, L. S., Waldresn, R. P., Teare, I. D. 1973. *Rapid Determination of Free Proline For Water-Stress Studies*. Plant Soil. 39: 205–207.

- Beek, T. A. van, & Joulain, D. 2018. *The essential oil of patchouli, Pogostemon cablin: A review*. Flavour and Fragrance Journal. 33 (1): 6–51. <https://doi.org/10.1002/ffj.3418>
- Beutel, S., Aguilar, F., Ekramzadeh, K., Scheper, T. 2020. *Whole-cell Production of Patchouli Oil Sesquiterpenes in Escherichia coli: Metabolic Engineering and Fermentation Optimization in Solid-Liquid Phase Partitioning Cultivation*. ACS Omega. 5 (50): 32436–32446. <https://doi.org/10.1021/acsomega.0c04590>
- Budi, G. P. 2012. *Kompetisi Gulma dengan Tanaman Budidaya Dalam Sistem Pertanaman Multiple Cropping*. Jurnal Online Universitas Muhammadiyah Purwokerto: 29–35.
- Czarnocka, W., & Karpiński, S. 2018. Friend or Foe? *Reactive Oxygen Species Production, Scavenging and Signaling in Plant Response to Environmental Stresses*. Free Radical Biology and Medicine. 122: 4–20. Elsevier Inc. <https://doi.org/10.1016/j.freeradbiomed.2018.01.011>
- Damalas, C. A., Koutroubas, S. D. 2022. *Weed Competition Effects on Growth and Yield of Spring-Sown White Lupine*. Horticulturae. 8 (5). <https://doi.org/10.3390/horticulturae8050430>
- Dar, M. I., Naikoo, M. I., Rehman, F., Naushin, F., Khan, F. A. 2015. Proline Accumulation in Plants: Roles in Stress Tolerance and Plant Development. Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies. 155-66. Springer India. https://doi.org/10.1007/978-81-322-2616-1_9
- Darmanti, S., Hastuti, E. D., & Suedy, S. W. A. 2021. Exogenous Hydrogen Peroxide Induces An Antioxidative Defense System Against Drought Stress In Soybean [*Glycine max* (L.) Merr.] Crops. Journal of Animal and Plant Sciences. 31 (1): 213–220. <https://doi.org/10.36899/JAPS.2021.1.0208>
- Darmanti, Sri. 2015. *Toleransi Kedelai [Glycine max (L.) Merr. cv. Grobogan] Terhadap Interferensi Teki (Cyperus rotundus L.) Pada Kondisi Cekaman Kekeringan*. Dissertation of Biological Sciences Study Program Gadjah Mada University.
- Direktorat Jenderal Perkebunan. 2017. *Statistik Perkebunan Indonesia Komoditas Nilam*. <http://ditjenbun.pertanian.go.id>
- Ejaz, Ahmad Waraich, Ahmad, R., & Ashraf, M. Y. 2011. *Role of Mineral Nutrition in Alleviation of Drought Stress in Plants*. AJCS, 5(6), 764–777.
- Fernandez, C., Monnier, Y., Santonja, M., Gallet, C., Weston, L. A., Prévosto, B., Saunier, A., Baldy, V., & Bousquet-Mélou, A. 2016. *The Impact of Competition and Allelopathy on the Trade-off between Plant Defense and Growth in Two Contrasting Tree Species*. Frontiers in Plant Science, 7. <https://doi.org/10.3389/fpls.2016.00594>
- García-Caparrós, P., Romero, M. J., Llanderal, A., Cermeño, P., Lao, M. T., & Segura, M. L. 2019. *Effects of Drought Stress on Biomass, Essential Oil Content, Nutritional Parameters, and Costs of Production in Six Lamiaceae Species*. Water (Switzerland). 11 (3):

- 1-12. <https://doi.org/10.3390/w11030573>
- Gorgini S.H., Khorasaninejad, S., Soltanloo, H., Shariati, V. 2021. *Physiological Response and Secondary Metabolites of Three lavender Genotypes under Water deficit*. Scientific Reports. 11(1). <https://doi.org/10.1038/s41598-021-98750-x>
- Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J., Ahmad, A. 2012. *Role of Proline under Changing Environments: A Review*. Plant Signaling and Behavior. 7 (11). <https://doi.org/10.4161/psb.21949>
- Isda, Mayta N., Fatonah S., Fitri. R. 2016. *Potensi Ekstrak Daun Gulma Babadotan (Ageratum conyzoides L.) terhadap Perkecambahan dan Pertumbuhan Paspalum conjugatum Berg.* Al-Kauniah Jurnal Biologi. 6 (2): 120–125. <https://doi.org/10.15408/kauniah.v6i2.2752>
- Kasmiyati, S., & Sucahyo. 2014. *Detection of Oxidative Stress Due to Chromium Toxicity on Sonchus oleraceus L.* By Spectrophotometrically and Histochemical Determination of Reactive Oxygen Species. 26 (1): 85–98.
- Khalid, K. Ali. 2006. *Influence of Water Stress on Growth, Essential Oil, and Chemical Composition of Herbs (Ocimum sp.)*. International Agrophysics. <https://www.researchgate.net/publication/26551760>
- Kilkoda, A. K., Nurmala, T., Widayat, D. 2015. Pengaruh Keberadaan Gulma (*Ageratum conyzoides* dan *Boreria alata*) Terhadap Pertumbuhan dan Hasil Tiga Ukuran Varietas Kedelai (*Glycine max* L. Merr) Pada Percobaan Pot Bertingkat. Kultivasi. 14 (2): 1-9. <https://doi.org/10.24198/kltv.v14i2.12072>
- Kishor, P. B. K., Hima Kumari, P., Sunita, M. S. L., Sreenivasulu, N. 2015. *Role of Proline in Cell Wall Synthesis and Plant Development and Its Implications in Plant Ontogeny*. Frontiers in Plant Science. 6: 1-17. Frontiers Research Foundation. <https://doi.org/10.3389/fpls.2015.00544>
- Kumar, N., Kumar, K., Asma, Kumar, A. 2018. *Allelopathic Potential Of Ageratum conyzoides L. On Growth and Development of Pisum sativum L.* International Journal of Current Research. 10 (7): 71659–71663. <https://doi.org/10.24941/ijcr.31421.07.2018>
- Kurosaki, F., Kato, T., Misawa, N., Taura, F. 2016. *Efficient Production of δ -Guaiene, an Aroma Sesquiterpene Compound Accumulated in Agarwood, by Mevalonate Pathway-Engineered Escherichia coli Cells*. Advances in Bioscience and Biotechnology. 07 (11): 435–445. <https://doi.org/10.4236/abb.2016.711042>
- Lee, H. S., Lee, J., Smolensky, D., Lee, S. H. 2020. *Potential Benefits of Patchouli Alcohol in Prevention of Human Diseases: A Mechanistic Review*. International Immuno-pharmacology. 89. Elsevier B.V. <https://doi.org/10.1016/j.intimp.2020.107056>
- Liang, X., Zhang, L., Natarajan, Sathish K., Becker, Donald F. 2013. *Proline Mechanisms of Stress Survival*. Antioxidants & Redox Signaling. 19 (9): 998-1011.

- Mahanta, J. J., Chutia, M., & Sarma, T. C. 2007. *Study on Weed Flora and Their Influence on Patchouli (Pogostemon cablin Benth.) Oil and Patchoulol*. Journal of Plant Sciences. 2 (1): 96-101.
- Manguntungi, B., Kusuma, A. B., Yulianti, Asmawati, Yunianti. 2017. *Pengaruh Kombinasi Ekstrak Kirinyuh (Chromolaena odorata) dan Sirih (Piper betle L.) dalam Pengendalian Penyakit Vibriosis pada Udang*. Journal of Biota. 1 (3). <https://doi.org/10.24002/biota.v1i3.1231>
- Mittler, R. 2017. *ROS Are Good*. Trends in Plant Science. 22 (1): 11–19. Elsevier Ltd. <https://doi.org/10.1016/j.tplants.2016.08.002>
- Moelyaandani, D. Q., & Setiyono, S. 2020. *Kompetisi Beberapa Jenis Gulma Terhadap Pertumbuhan Awal Beberapa Varietas Tanaman Tebu (Saccharum officinarum L.)*. Jurnal Proteksi Tanaman Tropis. 1 (1): 21. <https://doi.org/10.19184/jptt.v1i1.15585>
- Mukti Prabowo, S., Annisa Dewi, S., Hatmini, D. 2020. *Identification of Local Seeds Bank Weed and The Effect of Weeding Frequency on Growth and Results Of Cayenne (Capsicum frutescens)*. Agric. 32 (2): 121–128. <https://doi.org/https://doi.org/10.24246/agric.2020.v32i2.p121-128>
- Naikoo, M. I., Dar, M. I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., Khan, F. A., Naushin, F. 2019. *Role and Regulation of Plants Phenolics in Abiotic Stress Tolerance: An Overview*. Plant Signaling Molecules: Role and Regulation under Stressful Environments. 157–168). Elsevier. <https://doi.org/10.1016/B978-0-12-816451-8.00009-5>
- Nasrin, S., Saha, S., Begum, H. H., Samad, R. 2020. *Impacts of Drought Stress on Growth, Protein, Proline, Pigment Content and Antioxidant Enzyme Activities in Rice (Oryza sativa L. var. BRRI dhan-24)*. Dhaka University Journal of Biological Sciences. 29 (1): 117-123. <https://doi.org/10.3329/dujbs.v29i1.46537>
- Nasruddin, N., Harahap, E. M., Hanum, C., Siregar, L. A. 2019. *The Level of Water Supply and its Effect on the Growth of Plants and Yields Patchouli [Pogostemon cablin (Blanco) Benth.]*. <https://doi.org/10.4108/eai.20-1-2018.2282082>
- Negi, B., Bargali, S. S., Bargali, K., & Khatri, K. 2020. *Allelopathic Interference of Ageratum conyzoides L. against Rice Varieties*. Current Agriculture Research Journal. 69-76. <https://doi.org/10.12944/carj.8.2.01>
- Parwata, I Made Oka A. 2015. *Bahan Ajar Uji Bioaktivitas (Antioksidan)*. Universitas Udayana (pp. 1–51).
- Patel, R. I., Saras, P. K., Patel, C. K., Patel, N. v, Rabari, K. v. 2017. *Influence of Nutrient, Weed and Pest Management Practices on Performance of Mungbean (Vigna radiata L.)*. 6 (4): 2622-2630.
- Pratyusha, S. 2022. *Phenolic Compounds in the Plant Development and Defense: An Overview*. Intech Open. 1–17. <https://doi.org/https://doi.org/10.5772/intechopen.102873>
- Puspitasari, K., Sebayang, H. T., & Guritno, B. 2013. *Pengaruh Aplikasi Herbisida Ametrin Dan 2, 4-D Dalam Mengendalikan Gulma Tanaman Tebu (Saccharum officinarum L.)* The

- Effect of Herbicide Ametrin and 2, 4-D Application in Weed Control of Sugar Cane (Saccharum Officinarum L.). Jurnal Produksi Tanaman.* 1 (2): 72-80.
- Rana, V., Ram, S., Nehra, K. 2017. *Review Proline Biosynthesis and its Role in Abiotic Stress.* International Journal of Agriculture Innovation and Research. 6 (3): 473-478.
- Rao, B. R. R., Rajput, D. K., Sastry, K. P., Bhattacharya, A. K., Patel, R. P., & Ramesh, S. 2007. *Effect of Crop-Weed Mixed Distillation on Essential Oil Yield and Composition of Five Aromatic Crops.* Journal of Essential Oil-Bearing Plants. 10 (2): 127-132. <https://doi.org/10.1080/0972060X.2007.10643530>
- Rodriguez, A., Martínez, J. A., Flores, N., Escalante, A., Gosset, G., Bolivar, F. 2014. *Engineering Escherichia coli to overproduce aromatic amino acids and derived compounds.* Microbial Cell Factories. 13 (1): 1-15. <https://doi.org/10.1186/s12934-014-0126-z>
- Rosmanah, S., Kusnadi, H., Harta, L. 2017. *Identifikasi dan Dominansi Gulma Pada Lahan Kering Dataran Tinggi Di Kabupaten Kepahiang Provinsi Bengkulu.* Page 35 – 41. In Prosiding Seminar Nasional Agroinovasi Spesifik Lokasi Untuk Ketahanan Pangan Pada Era Masyarakat Ekonomi ASEAN.
- Santos-Sánchez, N. F., Salas-Coronado, R., Hernández-Carlos, B., & Villanueva-Cañongo, C. 2019. *Shikimic Acid Pathway in Biosynthesis of Phenolic Compounds.* Intech Open. 1-15. <https://doi.org/https://doi.org/10.5772/intechopen>
- Schandry, N., Becker, C. 2020. *Allelopathic Plants: Models for Studying Plant-Interkingdom Interactions.* Trends in Plant Science. 25 (2): 176-185. <https://doi.org/10.1016/j.tplants.2019.11.004>
- Setiawan, A., Armita, D., Rahayu, A. P., Barunawati, N. 2021. *Land Suitability Assessment For Patchouli (Pogostemon cablin) Development And Essential Oil Production.* Biotropia. 28 (1): 74-83. <https://doi.org/10.11598/btb.2021.28.1.1092>
- Sharma, P., Jha, A. B., Dubey, R. S., Pessarakli, M. 2012. *Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanisme in Plants under Stressful Conditions.* Hindawi Publishing Corporation Journal of Botany. 2012. 1-26. <https://doi.org/10.1155/2012/217037>
- Singh, Harminder P., Daizy R. Batish, Shalinder Kaur, Ravinder K. Kohli, Kuldeep S. Dogra. 2004. *Alleopathic Interference of Ageratum conyzoides L. Against Some Crop Plants.* Fourteenth Australian Weeds Conference. 558-561.
- Singleton, V. L., Orthofer, R., M. Rosa, Lamuela-Raventos. 1999. *Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent.* Academic Press. 299: 152-178.
- Souhoka, F. A., al Aziz, A. Z., Nazudin, N. 2020. *Patchouli Oil Isolation and Identification of Chemical Components Using GC-MS.* Indo. J. Chem. Res. 8 (2):108-113. <https://doi.org/10.30598/ijcr.2020.8-fas>

- Swamy, M. K., Sinniah, U. R. 2015. *A Comprehensive Review On The Phytochemical Constituents And Pharmacological Activities Of Pogostemon cablin Benth.: An Aromatic Medicinal Plant Of Industrial Importance*. *Molecules*. 20 (5): 8521–8547. MDPI AG. <https://doi.org/10.3390/molecules20058521>
- Widyasanti, A., Nurjanah, S., Nurhadi, B., Osman, C. P. 2021. *Isolation of Guaiene from Crude and Distillate Patchouli Oil Extracted by Molecular Distillation*. *IOP Conference Series: Earth and Environmental Science*. 924 (1). <https://doi.org/10.1088/1755-1315/924/1/012008>
- Yamawo, A. 2015. *Relatedness of Neighboring Plants Alters the Expression of Indirect Defense Traits in an Extrafloral Nectary-Bearing Plant*. *Evolutionary Biology*. 42 (1): 12–19. <https://doi.org/10.1007/s11692-014-9295-2>
- Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z., Chen, S. 2021. *Response Mechanism of Plants To Drought Stress*. *Horticulturae*. 7 (3). <https://doi.org/10.3390/horticulturae7030050>
- Zhang, X., & Liu, C. J. 2015. *Multifaceted Regulations of Gateway Enzyme Phenylalanine Ammonia-Lyase In The Biosynthesis Of Phenylpropanoids*. *Molecular Plant*. 8 (1): 17–27. <https://doi.org/10.1016/j.molp.2014.11.001>
