Australian Journal of Crop Science AJCS

Aust J Crop Sci. 19(07):758-764 (2025) | https://doi.org/10.21475/ajcs.25.19.07.p34 ISSN:1835-2707

The effect of shading and vermicompost on the growth, yield, antioxidant activity, and flavonoid content of two *Medinilla* species

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Submitted: 16/05/2025

Revised: 09/07/2025

Accepted: 11/07/2025

Abstract: One of the medicinal plants with significant beneficial potential is *Medinilla*, locally known as *parijoto* in Java and *bunga trijata* in Bali. This plant is originally from the Philippines. *Medinilla* fruit is rich in bioactive compounds, including alkaloids, anthocyanins, antioxidants, flavonoids, glycosides, saponins, and tannins. This study aims to investigate the effects of cultivation techniques and microclimate conditions on the secondary metabolite content, especially antioxidants and flavonoids of *Medinilla* cultivated outside its natural habitat. This study utilized plant materials from two species, *Medinilla speciosa* and *Medinilla verrucosa*. The experiment employed a Nested Factorial Randomized Complete Block Design with three factors: *Medinilla* species, shading level, and vermicompost dose. This study also examined the effects of shading and vermicompost (kascing fertilizer) treatments on *Medinilla speciosa* and *Medinilla verrucosa* over a 12-month period. The results demonstrated that 75% shading significantly suppressed plant growth and flavonoid content, while enhancing antioxidant activity. *Medinilla verrucosa* exhibited more dominant adaptive responses to shading conditions. In contrast, vermicompost application did not improve growth, flavonoid levels, or antioxidant capacity in either species.

Keywords: antioxidant, flavonoid, *Medinilla*, shading, vermicompost.

Introduction

Indonesia is among the ten countries classified as having the highest biodiversity, known as megadiversity (Rasdianah Aziz et al., 2019). One of the medicinal plants with significant potential is *Medinilla*, locally known as *parijoto* in Java and *bunga trijata* in Bali. This plan is originally from the Philippines. Then, it spreaded to Indonesia, particularly in highland areas such as Gunung Muria, Kudus Regency, Central Java Province (Ananingsih et al., 2023; Maria et al., 2012). Morphologically, *Medinilla* grows to a height of approximately 1–2 meters, with leaves measuring 10–20 cm in length. Its fruits are round, reddish-purple, and have a slightly sour and astringent taste. The fruits typically ripen between March and May (Toni et al., 2022). In Indonesia, this plant has two main species: *Medinilla speciosa* and *Medinilla verrucosa*.

Medinilla is primarily consumed for its fruit, both in fresh and dried forms. According to indigenous knowledge of local communities in Gunung Muria, Central Java, *Medinilla* fruit is beneficial for reproductive health and is particularly recommended for pregnant women (Imaduddin et al., 2023). Scientific studies have confirmed the content of *Medinilla* fruit as it is rich in bioactive compounds, including alkaloids, anthocyanins, antioxidants, flavonoids, glycosides, saponins, and tannins (Sa'adah et al., 2019).

The increasing acceptance of traditional medicine, driven by cultural beliefs, scientific research, and a shift toward natural remedies, has enhanced public confidence in *Medinilla* as a viable health remedy. Due to its beneficial phytochemical properties, *Medinilla* has been widely processed into various consumer products, including beverages, snacks, and sweets. This variety facilitates the increasing consumption of this plant. It has also become a popular local souvenir product (Akhsanitaqwim et al., 2024).

Despite the high demand for fresh and processed *Medinilla* products, developing its agro-industry faces several challenges. One major limitation is the insufficient supply of *Medinilla* fruit, primarily due to the limited cultivation area in its native habitat in the Muria region. Additionally, the cultivation technique is not well-established. As a result, current cultivation efforts remain suboptimal and unable to meet the needs for continuous production. Consequently, producers are unable to maintain a steady supply due to irregular fruit availability (Radyanto et al., 2024). Other challenges are land-use conversion and climate change that might threaten the development of this medicinal plant and further impact its availability. Previous studies predict the number of threatened medicinal plant species will increase under future climate scenarios. Therefore, conservation efforts, particularly ex-situ cultivation, are highly needed to preserve these valuable plant resources (Cahyaningsih et al., 2021). Moreover, standardizing cultivation practices is crucial to addressing these challenges, while ensuring that the secondary metabolite content of cultivated *Medinilla* remains comparable to that of plants grown in their natural habitat in Muria.

To support successful cultivation outside its natural habitat, it is essential to understand how microclimatic factors affect growth, yield, and metabolite content. Since fruit quality is influenced by both genetic and environmental factors, factors like light intensity and nutrient availability play a crucial role in the plant's morphology, physiology, and biochemistry. Therefore, this study aims to investigate the effects of cultivation techniques and microclimate conditions on the secondary metabolite content, especially antioxidants and flavonoids of *Medinilla* cultivated outside its natural habitat. To achieve this, the study examines the cultivation of two *Medinilla* species, *Medinilla speciosa* and *Medinilla verrucosa*, utilizing shading level treatments and vermicompost application to manipulate microclimatic conditions at an elevation of 900 meters above sea level. Shading level treatments influence the surrounding environment by regulating light penetration, which in turn, affects temperature and humidity. The shading treatments are expected to enhance plant growth, fruit production, and the *Medinilla*'s antioxidant and flavonoid content. Meanwhile, vermicompost is a nutrient-rich organic fertilizer containing plant growth regulators that improve soil fertility and enhance plant growth.

Results and Discussion

Effects of shading and vermicompost fertilizer treatments on the growth of Medinilla species

In this study, the shading levels significantly influenced the morpholog of the plant such as height, leaf number, leaf area, root volume, fresh biomass weight, and dry biomass weight in both *Medinilla speciosa* and *Medinilla verrucosa. M. speciosa* with a 25% shading level treatment resulted in notable differences in these growth parameters (Table 2). Meanwhile, for *M. verrucosa*, the 50% shading level treatment had a more pronounced effect. Specifically, the former level treatment in *M. speciosa* exhibited specific changes, e.g., greater leaf number or larger leaf area, whereas the latter in *M. verrucosa* showed fewer leaves and smaller leaf area. These morphological variations suggest that the two species respond differently to shading, with *M. verrucosa* potentially being more adaptable to certain light conditions compared to *M. speciosa*.

Plants grown under low light intensity conditions exhibited suppressed biomass growth in the stems compared to those grown under optimal light conditions (Table 1). In response to this environmental stress, plants accumulate auxin hormones, which play a critical role in regulating growth. The activation of auxin under low light conditions often induces etiolation, a physiological response characterized by elongated, slender stems that are structurally fragile and prone to breakage, as well as a reduction in leaf number (Amissah et al., 2015).

As the primary organ capturing light, leaves play a crucial role in photosynthesis. Researchers suggest that a decline in assimilate production under low-light conditions negatively impacts leaf primordia development, thereby limiting overall plant growth (Feng et al., 2019). When the exposure to low light intensity is done in an extended period, the effect has been associated with accelerated leaf senescence, leading to premature aging in plants (Li et al., 2017). In response to such conditions, plants exhibit an adaptive mechanism by increasing leaf surface area, which facilitates greater light absorption to optimize the photosynthetic process. The limitations imposed by low light conditions also extend to carbohydrate metabolism, as reduced photosynthetic activity leads to lower carbohydrate accumulation.

Table 1. Effects of shading treatments on plant height and root volume.

Treatments	Plant height	Root volume
25% shading	167.00	134,44^{B}
50% shading	87.55	101,11^{AB}
75% shading	90.44	81,11^{A}

Note: Treatments labelled with the same letter indicate no significant differences at p ≤ 0.05.

Table 2. Effect of shading of shading treatments on the growth of *Medinilla* spesies.

Treatments	Leave number	Leave area	Fresh biomass weight	Dry biomass weight
25% shading *Medinilla speciosa*	87.5^{AB}	7220^{AB}	433^{A}	88.36^{A}
25% shading *Medinilla verrucosa*	113.3^{BC}	5108^{A}	496.55^{A}	83.34^{A}
50% shading *Medinilla speciosa*	48.7^{A}	17401^{B}	400.66^{A}	79.71^{A}
50% shading *Medinilla verrucosa*	147.2^{C}	5494^{A}	660^{A}	96.62^{A}
75% shading *Medinilla speciosa*	62.8^{A}	9227^{AB}	414.55^{A}	84.80^{A}
75% shading *Medinilla verrucosa*	108.8^{BC}	4512^{A}	582.44^{A}	96.21^{A}

Note: Treatments labelled with the same letter indicate no significant differences at p ≤ 0.05.

Similarly, the application of vermicompost doses nested within different shading level treatments demonstrated significant variations in dry biomass weight parameters for *Medinilla* (Table 3).

Table 3. Effects of shading and vermicompost fertilizer treatments on dry biomass weight.

Treatments	Dry biomass weight
25% shading vermicompost dose 5 ton/ha	73.69^{A}
25% shading vermicompost dose 7,5 ton/ha	105.88^{A}
25% shading vermicompost dose 10 ton/ha	77.98^{A}
50% shading vermicompost dose 5 ton/ha	93.20^{A}
50% shading vermicompost dose 7,5 ton/ha	63.91^{A}
50% shading vermicompost dose 10 ton/ha	107.38^{A}
75% shading vermicompost dose 5 ton/ha	71.66^{A}
75% shading vermicompost dose 7,5 ton/ha	115.53^{A}
75% shading vermicompost dose 10 ton/ha	84.32^{A}

Note: Treatments labelled with the same letter indicate no significant differences at $p \leq 0.05$.

Among the treatment combinations, the highest average growth parameters for dry biomass weight were observed in the combination of 7.5 tons/ha vermicompost dose and 75% shading level. In contrast, the lowest average growth parameters were recorded in the combination of 7.5 tons/ha vermicompost dose and the 50% shading level. These findings indicate that the application of 7.5 tons/ha of vermicompost represents the optimal dose of fertilizer under low light intensity conditions. Vermicompost supplies plants with essential nutrients, thereby facilitating efficient photosynthesis. Interestingly, these results are consistent with the findings of Kaundal and Kumar (2020) in their study on *Valeriana jatamansi*.

Effects of shading treatments on chlorophyll and flavonoid content

In addition to morphological changes, the shading levels also had a significant impact on chlorophyll content. In this study, marked increases in chlorophyll a, chlorophyll b, and total chlorophyll content were observed in the 75% shading level treatment (Table 4).

Table 4. Effects of shading treatments on chlorophyll and flavonoid content.

p	Ca	Cb	Ct	F
S1	0.47^{AB}	0.24^{A}	0.71^{A}	29.31^{B}
S2	0.45^{A}	0.21^{A}	0.66^{A}	5.67^{A}
S3	0.52^{B}	0.39^{B}	0.91^{B}	11.62^{A}

Note: S1 = 25% shading, S2 = 50% shading, S3 = 75% shading. The value indicates mean (n = 3 replicates). Ca= chlorophyll a, Cb = chlorophyll b, Ct = chlorophyll total, FW = fruit weight, F = flavonoid. Treatments labelled with the same letter indicate no significant differences at $p \leq 0.05$.

Chlorophyll, a fundamental pigment in photosynthesis, is crucial for absorbing and converting solar energy into biochemical energy (Fan et al., 2019). Under low-light conditions, plants tend to increase chlorophyll concentration to compensate for reduced light availability. In this way, the photosynthetic activity can be sustained (Mensah et al., 2022). This study supports previous findings that lower light intensity increases chlorophyll content, while excessive light exposure reduces it due to photodamage and degradation (Liu et al., 2018).

The shading levels also had a significant impact on flavonoid content. A marked increase in flavonoid content was observed in the 25% shading level treatment. This finding indicates that shading levels play a crucial role in modulating the production of secondary metabolites, such as flavonoids. It is similar to a previous study using a comparable shading level and vermicompost application model on Sonchus arvensis, a medicinal plant valued for its leaves. The study demonstrated that the highest flavonoid content was achieved under 75% shade and the application of 0.5 kg of vermicompost fertilizer per polybag, resulting in a 22% flavonoid content (Putri et al., 2018).

Effects of shading treatments on fruit weight

Another variable examined in this study is the shading's effect on the fruit weight. For *M. speciosa*, a marked increase in fruit weight was observed at the 25% shading level treatment, while for *M. verrucosa*, a marked increase in fruit weight was observed at the 50% shading. Figure 1 shows that the shading levels significantly influenced fruit weight in *Medinilla speciosa* and *Medinilla verrucosa*. This aligns with the findings of previous studies reporting that optimal light availability significantly impacts fruit maturation, size, and composition (Getachew et al., 2022).

Effects of shading and vermicompost fertilizer treatments on antioxidant content

The antioxidant of *M. speciosa* markedly increased, especially in the combination of 7.5 tons/ha vermicompost dose and 75% shading. Meanwhile, for *M. verrucosa*, a marked increase in antioxidant was observed in the combination of 5 tons/ha vermicompost dose and 50% shading. The details of these increases are provided in Fig. 2. These findings indicate that shading levels are crucial in modulating the production of secondary metabolites, such as antioxidants. Plants produce higher levels of reactive oxygen species (ROS) under low light intensity. This condition not only stimulates but also enhances the activity of antioxidant enzymes, including *Peroxidase, Catalase, Superoxide Dismutase*, and *Ascorbate Peroxidase*, which collectively play essential roles in the plant's antioxidant defense system (Zaman et al., 2022). Aligning with the previous

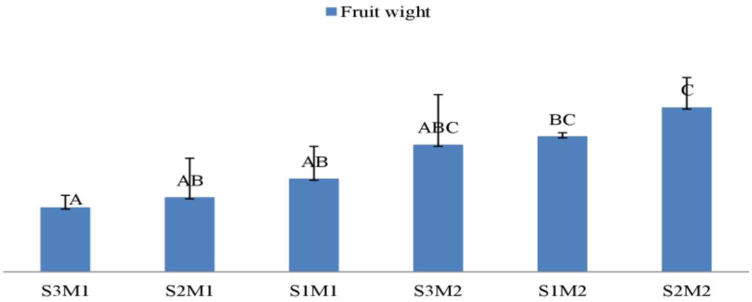

Fig. 1. Effects of shading treatments on the fruit weight. Note: S1 = 25% shading, S2 = 50% shading, S3 = 75% shading, M1 = *Medinilla speciosa*, M2 = *Medinilla verrucosa*. The value indicates mean (n = 3 replicates). Treatments labelled with the same letter indicate no significant differences at p ≤ 0.05.

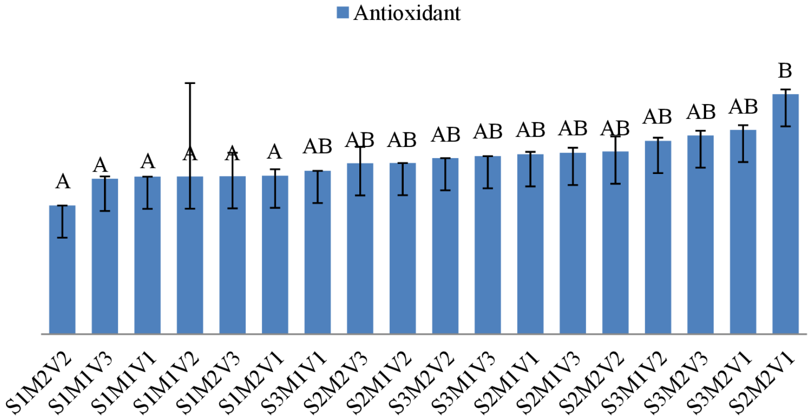

Fig. 2. Effects of shading and vermicompost fertilizer Treatments on the antioxidant content. Note: S1= 25% shading, S2= 50% shading, S3= 75% shading, M1= *Medinilla speciosa*, M2= *Medinilla verrucosa*, V1= vermicompost dose 5 ton/ha, V2= vermicompost dose 7.5 ton/ ha, V3= vermicompost dose 10 ton/ha. The value indicates mean (n = 3 replicates). Treatments labelled with the same letter indicate no significant differences at p ≤ 0.05.

studies, the findings of this study indicate that low light intensity induces oxidative stress in plants (Dong et al., 2014; Liu et al., 2019; Zhu et al., 2017).

Correlations and path analysis

Correlation analysis was employed to examine the relationship between two variables by estimating the strength of their association. As shown in Table 5, among all tested microclimate factors, only shading was found to be correlated with fruit weight. Specifically, 50% shading exhibited a moderate positive correlation with fruit weight (r = 0.64*), similar to 25% shading (*r* = 0.63*). Both correlation values fell within the moderate range (*r* = 0.40–0.69). In addition, path analysis was conducted to determine both the direct and indirect effects of microclimate factors on yield.

Table 5. Correlation and path analysis of microclimate factors with fruit weight, antioxidant content, and flavonoid levels.

Variabl	Paramete	Direct effect	Indirect effect					Correlation coefficient
			T	H	S1	S2	S3	
FW	T	0.04	-	-0.00	0.00	0.01	0.00	-0.15
	H	0.04	-0.00	-	0.00	0.00	0.00	-0.02
	S1	0.05	-0.03	-0.03	-	0.08	0.00	0.63*
	S2	0.32	-0.08	-0.07	0.08	-	0.01	0.64*
	S3	0.00	0.00	-0.00	0.00	0.00	-	0.24
F	T	0.10	-	-0.00	0.00	0.00	-0.00	0.07
	H	0.01	0.00	-	0.00	0.00	0.00	0.03
	S1	0.10	0.04	0.01	-	0.05	0.05	0.44
	S2	0.14	0.04	0.01	0.04	-	0.05	0.38
	S3	0.12	0	0	0	0	-	0
A	T	0.32	-	0.00	0.00	0.04	0.07	-0.30
	H	0.00	0.00	-	0.00	0.00	0.00	-0.15
	S1	0.00	0.00	0.00	-	0.00	0.00	0
	S2	0.07	0.02	0.00	0.00	-	0.01	-0.16
	S3	0.21	0.01	0.00	0.00	0.00	-	-0.06

Note: FW = fruit weight, A= antioxidant, F= flavonoid, T= temperature, H= humidity, S1=shading level 25%, S2= shading level 50%, S3= shading level 75%.

Table 5 shows that the microclimate parameter of 50% shading (0.32) had the strongest direct positive effect on fruit weight of *Medinilla*. Path analysis also revealed that the microclimate parameter with the strongest direct positive effect on antioxidant content was temperature (0.32), followed by 75% shading (0.21). Furthermore, the path analysis examining the association between microclimate parameters and flavonoid content revealed that the strongest direct positive effect on flavonoid content was observed under 50% shading (0.14), followed by 75% shading (0.12).

Materials and methods

Plant material

This study utilized plant materials from two species, *Medinilla speciosa* and *Medinilla verrucosa*. The seedlings were obtained from Alammuria in Kudus City. Cultivation was carried out in South Kaliurang, Sleman Regency, Yogyakarta. The plants grew at an elevation of 900 meters above sea level. The study area receives an average annual rainfall of approximately 220.3 mm, with an average daily temperature of 22°C to 27°C, and air humidity between 59% and 77%. The research was conducted over 16 months, from April 2022 to August 2023.

Research design

The experiment employed a Nested Factorial Randomized Complete Block Design with three factors: *Medinilla* species, shading level, and vermicompost dose. The shading treatment was nested within the combinations of *Medinilla* species and vermicompost dosage. The *Medinilla* species factor consisted of two levels: *Medinilla speciosa* (M1) and *Medinilla verrucosa* (M2). The shading treatment included three levels: 75% light intensity (S1), 50% light intensity (S2), and 25% light intensity (S3), while the vermicompost dose treatment comprised of three levels: 5 tons/ha (V1), 7.5 tons/ha (V2), and 10 tons/ha (V3). As a result, a total of 18 treatment combinations were generated, with each combination replicated three times, resulting in 54 experimental units. Plant height and leaf count were recorded monthly throughout the study. Meanwhile, leaf area, root volume, fresh biomass weight, and dry biomass weight were measured at the end of the experiment. Additionally, measurements of fruit weight, chlorophyll content, antioxidant, and flavonoid were conducted following the harvest period.

Chlorophyll analysis

Chlorophyll analysis in *Medinilla* was conducted following the method described by Rasyidi et al. (2024). The analysis utilized a spectrophotometer at wavelengths of 645 nm and 663 nm. Prior to analysis, the *Medinilla* leaves were washed in the laboratory using water and air-dried to eliminate any surface contaminants. A 0.5-gram sample of the leaves was weighed and homogenized using a mortar and pestle. The homogenized leaf tissue was then mixed with 10 ml of acetone. The mixture was filtered using filter paper, and the filtrate was collected in a beaker. The resulting filtrate was transferred into a test tube and analyzed using a spectrophotometer at wavelengths of 645 nm and 663 nm, with acetone serving as the blank control.

The absorbance values obtained were used to calculate chlorophyll a, chlorophyll b, and total chlorophyll concentrations using the following equations:

Chlorophyll a = ((12.7 × A663) – (2.69 × A645)) × 0.02

Chlorophyll b = ((22.9 × A645) – (4.68 × A663)) × 0.02

Total chlorophyll = ((20.2 × A645) + (8.02 × A663)) × 0.02

The chlorophyll analysis was performed at the Plant Production Management Laboratory, Department of Agricultural Cultivation, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, on 18 January 2023.

Antioxidant analysis

The antioxidant analysis of *Medinilla* fruit was conducted using the method described by Herawati et al. (2021) with several modifications. Prior to analysis, the fruit samples were thoroughly cleaned to remove dirt and any impurities. The cleaned samples were then homogenized to obtain *Medinilla* fruit extract using ethanol. A 10 mg portion of the extract was weighed and dissolved in 10 mL of methanol. Subsequently, 1 mL of the methanol solution was mixed with 1 mL of 1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in a test tube. The mixture was incubated in a dark place for 30 minutes. After the incubation period, the antioxidant activity was measured using a spectrophotometer at a wavelength of 520 nm. The absorbance measurements were repeated three times for accuracy. The results were then calculated using the DPPH radical scavenging activity formula:

$$\%\,Scavenging\ activity = \frac{A_{blank} - A_{sample}}{A_{blank}}\ x\ 100\%$$

where A_{blank} is the absorbance of the control (DPPH solution without sample) and A_{sample} is the absorbance of the sample. The antioxidant analysis was carried out at the Food Chemistry and Biochemistry Laboratory, Faculty of Agriculture, Sebelas Maret University, Central Java on 22 February 2023.

Flavonoid analysis

The flavonoid content of *Medinilla* fruit was analyzed using the method described by Purnamasari et al. (2022), with some modifications to UV-Vis. The analysis began with weighing a 0.1 mg/ml portion of the *Medinilla* fruit extract and dissolving it in 10 ml of methanol. The sample was then reacted with 2 mL of 2% $AlCl_3 \bullet 6H_2O$ (aluminum chloride hexahydrate) and incubated for 1 hour. The absorbance of the sample was then measured using a UV-Vis spectrophotometer at a wavelength of 415 nm.

Statistical analysis
All the data were analyzed using Analysis of Variance (ANOVA) at a 95% confidence level, followed by the Duncan Multiple Range Test (DMRT) for mean comparisons at significance levels of 5% and 1%. After that, regression-correlation analysis and path analysis were conducted to examine the relationships among the observed variables. In this study, the statistical analyses were performed using SPSS software version 23.

Conclusion

This study confirms the capability of *Medinilla* plants to grow and develop fruits outside their natural habitat. The 50% shading treatment enhanced growth, fruit production, and the accumulation of secondary metabolites (including antioxidants and flavonoids) in *Medinilla* plants. Among the two *Medinilla* species tested, *Medinilla verrucosa* demonstrated a better adaptive response than *Medinilla speciosa*.

Acknowledgmenets

The authors would like to express their sincere gratitude to Mr. Suradi, a local farmer, for his generous support in providing land and maintaining the plants throughout the study.

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