Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Effects of Shading and Nitrogen Fertilizer on Growth and Physiology of Gandarusa (*Justicia gendarussa* Burm. F.)

Syaefudin SUMINTO*[1], Syahrul RAMADHAN[2], Uswatun HASANAH[3], Waras NURCHOLIS[4]

[1,2,4]IPB University, Faculty of Mathematics and Natural Sciences, Department of Biochemistry, 16680, Bogor, Indonesia
[1,4]IPB University, Tropical Biopharmaca Research Center, 16128, Bogor, Indonesia
[3]IPB University, Faculty of Agricultural Engineering and Technology, Department of Food Science and Technology, 16680, Bogor, Indonesia
[3]IPB University, South-East Asia Food and Agricultural Science and Technology Center, 16680, Bogor, Indonesia

[1]https://orcid.org/0000-0002-4246-9048, [2]https://orcid.org/0000-0002-6477-3702, [3]https://orcid.org/0000-0002-2202-1924
[4]https://orcid.org/0000-0001-7047-5093

*Corresponding author e-mail: wnurcholis@apps.ipb.ac.id

Article Info

Received: 09.11.2022
Accepted: 07.04.2023
Online published: 15.06.2023
DOI: 10.29133/yyutbd.1201543

Keywords

Chlorophyll,
Justicia gendarussa,
Nitrogen fertilizer,
Shading,
Sugar leaves

Abstract: Gandarusa (*Justicia gendarussa* Burm. F.) is a shrub used in herbal medicine, but knowledge of optimal cultivation methods for enhancing plant growth and metabolite yield is limited. This research aimed to evaluate the effect of shading and nitrogen fertilizer on the growth, photosynthetic parameters, and total sugar content of gandarusa. A split-plot experimental design was used with shading (S) (0% (S_0), 25% (S_{25}), and 50% (S_{50})) as the main plots and nitrogen fertilizer (N) (0 (N_0), 90 (N_{90}), 180 (N_{180}), and 270 (N_{270}) kg ha^{-1}) as the subplots. The results showed that the combination of S_0 and N_{270} was the most effective treatment for plant growth, indicated by the highest values of plant height and the number of leaves and branches. It also yielded high sugar content, with a value range of 72-76 mg g^{-1} leaves wet weight. The combination of S_0 and N_0 produced the highest photosynthetic rate (Pn) in the plant at 23.91 mol CO_2 m^{-2} s^{-1}, and total chlorophyll content was highest with S_{25} and N_{270}. Based on the results, shading decreased Pn, sugar production, and growth of gandarusa, while nitrogen fertilizer enhanced them. However, there was no interaction between shading and fertilizer on sugar production and growth of gandarusa, except for Pn.

To Cite: Suminto, S, Ramadhan, S, Hasanah, U, Nurcholis, W, 2023. Treatment of Shade and Nitrogen Fertilizer on Growth and Physiology of Gandarusa (*Justicia gendarussa* Burm. F.). *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(2): 192-206.
DOI: https://doi.org/10.29133/yyutbd.1201543

1. Introduction

Gandarusa (*Justicia gendarussa* Burm. F.) is a shrub found in forests and along river embankments, thriving in areas with sufficient water. It belongs to the class Magnolipsida, order Scrophulariales, family Acanthaceae, genus *Justicia*, and species *gendarussa* (Kavitha et al., 2014). Empirically, gandarusa has been widely used in herbal medicine for various conditions, including rheumatism, eczema, bronchitis, jaundice, and the common cold by Indians. In Papua, males use gandarusa as a contraceptive herb (Ratih et al., 2019). It reportedly has bioactive compounds, such as phenols, flavonoids, tannins, alkaloids, steroids, glycosides, saponins, stigmasterol, lupeol, 16-

hidroxilupeol, triterpenoids, and justicin (Hesturini et al., 2017). Previous research also revealed that gandarusa exhibited anticancer, antidiabetic, and anti-inflammatory activities (Nirmalraj et al., 2015; Adelina, 2020; Subbiah et al., 2021).

Identifying the best cultivation method to increase the yield of bioactive compounds is crucial for maximizing the potential of gandarusa as a medicinal plant. According to Moo-Young (2011), the growth and development of plants are dominantly affected by light intensity, nutrients, soil moisture, and substrate. Although sufficient light intensity is essential for photosynthesis, certain plants require proper cultivation methods to enhance their growth, such as using shading treatment. Shading was shown to reduce microclimate, radiation, temperature, and absorption of water and nutrients (Semchenko et al., 2012; Masabni et al., 2016; Arevalo-Gardini et al., 2021). Fan et al. (2018) reported that under the full intensity of light, corn showed higher biomass productivity than with shading treatment. In contrast, Khalid et al. (2019) reported enhanced bell pepper yield with shading treatment.

Another factor involved in improving plant productivity is nitrogen (N). Nitrogen fertilizer use on crops has increased in the past three centuries. Along with phosphorus (P) and potassium (K), nitrogen is classified as an essential macronutrient (Xu et al., 2012). Tian et al. (2020) reported that nitrogen fertilizer could increase biomass productivity, antioxidant enzyme activity, and photosynthetic rate (Pn) of corn. However, Zhang et al. (2016) found only a slight improvement in crop production after its application. Rahmah et al. (2021) reported that the application of manure and NPK fertilizer could improve growth, stomatal conductivity (Sc), intercellular CO_2 concentration, transpiration rate (Tr), leaves yield, and sugar content in gandarusa. However, the cultivation method to enhance plant growth and metabolite yield of gandarusa has not been thoroughly researched. There is also no research available on the influence of shading and nitrogen fertilizer treatments on its growth and physiology. Therefore, this research evaluated the effect of shading and nitrogen fertilizer treatments on the growth, Pn, total chlorophyll content, and carbohydrate production of gandarusa. This research will be vital for the development of gandarusa as a medicinal plant.

2. Material and Methods

2.1. Instrument and material

The instruments used in this research included Li-Cor portable photosynthesis system (L1-6400XT, Li-Cor Inc., Lincoln, NE), UV-Visible spectrophotometer (T60UV, PG Instruments, UK), and microcentrifuge (KITMAN-T24, Tomy Kogyo Co., Ltd., Tokyo, JP). Several materials were used in this research, including gandarusa (*Justicia gendarussa* Burm. F. local variety), urea (nitrogen fertilizer), dimethylsulfoxide (DMSO), EtOH, H_2SO_4, phenol, and glucose. All chemical materials used were commercially available. This research was conducted for five months, from November 2021 to April 2022, at the Biopharmaca Cultivation Conservation Unit Garden, Tropical Biopharmaca Research Center of the Institute for Research and Community Service (LPPM), IPB University, Cikabayan Garden Block C, Dramaga IPB Campus, Bogor (6o3'49"S and 106o42'57"T) at an elevation of 141 m above sea level. It was also conducted in the Biochemistry Department Laboratory at the IPB University, West Java, Indonesia.

2.2. Experimental design

A split-plot design was used, where seedlings obtained from 15 cm stem cuttings of gandarusa were planted into 10 × 15 cm polybags. After one month, the gandarusa seeds were transferred into 25 × 30 cm polybags consisting of soil, rice husk, and manure (1:1:1) for the treatments. The two parameters used for this research included shading as the main plot and nitrogen fertilizer (urea) as the subplot. Table 1 shows the treatment design for shading and nitrogen fertilizer. Furthermore, the experiment was carried out in triplicate, with each replicate consisting of six plants.

Table 1. Treatment design of shading and nitrogen fertilizer on gandarusa

Subplot (N Fertilizer (kg ha^{-1}))	Main Plot - Shading (%)		
	0	25	50
0	S_0N_0	$S_{25}N_0$	$S_{50}N_0$
90	S_0N_{90}	$S_{25}N_{90}$	$S_{50}N_{90}$
180	S_0N_{180}	$S_{25}N_{180}$	$S_{50}N_{180}$
270	S_0N_{270}	$S_{25}N_{270}$	$S_{50}N_{270}$

Note: "S" = shading (%), "N"= nitrogen fertilizer (kg ha^{-1}).

2.3. Photosynthetic parameters measurement

The photosynthetic parameters, including Pn, Sc, CO_2 intercellular (Ci), and Tr, were measured according to the method previously described by Zhang et al. (2016).

2.4. Chlorophyll content measurement

Chlorophyll was extracted from gandarusa following the method described by Parry et al. (2014). Leaves from each treatment were cut into small discs of 0.1 g and soaked with 7 mL DMSO solution before being boiled at 65°C for 25 minutes. The chlorophyll content was then measured using a spectrophotometer at λ_{649} and λ_{665} by a method developed by Arnon (1949). Furthermore, DMSO was used as a control in this experiment.

$$chlorophyll\ a\ (mg\ g^{-1}) = [(12.7 \times A_{663}) - (2.69 \times A_{645})] \cdot \frac{V}{1000 \cdot W} \quad (1)$$

$$chlorophyll\ b\ (mg\ g^{-1}) = [(22.9 \times A_{645}) - (4.68 \times A_{663})] \cdot \frac{V}{1000 \cdot W} \quad (2)$$

$$Total\ chlorophyll\ (mg\ g^{-1}) = chlorophyll\ a + chlorophyll\ b \quad (3)$$

Note: A : Absorbance
V : Volume of solution (mL)
W : Weight of sample (g)

2.5. Sugar content measurement

Sugar production in the leaves was evaluated using the phenol sulfuric acid method described by Pandey 2018. Fresh gandarusa leaves were crushed and ground using a mortar. A total of 0.1 g of the mashed leaves was dissolved in 1 mL of 80% EtOH and homogenized for one minute. The resulting suspension was centrifuged at 1000 RPM and 4°C for 15 minutes, and the obtained pellet was mixed with the 80% EtOH until the volume reached 10 mL. Subsequently, 1 mL of the sample solution was added to 1 mL of 5% phenol and 5 mL of H_2SO_4 before measuring the absorbance of the sample using a spectrophotometer at λ_{480}. Carbohydrate concentration was determined by comparing sample data with a standard curve. The standard curve (R^2 = 0.9945) was created using glucose solution with concentrations of 0, 5, 10, 15, 20, 25, and 50 µg mL^{-1}.

2.5. Data analysis

The effects of the treatment on the split-plot design were evaluated based on plant growth observation, photosynthetic parameters measurement, chlorophyll content measurement, and sugar production evaluation. The data were analyzed using ANOVA to determine the difference between each treatment. The significant differences ($p<0.05$) in the data were then subjected to the Duncan Multiple Range Test (DMRT) by SAS and SPSS software with a test level of 95%.

3. Results

3.1. Plant growth parameters

Figure 1 shows that shading treatment significantly decreased plant height, while nitrogen fertilizer did not. The greatest height was observed in S_0 at 39.8 cm. Each level of fertilizer treatment did not show any significant difference in enhancing plant height, except for N_{270} which produced the greatest height than those of lesser concentrations. There was no interaction between shading and nitrogen fertilizer treatments on plant height, as shown in Table 2.

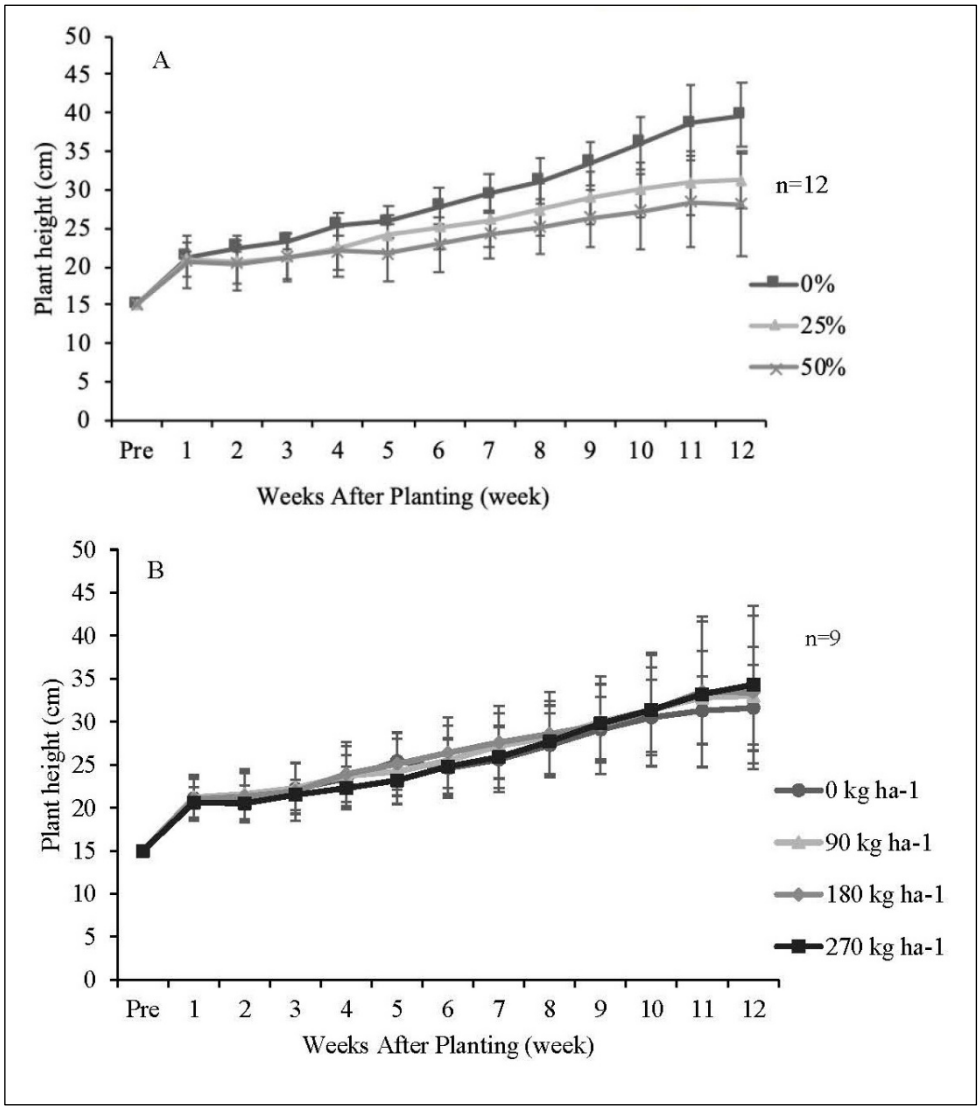

Figure 1. Effects of (A) shading and (B) nitrogen fertilizer on the plant height of gandarusa.

Table 2. The height of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Week After Planting (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
Shading (%)	Height of Plant (cm)											
0	21.4^{a}	22.4^{a}	23.4^{a}	25.5^{b}	25.9^{b}	27.9^{b}	29.5^{b}	31.2^{b}	33.5^{b}	36.2	38.7^{b}	39.8^{b}
25	20.9^{a}	20.6^{a}	21.3^{a}	22.0^{a}	21.8^{a}	22.9^{a}	24.2^{a}	25.2^{a}	26.4^{a}	27.3^{a}	28.5^{a}	28.2^{a}
50	20.6^{a}	20.4^{a}	21.3^{a}	22.4^{a}	24.1^{ab}	25.2^{a}	26.0^{a}	27.4^{a}	29.0^{a}	30.1^{a}	30.9^{a}	31.3^{a}
Nitrogen fertilizer (kg/ha)	Height of Plant (cm)											
0	21.1^{a}	21.2^{a}	22.2^{a}	23.4^{a}	25.3^{a}	24.6^{a}	25.6^{a}	27.3^{a}	29.1^{a}	30.5^{a}	31.3^{a}	31.6^{a}
90	21.2^{a}	21.6^{a}	22.3^{a}	23.7^{a}	24.2^{a}	25.5^{a}	27.2^{a}	28.2^{a}	30.0^{a}	31.4^{a}	32.8^{a}	33.0^{a}
180	21.0^{a}	21.3^{a}	21.9^{a}	23.9^{a}	25.1^{a}	26.4^{a}	27.6^{a}	28.6^{a}	29.6^{a}	31.3^{a}	33.5^{a}	33.4^{a}
270	20.6^{a}	20.5^{a}	21.5^{a}	22.3^{a}	23.2^{a}	24.8^{a}	25.9^{a}	27.7^{a}	29.8^{a}	31.4^{a}	33.2^{a}	34.3^{a}
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on the height of the plant.

Based on the result, shading treatment significantly reduced the number of leaves each week. As shown in Figure 2, the number of leaves increased following an increase in nitrogen fertilizer concentration. However, there was no difference between treatments at different concentrations. There was an interaction between shading and nitrogen fertilizer in the number of leaves, particularly within 2-5 weeks after treatment (WAT), no interaction was found at 6-12 WAT This can be attributed to the optimal growth conditions that led to faster leaves growth. The best treatment at 2-5 WAT was the combination S_0N_{90}. The interaction between shading and nitrogen fertilizer on the number of leaves is shown in Table 3.

Table 3. Number of leaves of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Week After Planting (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
Shading (%)	leaf											
0	11.7	23.9^{b}	27.5^{b}	36.3^{c}	38.0^{c}	45.1^{b}	49.4^{b}	52.9^{b}	56.4^{b}	58.4^{b}	60.4^{b}	60.8^{b}
25		13.3^{a}	14.1^{a}	16.3^{a}	18.3^{a}	21.5^{a}	23.9^{a}	24.9^{a}	26.0^{a}	25.3^{a}	25.7^{a}	23.5^{a}
50	9.0	15.9^{a}	18.1^{a}	21.7^{b}	25.3^{b}	27.6^{a}	30.1^{a}	29.8^{a}	29.2^{a}	27.7^{a}	24.7^{a}	23.1^{a}
Nitrogen fertilizer (kg ha^{-1})	leaf											
0		16.0^{a}	17.4^{a}	21.8^{a}	22.9^{a}	26.2^{a}	29.3^{a}	31.6^{a}	33.1^{a}	31.8^{a}	29.6^{a}	28.6^{a}
90	10.1	18.3^{a}	20.1^{ab}	26.2^{ab}	28.4^{ab}	32.3^{a}	35.6^{a}	36.4^{a}	37.2^{a}	36.7^{a}	35.9^{ab}	33.7^{ab}
180	11.1	19.7^{a}	23.0^{b}	28.2^{b}	30.4^{b}	34.1^{a}	37.4^{a}	37.1^{a}	37.6^{a}	36.3^{a}	36.4^{ab}	35.2^{ab}
270	9.2^{a}	16.8^{a}	19.0^{ab}	22.9^{ab}	27.0^{ab}	32.9^{a}	35.6^{a}	38.4^{a}	40.9^{a}	43.7^{a}	45.9^{b}	45.8^{b}
Interaction	ns	s	s	s	s	ns	ns	ns	ns	ns	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on the number of plant leaves, s = significant or there is an interaction between shading and nitrogen fertilizer on the number of plant leaves.

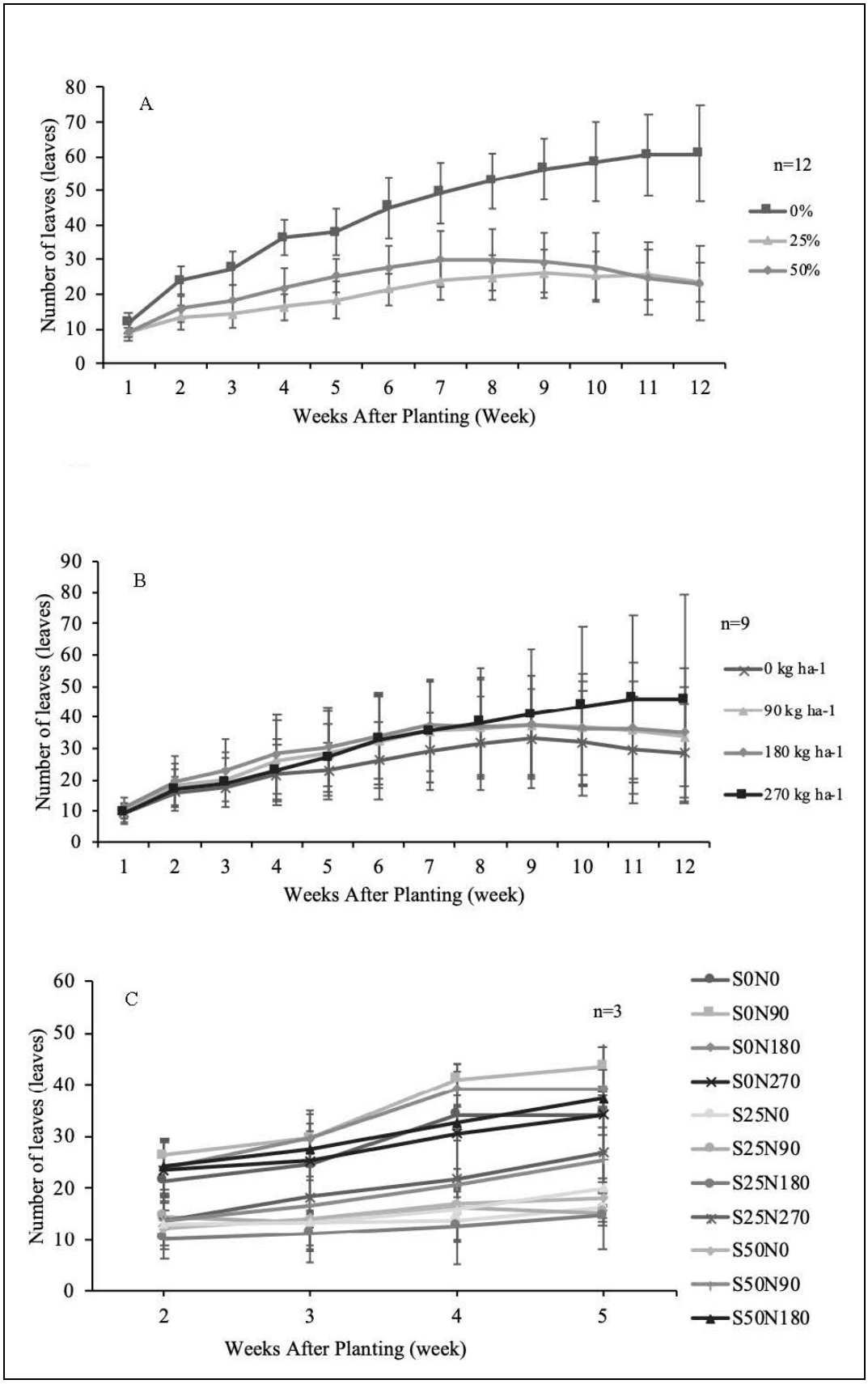

Figure 2. Effects of (A) shading, (B) nitrogen fertilizer, and (C) interaction between shading and nitrogen fertilizer on the number of leaves of gandarusa.

A further experiment on the number of branches revealed that both shading and nitrogen fertilizer treatments had significant effects, as shown in Figure 3. Shading treatment decreased the number of branches, indicated by a higher number of branches with the un-shading treatment (11.4 branches). This value was significantly different from S_{25} and S_{50}, with 4.3 and 4.2 branches, respectively. Based on nitrogen fertilizer treatment, N270 had the highest value of 10.8 branches, and the number decreased with concentration. As shown in Table 4, an interaction between the two treatments was observed at 2, 5, and 12 WAT. Additionally, at 5 WAT, the greatest treatment was observed in S_0N_{90}, while at 12 WAT, it was found in S_0N_{270}.

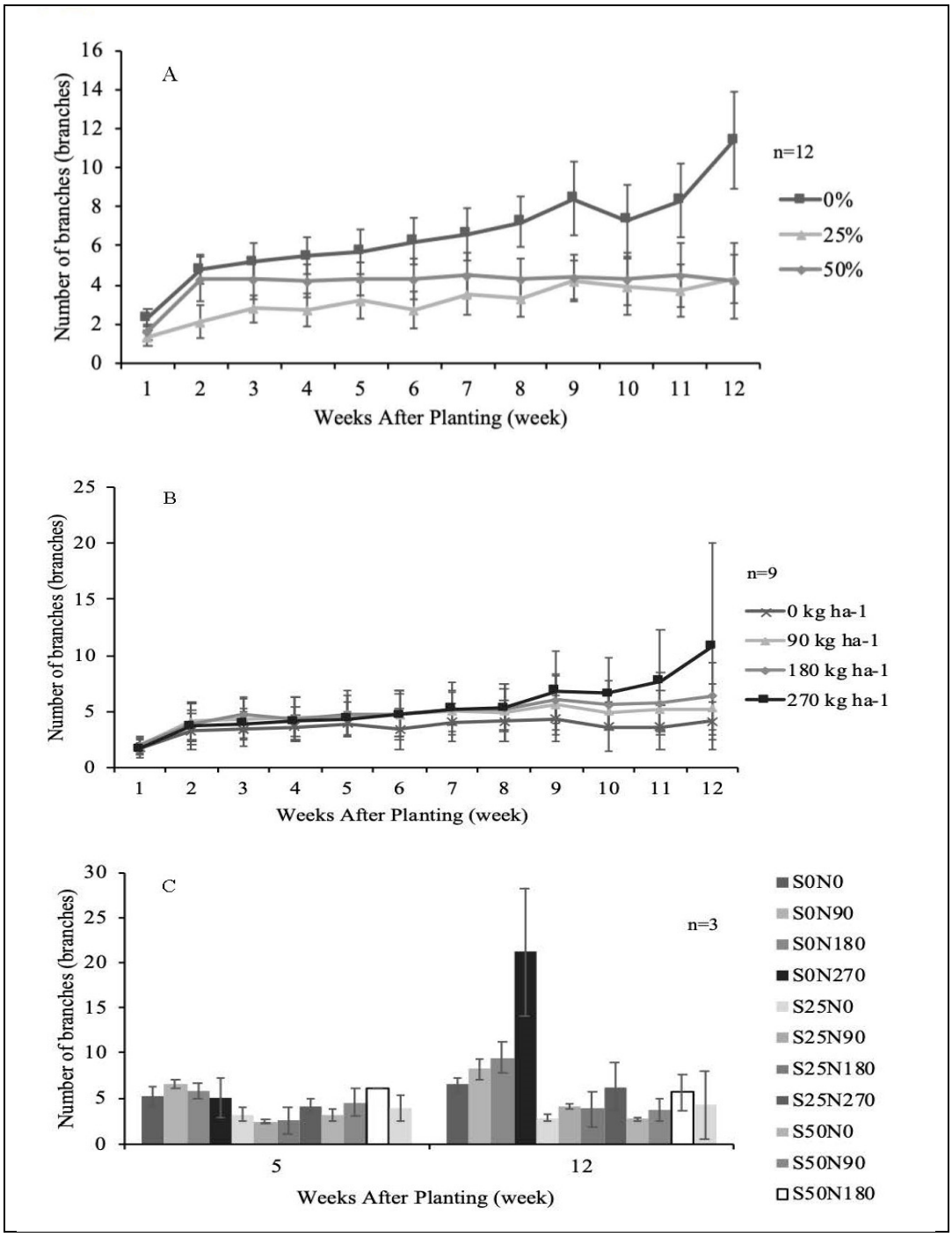

Figure 3. Effects of (A) shading, (B) nitrogen fertilizer, and (C) interaction between shading and nitrogen fertilizer on the number of branches of gandarusa.

Table 4. Number of branches of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Week After Planting (week)											
	1	2	3	4	5	6	7	8	9	10	11	12
Shading (%)	branch											
0	2.3^{b}	4.8^{b}	5.2^{b}	5.5^{c}	5.7^{c}	6.2^{c}	6.6^{b}	7.2^{b}	8.4^{b}	7.3^{b}	8.3^{b}	11.4^{a}
25	1.3^{a}	2.1^{a}	2.8^{a}	2.7^{a}	3.2^{a}	2.7^{a}	3.5^{a}	3.3^{a}	4.2^{a}	3.9^{a}	3.7^{a}	4.3^{a}
50	1.6^{a}	4.3^{b}	4.3^{b}	4.2^{b}	4.3^{b}	4.3^{b}	4.5^{a}	4.3^{a}	4.4^{a}	4.3^{a}	4.5^{a}	4.2^{a}
Nitrogen fertilizer (kg ha^{-1})	branch											
0	1.7^{a}	3.2^{a}	3.4^{a}	3.6^{a}	3.9^{a}	3.4^{a}	4.0^{a}	4.1^{a}	4.3^{a}	3.6^{a}	3.6^{a}	4.1^{a}
90	1.9^{a}	4.1^{a}	4.3^{ab}	4.4^{a}	4.6^{a}	4.7^{b}	5.0^{a}	4.9^{a}	5.6^{ab}	4.9^{ab}	5.1^{ab}	5.2^{a}
180	1.9^{a}	3.9^{a}	4.7^{b}	4.3^{a}	4.8^{a}	4.8^{b}	5.2^{a}	5.2^{a}	6.0^{ab}	5.6^{b}	5.7^{bc}	6.4^{a}
270	1.6^{a}	3.7^{a}	3.9^{ab}	4.1^{a}	4.3^{a}	4.7^{b}	5.2^{a}	5.3^{a}	6.8^{b}	6.6^{b}	7.7^{c}	10.8^{b}
Interaction	ns	s	ns	ns	s	ns	ns	ns	ns	ns	ns	s

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on the number of branches, s = significant or there is an interaction between shading and nitrogen fertilizer on the number of branches.

3.2. Photosynthetic parameters

The investigation of the photosynthetic parameters showed an interaction between shading and nitrogen fertilizer (S*N) in Pn and intercellular CO_2 (Ci), but none in Sc and Tr, as shown in Tables 5 and 6, respectively. As depicted in Figure 4, S_0N_0 had a high Pn of 23.91 µmol CO_2 m^{-2} s^{-1}, while $S_{50}N_0$ resulted in a high Ci with a value of 174.45 µmol CO_2 µmol air^{-1}. It was also noted that the Ci increased as shading concentration increased.

Sc was significantly different in the fertilizer treatment (N) alone but not significantly different in shading treatment alone (S). There was also no treatment interaction between the treatments (S*N). According to the results, N_{90} and S_0 had a high Sc with values of 0.1516 H_2O m^{-2} s^{-1} and 0.1504 H_2O m^{-2} s^{-1}, respectively. On the contrary, Tr was significantly different in both shading and nitrogen treatments. The result revealed that the highest Tr based on shading was in S_0 (0.0050 mol H_2O m^{-2} s^{-1}), while based on nitrogen fertilizer, it was in N_{90} (0.0048 mol H_2O m^{-2} s^{-1}). This research demonstrated that Tr decreased as shading level increased.

Table 5. Photosynthetic rate (Pn) and intercellular CO_2 (Ci) of gandarusa under various levels of shading and nitrogen fertilizer

Treatment		Photosynthetic rate (Pn) (µmol CO_2 $m^{-2}s^{-1}$)	Intercellular CO_2 (Ci) (µmol CO_2 mol air^{-1})
S_0	**N_0**	23.91^{a}	94.48^{d}
	N_{90}	21.55^{bc}	151.08^{abc}
	N_{180}	22.29^{b}	116.94^{cd}
	N_{270}	23.05^{ab}	138.91^{abc}
S_{25}	**N_0**	21.47^{bc}	133.16^{abcd}
	N_{90}	22.08^{b}	138.78^{abc}
	N_{180}	19.59^{d}	115.71^{cd}
	N_{270}	18.76^{d}	133.79^{abcd}
S_{50}	**N_0**	18.71^{d}	174.45^{a}
	N_{90}	20.08^{cd}	160.68^{ab}
	N_{180}	19.72^{d}	134.65^{abcd}
	N_{270}	20.28^{cd}	126.89^{bcd}

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. S = shading (%) and N = nitrogen fertilizer (kg ha^{-1}).

Table 6. Stomatal conductivity (Sc) and transpiration rate (Tr) of gandarusa under various levels of shading and nitrogen fertilizer

Treatment	Stomatal conductivity (Sc) (mol H_2O $m^{-2}s^{-1}$)	Transpiration rate (Tr) (mol H_2O $m^{-2}s^{-1}$)
Shading (%)		
0	0.1504[a]	0.0050[a]
25	0.1361[b]	0.0045[b]
50	0.1389[b]	0.0038[c]
Nitrogen fertilizer (kg ha^{-1})		
0	0.1454[a]	0.0046[ab]
90	0.1516[a]	0.0048[a]
180	0.1309[b]	0.0041[c]
270	0.1392[ab]	0.0043[bc]
Interaction	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on stomatal conductivity or transpiration rate.

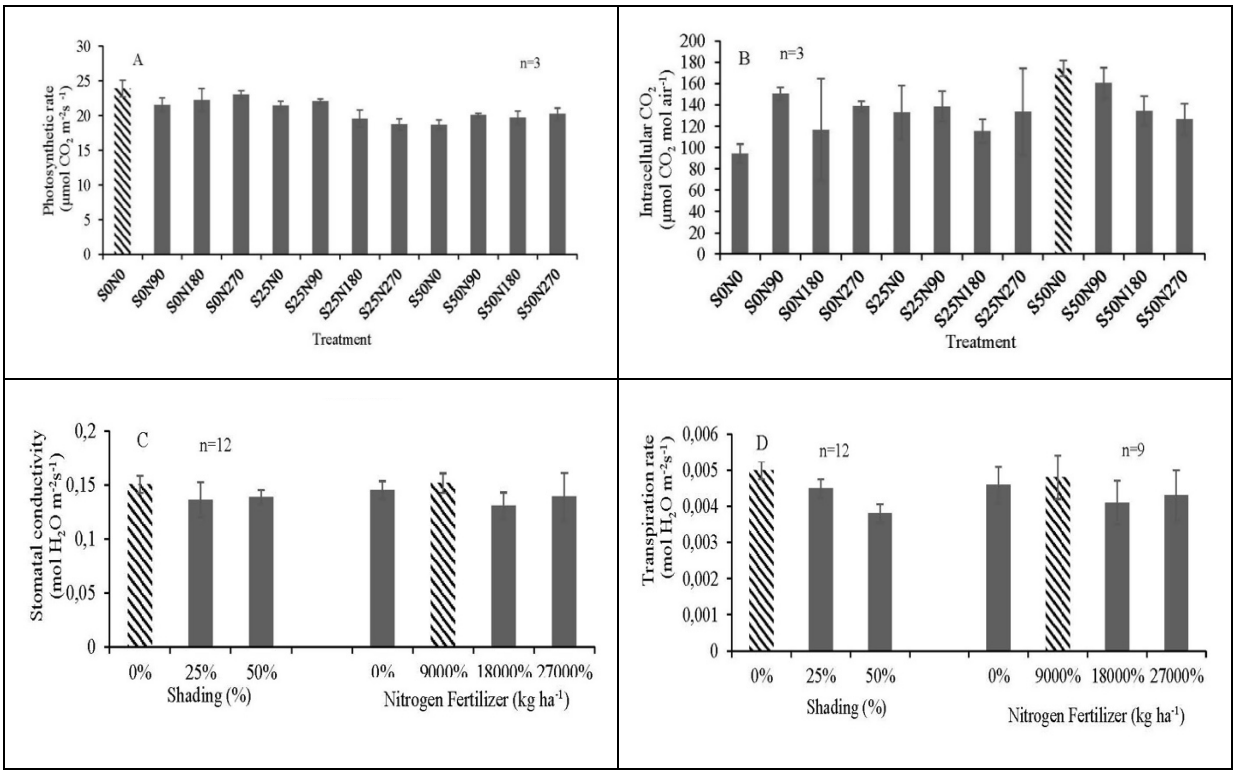

Figure 4. Effects of shading and nitrogen fertilizer on photosynthetic rate, intracellular CO_2, stomatal conductivity, and transpiration rate of gandarusa.

3.3. Chlorophyll content

Figure 5 shows the chlorophyll content of gandarusa leaves, including chlorophyll a, b, and total chlorophyll. The results indicated that shading treatment increased chlorophyll content, while nitrogen fertilizer treatment had no effect. Chlorophyll a in gandarusa leaves was also observed to be higher than chlorophyll b. As shown in Table 7, there was no interaction between the two treatments on the levels of chlorophyll a, b, and total chlorophyll. However, there was a significant difference in the levels of chlorophyll a in shading alone ($p<0.05$), and no significant difference with the fertilizer treatment ($p>0.05$).

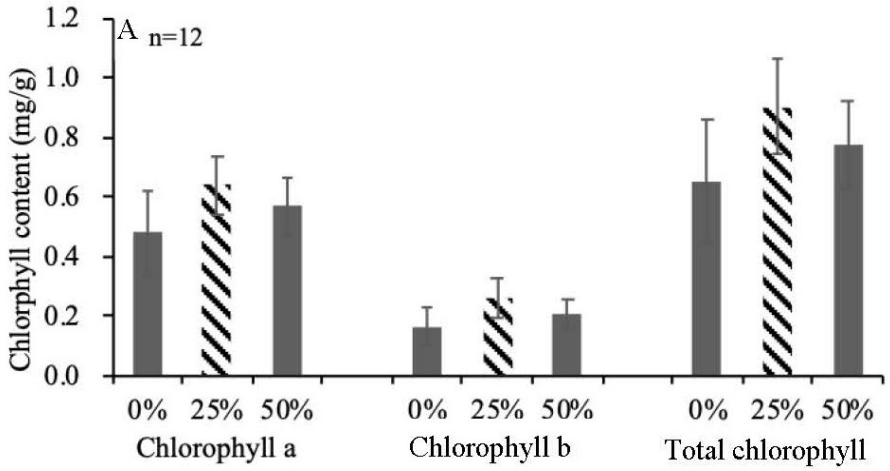

Figure 5. Effects of (A) shading and (B) nitrogen fertilizer on the chlorophyll a, chlorophyll b, and total chlorophyll contents of gandarusa.

Table 7. Chlorophyll content of gandarusa at various levels of shading and nitrogen fertilizer

Treatment		Chlorophyll a	Chlorophyll b	Total Chlorophyll
Shading (%)			(mg/g)	
	0	0.481^{b}	0.167^{b}	0.648^{b}
	25	0.639^{a}	0.263^{a}	0.902^{a}
	50	0.568^{ab}	0.207^{ab}	0.776^{ab}
Nitrogen fertilizer (kg ha^{-1})			(mg/g)	
	0	0.585^{a}	0.209^{a}	0.794^{a}
	90	0.532^{a}	0.181^{a}	0.712^{a}
	180	0.551^{a}	0.213^{a}	0.763^{a}
	270	0.584^{a}	0.247^{a}	0.832^{a}
Interaction		ns	ns	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on chlorophyll content.

3.4. Total sugar content

The determination of the total sugar content in gandarusa leaves revealed that there was no significant interaction between shading and nitrogen fertilizer (S*N), as shown in Figure 6 and Table 8. There was also no significant difference in sugar content in shading-only treatment (S). However, a significant difference was found in nitrogen fertilizer treatment alone (N). The highest sugar content in nitrogen and shading treatments was observed in N_{270} and S_0 with values of 76.25 mg g^{-1} and 72.88 mg g^{-1}, respectively. These results suggested a decrease in leaves sugar content due to higher levels of shading.

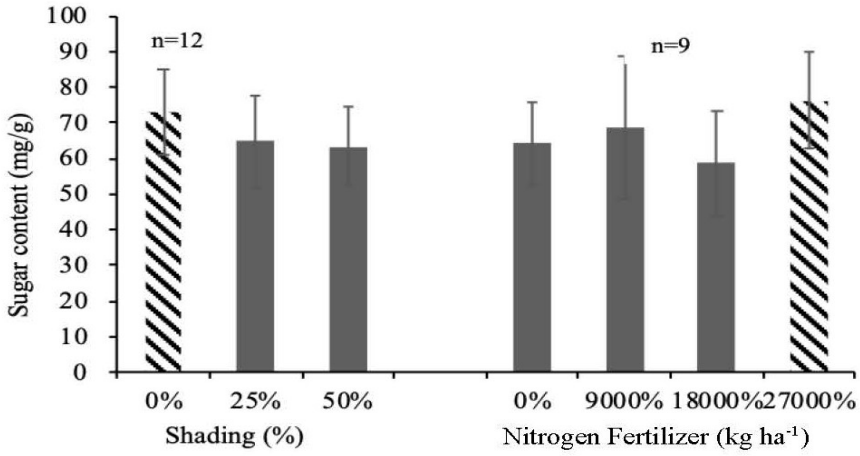

Figure 6. Effects of shading and nitrogen fertilizer on the total sugar contents of gandarusa leaves.

Table 8. Sugar content of gandarusa at various levels of shading and nitrogen fertilizer

Treatment	Sugar content (mg g^{-1})
Shading (%)	
0	72.88[a]
25	64.69[a]
50	63.19[a]
Nitrogen fertilizer (kg ha^{-1})	
0	64.08[ab]
90	68.70[ab]
180	58.64[b]
270	76.25[a]
Interaction	ns

Note: Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant or no interaction between shading and nitrogen fertilizer on sugar content.

4. Discussion

4.1. Plant growth

Shading treatment decreases photosynthetic capacity, which is essential for plant growth and energy production (Wang et al., 2020). The evaluation of gandarusa growth also showed a decrease in plant height, number of leaves, and number of branches. Raai et al. (2020) reported a decrease in the height and number of leaves and branches of *Psophocarpus tetragonolobus* due to shading and nitrogen fertilizer treatment. Lu et al. (2021) also reported that light deficiency inhibited plant growth by directly affecting photosynthesis, phytohormone transduction, stress-related transcription factor, and R genes that are responsible for the immune system in *Magnolia sinostellata*. On the contrary, Yasoda et al. (2018) found an increase in the number of cauliflower leaves due to 50% shading. These findings implied that the use of shading to increase plant growth would depend on the type of plant.

It has been known that nitrogen fertilizer contributes to plant growth. In this research, many branches were affected significantly by nitrogen fertilizer, while plant height and the number of leaves were not. Research on Egyptian cotton (Ibrahim et al., 2022), eggplant (Tanko et al., 2015), and maize (Tian et al., 2020) demonstrated the essential role of nitrogen in vegetative growth, number of tillers, biosynthesis of chlorophyll, amino acid, and protein synthesis. Further investigation revealed an interaction between shading and nitrogen fertilizer, indicating that both were required to achieve specific plant growth parameters and could not be separated (Vargas et al., 2015). Previous research explained that shading could decrease the absorption of water and nutrients, leading to a decrease in the concentration of nitrogen fertilizer required for optimal productivity (Semchenko et al., 2012; Arevalo-

Gardini et al., 2021). This interaction was most prominent in the middle of the observation, indicating an optimum growth phase of gandarusa.

4.2. Photosynthetic parameters

Photosynthetic parameters are necessary to understand plant physiology during treatment (Manjarrez-Sanchez et al., 2020). In this research, shading significantly decreased some photosynthetic parameters, such as Pn, Sc, and Tr, while Ci increased as shading levels increased. Lu et al. (2021) stated that shading for an extended period could reduce rubisco levels, limit the light intensity absorbed by photosynthetic antennae, and inhibit photo-system II (PSII) and photo-system I (PSI) expressing genes. Meanwhile, nitrogen fertilizer treatment increased Pn. Zhang et al. (2021) reported that high nitrogen application on crabapple plants could increase the net photosynthesis rate and growth rate of shoot tips.

Several photosynthetic parameters have been reported to contribute to plant growth (Moo-Young, 2011; Kirschbaum et al., 2011). For example, Sc measured the ability of stomate to regulate gas (CO_2) and water exchange in and out of leaves and was significantly affected by nitrogen fertilizer in this research. Additionally, a sufficient amount of nitrogen fertilizer could optimize the leaves' anatomy and physiology by activating the aquaporin enzyme (AQP) and carbonic anhydrase (CA). AQP was responsible for mediating genes in plasma membrane intrinsic protein and increasing the permeability of CO_2 through the membrane, and CA regulated mesophyll conductivity by converting CO_2 into HCO_3^- . Zhu et al. (2020) revealed that a high concentration of nitrogen fertilizer decreased rubisco activity, leading to Sc reduction.

Tr is another parameter which represented how much water transpired per unit area of leaves in a given time. In this research, increased shading levels decreased Tr, while nitrogen fertilizer (N90) produced the highest Tr value. The light intensity increased temperature and air drought, which removed water from leaves and increased Tr value. This result is consistent with the research by Zhu et al. (2020), which reported a decrease in transpiration and Sc in leaves with excessive nitrogen fertilizer (>90 kg ha^{-1}) use. Meanwhile, Ma et al. (2022) found that the addition of 90 kg ha^{-1} nitrogen fertilizer to the sunflower plant increased Sc and Tr values.

4.3. Chlorophyll content

Based on the results, the chlorophyll content of gandarusa increased by applying shading treatment. This is consistent with a result obtained by Juhaeti et al. (2021) in millet crops. Chen et al. (2021) proposed that there was a gene responsible for chlorophyll biosynthesis, known as CsPOR, which can be stimulated by decreasing light intensity. This research found no significant difference in chlorophyll content enhancement with nitrogen fertilizer treatment Similarly, Saparso et al. (2020) reported that nitrogen fertilizer had no significant effect on the chlorophyll content in red onion.

4.4. Total sugar content

The total sugar content was observed to be higher in un-shaded treatment compared to shading treatment. However, there was no significant difference among the treatments. Nitrogen fertilizer indicated a significant difference between the different concentrations, and increasing its concentration resulted in higher sugar content in leaves, as nitrogen is essential for ADP-glucose pyrophosphorylase involved in starch biosynthesis. Widodo et al. (2019) reported that applying nitrogen fertilizer to *Pennisetum purpureum* could increase its total digestible nutrients, such as protein and carbohydrates. Zhang et al. (2021) also reported a similar result which showed an increase in sucrose and sorbitol levels in crabapple shoots due to a high concentration of nitrogen Chang and Zhu (2017) revealed that low nitrogen fertilizer concentration caused the cells to be unable to increase the size of leaves cell. Leaves cell weight is mostly covered by vacuole weight, and the vacuole also acts as a storage place for sugar in the sink tissue. However, Previous research reported an opposite result or decreased sugar content of the leaves due to high nitrogen application (Kano et al., 2007; Braun et al., 2016).

Conclusion

Based on this research, shading treatment decreased the growth of gandarusa by reducing photosynthetic capacity, as indicated by plant height, number of leaves, and number of branches.

However, nitrogen fertilizer significantly affected the number of branches, Pn, Sc, and Tr. It was found that optimal plant growth was achieved through the interaction between these treatments. Chlorophyll content was significantly affected by shading treatment but not by nitrogen fertilizer, and total sugar content was not significantly affected by either treatment. These findings suggested that the effects of shading and nitrogen fertilizer on plant growth and physiology vary depending on the plant species, and their interaction should be considered for optimal growth.

Acknowledgments

The authors are grateful to the Ministry of Education, Culture, Research, and Technology of Indonesia for the financial assistance provided through the National competitive basic research grant (No: 3615/IT3.L1/PT.01.03/P/B/2022) and the Superior basic research college grant (No: 3554/IT3.L1/PT.01.03/P/B/2022).

References

Adelina, R. (2020). Simulasi docking molekuler senyawa potensial tanaman *Justicia gendarussa* Burm.f. sebagai antidiabetes. *Bulletin of Health Research*, 48(2), 117-122. doi: 10.22435/bpk.v48i2.3139.

Arevalo-Gardini, E., Farfan, A., Barraza, F., Arevalo-Hernandez, C. O., Zuniga-Cernades, L. B., Alegre, J., & Baligar, V.C. (2021). Growth, physiological, nutrient-uptake-efficiency and shade-tolerance responses of cacao genotypes under different shades. *Agronomy*, 11(8), 1-22. doi: 10.3390/agronomy11081536.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. *Plant Physiology*, 24(1), 1-15. doi:10.1104%2Fpp.24.1.1.

Braun, H., Fontes, P. C. R., da Silva, T. P., Finger, F. L., Cecon, P. R., & Farreira, A. P. S. (2016). Carbohydrates concentration in leaves of potato plants affected by nitrogen fertilization rates. *Revista Ceres*, 63(2), 241-248. doi:10.1590/0034-737X201663020016.

Chang, T. G., & Zhu, X.G. (2017). Source–sink interaction: a century old concept under the light of modern molecular systems biology. *Journal of Experimental Botany*, 68(16), 4417-4431. doi: 10.1093/jxb/erx002.

Chen, J., Wu, S., Dong, F., Li, J., Zeng, L., Tang, J. & Gu, D. (2021). Mechanism underlying the shading induced chlorophyll accumulation in tea leaves. *Frontier in Plant Science*, 12, 779819. doi:10.3389/fpls.2021.779819.

Fan, Y., Chen, J., Cheng, Y., Raza, M. A., Wu, X., Wang, Z., Liu, Q., Wang, R., Wang, X., Yong, T., Liu, W., Liu, J., Du, J., Shu, K., Yang, W., & Yang, F. (2018). Effect of shading and light recovery on the growth, leaf structure, and photosynthetic performance of soybean in a maizesoybean relay-strip intercropping system. *PLoSONE*, 13(5), e0198159. doi: 10.1371/journal.pone.0198159.

Hesturini, R. J., Herowati, R., & Widodo, G. P. (2017). Uji aktivitas analgetika fraksi-fraksi ekstrak etanol daun gandarusa (*Justicia gendarussa* Burm. f) dengan metode tail flick. *Jurnal Farmasi Indonesia*, 15(1), 13-17. https://oasis.iik.ac.id:9443/repo/items/show/5545. Access date: 10.09.2022.

Ibrahim, I. A. E., Yehia, W. M. B., Saleh, F. H., Lamlom, S. F., Ghareeb, R. Y., El-Banna, A. A. A., & Abdelsalam, N. R. (2022). Impact of plant spacing and nitrogen rates on growth characteristics and yield attributes of Egyptian cotton (*Gossypium barbadense* L.). *Frontiers in Plant Science*, 13, 916734. doi:10.3389/fpls.2022.916734.

Juhaeti, T., Adisyahputra, A., Hidayati, N., & Florina, R. (2021). Evaluation on foxtail millet (*Setaria italica* L.) responses to different light intensities and fertilizations. *Berita Biologi Jurnal Ilmu-Ilmu Hayati*, 21(3), 313-324. doi:10.14203/beritabiologi.v21i3.4233.

Kano, Y., Nakagawa, H., Sekine, M., Goto, H., & Sugiura, A. (2007). Effect of nitrogen fertilizer on cell size and sugar accumulation in the leaves of cabbage (*Brassica oleracea* L.). *Hortscience*, 42(6), 1490-1492. doi:10.21273/HORTSCI.42.6.1490.

Kavitha, K., Sangeetha, K. S. S., Sujath, K., & Umamaheswari, S. (2014). Phytochemical and pharmalogical profile of *Justicia gendarussa* Burm. F. - Review. *Journal of Pharmacy*

Research, 8(7), 990-997. https://www.researchgate.net/profile/Sridevi-Sangeetha/publication/280385739_Phytochemical_and_Pharmacological_Profile_of_Justicia_gendarussa_Burm_f_-_Review/links/55b3d07308ae092e9653b1bd/Phytochemical-and-Pharmacological-Profile-of-Justicia-gendarussa-Burm-f-Review.pdf. Access date: 10.09.2022.

Khalid, M. H. B., Raza, M. A., Yu, H. Q., Sun, F. A., Zhang, Y. Y., Lu, F. Z., Si, L., Iqbal, N., Khan, I., Fu, F. L., & Li., W. C. (2019). Effect of shade treatments on morphology, photosynthetic and chlorophyll fluorescence characteristics of soybeans (*Glycine max* L. Merr.). *Applied Ecology and Environmental Research*, 17(2), 2551-2569. doi:10.15666/aeer/1702_25512569.

Kirschbaum, M. U. F. (2011). Does enhanced photosynthesis enhance growth? lessons learned from CO_2 enrichment studies. *Plant Physiology*, 155(1), 117-124. doi: 10.1104/pp.110.166819.

Lu, D., Liu, B., Ren, M., Wu, C., Ma, J., & Shen, Y. (2021). Light deficiency inhibits growth by affecting photosynthesis efficiency as well as JA and ethylene signaling in endangered plant *Magnolia sinostellata*. *Plants*, 10(11), 1-21. doi: 10.3390/plants10112261.

Ma, T., Chen, K., He, P., Dai, Y., Yin, Y., Peng, S., Ding, J., Yu, S., & Huang, J. (2022). Sunflower photosynthetic characteristics, nitrogen uptake, and nitrogen use efficiency under different soil salinity and nitrogen applications. *Water*, 14, 982. doi: 10.3390/w14060982.

Manjarrez-Sanchez, J. M., & Martinez-Carrillo, G. (2020). Dataset of photosynthesis and photosynthetic factors measurements of greenhouse tomato. *Data Brief*, 32(10), 1-4. doi: 10.1016/j.dib.2020.106274.

Masabni, J., Sun, Y., Niu, G., & Valle, P. D. (2016). Shade effect on growth and productivity of tomato and chili pepper. *Horttechnology*, 26(3), 344-351. doi: 10.21273/HORTTECH.26.3.344.

Moo-Young, M. (2011). *Comprehensive Biotechnology* (2nd Ed.). Oxford, UK: Elsevier.

Nirmalraj, S., Ravikumar, M., Mahendrakumar, M., Bharath, B., & Perinbam, K. (2015). Antibacterial and anti-inflammatory activity of *Justicia gendarussa* Burm. f. leaves. *Journal of Plant Sciences*, 10(2), 70-74. doi:10.3923/jps.2015.70.74.

Pandey, S. 2018. Physiological and biochemical changes associated with the development of brown spot diseases in rice leaves. *International Journal of Advances in Agricultural Science and Technology*, 5(4), 69-78. https://ijaast.com/publications/vol5issue4/V5I408.pdf. Access date: 10.09.2022.

Parry, C., Blonquist, J. M., & Bugbee, B. (2014). In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. *Plant, Cell, & Environment*, 37(11), 2508–2520. doi: 10.1111/pce.12324.

Raai, M. N., Zain, N. A. M., Osman, N., Rejab, N. A., Sahruzaini, N. A., & Cheng, A. (2020). Effects of shading on the growth, development and yield of winged bean (*Psophocarpus tetragonolobus*). *Ciencia Rural*, 50(2), e20190570. doi:10.1590/0103-8478cr20190570.

Rahmah, N. N., Rafi, M., Syukur, M., & Nurcholis, W. (2021). Influence of combined NPK and manure on improving growth, photosynthetic characteristic and yield of *Justicia gendarussa* Burm. F.. *Pakistan Journal of Biological Sciences*, 24(11), 1162-1168. doi: 10.3923/pjbs.2021.1162.1168.

Ratih, G. A. M., Imawati, M. F., Nugroho, R. R., Purwanti, D. I., Wongso, S., Prajogo, B., & Indrayanto, G. (2019). Phytochemicals of gandarusa (*Justicia gendarussa*) and its preparations. *Natural Product Communications*, 2019, 1-10. doi:10.1177/1934578X19851406.

Saparso, Kartini, & Apriliyanto, I. D. (2020). Growth and yield of shallots (*Allium ascalonicum* L.) Lokananta in various doses of nitrogen fertilizers and number of plant per hole in coastal sandy land. *IOP Conference Series: Earth and Environmental Science*, 653, 012145. doi: 10.1088/1755-1315/653/1/012145.

Semchenko, M., Lepik, M., Gotzenberger, L., & Zobel, K. (2012). Positive effect of shade on plant growth: Amelioration of stress or active regulation of grown rate? *Journal of Ecology*, 100(2), 459-466. doi:10.1111/j.1365-2745.2011.01936.x.

Subbiah, V. A., Mani, J., & Muniraj, S. (2021). Cytotoxicity activity of kidney cell line (PK-15) using ethanol extract of *Justicia gendarussa* Burm. f leaves. *International Journal of Botany Studies*, 6(3), 299-302. https://www.botanyjournals.com/download/1317/6-3-39-670.pdf. Access date: 09.09.2022.

Tanko, U. M., Momoh, & Yusuf, J. (2015). Influence of N-fertilizer application on the yield and yield components of two varieties of eggplant (*Solanum melongena* L.) in Anyigba, Kogi State. *Journal of Biology, Agriculture and Healthcare*, 5(17), 201-210.

https://www.iiste.org/Journals/index.php/JBAH/article/view/25651/26519. Access date: 10.09.2022.

Tian, G., Qi, D., Zhu, J., & Xu, Y. (2020). Effects of nitrogen fertilizer rates and water logging on leaf physiological characteristics and grain yield of maize. *Archives of Agronomy and Soil Science*, 67(7), 1-15. doi: 10.1080/03650340.2020.1791830.

Vargas, M., Glaz, B., Alvarado, G., Pietragalla, J., Morgounov, A., Zelenskiy, Y., & Crossa, J. (2015). Analysis and interpretation of interactions in agricultural research. *Agronomy*, 107(2), 748-762. doi: 10.2134/agronj13.0405.

Wang, J., Shi, K., Lu, W., & Lu, D. (2020). Post-silking shading stress affects leaf nitrogen metabolism of spring maize in Southern China. *Plants*, 9(210), 1-15. doi: 10.3390/plants9020210.

Widodo, A., Khumaidi, A., & Lasongke, P. F. A. (2019). Toksisitas ekstrak etanol dan ekstrak air dari daun jotang kuda (*Synedrella nodiflora* (L.) Gaertn.), daun gandarusa (*Justicia Gendarussa* Burm.F.), dan daun pulutan (*Urena lobata* L.) dengan *brine shrimp lethality test*. *Galenika Journal of Pharmacy*, 5(2), 198-205. doi: 10.22487/j24428744.2019.v5.i2.13935.

Xu, G., Fan, X., & Miller., A. J. (2012). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, 63, 153–182. doi:10.1146/annurev-arplant-042811-105532.

Yasoda, P. G. C., Pradheeban, L., Nishanthan, K., & Sivachandiran, S. (2018). Effect of different shade levels on growth and yield performances of cauliflower. *International Journal of Environment, Agriculture and Biotechnology*, 3(3), 948-956. doi:10.22161/ijeab/3.3.30.

Zhang, J., Liu, J., Yang, C., Du, S., & Yang, W. (2016). Photosynthetic performance of soybean plants to water deficit under high and low light intensity. *South African Journal of Botany*, 105(1), 279-287. doi: 10.1016/j.sajb.2016.04.011.

Zhang, L., Sun, S., Liang, Y., Li, B., Ma, S., Wang, Z., Ma, B., & Li, M. (2021). Nitrogen levels regulate sugar metabolism and transport in the shoot tips of crabapple plants. *Frontiers in Plant Science*, 12, 626149. doi: 10.3389/fpls.2021.626149.

Zhu, K., Wang, A., Wu, J., Yuan, F., Guan, D., Jin, C., Zhang, Y., & Gong, C. (2020). Efects of nitrogen additions on mesophyll and stomatal conductance in Manchurian ash and Mongolian oak. *Science Reports*, 10, 10038. doi: 10.1038/s41598-020-66886-x.