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# Effects of Nitrogen Application Doses on the Growth and Yield of Jiaogulan under Shading Conditions in the Spring-Summer Season

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## Abstract

Jiaogulan (*Gynostemma pentaphyllum* (Thunb.) Makino) is distributed mostly in mountainous areas, in shaded and humid places at 300-3200 meters above sea level. This study was conducted to determine the appropriate application dose of nitrogen for the growth of Jiaogulan under different shading conditions. The experiment was arranged in a split-plot design with three replications in 2021. Nitrogen application dose was the main factor with three levels: N1, N2, N3 of 90, 120 and 150kg of N ha$^{-1}$, respectively. The shading condition was the sub-factor with two regimes: S1 - no shading and S2 - 30% shading (30% reduction in solar radiation using black netting). The observation parameters included main stem length, number of leaves, and number of primary branches. The physiological indicators (LAI, SPAD, dry mass) were measured at 30, 60, 90, and 120 days after transplanting. Yield and total content of saponins and flavonoids were determined at harvesting time. The results showed that the application of the 30% shading condition combined with increasing nitrogen application doses had positive effects on the growth and yield of Jiaogulan. The highest yield was obtained in the treatment combining the 30% shading condition and N3 nitrogen application level. The total flavonoid and total saponin contents did not significantly differ among the treatments. Thus, in the spring-summer season, a combination of a 30% shading condition and nitrogen application of 150 kg N ha$^{-1}$ should be applied to produce good growth and high yield as well as ensuring the quality of Jiaogulan.

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## Keywords

Jiaogulan, nitrogen fertilizer, shade, yield, quality

## Introduction

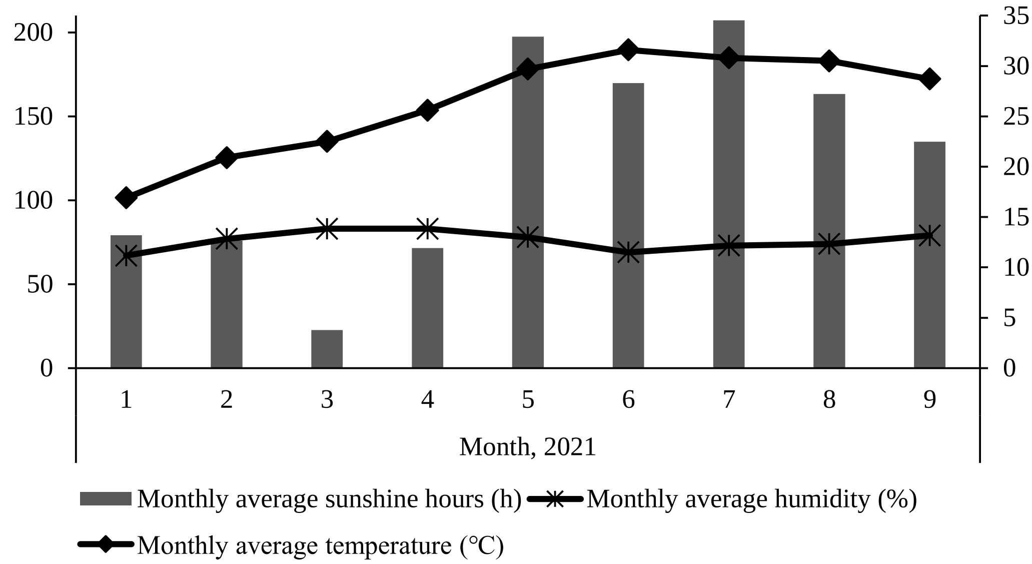
*Gynostemma pentaphyllum* (Thunb.) Makino is a perennial climbing plant belonging to the Cucurbitaceae family (China Pharmaceutical University, 1996). This crop is also known by other names including Jiaogulan, Qi Ye Dan, Gong Luo Guo Di, Pian Di Sheng Gen, Xiao Ku Yao, Amachazuru, and Penta (Valentina *et al.*, 2005). Jiaogulan is a medicinal herb with great therapeutic effects on many diseases such as inflammation, hepatitis, cancer, hematuria, pharyngeal edema, and trauma (Wu *et al.*, 1998; Blumert & Liu, 1999; Aftab *et al.*, 2023). In Japan, *G. pentaphyllum* is indicated as a medicine with diuretic, antipyretic, anti-inflammatory, and tonic properties (Tanner *et al.*, 1999; Chen, 2000). These effects are due to Jiaogulan having many pharmacological compounds, especially the abundance of saponins. The biological activity of G. pentaphyllum has been attributed to its saponins of a dammarane nature called gypenosides or gynosaponins (Cui *et al.*, 1999; Aftab *et al.*, 2023; Nizomiddin *et al.*, 2023; Wang *et al.*, 2023). Over 189 types of saponins were isolated and identified from Jiaogulan, which are known as gypenoside saponins (Gyps) (Li *et al.*, 2016). With so many great effects, Jiaogulan is used more and more widely as a medicine, functional food, herbal tea, and vegetable. As a result, natural sources of this plant are being over-exploited. Thus, research to find out the technical measures for its expanding planting areas as well as increasing its crop seasons to produce enough materials for production and consumption is essential.

*G. pentaphyllum* is distributed mostly in mountainous areas, in shaded and humid places at 300-3200 meters above sea level. In Vietnam, *G. pentaphyllum* is found naturally on Fansipan mountain, and in Lao Cai, Ha Giang, Cao Bang, Hoa Binh, and Ninh Binh provinces (Do Tat Loi, 2004). Guo & Wang (1993) indicated the ideal soil for Jiaogulan cultivation was a fertile sandy loam more than 30 cm deep, rich in humus, nitrogen, and phosphorus, and having a pH of 6.5-7.0. Jiaogulan plants prefer shady areas with air temperatures of 15-30℃. In order to increase the planting area, it is necessary to study cultivation techniques to create suitable conditions to meet the ecological requirements and nutrient needs of Jiaogulan. The Vietnam Red River Delta is a region with a hot and humid tropical climate, especially in May, June, July, and August with high radiation levels, which are unfavorable conditions for the growth of Jiaogulan. Shading, a technique to reduce solar radiation, is applied to many plants that require weak light conditions but are grown in strong light ecological areas (Moniruzzaman *et al.*, 2009; Meenakshi *et al.*, 2019; Pavithira 2022; Hongzhi *et al.*, 2023; Ornprapa *et al.*, 2023). Therefore, shading should be tested and applied to Jiaogulan in order to grow it in the Red River Delta ecological conditions. In addition, because the vegetative parts of Jiaogulan are used as medicines and dietary supplements, the application of nitrogen fertilizers needs to be carefully considered to ensure the content of useful active ingredients as well as to increase yield. Besides, many studies have shown that the combination of shading conditions with increased nitrogen fertilization brings beneficial effects on the growth of agricultural crops (Hongzhi *et al.*, 2023; Ornprapa *et al.*, 2023). Most studies of Jiaogulan have focused on extraction, chemistry, and pharmacology, while research on fertilizer application and shading techniques is still limited. Therefore, the present study was conducted to determine the appropriate application level of nitrogen and shading conditions for the growth, yield, and quality of Jiaogulan.

## Materials and Methods

### Experiment site, plant materials, and cultivation practices

The experiment was conducted at Vietnam National University of Agriculture in the Spring-Summer season of 2021, in Hanoi, Vietnam (21°0’ N, 105°55’ E). The measured initial chemical properties of the experimental soil included total N (0.09%), total P (0.18%), total K (1.33%), exchangeable N (4.25 mg/100 g), exchangeable P (50.05 mg/100 g), exchangeable K (11.75 mg/100 g), organic matter (1.65%), and pH 6.35. The climate in this region is tropical, with an average annual temperature of 25.3℃ and an average annual rainfall of 1952.2 mm. Meteorological data for the research area during the field experiment were collected at a weather station and are shown in **Figure 1**.




*Note: The right vertical axis refers to the data of monthly average temperature. The left vertical axis refers to the data of monthly sunshine hours and monthly average humidity.*

**Figure 1.** Climatic data during the entire experiment in Hanoi, Vietnam

Five-leaf Jiaogulan seedlings were used in this study. Seedlings were prepared in a greenhouse on sandy soil. The planting density was 20 plants $m^{-2}$. The applied doses of fertilizers for 1ha were 2 tons of microbial organic fertilizer, 60kg of $P_2O_5$, and 60kg of $K_2O$. The applied nitrogen doses were according to the experimental treatments. The types of fertilizers used in the experiment were Song Gianh microbial organic fertilizer (organic matter 15%, humic acid 2.5%, Ca 1.0%, strains of microorganisms: *Bacillus* $1\times10^6$ CFU/g; *Azotobacter* $1\times10^6$ CFU/g; *Aspergillus sp.* $1\times10^6$ CFU/g), urea (46% N), Lam Thao superphosphate (16.5% $P_2O_5$), and potassium chloride (60% $K_2O$). Basal applications of the microbial organic and phosphorous fertilizers were applied. The amounts of the nitrogen and potassium fertilizers were divided into two applications at 20 days and 50 days after planting.

### Experimental design, treatments, and parameters

The experiment was arranged in a split-plot design with three replications. The experimental main plot area was $10m^2$. The nitrogen application dose was the main factor with three levels: N1 - 90 kg of N $ha^{-1}$, N2 - 120 kg of N $ha^{-1}$, and N3 - 150 kg of N $ha^{-1}$. The shading conditions were the sub-factor with two conditions: S1 - No shading and S2 - 30% shading. Black netting was used to provide the 30% shading condition (30% reduction in solar radiation) and kept about 50 cm away from the surface of the Jiaogulan canopy. The observation parameters included the main stem length (measured from the base to the top of the main stem), number of leaves, and number of primary branches (branches grown from the main stem). The physiological traits (leaf area index (LAI) and dry mass) were measured at 30, 60, 90, and 120 days after transplanting (DAT). LAI ($m^2$ of leaves $m^{-2}$ of land) was determined according to the formula: LAI= (A1* Number of plants $m^{-2}$ of land)/A2*100 where A1 is the weight of whole fresh leaves of one plant (g) and A2 is the mass of $1dm^2$ of fresh leaves (g). The shoots were cut at 5 cm from the base and oven-dried at 80℃ for 48h to determine the dry mass (weight method), and saponin and flavonoid contents. The total content of saponins was measured by the recommended method of the Vietnam Ministry of Health (Vietnam Ministry of Health, 2017). The total content of the flavonoids was determined according to Pan Zhi Hao *et al.* (2012).

**Statistical analysis**

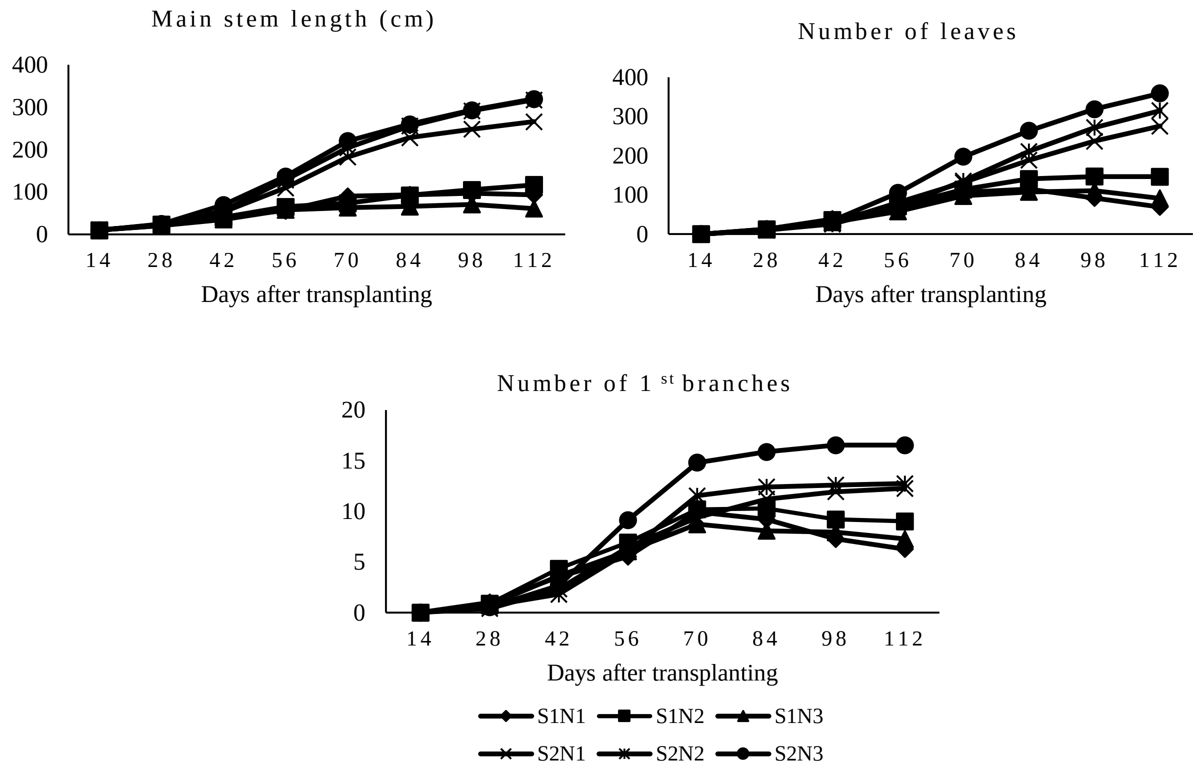
The data were subjected to ANOVA for the shading conditions, nitrogen application doses, interaction of shading condition and nitrogen application dose, and replication, using IRRISTAT 5.0. The treatment mean differences were analyzed using least significant differences (LSD) at the 5% significance level.

## Results

### Effect of nitrogen application doses and shading conditions on the growth characteristics of Jiaogulan

Changes in the plant growth characteristics of Jiaogulan due to the effects of the nitrogen fertilizer and shading conditions are shown in **Figure 1** and **Table 1**. The length of the main stem, the number of leaves, and the number of primary branches increased rapidly in all the treatments from 42 to 84 DAT. Under S1, Jiaogulan gave better growth when nitrogen was applied at N2. Under S2, the growth results of Jiaogulan with the N2 and N3 nitrogen applications were better than that of N1. The application doses of nitrogen did not significantly affect the main stem length. In contrast, the 30% shading treatment showed a statistically significant difference in this parameter of Jiaogulan in comparison with the no-shading treatment. Regarding the interaction between doses of nitrogen application and shading conditions, the S2N3 treatment showed the longest main stem among the treatments.

Concerning the number of leaves, the results show that Jiaogulan produced more leaves when nitrogen application levels were increased. Different shading conditions also had significantly different effects on the number of leaves. Under the 30% shading condition, applying 150 kg of N $ha^{-1}$ resulted in the highest number of leaves among the treatments. Regarding the number of primary branches, the nitrogen application of 150 kg $ha^{-1}$ gave the highest value (11.90), followed by 120 kg N $ha^{-1}$ (10.87), and finally 90kg of N $ha^{-1}$ (9.27).




*Note: S1 - no shading, S2 - 30% shading, N1 - 90 kg of N $ha^{-1}$, N2 - 120 kg of N $ha^{-1}$, and N3 - 150 kg of N $ha^{-1}$.*

**Figure 2.** Changes in the growth characteristics of Jiaogulan affected by nitrogen application doses and shading conditions

**Table 1.** Effects of nitrogen application doses and shading conditions on the growth of Jiaogulan

| Treatments | | Main stem length (cm) | Number of leaves | Number of primary branches |
|---|---|---|---|---|
| S1 | N1 | 93.21[cd] | 69.33[d] | 6.27[d] |
| | N2 | 116.45[c] | 146.40[c] | 9.00[c] |
| | N3 | 60.77[d] | 91.13[cd] | 7.27[d] |
| S2 | N1 | 265.75[b] | 275.27[b] | 12.27[b] |
| | N2 | 317.16[a] | 314.8[ab] | 12.73[b] |
| | N3 | 319.63[a] | 358.93[a] | 16.53[a] |
| Shading (S) | S1 | 90.14[b] | 102.29[b] | 7.51[b] |
| | S2 | 300.85[a] | 316.33[a] | 13.84[a] |
| Nitrogen application dose (N) | N1 | 179.48[a] | 172.3[b] | 9.27[c] |
| | N2 | 216.81[a] | 230.60[a] | 10.87[b] |
| | N3 | 190.20[a] | 225.03[a] | 11.90[a] |
| $LSD_{0.05}$(S) | | 51.72 | 92.23 | 2.70 |
| $LSD_{0.05}$(N) | | 28.39 | 48.05 | 0.91 |
| $LSD_{0.05}$(SxN) | | 40.14 | 67.95 | 1.28 |
| CV% | | 10.90 | 17.20 | 6.40 |
| CV% (S) | | 12.00 | 15.20 | 9.20 |

*Note: Means followed by different letters in each column for the single factors (shading condition or nitrogen application dose) or the interaction of shading condition and nitrogen application dose were significantly different in the LSD tests.*

Jiaogulan gave a statistically significantly higher number of primary branches when grown under the 30% shading condition in comparison with the no-shading condition. The interaction of different application levels of nitrogen and shading conditions also significantly affected the number of primary branches. The combination of 30% shading and a nitrogen dose of 150 kg $ha^{-1}$ gave the best effects on the number of primary branches (16.53).

## Effect of nitrogen application doses and shading conditions on the physiological characteristics of Jiaogulan

*Effect of nitrogen application doses and shading conditions on LAI*

The data in **Table 2** show that the LAI gradually increased with growing time. It can be seen that the 30% shading condition significantly increased the LAI in comparison with no shading. LAI in the 30% shading treatment was doubled compared with that in the no shading condition. Increasing the application dose of nitrogen from 90 to 120 to 150 kg $ha^{-1}$ also increased the LAI from 1.80 to 3.00 to 3.24, respectively. Statistical analysis of the interaction effect between the nitrogen application levels and shading conditions indicated that increasing the application dose of nitrogen in both the no-shading and 30% shading conditions increased LAI. Nitrogen application at the N3 level in the 30% shading condition gave the highest LAI among the treatments.

*Effect of nitrogen application doses and shading conditions on the SPAD values*

The results of **Table 3** show that the application doses of nitrogen and shading conditions clearly affected the SPAD values at 120 days after planting. The SPAD value when plants were grown under the 30% shading condition (44.44) was higher in comparison with the no-shading condition (38.14). Fertilizing with increased nitrogen application doses also increased the SPAD values; however, no statistical differences were recorded among the treatments. Although the interaction between shading conditions and nitrogen application doses had a statistically significant effect on the SPAD values, under the 30% shading condition, Jiaogulan did not show statistically significant

**Table 2.** Effects of nitrogen application doses and shading conditions on LAI

*Unit: $m^2$ of leaves $m^{-2}$ of land*

| Treatments | | Days after transplanting | | |
|---|---|---|---|---|
| | | 60 | 90 | 120 |
| S1 | N1 | $0.89^c$ | $0.98^e$ | $1.05^d$ |
| | N2 | $0.89^c$ | $1.31^d$ | $1.80^c$ |
| | N3 | $0.88^c$ | $1.98^c$ | $2.11^c$ |
| S2 | N1 | $1.63^b$ | $2.50^b$ | $2.54^b$ |
| | N2 | $2.30^a$ | $3.81^a$ | $4.19^a$ |
| | N3 | $2.34^a$ | $3.64^a$ | $4.37^a$ |
| Shading (S) | S1 | $0.89^b$ | $1.42^b$ | $1.66^b$ |
| | S2 | $2.09^a$ | $3.42^a$ | $3.70^a$ |
| Nitrogen application dose (N) | N1 | $1.26^b$ | $1.74^c$ | $1.80^b$ |
| | N2 | $1.60^a$ | $2.56^b$ | $3.00^a$ |
| | N3 | $1.61^a$ | $2.81^a$ | $3.24^a$ |
| $LSD_{0.05}$(S) | | 0.14 | 0.52 | 0.28 |
| $LSD_{0.05}$(N) | | 0.14 | 0.15 | 0.25 |
| $LSD_{0.05}$(SxN) | | 0.20 | 0.21 | 0.35 |
| CV% (S) | | 13.4 | 14.0 | 15.0 |
| CV% | | 7.10 | 4.80 | 7.00 |

*Note: Means followed by different letters in each column for the single factors (shading condition or nitrogen application dose) or the interaction of shading condition and nitrogen application dose were significantly different in the LSD tests.*

differences in SPAD values among the different nitrogen level treatments.

*Effect of nitrogen application doses and shading conditions on dry mass*

There were significant differences in the dry mass of Jiaogulan among the treatments at all observation stages (30, 60, 90, and 120 DAT) (**Table 4**). The dry mass increased most rapidly during the stage of 60 to 90 DAT. Dry mass ranged from 170.77 g $m^{-2}$ (S1N3) to 381.37 g $m^{-2}$ (S2N3). The 30% shading condition provided higher dry mass than the no shading condition at all growth stages. Under the 30% shading condition, increasing the application amount of nitrogen also increased the dry mass of Jiaogulan. However, under the no-shading condition, in the period of 90 to 120 DAT, due to hot weather conditions, the growth of plants in the N3 treatment was seriously affected, and dry weight was greatly reduced. The interaction between shading conditions and nitrogen application doses had a statistically significant effect on the dry mass of Jiaogulan. The highest dry mass was recorded when applying nitrogen at the N3 level under the 30% shading condition among the treatments (381.37 g $m^{-2}$).

**Effect of nitrogen application doses and shading conditions on the yield and quality of Jiaogulan**

The yield of Jiaogulan was significantly affected by the interaction of shading conditions and different application doses of nitrogen during the growth period. Under the no-shading condition, increasing the application levels of nitrogen from N1 to N2 increased the dry yield from 2.05 to 2.55 tons of dry mass $ha^{-1}$, while the yield decreased dramatically in the N3 treatment (1.71 tons of dry mass $ha^{-1}$). Under the 30% shading condition, increasing the nitrogen application doses from N1 to N2 to N3 increased dry yield from 2.64 to 2.81 to 3.81 tons of dry mass $ha^{-1}$, respectively (**Table 5**). The different yields among the treatments under the interaction effect might have been driven by the different influences of both the two single factors of

**Table 3.** Effects of nitrogen application doses and shading conditions on SPAD values

| Treatments | | Days after transplanting | | | |
|---|---|---|---|---|---|
| | | 30 | 60 | 90 | 120 |
| Shading (S) | S1 | 41.48$^{a}$ | 42.38$^{a}$ | 44.00$^{a}$ | 38.14$^{b}$ |
| | S2 | 40.43$^{a}$ | 39.32$^{a}$ | 43.60$^{a}$ | 44.44$^{a}$ |
| Nitrogen application dose (N) | N1 | 40.23$^{a}$ | 39.06$^{a}$ | 42.32$^{a}$ | 39.67$^{a}$ |
| | N2 | 41.14$^{a}$ | 40.94$^{a}$ | 44.41$^{a}$ | 42.50$^{a}$ |
| | N3 | 41.50$^{a}$ | 42.47$^{a}$ | 44.67$^{a}$ | 41.70$^{a}$ |
| S1 | N1 | 40.72$^{a}$ | 39.82$^{a}$ | 41.10$^{a}$ | 35.78$^{b}$ |
| | N2 | 42.80$^{a}$ | 43.40$^{a}$ | 46.34$^{a}$ | 40.25$^{ab}$ |
| | N3 | 40.93$^{a}$ | 43.74$^{a}$ | 44.55$^{a}$ | 38.38$^{ab}$ |
| S2 | N1 | 39.73$^{a}$ | 38.30$^{a}$ | 43.53$^{a}$ | 43.55$^{a}$ |
| | N2 | 39.48$^{a}$ | 38.45$^{a}$ | 43.85$^{a}$ | 44.25$^{a}$ |
| | N3 | 42.08$^{a}$ | 41.20$^{a}$ | 44.78$^{a}$ | 45.02$^{a}$ |
| $LSD_{0,05\%}$(S) | | 3.74 | 13.23 | 9.68 | 1.84 |
| $LSD_{0,05\%}$(N) | | 2.52 | 4.03 | 3.75 | 5.07 |
| CV%(S) | | 4.56 | 16.20 | 11.05 | 7.00 |
| $LSD_{0,05\%}$(CxN) | | 3.57 | 5.7 | 5.31 | 7.16 |
| CV% | | 4.60 | 7.40 | 6.40 | 9.20 |

*Note: Means followed by different letters in each column for the single factors (shading condition or nitrogen application dose) or the interaction of shading condition and nitrogen application dose were significantly different in the LSD tests.*

shading condition and nitrogen application level. The 30% shading condition provided 9.23 tons of fresh mass ha$^{-1}$ and 0.99 tons of dry mass ha$^{-1}$ of yield of Jiaogulan, which were higher than plants under the no shading condition. Increasing the application doses of nitrogen from N1 to N2 to N3 increased the yield from 2.35 to 2.68 to 2.76 tons of dry mass ha$^{-1}$. Therefore, the results showed that combining the 30% shading condition and the nitrogen application of 150 kg ha$^{-1}$ gave the highest yield of Jiaogulan.

**Table 5** shows that there were no significant differences in the total saponin and total flavonoid contents among the treatments. This indicates that the shading conditions or different application doses of nitrogen did not cause statistical differences in the contents of saponins and flavonoids. However, due to the increase of yield in N2 and N3 under the 30% shading condition, the amount of saponins and flavonoids per cultivated unit area were higher than under the no shading condition.

## Discussions

### Application of the 30% shading condition created favorable ecological conditions for Jiaogulan to grow well and produce a high yield

Jiaogulan is a medicinal plant that prefers shaded and cool climates with temperatures ranging from 15 to 30°C. If Jiaogulan is grown in such outdoor conditions, the plants will grow well, producing large biomasses and high active ingredient contents (Guo & Wang, 1993). In our experiment, the 30% shading condition created suitable lighting regimes with Jiaogulan's ecological demands, and thus, the growth, development, and yield indicators in the 30% shading condition were higher than those in the no shading condition.

**Table 4.** Effects of nitrogen application doses and shading conditions on dry mass

*Unit: g $m^{-2}$*

| Treatments | | Days after transplanting | | | |
|---|---|---|---|---|---|
| | | 30 | 60 | 90 | 120 |
| S1 | N1 | $16.67^{b}$ | $81.60^{d}$ | $221.79^{c}$ | $204.68^{c}$ |
| | N2 | $15.47^{b}$ | $80.80^{d}$ | $218.59^{c}$ | $25523^{b}$ |
| | N3 | $10.80^{c}$ | $116.47^{c}$ | $354.03^{a}$ | $170.77^{c}$ |
| S2 | N1 | $16.47^{b}$ | $123.40^{bc}$ | $209.11^{c}$ | $281.47^{b}$ |
| | N2 | $14.13^{bc}$ | $137.73^{b}$ | $251.16^{b}$ | $263.88^{b}$ |
| | N3 | $24.80^{a}$ | $180.13^{a}$ | $269.69^{b}$ | $381.37^{a}$ |
| Shading (S) | S1 | $14.31^{b}$ | $92.96^{b}$ | $264.80^{b}$ | $210.23^{b}$ |
| | S2 | $18.47^{a}$ | $147.09^{a}$ | $243.32^{a}$ | $308.91^{a}$ |
| Nitrogen application dose (N) | N1 | $16.57^{ab}$ | $102.50^{b}$ | $215.45^{b}$ | $243.07^{b}$ |
| | N2 | $14.80^{b}$ | $109.27^{b}$ | $234.87^{b}$ | $259.55^{ab}$ |
| | N3 | $17.80^{a}$ | $148.30^{a}$ | $311.86^{a}$ | $276.07^{a}$ |
| $LSD_{0.05}$(S) | | 4.20 | 3.97 | 26.00 | 75.61 |
| $LSD_{0.05}$(N) | | 2.37 | 11.66 | 19.74 | 30.05 |
| $LSD_{0.05}$(SxN) | | 3.34 | 16.49 | 27.91 | 42.50 |
| CV% | | 10.80 | 7.30 | 5.80 | 8.70 |
| CV% (S) | | 14.3 | 13.3 | 12.8 | 12.7 |

*Note: Means followed by different letters in each column for the single factors (shading condition or nitrogen application dose) or the interaction of shading condition and nitrogen application dose were significantly different in the LSD tests.*

**Table 5.** Effects of nitrogen application doses and shading conditions on the contents of flavonoids and saponins, and the yield of Jiaogulan

| Treatments | | Total flavonoids (%) | Total saponins (%) | Fresh yield (tons of fresh mass $ha^{-1}$) | Dry yield (tons of dry mass $ha^{-1}$) |
|---|---|---|---|---|---|
| S1 | N1 | 2.36 | 2.00 | $7.58^{e}$ | $2.05^{c}$ |
| | N2 | 2.39 | 2.30 | $11.00^{d}$ | $2.55^{b}$ |
| | N3 | 1.63 | 2.30 | $7.08^{e}$ | $1.71^{c}$ |
| S2 | N1 | 2.47 | 2.23 | $15.67^{c}$ | $2.64^{b}$ |
| | N2 | 2.52 | 2.73 | $17.38^{b}$ | $2.81^{b}$ |
| | N3 | 2.53 | 2.83 | $20.33^{a}$ | $3.81^{a}$ |
| Shading (S) | S1 | 2.20 | 2.28 | $8.56^{b}$ | $2.10^{b}$ |
| | S2 | 2.51 | 2.60 | $17.79^{a}$ | $3.09^{a}$ |
| Nitrogen application dose (N) | N1 | 2.42 | 2.12 | $11.63^{b}$ | $2.35^{b}$ |
| | N2 | 2.46 | 2.52 | $14.19^{a}$ | $2.68^{ab}$ |
| | N3 | 2.08 | 2.68 | $13.71^{a}$ | $2.76^{a}$ |
| $LSD_{0.05}$(S) | | - | - | 1.73 | 0.75 |
| $LSD_{0.05}$(N) | | - | - | 0.95 | 0.30 |
| $LSD_{0.05}$(SxN) | | - | - | 1.34 | 0.43 |
| CV% | | 6.03 | 9.36 | 5.40 | 8.80 |
| CV% (S) | | 12.05 | 12.68 | 10.03 | 14.49 |

*Note: Means followed by different letters in each column for the single factors (shading or nitrogen application dose) or the interaction of shading condition and nitrogen application dose were significantly different in the LSD tests.*

The plant height, number of leaves, number of primary branches, LAI, dry mass, and yield of Jiaogulan in the 30% shading condition were significantly higher in comparison with the no-shading condition. This is similar to the research results of Chen *et al.* (1991) in China who reported that the dry weight of Jiaogulan was 0.15 kg $m^{-2}$ under a shaded treatment while 0.035 kg $m^{-2}$ in an open area treatment. The dry mass and yield of Jiaogulan in this experiment were higher than plants grown in Beijing, China (Chen *et al.*, 1991), and similar to the research results of Luu *et al.* (2021) in Vietnam. Meenakshi *et al.* (2019) reported that damask rose's LAI values increased when plants were grown under 25% and 50% shading conditions in comparison with the control (0% shade). Guenni *et al.* (2008) indicated that shade as a single factor can improve shoot growth in C4 perennial grasses commonly used as forage plants in the tropics. Kirk *et al.* (2002) also reported that applying a 33% shaded condition increased the growth, development, and yield parameters of pawpaw plants such as plant height, number of leaves, LAI, dry mass, and yield. Pavithira (2022) indicated that cabbage grown at a 70% shading level obtained higher growth and yield in a low country dry zone. The results in our study also clearly showed that Jiaogulan grew well and had high yields when grown in shaded conditions. Thus, shaded conditions should be applied for planting Jiaogulan in areas with ecological conditions of strong light. This will create opportunities to expand the planting area and increase productivity to meet the needs of humans.

**Under the 30% shading condition, increasing the application dose of nitrogen had good effects on Jiaogulan growth and yield while ensuring quality**

The positive effects of nitrogen fertilizer on plant growth and yield have been reported in many published papers, especially for plants in which the harvestable parts are the stems and leaves. The yield of cabbage has been shown to increase when the amount of nitrogen fertilizer is increased (Timothy *et al.*, 2022). Wenping *et al.* (2022) reported that nitrogen nutrition improved the weight of Chinese flowering cabbage (*Brassica campestris* L. ssp. chinensis var. utilis Tsen et Lee). The addition of nitrogen also stimulated a potato plant's source capacity and consequently increased tuber yield (Zewide *et al.*, 2012; Li *et al.*, 2016). The green color of plant leaves, cell elongation, growth of vegetative parts, and chlorophyll content were encouraged by nitrogen applications (Brady, 1990), resulting in Bangladhonia leaves that were succulent and soft (Islam *et al.*, 2003). In addition, if creating environmental conditions close to the plant's ecological demands when planting in unsuitable regions or seasons, producers can combine other cultivation measures to promote growth and development, and increase productivity. Moniruzzaman *et al.* (2009) indicated that for optimizing yield and quality, Bangladhonia (*Eryngium foetidum* L.) needs to be grown in places where 25-50% shade is available coupled with the application of 161kg of N $ha^{-1}$. Domingos *et al.* (2011) reported that the application of shading combined with nitrogen fertilizer increased leaf length and the leaf elongation rate of *Brachiaria* species. Shading stimulates the response of the plants to the application of nitrogen which increases leaf growth. Combining shading conditions and increasing the amount of nitrogen fertilizer also increased the yield of vegetable fern (Ornprapa *et al.*, 2023).

The results of our experiment are in agreement with previous studies (Domingos *et al.*, 2011; Timothy *et al.*, 2022; Wenping *et al.*, 2022), in that the growth and yield of Jiaogulan were better in treatments combining the 30% shading condition and nitrogen application doses, and yields were higher in treatments with higher nitrogen fertilizer concentrations. Concerning quality, in our study, the total flavonoid and total saponin contents of Jiaogulan did not significantly differ among the treatments. However, under the 30% shading condition, the total contents of these two substances in the N2 and N3 nitrogen levels were slightly higher than in the N1 treatment. Thus, growing Jiaogulan in the 30% shading condition combined with nitrogen fertilization both increased productivity and ensured the medicinal contents. This will be meaningful in practical production as it can both expand growing areas and increase productivity while still ensuring the quality of Jiaogulan to meet the purpose of medicinal herb production as well as daily consumption.

## Conclusions

Jiaogulan growth was much affected by the interaction of shading conditions and nitrogen application doses. At the early growth stages, increasing the nitrogen application dose increased plant growth under both the 30% shading and no shading conditions. At the later growth stages, under the 30% shading condition, increasing the nitrogen application dose continuously increased plant growth. However, the N3 treatment dramatically decreased plant growth under the no-shading condition. The highest Jiaogulan yield was recorded in the combination treatment of N3 with the 30% shading condition. There were no significant differences in the total flavonoid and total saponin contents of Jiaogulan among the treatments. Thus, in the spring-summer crop season, a combination of a 30% shading condition and nitrogen application of 150 kg N $ha^{-1}$ should be applied to produce great growth and high yield as well as ensuring the quality of Jiaogulan.

## References


Aftab A., Muhammad A. S., Farhan S., Muhammad A., Ali I., Muhammad N., Saadia A., Muhammad I., Muzzamal H. & Entessar A. J. (2023). *Gynostemma pentaphyllum* an immortal herb with promising therapeutic potential: a comprehensive review on its phytochemistry and pharmacological perspective. International Journal of Food Properties. 26(1): 808-832.

Blumert M. & Liu J. L. (1999). Jiaogulan: China's ''Immortality Herb". Torchlight Publishing, Inc., Badger, CA 93603.

Brady N. C. (1990). The Nature and Properties of Soils, 10th Ed. MacMillan Pub.Co., New York.

Chen J. (2000). A novel Cdc2-related protein kinase, is required for hyphal development and virulence in *Candida albicans*. Molecular Cell Biology. 20(23): 696-708.

Chen Z., Zhao Y., Ma X., Lu R. & Song J. (1991). Introduction and cultivation of *Gynostemma pentaphyllum* Mark. in Beijing. Zhongguo Zhong Yao Za Zhi. 16(4): 208-211.

China Pharmaceutical University (1996). Encyclopedia of Chinese Herbs Vol2, 1st Ed. China Medicine, Science and Technology Publisher, China.

Cui J., Eneroth P. & Bruhl. J. G. (1999). *Gynostemma pentaphyllum*: identification of major sapogenins and differentiation from Panax species. European Journal of Pharmaceutical Science. 8(3):187-91.

Deng M., Zhong S., Ren B. & Tu Z. M. (2000). Studies on the effect of illumination intensity on the content of total saponin in *Gynostemma pentaphylllum* (Thunb.) Makino. Acta Academiael Hubei. 102-103.

Do Tat Loi (2004). Medicinal plants and medicine in Vietnam. Medicine Publisher. Viet Nam. (in Vietnamese).

Domingos S. C. P., Priscila B. F., Carlos A. de M. G., Carlos R. T. de C., Fausto de S. S. & Carlos A. B. de C. (2011). The growth dynamics in Brachiaria species according to nitrogen dose and shade. Revista Brasileira de Zootecnia. 40 (2): 270-276.

Guenni O., Seiter S. & Figueroa S. (2008). Growth responses of three Brachiaria species to light intensity and nitrogen supply. Tropical Grasslands. 42: 75-87.

Guo W. Y. & Wang W. X. (1993). Cultivation and utilization of *Gynostemma pentaphyllum*. Publishing House of Electronics. Science and Technology University. China.

Hongzhi Z., Qi Z., Zhong W., Lihong W., Xiaorong L., Zheru F., Yueqiang Z., Jianfeng L., Xin G., Jia S. & Fu C. (2023). Effects of Nitrogen Fertilizer on Photosynthetic Characteristics, Biomass, and Yield of Wheat under Different Shading Conditions. Agronomy. 11: 1989.

Islam, M. R., Mozumder S. N., Moniruzzaman M. & Alam S. N. (2003). Effects of N, P, K on yield and profitability of Bilatidhonia in the hilly region. Bangladesh Journal of Agricultural Research. 28(1): 105-110.

Kirk W. P., Desmond R. L. & Snake C. J. (2002). Incident Irradiance and Cupric Hydroxide Container Treatment Effects on Early Growth and Development of Container-growth Pawpaw Seedlings. Journal of the American Society for Horticultural Science. 127(1): 13-19.

Li W., Xiong B., Wang S., Deng X., Yin L. & Li. H. (2016). Regulation effects of water and nitrogen on the source-sink relationship in potato during the tuber bulking stage. PLoS ONE. 11: e0146877.

Li Y., Lin W., Huang J., Xie Y. & Ma W. (2016). Anticancer effects of *Gynostemma pentaphyllum* (Thunb.) Makino (Jiaogulan). Chinese Medicine. 11: 43.

Luu V. H., Nguyen T. T. & Vu M. L. (2021). Development and productivity of Gynostemma pubescens, Gymnema silvestre and Curculigo orchioides cultivated in Dai Dinh commune, Tam Dao district, Vinh Phuc province. Journal of Science on Natural Resources and Environment. 36: 57-63 (in Vietnamese).

Meenakshi T., Vinod B. & Rakesh K. (2019). Effect of shade level and mulch type on growth, yield and essential oil composition of damask rose *(Rosa damascena* Mill.) under mid hill conditions of Western Himalayas. PLoS One. 4(4):e0214672.

Moniruzzaman M., Islam M. S., Hossain M. M. & Miah M. G. (2009). Effects of shade and nitrogen levels on quality bangladhonia production. Bangladesh Journal of Agriculture Research. 34(2): 205-213.

Nizomiddin K., Elvira G., Alfina N., Natalya K., Renata G. & Svetlana R. (2023). Biological study of *Gynostemma pentaphyllum* (Thunb.) Makino. BIO Web of Conferences. 93:02001. Forestry Forum 2023.

Ornprapa T., Rantiya I. & Preuk C. (2023). The Effects of Shading and Nutrient Management on Yield Quality of Vegetable Fern. Horticulturae. 9: 259.

Pan Z. H., Ye Z. Z. & Sheng M. (2012). Study on extraction process of total flavonoids from *Gynostemma pentaphyllum*. Journal of Guangdong Pharmaceutical University. 28(1): 44-46.

Pavithira R. (2022). The Impact of Shading on Growth and Yield of Cabbage (*Brassica oleracea* L.) in the Low Country Dry Zone, Ampara, Sri Lanka. OUSL Journal. 17(1): 35-47.

Tanner M. A., Bu X., Steimle J. A. & Myers P. R. (1999). The direct release of nitric oxide by gypenosides derived from the herb *Gynostemma pentaphyllum*. Nitric Oxide. 3(5): 359-365.

Timothy C., Kate C. D. & Andre L. B. R. da S. (2022). Influence of Nitrogen Rate, Fertilizer Type, and Application Method on Cabbage Yield and Nutrient Concentrations. Horttech. 32(2): 134-139.

Valentina R. N., George Q. L. & Colin. C. D. (2005). *Gynostemma pentaphyllum* cultivation in Sydney, Australia and its comparison with products from China. Journal of Applied Horticulture. 7(2): 99-104.

Vietnam Ministry of Health (2017). Vietnam Pharmacopoeia. *Gynostemma pentaphyllum*. Medical publisher. Hanoi, Vietnam.

Wang Z., Yang J., Zhang Z., Zhu X., Xiang P., Sun J. & He X. (2023). Chemical constituents and biological activity of *Gynostemma pentaphyllum*: A review. Journal of Southern Agriculture. 54(6): 1741-1752.

Wei C., Sun Q., Peng Z. & Yan D. (2003). Light environment characteristics of forest gap in deciduous broad-leaved forest and its effects on growth features of *Gynostemma pentaphyllum* in Jianghuai watershed. The Journal of Applied Ecology. 665-670.

Wenping L., Małgorzata M. P. & Tomasz K. (2022). Effect of Nitrogen Nutrition and Planting Date on the Yield and Physicochemical Parameters of Flowering Chinese Cabbage. Agronomy. 12(11): 2869.

Wu Y., Zhang Y., Wu J. A., Lowell T., Gu M. & Yuan C. S. (1998). Effects of Erkang, a modified formulation of Chinese folk medicine Shi-Quan-Da-Bu-Tang, on mice. Journal of Ethnopharmacoly. 61: 153-159.

Zewide I., Mohammed A. & Tulu S. (2012). Effect of different rates of nitrogen and phosphorus on yield and yield components of potato (*Solanum tuberosum* L.) at Masha District, Southwestern Ethiopia. International Journal of Plant and Soil Science. 7: 146.