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Effects of light on growth and biomass of *Dendrobium officinale* (Kimura et Migo) grown in ThaiNguyen province, Vietnam

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Abstract

Dendrobium officinale Kimura et Migo (DO), one of the valuable and scarce medicinal orchids, in nature. Applying technical measures to regulating growth, increasing artificial plant biomass and conserving this herb is necessary. This research was conducted to evaluate the effect of shade treatments (30, 50, 70, and 90%) and light color (white, purple, blue, and red) on growth and biomass of the DO under greenhouse conditions in Thai Nguyen province, Vietnam. The results revealed that shading 50% and 70% significantly influenced on growth and biomass. Using red light is the best for growth, biomass, and increasing contents of polysaccharide and alkaloid. These results should be recommented in production technology of the DO.

Keywords: *Dendrobium officinale*; Light; Growth; Biomass; Greenhouse

1. Introduction

Dendrobium officinale Kimura et Migo is a scarce herbaceous plant belonging to the Orchidaceae family. In ChineseTraditional Medicine, the *Dendrobium officinale* (DO) has preeminent functions including benefiting the stomach, increasing body fruids and boosting immunity (1). It is reported that this herb contained about 190 compounds of which polysaccharides and alkaloids are main chemical compositions having potetial antioxidant effect and high efficiency in the treatment of diabetes, heart-related diseases and cancer (2,3,4).1 kg of dry stem trated for 80.000 Yuan (5,6). Although the artificial cultivation of the DO wasconcerned, the biomass production is still difficult due to the slow growth rate and exhibited the lower yield, and contents of main active ingredients compared to the nature growth.

The former studies reported that ligh quality affects on plant development and growth. The LED light is more suitable than fluorescent light for plant growth of Euphorbia milii, Potato, Zantedeschia, Chrysanthemum, Gossypium hirsutum (7,8,9,10,11). Liu et al. (2019) claimed that flavonoid biosynthesis of Syringa oblata Lindl. in response to different light intensity (12). Fritz and Klaus (1973) reported thatthe light has a role in stimulating the synthesis of flavonoid glycosides in cell culture of Petroselinum hortense (13).The effects of light on stimulation of anthocyanin inPerilla frutescens (14),artemisinin of Artemisia annua (15) were proven. Therefore, the regulating light quality to increase biomass and contents of main active ingredients of the DO is completelyfeasible. However, there was no study on suitable light quality for growth, biomass accumulation, and main active ingredients of the DO under greenhouse conditions in ThaiNguyen province (Vietnam) and similar conditions. Thus, the studyaimed to investigate the growth and biomass of *Dendrobium officinale* to light in ThaiNguyen province, Vietnam.

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2. Materials and methods

2.1. Plantmaterialsand growth conditions

The experiments were conducted consecutively from April 2021 to September 2022 in green house at the Institute of Life Science, located at ThaiNguyen city, Northern Vietnam(21°40′N 105°50′E). The averagesolarradiationper day during the experimental periodis 4.985 Wh/m^2/day.

The DO plants have one main stem with 8-8.5 cm long, 3-4 mature leaves, 2-3 roots used as experimental materials. Plants are fresh of pests and diseases, growing normally in pots of 10 x 12 cm. The substrated medium is with the proportion of 40 % coconut fiber, 30% fern roots and 30% charcoal. The greenhouse conditions consist temperature of 18-25 ^{o}C, humidity of 70-90%.

2.2. Experimental Design

Experiment 1: Determine effects of shade on growth, biomass, and contents of polysaccharides and alkaloids ofthe DO plant

The effect of shade treatments on growth and biomass of the DO plant was designed covering by shade sun cloth net(Shadow rate: 30-90%). Four kinds of shade sun cloth netswere used for treatments (T) to creat differences ofshade: 30% of full sunlight (T1), 50% of full sunlight (T2), 70% of full sunlight (T3), 90% of full sunlight (T4). The experiment was established inRandomized Complete Block, with each treatment replicated three times and ten DO plantsfor each replication per a treatment.

Experiment 2: Determine effects of monochromatic light on growth, biomass and content of polysaccharides and alkaloids in DO plant

The effect of light color on growth and biomass of the DO plant was designed by using colour light filters(British standard BS3944). Four treatments werecreated: white light-λ=400-760nm (T1); purple light -λ=400-430nm (T2); blue light-λ=510-560nm (T3) and red light-λ=610-730nm.All experiments used a Randomized Complete Block Designwith three replicates per treatment, and ten DO plants for each replication per a treatment.

2.3. Growth parameters

The growth parameters includingshoot multiplier (times), change in plant height (mm), stem diameter (mm), number of leaves (leaves) and number of roots (roots) were determined 90 days after planting.

2.4. Contents of polysaccharides and alkaloids

The stem of the DO after harvesting was washed by distilled water and dried by freeze-drying (-40 ^{o}C) for 24h. The moisture of herbal samples is 7-10%. The 50-mesh sieved dry powder were separately extracted with methanol at concentration 60% (vol/vol) at 50 ^{o}C for 90 min by ultrasonic and then was filtered. The filtrates were combined and concentrated in a vaccuum evaporator at 45 ^{o}C. The dehydrated fractionation was weighted to calculate yield, then dissolved in DMSO to a ragulator concentration and the contentsof polysaccharide and alkaloid were measured.

The polysaccharide content was determined according to the phenol- sulfuric acid method (16). The monosaccharides from hydrolysis reaction of polysaccharides is used for reacting with phenol in an acidic medium to produce a yellow-gold color. The absorbance was measured at 490 nm. Total polysaccharide contents were calculated from a calibration curve using various concentration of glucose (% of dry materials)

The alkaloid content was determined according to the method based on the reaction of alkaloid with bromocresol green, forming a yellow colored product (17).

2.5. Statistical analysis

The data were statistically analyzed to determine the difference of treatments. The level of least significane difference (LSD) was determined using Duncan's multiple range test at $p <0.05$ for comparing means of the treatments. All analyses were performed using the SAS statistical package (SAS Intitute, 1990).

3. Results and discussion

3.1. Effects of shadeon growth, biomass and polysaccharide, and alkaloid content of the DO plant

3.1.1. Growth parameters of DO plantresponse to shade:

Table 1 Effects of shade on growth of the DO plant (after 90 days)

Treat.	Shade treatments	Shoot multiplier (times)	Change in plant height (mm)	Changein stem diameter (mm)	Change in number of leaves (leaves)	Change in number of roots (roots)
T1	30%	1.30[b]	17.90[b]	1.34[ab]	1.07[a]	1.00[b]
T2	50%	2.07[a]	19.61[a]	1.40[a]	1.12[a]	1.10[a]
T3	70%	1.97[a]	19.00[a]	1.32[ab]	1.06[a]	1.04[ab]
T4	90%	1.13[b]	16.97[c]	1.26[b]	0.70[b]	0.85[c]
P ≤ 0,05		<0.05	<0.05	<0.05	<0.05	<0.05
CV%		9.05	2.85	3.70	3.62	3.62
LSD0,05		0.29	1.04	0.10	0.07	0.07

[a-c]values with different superscripts were significantly different at p <0.05.

The results show that using sunshade nets with 30-90% shade in June and August in ThaiNguyen province differently affects growth parameters of the DO plant (Table 1). The DO plant growing under 50% and 70% shade had higher parameters (parameters including shoot multiplier, change in plant height, stem diameter, number of leaves, and number of roots) compared to the DO plantunder 30% and 90% shade. The shoot multiplier, change in plant height, stem diameter, number of leaves, and number of roots for shading 50% and 70% of full sunlight are 2.07 times, 19.61 mm, 1.4 mm, 1.12 leaves, 1.1 roots and 1.97 times, 19.00 mm, 1.32 mm, 1.06 leaves, and 1.04 roots, respectively. There are no significant different between two for light covarge rate of 50% and 70% (p<0.05).

3.1.2. Biomass and contents of polysaccharides and alkaloids of the DO plant response to shade:

Table 2 Effects of shade on plant weight and contents of polysaccharides and alkaloids of the DO plant (after 90 days)

Treat.	Shade treatments	Change in plant weight(g)	Polysaccharide content(%dry materials)	Alkaloid content (mg/100 g dry materials)
T1	30%	15.00[b]	25.45[a]	141.73[c]
T2	50%	21.70[a]	26.10[a]	147.00[a]
T3	70%	20.50[a]	26.00[a]	146.57[ab]
T4	90%	12.00[c]	23.95[b]	130.50[d]
P ≤ 0.05		<0.05	<0.05	<0.05
CV%		3.92	1.55	0.62
LSD0.05		0.36	0.79	1.73

[a-d]values with different superscripts were significantly different at p <0.05.

The plant weight and content of main active ingredients of the DO were also affected by shade treatments (Table 2). The changes in plant weights of shade treatments havevalues from 12g to 21.7 g, of which, the highest change in plant weight was recored in 50% and 70% shading (21.7g and 20.5g, respectively) being higher than in 30% and 90% shading (15g and 12g, respectively) (Table 2).

The shade treatments significantly influenced to polysaccharides and alkaloid contents of the DO (p≤ 0.05).The polysaccharide contents ranged from 23.95% to 26.1%, of which the highest values are 26.1% and 26% in shading 50%

and 70% (T2 and T3, respectively). These are higher than T1 and T4 (Table 2). In addition, the alkaloid contents ranged from 130.5 and 147.0 mg/100 g of dry materials, ofwhich the highest and lowest values are 147.0 mg/100 g of dry materials and 130.5 mg/100 g of dry materials in T2 (shade 50%) and T4 (shade 90%). There are no significant difference between T2 (shade 50%) and T3 (shade 70%) (P<0.05).These results indicated that the DO is a sciophyte species. The too low light intensity (shade 30%) or too high light intensity (shade 90%) is unsuitable for its growth.

The light intensities are one of the important environmentfactors affectting to the growth and polysaccharide, and alkaloid content in plant (12,18,19,20,21,22). Photosynthetic activity of plants was depressed due to photoinhibition under too low or too hight shade (18). Shadingis an effective method to make a suitable environment for plant growth in greenhouse in hot and sunny regions (21). In this experiment, DO plant achieved the best growth and polysaccharides and alkaloid content in shading 50% and 70% of the nature light. The results demonstrate that suitable light shading (50% and 70% of nature light in ThaiNguyen province) has the effects of enhancing growth and accumulation of polysaccharides and alkaloid in DO plant.This result is consensus with characteristics of growth conditions of DO plant (a shade-tolerant species) under natural forest in Vietnam. In agreement, Zhang et al. (2004) reported that shade-tolerant plant (Hosta cv. Antioch và Golden Edger) achived maximal photosynthetic rate in 50 % of full irradiance (23).Similarly, shading above approximately 60% of full sunlight is optimal for photosynthesis and growth of the 'Bluecrop' blueberry.In constract, Yueping et al.(2012) reported that 85% or 88% shading isan effective method for growth and polysaccharide, and alkaloid content in Dendrobium candidum plants in Zhejiang Province in China (18), whereas Youn et al. (2012) claimed that 35% shade of nature light in Republic of Korea gave growth and biomass in Heracleum moellendorffii and Adenophora divaricata (24).This observed difference may be the related response of the genotypes of the plant materials used to light intensities.

3.2. Effects of monochromatic light on growth, biomass and content of polysaccharide and alkaloidof the DO plant.

3.2.1. Growth parameters of DO plant response to monochromatic light:

Table 3 Effect of monochromatic light on growth of the DO plant (after 90 days)

Treat.	**Type of monochromatic light(λ)**	**Shoot multiplier (times)**	**Change in plant height (mm)**	**Change in stem diameter (mm)**	**Change in number of leaves (leaves)**	**Change in number of roots (roots)**
T1	White (λ=400-760nm)	2.23[a]	17.81[a]	1.33[a]	1.08[a]	0,98[a]
T2	Purple (λ=400-430nm)	1.27[c]	16.61[b]	1.10[c]	0.73[c]	0,90[a]
T3	Blue (λ=510-560nm)	1.73[b]	16.80[b]	1.20[b]	0.98[b]	0,97[a]
T4	Red (λ=610-730nm)	2.10[a]	18.45[a]	1.36[a]	1.11[a]	0,95[a]
P ≤ 0.05		<0,05	<0.05	<0.05	<0.05	<0.05
CV%		8.13	2.77	4.37	4.79	2.37
LSD0,05		0.30	0.96	0.09	0.14	0.06

[a-d]values with different superscripts were significantly different at p <0.05.

The parameters as shoot multiplier, change in plant height, stem diameter, number of leaves, and number of roots varied significantly with respects to kind of monochromatic light (P<0.05). The best results of shoot multiplier, change in plant height, stem diameter, number of leaves, number of roots of DO plants under white and red light were abtained by 2.23 times, 17.81 mm, 1.33 mm, 1.08 leaves, 0.98 roots and 2.10 times, 18.45 mm, 1.36 mm, 1.11 leaves, and 0.95 roots, respectively, which was shown in Table 3. And there is no significant difference in growth parameters between red and white light treatments.For shoot multiplier, change in plant height, stem diameter, number of leaves, and the growth of DO plant in blue and purple light treatments was lower than in white and red light treatments (p< 0.05). For change in number of roots of DO plant, no significant difference was found in all of light treatments (Table 3).

3.2.2. Biomass and contents of polysaccharides and alkaloids of the DO plantresponse to monochromatic light:

Table 4 Effects of monochromatic light to biomass and contents of polysaccharides and alkaloids of the DO plant(after 90 days)

Treat.	**Type of monochromatic light (λ)**	**Change in plant weight(g)**	**Polysaccharide content (% dry materials)**	**Alkaloid content (mg/100 g dry materials)**
T1	White (λ=400-760nm)	19.30[ab]	25.60[b]	142.12[b]
T2	Purple (λ=400-430nm)	12.50[c]	19.08[d]	125.20[c]
T3	Blue (λ=510-560nm)	16.20[b]	22.27[c]	127.25[c]
T4	Red (λ=610-730nm)	20.50[a]	27.22[a]	143.45[a]
P ≤ 0,05		<0.05	<0.05	<0.05
CV%		6.65	3.14	1.06
LSD0,05		0.11	1.48	2.85

[a-d]values with different superscripts were significantly different at p <0.05.

The monochromatic light affected change biomass, polysaccharide, and alkaloid content of the DO plant (Table 4).Change in plant weight varied from 12.5 g to 20.5 g under monochromatic light treatments (white, purple, blue, and red light). In the white and red light, changes in plant weight have the best values with 19.30 g and 20.50 g, respectively (P<0.05). The lower changes of plant weight were in blue and purple light treatments with 16.2 g and 12.50 g, respectively.

The polysaccharide and alkaloid contents ranged from 19.08% to 27.22% and from 125.2 mg/100 of dry materials to 143.45 mg/100 g of dry materials, respectively (Table 4), in which, T4 (the red light) has the highest polysaccharide and alkaloid contents (27.22% and 143.45 mg/100 g dry materials). The T1 (white light) has second polysaccharide and alkaloid contents being higher T2 (purple light) and T3 (blue light) (P<0 .05).

Monochromatic light affects the plant growth and development (25,26,27,28,29). More than providing the energy for photosynthesis, light also regulates plant development, shaping, and metabolism, in the complex phenomenon of photomorphogenesis, driven by light colours (30,31). Using coloured glass filters to provide plants with light of diferent colours for controlling plant growth and development wasreported by Kasperbauer and Kaul (1996), McCree (1972) (32,33). In the early studies, plant response to monochromatic light was investigated mainly in vegetable, flower and ornamental crops (31).

These studied resultsshown that using colour light filters creating red light alone is the most suitablest for shoot multiplier, change in plant height, stem diameter, number of leaves and roots, biomass and polysaccharide and alkaloid accumulation in DO plant cultivated greenhouse in ThaiNguyen province, Vietnam compared with white, blue and purple light. Many reports showed as red light alone could promote the synthesis of pigments and active metabolites in diferent species, improving the product nutritional quality. In agreement, Wang et al. (2017) claimed that the growth and development of *Dendrobium officinale* seedlings were affected by the light quality and red light increased seedling height, extracted content in the China greenhouse conditions (34);and José et al. (2020) reported that the highest germination and the optimum early seedling development were observed in red light for Dendrobium phalaenopsis. In agreementwith this opinion (35), Yavari et al. (2021) claimed that red light significantly increased leaf area growth, biomass, and promoted net photosynthetic rate in Arabidopsis thaliana (36). In constract, many other authors demonstrated thatmany other authors demonstrated that the optimal red:blue ratio enhances photosynthetic capacity and improves growth and yield (25,37,38,39).These differences in views of the optimal light quality for plant growth and development endorsed the assumption that the response of the genotypes to monochromatic light is different.

4. Conclusion

The growth and biomassof the DO plant grown under greenhouse conditions in ThaiNguyen province, Vietnam were significantly influenced by light. In the shading 50% and 70%, the DO plant has the highest growth, biomass and polysaccharid, and alkaloid accumulation. The red light is the most suitable for the plant growth, biomass accumulation, polysaccharide and alkaloid synthesiscompared to purple, blue and white light. Thus, light treatment (shade 50-70% and use red light) is meansure usfully for *Dendrobium officinale* cultivation in greenhouse horticulture.

Compliance with ethical standard

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Disclosure of conflict of interest

No conflict of interest exists.

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