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Effect of Different Light Qualities on Essential Oil and Asarinin in *Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag

Zhiqing Wang [1,*,†], Guanghui Wang [1,†], Xingzhou Quan [1], Min Zhang [1], Yanhong Wang [2], Lili Cui [3], Huiling Li [1], Ze Zhang [1] and Wei Hou [3,*]

[1] College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun 130118, China; wguanghui1020@163.com (G.W.); quanxingzhou697@139.com (X.Q.); zm15567620935@163.com (M.Z.); lhl6162023@163.com (H.L.); zz51998@126.com (Z.Z.)

[2] Ginseng and Antler Product Quality and Safety Risk Assessment Laboratory, Ministry of Agriculture and Rural Affairs, Jilin Agricultural University, Changchun 130118, China; yanhong-w@163.com

[3] Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agriculture Sciences, Changchun 130112, China; cbscui@126.com

* Correspondence: wangzhiqing@jlau.edu.cn (Z.W.); jilinhouwei@163.com (W.H.)

† These authors contributed equally to this work.

Abstract: *Asarum heterotropoides* Fr. var. *mandshuricum* (Maxim.) is a perennial herb native to China. Its essential oil components and asarinin are health-promoting compounds. However, cultivation under natural light can affect the growth and secondary metabolite yield of this sciophyte. Adjustment of light irradiance may be beneficial in this respect. Here, we applied six types of filtered solar irradiance by using filter films of red (I), yellow (II), blue (III), green (IV), purple (V) and 50% sunlight (VI). We measured net photosynthetic rate (APn), activity of phenylpropanoid biosynthetic enzymes, asarinin content, and essential oil content and composition. Light quality treatments V and VI resulted in increased APn, enzyme activities, asarinin content and essential oil content. Samples harvested in September 2020 had relatively higher contents and enzyme activities compared to those harvested in July 2020. Elemicin, 2,5-dimethoxytoluene and methyleugenol accumulated at higher levels in response to light quality V. Light qualities III and IV showed promising results for several compounds. Overall, we conclude that controlling the light conditions in growing environments promotes the amount of asarinin and essential oil by regulating photosynthesis and the activities of phenylpropanoid biosynthetic enzymes.

Keywords: asarinin; essential oil; phenylpropanoids; colored filter films; light quality; selective wavelength transmittance

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1. Introduction

Asarum heterotropoides Fr. var. *mandshuricum* (Maxim.) is a perennial herb native to China. Its fibrous roots are used in traditional Chinese medicine and are commonly known as Xixin. Its extracts have been used for their health improving effects antifungal, antibacterial, antiallergic, analgesic, antitussive, anti-inflammatory, and antipyretic properties [1]. Major bioactive components in Asarum are essential oil and asarinin, which are key indicators for assessing medicinal herb quality [2]. Asarum essential oil mainly consists of methyleugenol, safrole, myristicin, elemicin and 3,5-dimethoxytoluene, whose biosynthesis is derived from the shikimic acid and cinnamic acid pathways [2,3]. Asarinin, a lignin (furofuran) is the epimer of sesamin, which is derived from phenylalanine through phenylpropanoid biosynthetic pathway [4]. The essential oil derived from *A. heterotropoides* var. *mandshuricum* is mainly composed of phenylpropanoid compounds. Studies have reported that the essential oil majorly consists of methyleugenol, eucarvone, 5-allyl-1,2,3-trimethoxybenzene,

3,7,7-trimethylbicyclo(4.1.0)hept-3-ene, 2,6,6-trimethylbicyclo(3.1.1)hept-2-ene, (1S)-(1)-beta-pinene, and 1,3-dimethoxy-5-methylbenzene, benzene, 1,2-dimethoxy-4-(2-propenyl)-, 1,3-benzodioxole,5-(2-propenyl)- and 1,3-benzodioxole, 4-methoxy-6-(2-propenyl) [5]. The phenylpropanoid biosynthesis pathway starts with the products of phenylalanine, tyrosine and tryptophan biosynthesis, i.e., phenylalanine and cinnamic acid, which are then converted into lignins and other products [6]. In this pathway, several key enzymes 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS), phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL) [6–8]. DAHPS, CM, PAL, C4H and 4CL are therefore the key enzymes catalyzing the biosynthesis of phenylpropene precursors. Similarly, cinnamic acid and *p*-coumaric acid are crucial secondary metabolites for the biosynthesis of essential oil in *A. heterotropoides* var. *mandshuricum* [6–8].

The accumulation of essential oil is influenced by several factors, including agro-nomic practices, climatic conditions, plant growth stage and tissue, harvest time and the onset of biotic and abiotic stresses. An important factor among growth conditions is light quality. Studies have shown that light quality affects essential oils, volatile compounds and other compounds with medicinal properties [9]. Research on the content of these compounds in aromatic plant species is taking place in farmers' fields, and more parameters are being tested.

A more practical and farmer-friendly approach to control the quality and quantity of light is by using shading nets (and filter films) with different colors and combinations. Studies have reported increased antioxidant content in thyme [10], marjoram, oregano [9], coriander [11], Asarum [12], basil [13] and several others. The filter films of various colors have different abilities to modify the transmitted light spectrum in the ultraviolet, visible and far-red regions [14]. Thus, the use of colored filter films enriches the relative content of scattered light and ratios at the luminous environment of the plant species being grown. This differently scattered light is ultimately perceived by the photosynthetic antenna proteins and affects photosynthesis, carbon assimilation [15,16], and ultimately the compositions of essential oils and secondary metabolites [10].

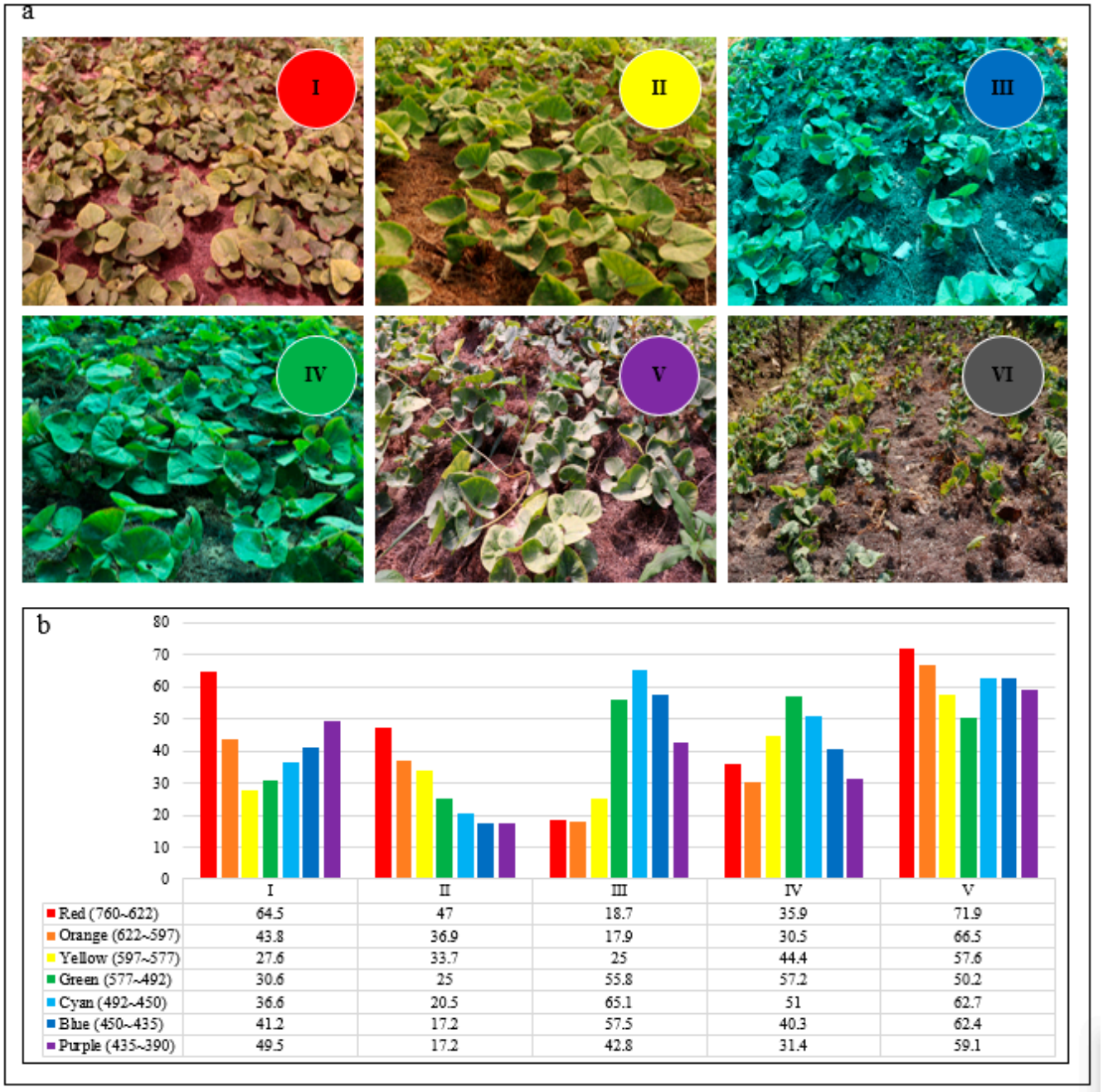
Therefore, the use of different colored films is a useful strategy to manipulate and optimize the biosynthesis and content of essential oils and secondary metabolites. Considering this, this work investigates the effect of light-quality on net photosynthetic rate, essential oil, asarinin, methyleugenol, safrole, 3,5-Dimethoxytoluene myristicin, elemicin, and eucarvone accumulation in Asarum. We also studied the activities of enzymes catalyzing biosynthesis of phenylpropanoids in Asarum.

2. Materials and Methods

Asarum heterotropoides Fr. var. *mandshuricum* (Maxim.) Kitag cultivar "Zhongnong xixin 1" was used as plant material. At the end of September 2019, 4-year-old Asarum was transplanted into the medicinal herb garden of Jilin Agricultural University (43.80′ N, 125.42′ E) similar to earlier work [12]. Light quality treatment was carried out until mid-May 2020. Five light quality treatments (I–V) for different colors i.e., red, yellow, blue, green, and purple were given by covering the plant material with five different colored filter foils (Figure 1). The plant material was covered with different black nylon shade nets to receive about 50% sunlight as light quality VI.

2.1. Photosynthesis Measurement and Enzyme Assays

The diurnal variation of photosynthesis was measured on 12 June, 15 July, 10 August and 10 September on sunny days. Measurements were taken once every two hours from 6.20 am to 5.30 pm using the CIRAS-2 portable photosynthesis system (PP Systems, Hertfordshire, UK). Enzyme activities of 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (DAHPS), phenylalanine ammonia lyase (PAL), transcinnamate 4-monooxygenase (C4H) and 4-coumarate-CoA ligase (4CL) were determined according to Wang et al. [1].



	I	II	III	IV	V
Red (760~622)	64.5	47	18.7	35.9	71.9
Orange (622~597)	43.8	36.9	17.9	30.5	66.5
Yellow (597~577)	27.6	33.7	25	44.4	57.6
Green (577~492)	30.6	25	55.8	57.2	50.2
Cyan (492~450)	36.6	20.5	65.1	51	62.7
Blue (450~435)	41.2	17.2	57.5	40.3	62.4
Purple (435~390)	49.5	17.2	42.8	31.4	59.1

Figure 1. Light quality treatment. (**a**) Asarum plants growing under filter films with different colors; (**b**) light quality conditions under different colors. The bars represent the wavelength-permeable rate (%) under different light quality treatments. The roman letters I–VI refer to red, yellow, blue, green, purple, and black (50% sunlight).

2.2. Analysis of Asarinin

Asarinin standards were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. (Shanghai, China). The plant material was oven-dried at 40 °C and ground to fine powder. The crude product was obtained according to the methods described in the Chinese Pharmacopoeia (2020) [2]. The crude product was filtered through a 0.22 μm microporous membrane (Jinteng Experiment Equipment Co., Ltd., Tianjin, China) and used for chromatographic analysis on an H-class ultra-high-performance liquid chromatograph (Waters Corporation, Milford, MA, USA) (ACQUI-TYC18 column (50 mm × 2.1 mm, 1.7 μm). The mobile phase was acetonitrile (A)-water (B), gradient elution (0~7 min: 25% (A); 7~20 min:

25~33% (A); 20~30 min: 33~47% (A); 30~60 min: 47% A~55% A; 60~80 min: 60% A. The detection wavelength was 287 nm, the flow rate was 0.3 mL/min, the column temperature was 30 °C, and the injection volume was 2 μL.

2.3. Extraction and GC-MS Analysis of Essential Oil

Fibrous roots were harvested for each treatment and control on 10 July and 15 September 2020, washed three times with distilled water, dried in the shade, ground to powder and used for essential oil extraction.

Approximately 20 g of sample for each replicate of each treatment was separately hydrodistilled for 3 h according to the method of Chinese Pharmacopoeia (2020) and used to determine the yield of essential oil. A total of 10 μL of essential oil was collected from the solution and diluted 50 times with petroleum ether. A total of 1 μL of this diluted sample was analysed by gas chromatography–mass spectrometry (Agilent-7890A/5975C, Agilent Technologies Inc., Santa Clara, CA, USA). An HP-5MS highly polar capillary column (30 m × 0.32 mm × 0.25 μm, Hewlett-Packart, Palo Alto, CA, USA) coated with a 100% polyethylene glycol stationary phase was used. The following oven temperatures and times were used: 40 °C (held for 2 min), increased from 40 °C to 160 °C at a rate of 2.5 °C/min, from 160 °C to 280 °C at a rate of 8 °C/min, and finally held at 280 °C for 10 min. The injection temperature was set at 280 °C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. An injection volume of 1 μL was used with a split ratio of 100:1. The mass spectrometer was operated in an electron impact (EI) mode at 70 eV with scan ranges between 30 and 550 amu. The ion source temperature was maintained at 230 °C and the quadrupole at 150 °C.

Compound identification was performed by comparing the National Institute of Standards Technology (NIST), United States Department of Commerce (https://www.nist.gov/; accessed on 5 December 2023) library data of the peaks with those reported in the literature, and mass spectra were compared with peaks from literature data. Percentage composition was computed from GC peak areas on with DB-5 ms column without applying correction factors.

2.4. Determination of Methyleugenol, Safrole, 3,5-Dimethoxytoluene, Myristicin, Elemicin and Eucarvone

Standards of methyleugenol, safrole, 3,5-Dimethoxytoluene, myristicin, elemicin, and eucarvone were purchased from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). The methods of determination of methyleugenol, safrole, 3,5-Dimethoxytoluene, myristicin, elemicin, eucarvone according to GC-MS analysis given in Section 2.3.

2.5. Statistical Analysis

Data were analyzed using a one-way analysis of variance in SPSS statistical software version 22.0 (https://www.ibm.com/; accessed on 5 December 2023). Tests of significant differences among treatments were analyzed using the least significant difference (LSD) test. Relative standard deviation (RSD) was computed. The significance level was set at $p < 0.05$. Principal component analysis (PCA) was conducted by OriginPro software 2021 (OriginaLab Corporation, Northampton, NC, USA; https://www.originlab.com; accessed on 5 December 2023).

3. Results

3.1. Effect of Different Light Qauality on Photosynethesis and Enzyme Activities

Asarum heterotropoides var. *mandshuricum* was subjected to six light quality treatments, including 50% sunlight using a black shading net (Figure 1a). The diurnal variation of photosynthesis rate (APn) was measured on 18 June, 15 July, 7 August and 15 September, representing four growth stages, respectively. Our data indicated that the variation trends of APn in the different light treatments were similar across the four growing stages (Figure 2a). Generally, the APn values were observed in response to IV and V were significantly higher

than those in the four other light treatments. The lowest APn values were recorded for III and VI in all four growing stages.

As discussed in the introduction, the key metabolites in the biosynthetic pathway of essential oil and asarinin of *A. heterotropoides* var. *mandshuricum* are derived via the shikimic acid and cinnamic acid pathways. To this end, we determined the activities of four 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS), 4-coumarate-CoA ligase (4CL), cinnamate-4-hydroxylase (C4H) and phenylalanine ammonia lyase (PAL) under the different light treatments in Asarum lamina and fibrous roots at four stages (Figure 2). Across the four growth stages, DAHPS activity was strongly induced by light qualities V and IV in both lamina and root. Conversely, light quality III induced the least DAHPS activity in both lamina and root (Figure 2b). PAL, C4H and 4CL activities were strongly influenced by light quality V followed by IV in both lamina and root. On the other hand, light quality III had the least effect on PAL activity (Figure 2c,d). Among all the enzymes, DAHPS and C4H were more active in lamina than in root under different light treatments at all four stages. Conversely, PAL activity was higher in root than in lamina in different light treatments at all four stages (Figure 2b). Taken together, these observations indicate that light treatment V is the most effective, followed by VI, and light quality III is the least effective.

3.2. Effect of Different Light Qualities on Asarinin Content

To reveal the effect of light quality on asarinin, we measured the asarinin content of *A. heterotropoides* var. *mandshurium* in July and September 2020. Standard curve $y = 2358x + 1015.2$ ($r^2 = 0.9999$) established was used to calculate the content of asarinin (Figure 3, Figures S2 and S3). Methodological validation was carried out, including a precision test (RSD = 0.60%), a repeatability test (RSD = 0.90%) and a stability test (RSD = 1.0%); the sample average recovery rate was 98.09~102.41%, (RSD = 1.96%). The asarinin content of root samples grown in light quality V was higher than others ($p < 0.05$) which indicated that light quality V contributed to biosynthesis of asarinin in roots. Comparatively, light quality III had the lowest asarinin content in roots on both sampling times. Roots had 10 times higher asarinin content than leaves. Among the two sampling times, the root samples collected during September had higher asarinin content compared to those collected in July. Generally, both sampling times showed a similar accumulation trend in roots.

3.3. Essential Oil Content and Composition in A. heterotropoides var. mandshuricumin Plants Grown in Different Light Quality

3.3.1. Essential Oil Content

We extracted the essential oil from fibrous roots of *A. heterotropoides* var. *mandshuricum* grown in six different light quality in July and September 2020 and compared the yield. The results showed that the highest oil yield was obtained in fibrous roots of plants grown in light quality IV, followed by V. Contrarily, the lowest essential oil yield was obtained from fibrous roots of plants grown in light quality III. The highest and lowest essential oil contents were consistent in the two harvesting times for light qualities IV and V, and III, respectively (Figure 4a). Generally, the essential oil content in roots harvested in September was higher than July 2020 (Figure 4a). These results are consistent with the APn as well as enzyme activities (Figure 2). It should be noted that the IV and V light qualities had higher essential oil contents than the most commonly used shading net with 50% sunlight, indicating their potential practical application in Asarum cultivation for increased essential oil biosynthesis.

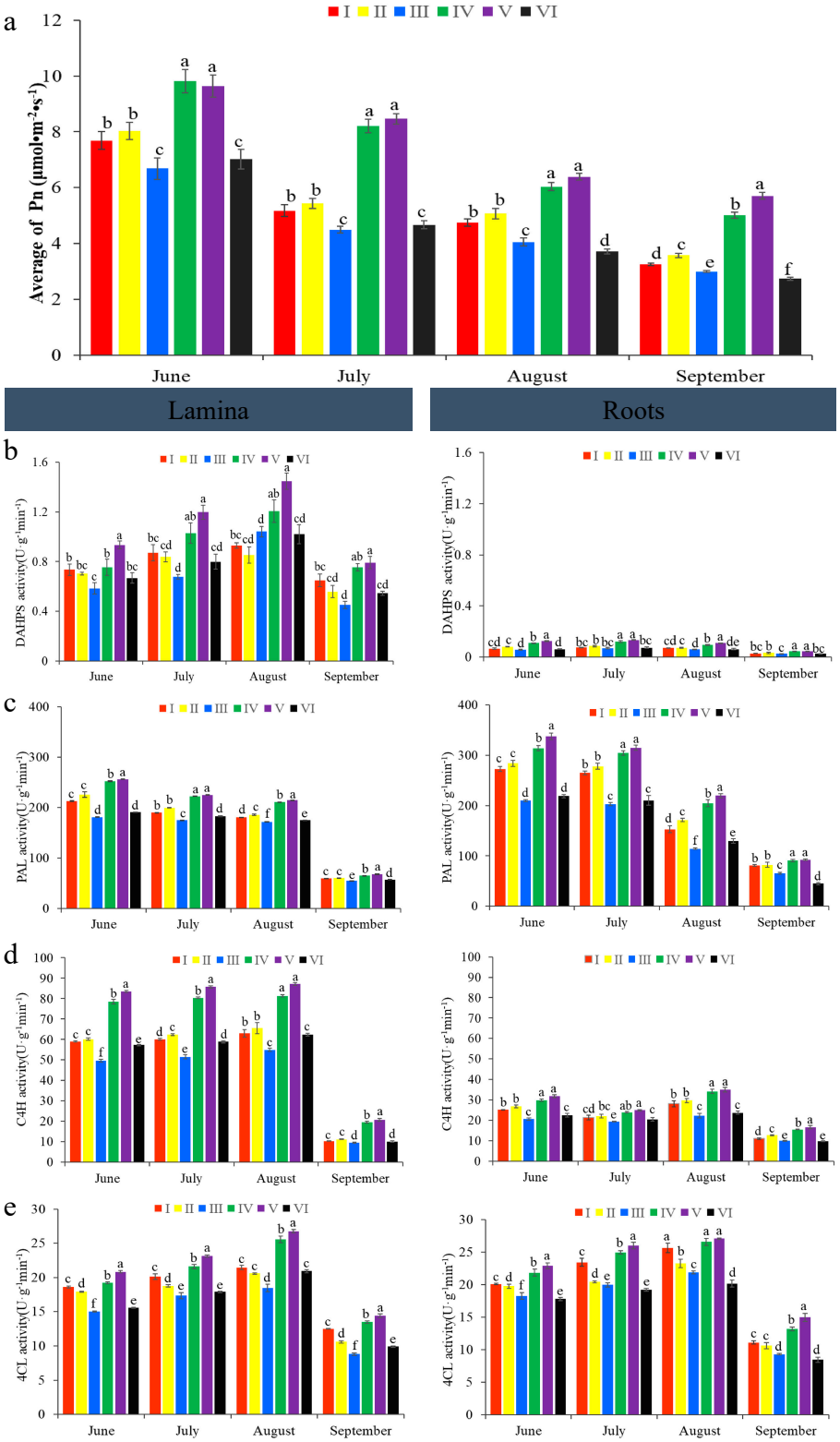


Figure 2. Effect of light quality on Asarum photosynthesis and enzyme activities. (**a**) The diurnal net photosynthetic rate average of four stages in six light qualities. (**b**) Activity of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS), (**c**) phenylalanine ammonia lyase (PAL), (**d**) cinnamate-4-hydroxylase (C4H), and (**e**) 4-coumarate: CoA ligase (4CL) in different tissues of *A. heterotropoides* var. *mandshuricum* plants grown in six light irradiations. I, II, III, IV and V are light qualities listed in Figure 1b. VI indicates 50% full sunlight. Error bar represents standard deviation (n = 5); different letters on the bars mean significant difference ($p < 0.05$).

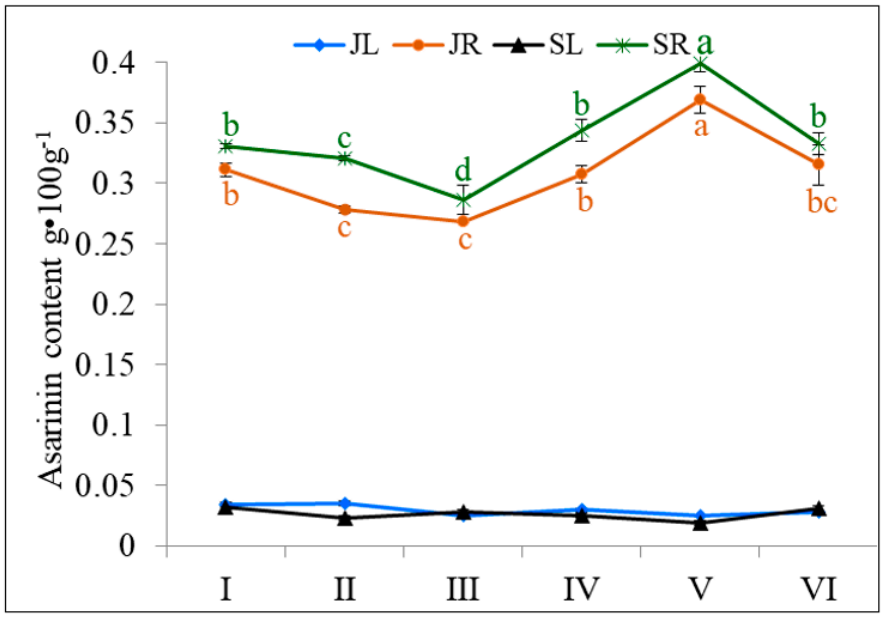


Figure 3. Asarinin content of *Asarum heterotropoides* var. *mandshuricum* grown in different light qualities. JL and JR indicate leaves and roots collected in July, respectively. SL and SR indicate leaves and roots collected in September, respectively. I, II, III, IV and V are light qualities listed in Figure 1b. VI indicates 50% full sunlight. Error bar represents standard deviation (n = 3); different letters on the bars mean significant difference ($p < 0.05$).

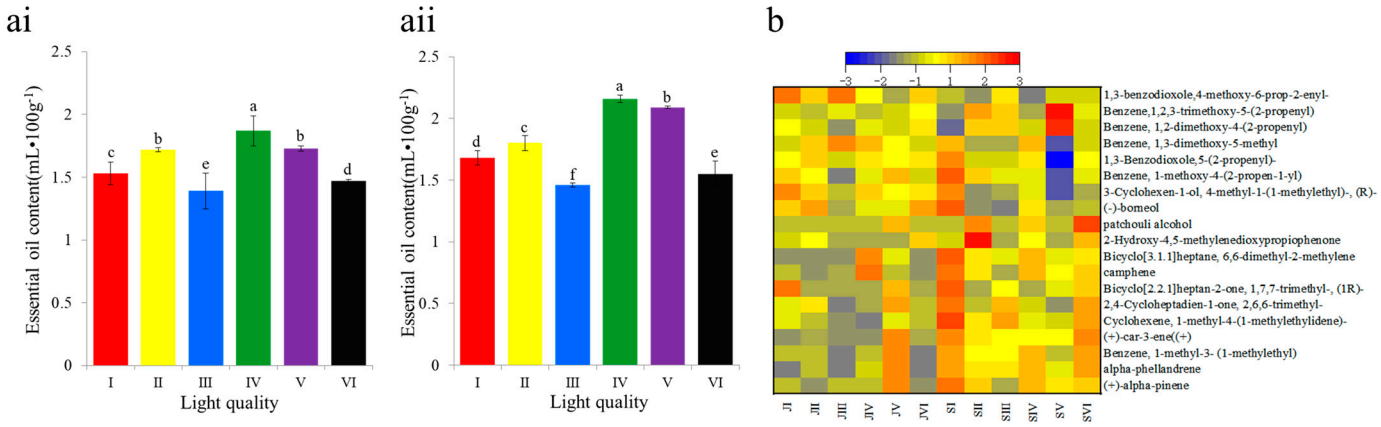


Figure 4. Essential oil content and composition in Asarum roots. Essential oil content in roots collected in (**ai**) July 2020 and (**aii**) September 2020. I, II, III, IV and V are light qualities listed in Figure 1b; VI indicates 50% full sunlight; error bar represents standard deviation (n = 3); different letters on the bars mean significant difference ($p < 0.05$). (**b**) Differences in the main chemical components of volatile oil in July (J) and September (S) 2020.

3.3.2. Essential Oil Composition

To further understand the effect of light quality on the composition of essential oil, through GC-MS analysis, only compounds with at least 90% similarity to NIST mass spectral library and relative content exceeding 0.1% were selected (Table S1). A marked difference was observed in the relative content of these compounds among the different light treatments (Figure 4; Table S1) and stages. According to the GC-MS analysis, benzene,1,2-dimethoxy-4-(2-propenyl), benzene, 1,3-dimethoxy-5-methyl, 1,3-benzodioxole, 5-(2-propenyl), 1,3-benzodioxole,4-methoxy-6-(2-propenyl), benzene,1,2,3-trimethoxy-5-(2-propenyl) and 2,4-cycloheptadien-1-one, 2,6,6-trimethyl- were major compounds in the Asarum essential oil (Figure 4b). In the case of the Asarum roots harvested in July, these

compounds accounted for 79.77% (JI), 83.72% (JII), 78.08% (JIII), 83.66% (JIV), 76.7% (JV) and 81.82% (JVI) of the total essential oil. Their % in the total essential oil in the samples harvested in September 2020 was 77.15% (SI), 76.36% (SII), 79.67% (SIII), 78.13% (SIV), 74.6% (SV) and 75.93% (SVI) (Table S1).

However, to determine the influence of the light quality treatments on their quantity, we measured their individual contents. Linearity was established using a dilution series of stock solutions of the metabolites. Calibration curves of benzene, 1,2-dimethoxy-4-(2-propenyl(methyleugenol), 1,3-benzodioxole,5-(2-propenyl)-(safrole), benzene, 1,3-dimethoxy-5-methyl(3,5-dimethoxytoluene), 1,3-benzodioxole,4-methoxy-6-prop-2-enyl-(myristicin), benzene, 1,2,3-trimethoxy-5-(2-propenyl)-(elemicin) and 2,4-cycloheptadien-1-one, 2,6,6-trimethyl-(eucarvone) were $y = 1.28e^5x + 2.98e^3$ ($r^2 = 0.999$), $y = 7.73e^4x + 1.11e^3$ ($r^2 = 0.999$), $y = 8.76e^4x + 4.38e^2$ ($r^2 = 1.000$), $y = 8.66e^4x + 3.02e^3$ ($r^2 = 0.998$), $y = 5.15e^4x - 1.82e^3$ ($r^2 = 0.998$), and $y = 1.83e^4x + 2.18e^2$ ($r^2 = 1.000$), respectively (Figures 5a and S1). Eucarvone content was highest in light quality treatment IV, followed by III in July 2020, whereas in September 2020, treatment III induced the highest eucarvone content, followed by both II and VI. Generally, September had a higher content for each light treatment compared to the samples collected in July 2020 (Figure 5a). 3,5-dimethoxytoluene content had a different accumulation trend compared to other observed essential oils (Figure 5b). Safrole content was significantly higher in treatment III in July 2020, and in treatment IV followed by III in September 2020 (Figure 5c). Methyleugenol content also exhibited the same accumulation trends as that of eucarvone in response to different treatments (Figure 5d). Myristicin followed the same accumulation trends as 3,5-dimethoxytoluene (Figure 5e). Generally, the myristicin content range was similar in both time points but differed within the light quality treatments. Finally, elemicin content was significantly highly accumulated in response to treatment III in July 2020 and treatment VI in September 2020 (Figure 5f).

Principal component analysis was also performed to reveal the correlation between eucarvone, 3,5-dimethoxytoluene, safrole, methyleugenol, myristicin, elemicin and light quality (Figure 5g), considering July 2020 and September 2020 (Figure 5(gi–giii)). The synthesis and accumulation of six compounds were positively affected by blue light (450~435 nm), purple light (435~390 nm) and cyan light (492~450 nm). Among these, myristicin, methyleugenol and 3,5-dimethoxytoluene were simulated by purple light, and safrole, elemicin and eucarvone were mainly simulated by blue light.

Overall, these results suggest that the percentage of major compounds in the total essential oil of Asarum roots is higher when grown under light treatment IV. Furthermore, the essential oil composition of Asarum roots can be manipulated by growing under different light quality treatments. The samples collected in September contain relatively higher levels of essential oil than those collected in July. In general, the light quality treatments IV and V can be a promising approach to induce a higher accumulation of essential oil and asarinin content.

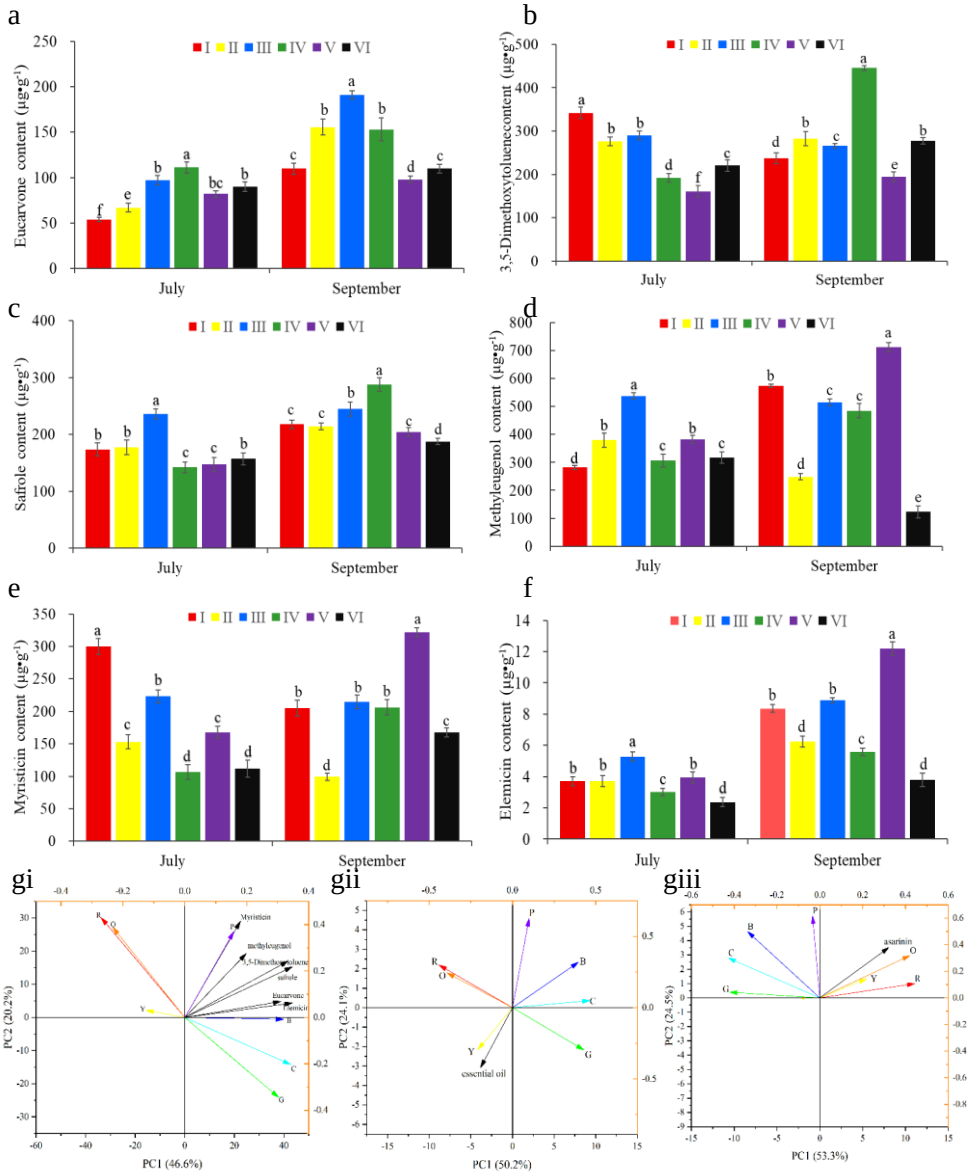


Figure 5. Levels of six essential oil compounds accumulated in Asarum (roots) ground under different light quality treatments during July and September 2020. (**a**) Eucarvone, (**b**) 3,5-dimethoxytoluene, (**c**) safrole, (**d**) methyleugenol, (**e**) myristicin, and (**f**) elemicin. Error bars on the column charts represent standard deviation (n = 3); different letters on the bars mean significant difference ($p < 0.05$). (**g**) Principal component analysis of the six essential oil compounds grown under different light quality treatments in July 2020 (**gi**), August 2020 (**gii**) and September 2020 (**giii**). The colors of the arrows indicate the wavelength, as given in Figure 1b.

4. Discussion

Asarinin and essential oil components with biological and pharmacological activities in *A. heterotropides* offer a range of health beneficial properties. Studies have reported antibacterial, antidepressant, antifungal, antiallergic, and other properties [17–19]. Efforts are underway to increase the levels of health-promoting compounds in Asarum using various strategies. Recently, research has focused on the use of colored films to modify the light quality. The altered light quality can in turn influence several plant secondary metabolite biosynthesis pathways, thereby improving their content or modifying their composition [12]. Here, we discuss the effect of different light qualities on APn, the activities of several enzymes involved in phenylpropanoid biosynthesis, asarinin, essential oil content and composition at different harvest times in leaves and roots. Our results are

useful for the selection of a colored shade net or filter film, since plant growth depends on it [20].

The colored filter films provide a tool for manipulating the quality of light. To carry this out, the different colored films have been shown to transmit selective wavelengths (Figure 1b) by modifying light in the ultraviolet, visible or far-red spectra. We used red (I), yellow (II), blue (III), green (IV) and purple (V) filter films and a black (50% sunlight, VI) shading net (Figure 1a). Our results, that IV and V treatments were useful for increasing APn and the activities of several enzymes, are consistent with the previous work, where green filter film showed the highest photosynthetic activity in cordyline leaves compared to red, black and the control [21]. This is probably because green light penetrates deeper than red and blue light, which are absorbed by the top few layers. This in turn stimulates photosystems in deeper cell layers and ultimately benefits leaf photosynthesis [22]. Similarly, treatment V (purple) caused an increase in the percentage of most light wavelengths (Figure 1b), and therefore an increase in photosynthetic activity and the expression of enzymes [23]. Photosynthetic activity is also associated with the increased expression of several genes involved in phenylpropanoid biosynthesis and associated pathways. For example, Park et al. [24] reported that green light resulted in the highest PAL gene expression in *Agastache rugosa*. Similarly, Balkhyour et al. [25] reported that increased biomass and photosynthesis is accompanied with increased expression of C4H, 4CL, DAHPS and other enzymes in the phenylpropanoid biosynthesis pathway. Moreover, the same study indicated a relationship between photosynthesis and increased essential oil contents. Therefore, a similar mechanism may be present in Asarum. Moreover, the increased asarinin content is also consistent with the activities of PAL, C4H, and 4CL [26]. Thus, these two light quality profiles (Figure 1b) can be adjusted to improve photosynthesis, increase enzyme activities and consequently asarinin and essential oil content.

Essential oils are important secondary metabolites in aromatic plants. Several factors including genetics [27], growth conditions and nutrition [28], physical environment such as light quality [29], onset of stresses [30], etc., can influence the content and composition of essential oils in plants. The observation that both light qualities V and IV produced the highest essential oil yields (Figure 4a) indicates the role of the transmittance of different lights. This could be related to the ratio of transmitted light. Dou et al. [31] highlighted the usefulness of blue and red ratios for manipulating essential oil content. Light quality treatments IV and V had >50% green light transmittance. In addition to this, they had a high % of red light transmittance, which was not the case for light quality III. Thus, the factor of transmittance of different lights has an impact on essential oil content [32]. However, the overall transmittance spectrum can also not be ignored. Nevertheless, our observations provide preliminary details on this aspect of Asarum growth and essential oil content. Essential oils composition has also been reported to be influenced with the light quality in different aromatic plant species, e.g., rosemary [33], Japanese mint [34], thyme, marjoram and oregano [9]. In Asarum essential oils, methyleugenol and eucarvone are the active ingredients [35,36], whereas safrole and myristicin are the toxic ingredients [37,38]. The results, that elemicin, 3,5-dimethoxytoluene and methyleugenol were accumulated in higher quantities in response to light quality V, can be a useful observation. However, light qualities III and IV also showed promising results for several compounds. Since multiple genes control the biosynthesis of these metabolites [39,40], it is premature to conclude why their contents showed variable accumulation pattern. Nevertheless, the fact that light qualities IV and V had higher total essential oil contents provide useful preliminary understanding.

5. Conclusions

In this study it was found that the different colors of filter films had a significant effect on the secondary metabolism of Asarum, especially the light quality conditions of the purple and green films had a promoting effect on the synthesis of active compounds in Asarum roots. Based on the results, we conclude that September is a suitable time to

obtain higher asarinin and essential oil yields from roots. The increased photosynthetic rate, higher expression of phenylpropanoid biosynthesis-related genes, asarinin and essential oil content are due to the influence of light qualities IV and V. Treatment with purple and green films may help as a complementary cultivation practice to improve the content and quality of active substances for the development and utilization of Asarum.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10030258/s1, Supplementary Table S1. Details of compounds identified in Asarum essential oil. Supplementary Figure S1. Calibration curves of benzene, 1,2-dimethoxy-4-(2-propenyl (methyleugenol), 1,3-benzodioxole,5-(2-propenyl)-(safrole), benzene, 1,3-dimethoxy-5-methyl(3,5-dimethoxytoluene), 1,3-benzodioxole,4-methoxy-6-prop-2-enyl-(myristicin), benzene, 1,2,3-trimethoxy-5-(2-propenyl)-(elemicin) and 2,4-cycloheptadien-1-one, 2,6,6-trimethyl-(eucarvone). Supplementary Figure S2. High-performance liquid chromatography chromatograms of asarinin: Chromatogram of asarinin standard. Supplementary Figure S3. Chromatogram of asarinin in Asarum grown in light quality IV. Number 1 indicates asarinin peak.

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