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Full Length Research Paper

Growth and accumulation of bioactive compounds in medicinal *Chrysanthemum morifolium* Ramat. cv. '*Chuju*' under different colored shade polyethylene

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Chrysanthemum morifolium **Ramat. is not only a famous floricultural crop but also a plant of pharmacological importance. An experiment was carried out to evaluate the effects of different colored polyethylene shade on the growth and accumulation of chlorogenic acid, total flavonoids, quercetin, apigenin and acacetin in *C. morifolium* capitulum. The results showed that colored polyethylene shade decreased the flower number and biomass yield while increasing the flower diameter and plant height, and the magnitude of effect was in the order of blue>red>yellow. The chlorogenic acid content was observed to be highest under yellow and blue polyethylene cover at different stage of flower development. Blue shaded polyethylene led to lower contents of total flavonoid, quercetin, apigenin, and acacetin. The contents of total flavonoids and quercetin under different colored shaded polyethylene peaked at different stage throughout the study period. The contents of acacetin and apigenin in chrysanthemum capitulum from four shaded polyethylene initially increased and then decreased throughout the study period. The results suggested that different color of polyethylene could induce changes in the microclimate that could act through natural regulatory system within chrysanthemum and affect growth and bioactive compounds accumulation.**

Key words: *Chrysanthemum morifolium* Ramat. cv. '*Chuju*', colored polyethylene shade, growth, bioactive compounds.

INTRODUCTION

Chrysanthemum morifolium Ramat. (Compositae) is globally the second economically most important floricultural crop following rose, and one of the most important ornamental species (Teixeira da, 2003; Van and Heuvelink, 2006). Besides being important floricultural and ornamental crops, *C. morifolium* is also a plant of culinary, medicinal and (ethno) pharmacological interest (Teixeira da, 2003). *C. morifolium* has been cultivated in China for over 2000 years, and its dried capitulum, Chrysanthemi Flos, is an important traditional Chinese medicine (TCM) used for "scattering cold", ''cleaning heat and toxin" and ''brightening eyes", and used as an important component in many TCM formulas. Additionally, it is also widely used as a food supplement, or herbal tea, and is considered a healthy food by many consumers (Teixeira da, 2003; Liu et al., 2010a, 2010b; Lin and Harnly, 2010). The chrysanthemum capitulum contains significant amounts of flavonoids and hydroxycinnamic acid derivatives (mainly represented by chlorogenic acid) that are considered to be the biologically active components (Liu et al., 2010a).

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Environmental conditions including temperature and light conditions (e.g. light intensity, light quality, photoperiod) influence significantly the growth of chrysanthemum and year-round production can be achieved in greenhouses by controlling climate conditions (Karlsson et al., 1989; Kim et al., 2004; Van and Heuvelink, 2006; Ma et al., 2007; Kjaer and Ottosen, 2011). Different colored polyethylene shade could have ability to reflect different spectra of the visible light that may affect crop development and yield by manipulating the light quality and air temperature around plants (Khandaker et al., 2010). Hence, a series of studies have been conducted to improve the ornamental value of chrysanthemum through choose of colored polyethylene shade, and those previous works revealed that colored polyethylene shade has substantial effects on the ornamental characteristics of chrysanthemum such as flower color, size and form, flowing time and vegetative height (Cathey and Borthwick, 1957; Cockshull and Hughes, 1971; Khattak et al., 1999; Oyaert et al., 1999; Li et al., 2003; Khattak and Pearson., 2006; Heo et al., 2010).

On the other hands, the employment of colored polyethylene shade was expected to affect the accumulation of bioactive compounds in some kinds of medicinal plants (e.g. *Panax ginseng* C.A. Mey., *Rhodiola sachalinensis*, *Camptotheca acuminate*, and *Amaranthus tricolor* L). And it has showed marked effects on increment of bioactive compound contents. For example, Yan et al. (2004) explored the effects of colored polyethylene shade on the growth and bioactive compounds contents in *R. sachalinensis* and found that red film slightly decreased root biomass of 3- and 4-year-old *R. sachalinensis* while it improved significantly salidroside contents in roots. By contrast, yellow, blue and green polyethylene shade decreased significantly salidroside contents. Dai et al. (2004) found that the shade treatment with films, especially blue film, accelerated the accumulation of camptothecin in the leaves of *C. acuminate.* Additionally, Khandaker et al. (2010) found that blue polyethylene has potentials to increase the yield with health beneficiary bioactive compounds betacyanins, polyphenol and antioxidant activity in *Amaranthus tricolor* L.

Due to its medicinal and industrial importance, the demand for *C. morifolium* has steadily increased in the world market. Choosing suitable colored polyethylene shade for the production of medicinal *C. morifolium* will help to achieve year-round production of medicinal chrysanthemum with optimum biomass yield and bioactive compounds. However, to our best knowledge, few reports were available on the accumulation of bioactive compounds in chrysanthemum under different colored polyethylene shade.Hence, the objective of this study was to evaluate the effects of different colored polyethylene shade on the growth and accumualtion of bioactive compounds (e.g. chlorogenic acid, total flavonoids, quercetin, apigenin and acacetin) in medicinal *C. morifolium* capitulum, with the ultimate goal to optimize the production conditions under controlled environments.

MATERIALS AND METHODS

Plant material and growth conditions

The genotype selected for this study was *C. morifloium* Ramat. cv. *Chuju*, one of the standard varieties of *C. morifolium* in Chinese Pharmacopeia (Chinese Pharmacopoeia Commission, 2010). The experiment was carried out at the research greenhouse of Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing, P.R. China during the month of April-November, 2008. On 9 May, uniformly rooted *C. morifolium* shoot cuttings with three to four leaves were planted in plastic pots (25 × 25 cm) in standard peat mixture，with two cuttings per pot. After one month, plants were moved into iron framed growth chambers (2.0 × 1.2 × 1.3 m^3), and each growth chamber was covered with colored polyethylene shade, namely, transparent, red, yellow, and blue polyethylene (Shanghai Weikang CO., LTD., Shanghai, P.R. China), respectively. All growth chambers were placed in the greenhouse. Each treatment was replicated thrice in a randomized block design. There were ten pots inside each growth chamber. Transmission spectra of colored polyethylene shade were analyzed by UV-Vis spectrophotometer (Lambda25, PerkinElmer Inc., Waltham, MA, USA), scanning from 1000 to 350 nm, with a step of 2 nm. During the experiment period, light intensity was measured with Testo 540 Lux Meter (Testo Inc., Sparta, NJ, USA).

Plant height, flower diameter, number of flower and branch, and dry weight of plant were measured at the end of the experiment (tubular-shaped florets and ray florets being all opened). The contents of bioactive compounds (chlorogenic acid, total flavonoids, quercetin, apigenin and acacetin) in the dried capitulum were determined at five stages of chrysanthemum flower development according to the method of Guo et al. (2008) as followed: Tubular-shaped florets and ray florets being not opened yet (A stage), ray florets being opened 30% while tubular flower being not opened (B stage), ray florets being opened 50% and tubular florets being opened 30% (C stage), ray florets being opened 70% and tubular florets being opened 50% (D stage), ray florets and tubular florets being fully opened (E stage). Fresh flowers were treated with 100℃ steam for 3 min to deactivate enzymes, oven-dried at 50℃, and stored in plastic bags at -20℃.

Determination of the chlorogenic acid

Chlorogenic acid was extracted and quantified by adaptation of the method of Wu and Zhang, (2007). Dried chrysanthemum capitulum powder dissolved in 10 ml of methanol in an ultrasonic bath for 1 h and filtered through 0.45 µm filter before injecting into the RP-HPLC (ÄKTA purifier). The operating conditions were Shim-pack C_{18} column (4.6 × 250 mm) at 25℃, acetonitrile (A)-water with 0.5% phosphoric acid solution (B) as mobile phase in gradient mode at a flow of 1.0 $ml \cdot min^{-1}$ and UV detection at 323 nm. The gradient elution program was: 84% A (0 to 15 min), 84 to 0% A (16 to 20 min), 0% A (21 to 30 min), 0 to 84% A (31 to 32 min), 84 to 16% A (33 to 35 min). The injection volume was 100 µl.

Determination of the total flavonoids, quercetin, apigenin and acacetin

The flavonoid extraction method was according to the method of Lee et al. (2005) with slight modification. Dried chrysanthemum capitulum powder dissolved in methanol and left at room temperature for 4 h, then in an ultrasonic bath for 1 h. One of the

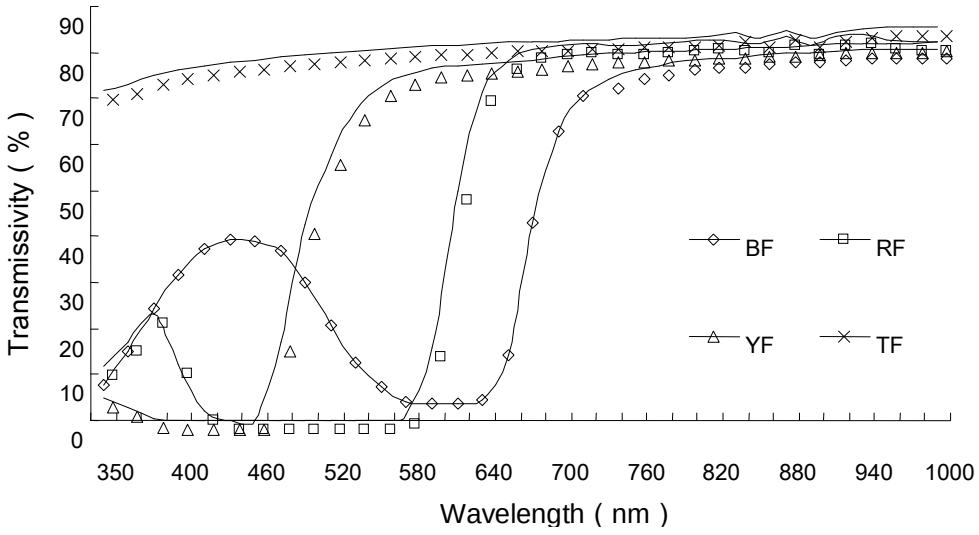


Figure 1. Transmissivity of different color polyethylene films used in the experiment. BF=blue polyethylene film, RF=red polyethylene film, YF=yellow polyethylene film, TF=transparent polyethylene film.

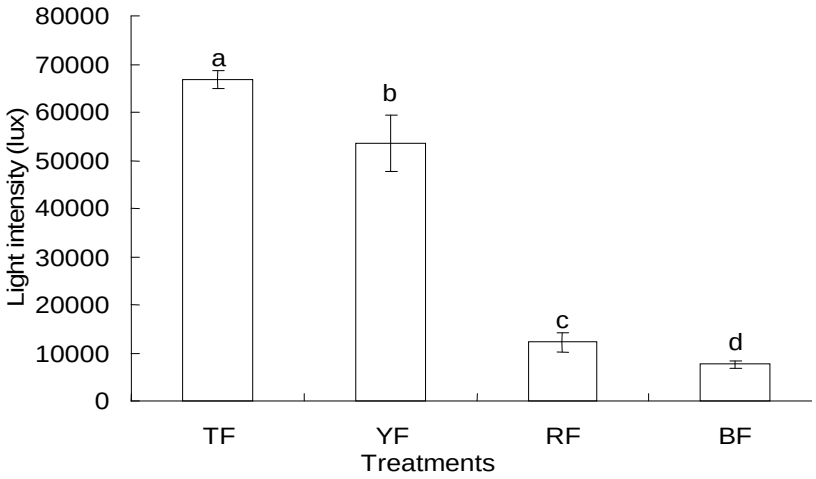


Figure 2. Light intensity of different colored polyethylene films used in the experiment. BF=blue polyethylene film, RF=red polyethylene film, YF=yellow polyethylene film, TF=transparent polyethylene film. The letters above the columns indicate significant differences at P<0.05.

upper phases was hydrolyzed with hydrochloric acid at 90℃ in a water bath for 60 min. Centrifuged for 20 min at 9000 rpm, and the supernatant was analyzed for flavonoids by HPLC (Agilent 1120 Compact LC). ZOBAX Eclipse XDB-C_{18} column (4.6 × 250 mm, 5 μm) was used at 25℃ with the mobile phase of methanol-0.2% phosphatic acid in a gradient manner. The flow rate was set at 1.0 ml·min^{-1}. The detection wavelength was 350 nm. A 15 ml sample was injected and quantified using external standards of quercetin, apigenin and acacetin. Total flavonoids were determined with a spectrophotometric method. One milliliter of the methanol extract was mixed with 5 ml of distilled water, followed by the addition of 1 ml of 5% (w/w) $NaNO_2$ solution. After 6 and 12 min, 1 and 10 ml, respectively, of 10% (w/w) $Al(NO_3)_3$ solution and 1 M NaOH were added. The mixture was brought to 25 ml with distilled water and determined at 510 nm using a spectrophotometer. The content of total flavonoids was calculated as rutin equivalents.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Statistical analyses were conducted using the statistical software package SPSS 11.5 for Windows. A level of P<0.05 was used as

Table 1. Growth of *C. morifolium* plants under different plastic films (mean±s, n=10). BF=blue polyethylene film, RF=red polyethylene film, YF=yellow polyethylene film, TF=transparent polyethylene film. Different letters in each column indicate significant differences at P<0.05 and the same letters indicate non-significant ones.

Treatment	Plant height (cm)	Branch No.	Flower No.	Flower diameter (cm)	Dry weight (g)
TF	45.00±6.05^{d}	9.90±5.38^{a}	32.60±10.46^{a}	4.38±0.26^{c}	22.05±2.68^{ab}
YF	52.67±4.88^{c}	10.60±3.24^{a}	24.70±7.63^{b}	4.51±0.07^{c}	23.64±1.23^{a}
BF	70.23±7.37^{a}	4.40±1.71^{b}	11.50±4.65^{c}	5.66±0.26^{a}	13.99±0.84^{c}
RF	60.92±12.35^{b}	7.60±2.55^{a}	19.40±8.76^{b}	5.22±0.14^{b}	19.65±1.20^{b}

the criterion for statistical significance.

RESULTS

Spectral filters

Colored polyethylene shade had distinct influence on light intensity and transmissivity throughout the study period (Figures 1 and 2). Transparent polyethylene shade had the highest light intensity followed by yellow and red polyethylene shade, while blue polyethylene shade showed the lowest intensity of light. In case of wavelength of transmission spectra, transparent polyethylene shade always had high light transmissivity, while yellow, red and blue polyethylene shade showed their specific spectral characteristics. The highest level of photosynthetically active radioation (400 to 700 nm) was found in transparent polyethylene shade, followed by yellow, red, and blue polyethylene shade.

Growth parameters under colored polyethylene shades

The different colored polyethylene cover manipulated the microclimate that significantly (P<0.05) influence the morphological parameters (Table 1). All colored polyethylene cover tested increased plant height significantly compared to control treatment (transparent polyethylene shade). The height of chrysanthemum plants under the blue, red and yellow polyethylene cover was increased by 56.07, 35.38 and 17.04% in comparison with transparent polyethylene shade, respectively.

The flower number of chrysanthemum plants from transparent polyethylene cover was found significantly higher than those under colored polyethylene cover whereas the plants under blue polyethylene shade had lowest flower number. An opposite pattern of results was observed in terms of flower diameter.

Chrysanthemum plants from blue polyethylene shade had the lowest biomass yield, which was 59.18, 63.45 and 71.20% of those from yellow, transparent, and red polyethylene shade, respectively. In case of branch number, the plants grown under blue polyethylene cover was lowest than those under other polyethylene cover, and there was no significant difference among transparent, yellow and red polyethylene cover treatments.

Bioactive component content at different flower development stages

The chlorogenic acid content was observed higher under the yellow polyethylene cover than under other and middle stage of flower development (from A to D stage). However, blue polyethylene cover led to higher chlorogenic acid content at the mature stage (E stage, Figure 3). The chlorogenic acid content in the chrysanthemum capitulum from red shaded polyethylene cover showed no significant variation during the different stages of flower development. While blue shaded polyethylene cover resulted in a lowest chlorogenic acid content at A, B and D stage.

The lower total flavonoids content was found in the *C. morifolium* plants under blue shaded polyethylene during the developmental period of chrysanthemum flower compared to other shade polyethylene (Figure 4). And the total flavonoids content under different colored shaded polyethylene peaked at different stage throughout the study period. Specifically, yellow polyethylene shade resulted in the highest total flavonoids content at B stage, while red and transparent shaded polyethylene led to the highest value at C stage and D stage, respectively. At the mature stage (E stage), the chrysanthemum capitulum from four colored shaded polyethylene showed no significant difference in total flavonoids content. And the variation tendency of quercetin content was similar to total flavonoids content, except that the lowest quercetin content at A and B stages was observed under transparent shaded polyethylene.

The acacetin content in chrysanthemum plants grown under four shaded polyethylene peaked at B stage (ray florets being opened 30%, while tubular flower being not opened) and then decreased. In generally, plants under transparent shaded polyethylene conditions showed higher acacetin content than other film treatment, while the chrysanthemum capitulum under blue colored shaded polyethylene contained lower acacetin content compared to other treatments. The variation of apigenin content

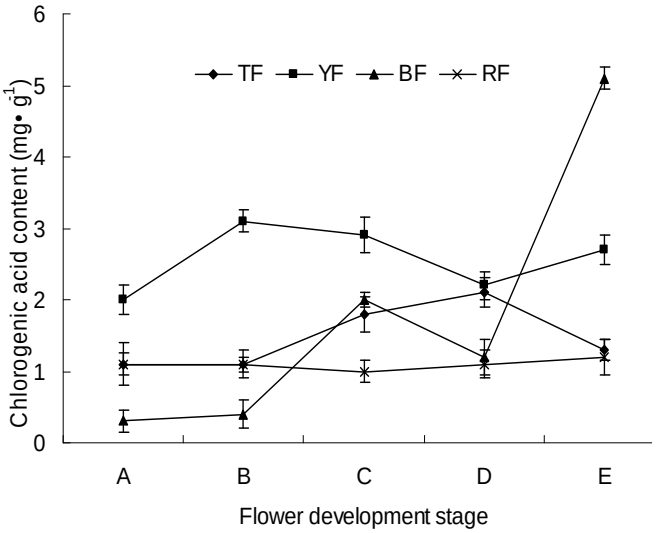


Figure 3. Chlorogenic acid content in *C. morifolium* capitulum at different flower developmental stage. BF=blue polyethylene film, RF=red polyethylene film, YF=yellow polyethylene film, TF=transparent polyethylene film. A=Tubular-shaped florets and ray florets being not opened yet, B=ray florets being opened 30% while tubular flower being not opened, C=ray florets being opened 50% and tubular florets being opened 30%, D=ray florets being opened 70% and tubular florets being opened 50%, E=ray florets and tubular florets being fully opened.

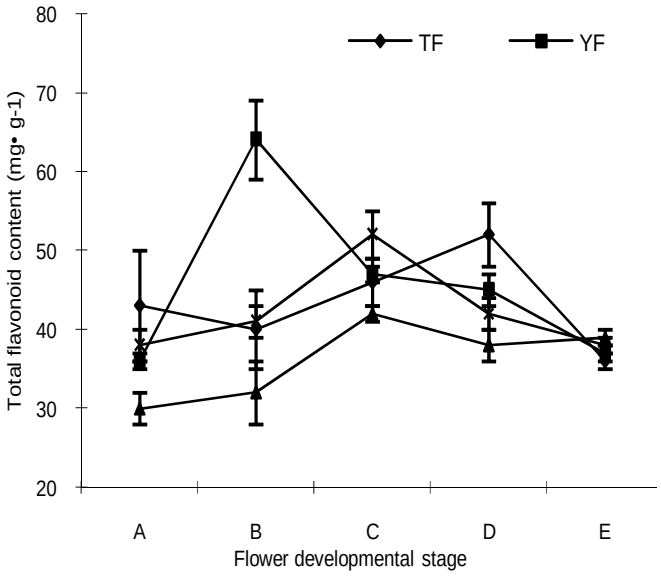
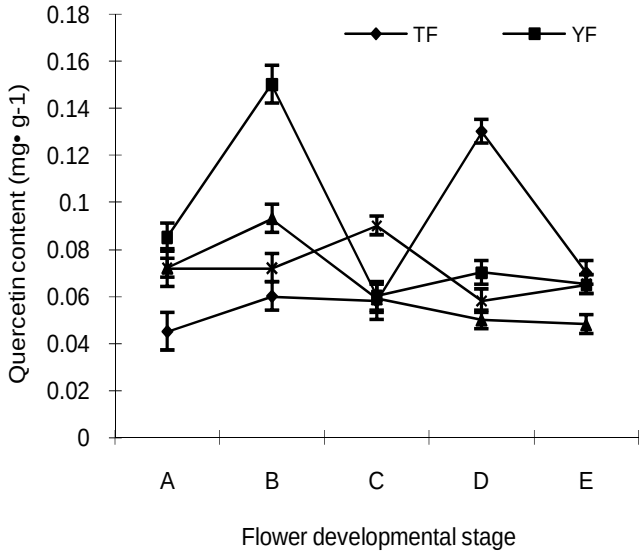


Figure 4. Contents of total flavonoid, quercetin, acacetin and apigenin in *C. morifolium* capitulum at different flower developmental stage. BF=blue polyethylene film, RF=red polyethylene film, YF=yellow polyethylene film, TF=transparent polyethylene film. A=Tubular-shaped florets and ray florets being not opened yet, B=ray florets being opened 30% while tubular flower being not opened, C=ray florets being opened 50% and tubular florets being opened 30%, D=ray florets being opened 70% and tubular florets being opened 50%, E=ray florets and tubular florets being fully opened.



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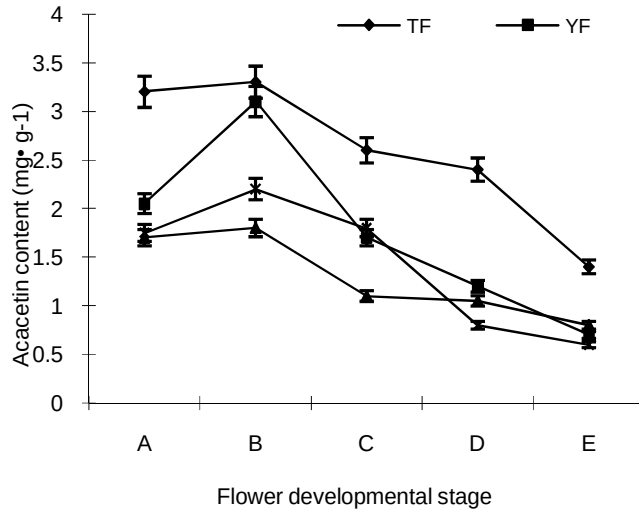

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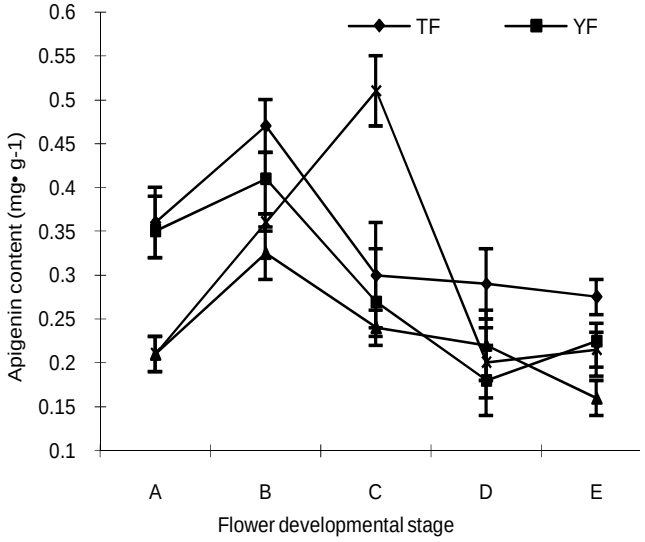
followed the similar pattern, except that the apigenin content under red shaded polyethylene peaked at C stage (ray florets being opened 50% and tubular florets being opened 30%).

DISCUSSION

Decoteau et al. (1989) reported that the color of poly ethylene shade largely determines its energy-radiating behavior and its influence on the microclimate around a vegetable plant. Current study found that colored polyethylene shade could increase the height of



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chrysanthemum in the order of blue > red > yellow. This result was in disagreement with the results of Khattak and Pearson (2006) who found that the spectral filters with low blue transmission produced the tallest plants whereas the spectral filters with relatively higher blue transmission resulted in shorter plants. And they speculated that the activity of phytochrome as well as a possible cryptochrome affects plant height in chrysanthemum. Shimizu and Ma (2006) showed that blue light from LEDs inhibits stem elongation of chrysanthemum *in vivo*. Previous work suggested that a blue acting photoreceptor (now known as cryptochrome) affects plant height in chrysanthemum, as well as phytochrome (Khattak and Pearson, 2006). The discrepancy of results may be mainly due to the different light intensity condition in those experiments. In current study, the magnitude of height increment under different colored shaded polyethylene was related negatively to the light intensity under corresponding polyethylene, indicating that the light intensity caused by colored cover had marked influence on the height of chrysanthemum.

Previous works showed that flower bud development in Chrysanthemum is affected by different light and temperature conditions. Cockshull and Hughes (1971) found that the number of florets formed on the receptacle was modified by light level during the initial period of floret formation. Karlsson et al. (1989) pointed out that flower area increases linearly as PPF increases and that it reaches its maximum under the optimal temperature conditions of about 18 to 20℃. Under the present study conditions, colored polyethylene shade decreased the flower number and increased the flower diameter, and the magnitude of effect was in the order of blue>red>yellow. Chrysanthemum growth is regulated by the light condition during irradiation (Ma et al., 2007). Blue polyethylene shade reduced significantly the biomass yield of chrysanthemum. This result was in agreement with the result of previous works (Oyaert et al., 1999; Ma et al., 2007). Oyaert et al. (1999) suggested that the decreased plant dry weight under the blue filters is probably a result of a decreased CO_2-assimilation rate. Additionally, Rajapakse and Kelly (1995) found that blue filters reduce the concentration of soluble sugars (sucrose, glucose and fructose) and starch in leaf and stem of chrysanthemum. Another explanation may be due to the low light intensity under blue shaded polyethylene conditions, though Ma et al. (2007) implied that the effect of light quality was more significant than that of light intensity and photoperiod on the growth of chrysanthemum.

Plant responses to red, blue, or UV radiation and induce different expression via signal transduction pathways using signal transducing photoreceptors which is a switch for controlling the expression of specific genes involved with growth, developmental processes and secondary metabolism in plants (Jenkins et al., 1995). Thus, photoselective films is an important method to optimize secondary metabolites accumulation through regulating the light condition and influencing the growth and secondary metabolism of medicinal plants (Dai et al., 2004; Yan et al., 2003, 2004; Khandaker et al., 2010).

In current study, the plants grown under blue shaded polyethylene had the lower contents of total flavonoid, quercetin, apigenin, and acacetin. Some previous researches have demonstrated the negative effect of blue light on the accumulation of some secondary metabolites. For example, Afreen et al. (2005) reported that under blue light the glycyrrhizin in the root tissues concentration was comparatively low to others. Blue light also inhibited the accumulation of total phenolic, flavonoid and chlorogenic acid compared with red, fluorescent and blue plus far-red light treatment (Shohael et al., 2006). Kurata et al. (2000) found that level of anthocyanin production by blue light was lower than that by the white light in strawberry cells.

Additionally, the extremely low light availability under blue polyethylene shade condition probably resulted in photosynthesis inhibition and adversely affected the secondary metabolism. The carbon/nutrient balance theory suggests if light becomes limiting (that is, shade) in nutrient-rich environments, the decline in photosynthesis may limit carbohydrates for growth and Carbon-based defenses (Bryant et al., 1983). For carbon-based secondary metabolites such as total flavonoid, quercetin, apigenin, and acacetin, this observation was coincided with the results in other medicinal plants (Briskin et al., 2001; Wang et al., 2007).

In conclusion, variable light transmitted by the different shade colors coupled with distinct light intensity variation in each shade color are implicated for the variability in growth pattern, chlorogenic acid, total flavonoids,

quercetin, acacetin and apigenin accumulation. Colored polyethylene shade decreased the flower number and biomass yield while increased the flower diameter and plant height. The health beneficiary bioactive compounds in chrysanthemum grown under different polyethylene shade peaked at different stage throughout the study period, and blue polyethylene shade adversely affected the biomass yield and bioactive compounds accumulation.

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