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滤光膜对喜树幼苗叶片生长和喜树碱含量的影响

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摘要：喜树（*Camptotheca acuminata*）为中国特有树种，因其次生代谢产物喜树碱具有抗癌作用而闻名。通过用黄色、红色、蓝色 3 种滤光膜对温室栽培的喜树幼苗进行遮光处理，研究了不同光照环境下喜树幼苗叶片生物量、叶绿素含量、光合作用和喜树碱含量的差异。结果表明在 30d 的遮光过程中，红膜和蓝膜遮光明显导致幼苗叶片生物量降低，黄膜遮光下幼苗叶片生物量在处理后 25d 才表现明显降低。不同滤光膜下幼苗叶片叶绿素含量先降低然后升高，遮光幼苗的叶绿素 a/b 明显低于日光幼苗。幼苗日最大净光合速率的顺序是：日光＞黄膜＞红膜＞蓝膜。处理后第 20 天，不同滤光膜下幼苗的光饱和光合速率（A_{max}）、光饱和点（I_s）、光补偿点（I_c）、最大表观量子效率（AQY_{max}）都不同程度的低于日光幼苗。处理后第 10 天至第 30 天，遮光幼苗叶片喜树碱含量均显著高于日光下幼苗，以蓝膜下幼苗的喜树碱含量最高。蓝膜和黄膜下幼苗的喜树碱产量在后期处理中显著高于日光下幼苗，蓝膜下幼苗喜树碱产量在第 30 天最高，是日光下幼苗的 2.49 倍。红膜下幼苗的喜树碱产量在第 10 天后与日光下幼苗差异不显著。通过滤光膜遮光促进喜树碱在幼苗叶片中的积累，提高了叶片喜树碱产量，对喜树碱的生产实践有一定的意义。
关键词：喜树；滤光膜；生物量；叶绿素含量；光合作用；喜树碱含量

Effects of color films on growth and camptothecin content in the leaves of *Camptotheca acuminata* seedlings

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Abstract: *Camptotheca acuminata* Decaisne (Nyssaceae) is a native Chinese tree that produces camptothecin (CPT), a pentacyclic quinoline alkaloid, which is known for its remarkable inhibitory activity against tumour cells. To investigate the effects of shading on the biomass, chlorophyll content, photosynthesis and CPT content on leaves of *C. acuminata*, the seedlings of *C. acuminata* with 4 pairs of leaves were shaded by yellow, red, and blue film in plots in a greenhouse. The resulting relative light intensity were 60.69%, 51.76%, and 26.96% compared to the full sunlight respectively. The biomass of leaves, chlorophyll, photosynthesis and CPT content in leaves were measured on the 5^{th}, 10^{th}, 15^{th}, 20^{th} and 25^{th} day respectively. Light-response curves were measured on Day 20.

The biomass accumulation of the leaves is significantly inhibited by the shade of yellow, red or blue film. The inhibition degree varies according to light quality and light intensity. The degree of inhibitions of the red film and blue film are gradually increased over 30 days of shade treatment; however the inhibition of yellow film does not approach significance until the 25^{th} day of treatment.

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The kinetics of chlorophyll contents in the leaves shows that total chlorophyll contents under various films are lower than those in full sunlight in the first five days. The contents increase gradually after ten days, and become higher than those in sunlight after fifteen days of the treatment. The ratio of chlorophyll a and chlorophyll b contents in the shaded leaves is consistently lower than that in sunlight over the study duration. The chlorophyll content in shaded leaves is quite different among these three films, and does not show regular characteristics.

The maximum net photosynthesis rates of all shaded seedlings decrease over time and being to become lower than that in sunlight around Day 5 of the treatment and is much less after day 10. The order of the maximum net photosynthesis rates from the highest to the lowest is under the condition of sunlight, yellow film, red film and blue film (No obvious difference between the red film treatment and the blue film treatment is found). After 20 days of treatment, the photosynthesis rate of light-saturated point (A_{max}), light-saturated point (I_s), light-compensated point (I_c), maximum apparent quantum yield (AQY_{max}) of shaded seedlings by various films are all lower than that in sunlight to different degrees. The A_{max}, I_s, I_c and AQY_{max} of seedlings under the yellow film treatment are 80.86%, 61.01%, 26.66% and 81.35% of those in sunlight respectively. The I_c of seedlings with the red film treatment is lower than that with the yellow film treatment, A_{max}, I_s and AQY_{max} however does not show obvious differences. The A_{max}, I_s and AQY_{max} of seedlings with the blue film treatment is lower than that with yellow and red film treatments, eventhough but I_c is higher.

The CPT content in leaves of all shaded seedlings shows no significant difference in the first five days of treatment from that in sunlight, but is remarkably increased and become higher from the 10th day to the 30th day of treatment. The leaves treated with blue film for 30 days have the highest CPT content, whereas the leaves treated with red and yellow films have similar CPT content. The CPT yield in leaves treated with blue and yellow films increases significantly and become higher than that in sunlight after ten days of treatment. The CPT yield under the red film is higher than that of sunlight only around day ten of treatment. The highest CPT yield is obtained under the blue film around Day 30 of treatment, which is 2.49 times higher than that in sunlight. CPT yield under the red film treatment, however, is higher than that in sunlight only in Day 10, and no obvious difference is observed in other periods. The above results demonstrate that the shade treatment with films, especially blue film, accelerate the accumulation of CPT in the leaves of seedlings, which may have practical significance in the production of CPT.

Key words: *Camptotheca acuminata*; color films; biomass; chlorophyll content; photosynthesis; camptothecin content

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光是植物生命活动中重要的环境因子之一，它不仅是植物生长发育的能量来源，而且作为信号因子调控植物的生长发育。人们通常从光强和光质两方面分析光照对植物的初生代谢过程、生长状态[1~3]和次生代谢过程[4,5]的影响规律。在科研和生产实践中，通过遮荫网或可控光源来获得不同的光强，通过各种颜色的荧光灯或滤光膜（滤光片）来获得不同的光质，从而实现对光照环境的控制。由于可控光源和纯光质荧光灯管或滤光片的成本较高，在大规模育苗生产实践中，普遍采用滤光膜遮光来获得特殊的光照环境[6~8]。很多植物的次生代谢产物具有重要药用或经济价值，研究滤光膜遮光环境下植物生长状况和次生代谢产物的变化，不仅可以为研究次生代谢的途径和机理提供依据，而且对在实践生产中提高这些次生代谢产物的产率具有重要意义[9,10]。

喜树（*Camptotheca acuminata* Decne）是珙桐科（Nyssaceae）喜树属（*Camptotheca*）多年生亚热带落叶阔叶树，是我国特有树种。喜树碱是喜树的一种重要的次生代谢产物，对胃癌、直肠癌、膀胱癌等多种癌症、白血病和艾滋病有较好的疗效[11,12]。对于喜树的生物学特性[13,14]、生长规律[15~18]、喜树碱的开发利用[11]，喜树碱在喜树幼苗中的分布规律[19,20]，以及遮荫、水渍对喜树中喜树碱含量的影响[21]等已有一些研究工作。研究表明喜树幼苗时期叶片中的喜树碱含量较高[20]，通过人工栽培喜树获得幼苗叶片是为喜树碱生产提供材料的有效手段。通过光照环境的改变进一步提高喜树幼苗叶片中喜树碱的含量对喜树碱的生产具有重要的意义。通过温室栽培实验，利用滤光膜进行处理，研究了不同光照环境下喜树幼苗生长和次生代谢产物喜树碱含量的差异，探讨了提高喜树幼苗叶片中喜树碱含量的光照环境条件。

1 研究方法

1.1 栽培处理实验

通过喜树种子获得实生幼苗，种源为四川金堂。幼苗在哈尔滨市（东经 126°38′，北纬 45°43′）东北林业大学阳光温室中培养。温室为自然采光，培养期间的昼间温度 23～26℃，夜间温度 17～20℃，昼夜温度自然过渡。湿度为 50%～60%。将幼苗栽

植于盛有普通花土,直径 15 cm,深 15 cm 的花盆中,每盆 1 株,建立喜树幼苗的实验种群。花土的有机质含量为 3.82%,全氮含量为 0.32%,全磷含量为 0.11%,全钾含量为 2.69%,pH 值为 6.9。待幼苗长出 4 对真叶后,进行滤光膜处理,分别遮以黄色、红色和蓝色的滤光膜,其相对光强分别为全光照的 60.69%、51.76%和 26.96%,3 种滤光膜对光质的影响见图 1。每个处理由每行 10 盆×每列 10 盆幼苗形成试验种群,在各取样时期,选取各处理中具代表性的幼苗进行测定。

1.2　叶片生物量、叶绿素含量、光合作用和喜树碱含量的测定

摘取每株幼苗的全株叶片,烘干测定干重后粉碎、混匀,测定喜树碱含量,喜树碱含量按照阎秀峰等[22]的方法测定。选取幼苗上部充分展开的叶片,利用 LI-6400 便携式光合作用系统(LI-COR 公司,美国)测定叶片的光合速率,而后摘取叶片进行叶绿素含量的测定。叶绿素含量按 Arnon[23]的方法测定。以上各项指标的测定,分别于处理后的第 5、10、15、20、25、30 天进行。在处理后的第 20 天测定各处理幼苗的光响应曲线。每个处理测定 3～5 株。

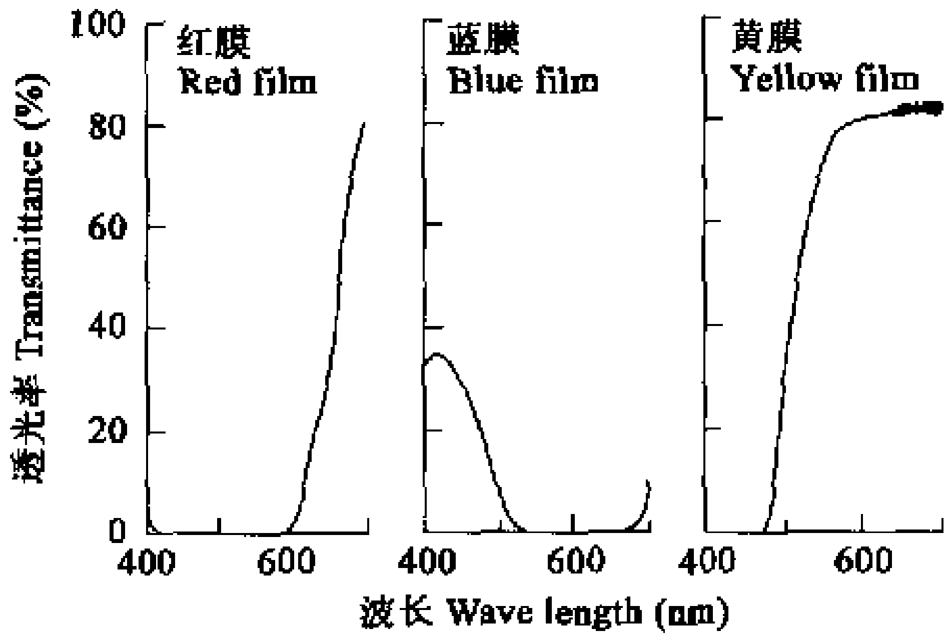

图 1　滤光膜对光质的影响

Fig. 1　Effect of film on light quality

2　结果与分析

2.1　滤光膜对喜树幼苗叶片生物量的影响

从图 2 中可以看出,在处理的前 10d,不同颜色滤光膜对喜树幼苗叶片的生物量影响不明显,而在第 15d 以后,这种影响逐渐明显。在处理的第 15 天,红膜和蓝膜下幼苗叶片的生物量(干重)分别为日光下的 83.9%和 80.1%,差异显著。第 15 天至第 30 天,这种差异不断加大,处理后的第 30 天,红膜和蓝膜下的叶片干重分别为日光下的 63.5%和 59.0%。虽然红膜下叶片的生物量要稍高于蓝膜,但两者间的差异不明显。黄膜下叶片生物量在处理第 25 天以后才表现出与日光下的明显差异,干重为日光下的 82.0%。由此可以看出,在整个 30d 的遮光过程中,蓝膜和红膜造成光照环境的差异对幼苗生物量的影响较明显,黄膜不明显。

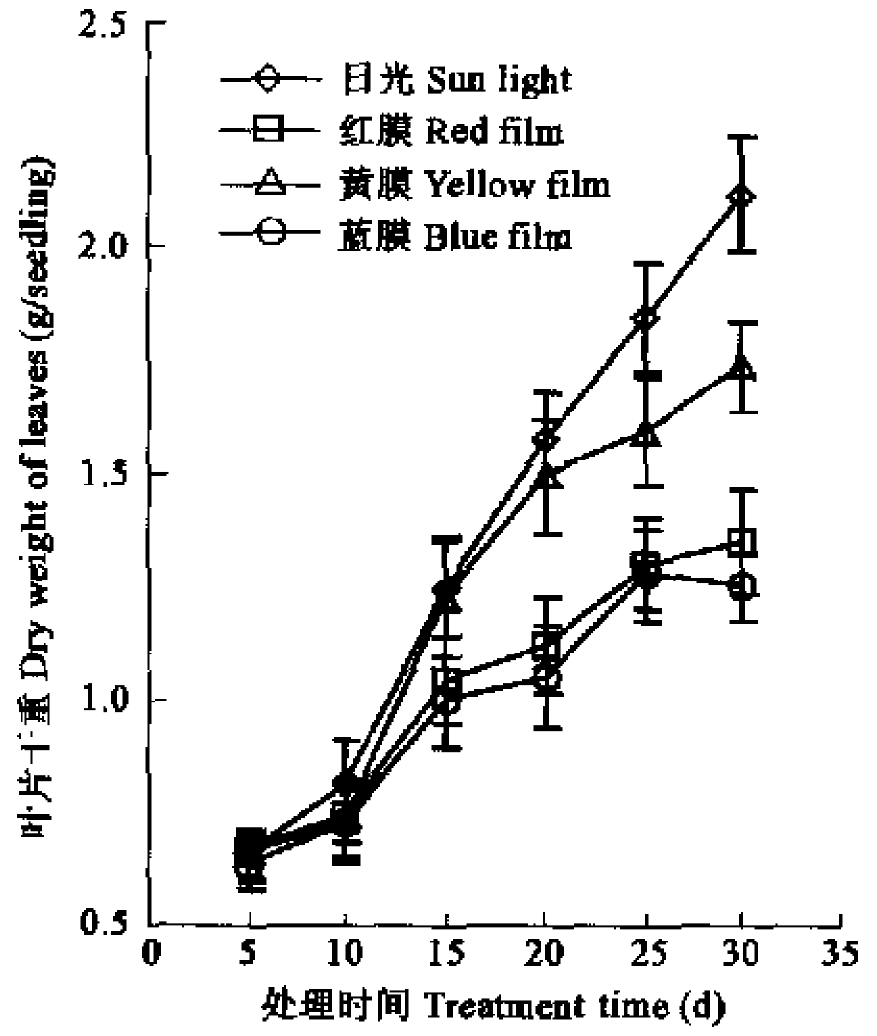

图 2　不同颜色滤光膜下喜树幼苗全株叶片的生物量

Fig. 2　The biomass of whole leaves of *Camptotheca acuminata* seedlings under different color films

2.2　滤光膜对喜树幼苗叶片叶绿素含量的影响

不同颜色的滤光膜遮光后,喜树幼苗叶片中的叶绿素含量与日光下幼苗明显不同。各遮光幼苗叶片的总叶绿素含量在处理后的第 5 天明显低于日光,第 10 天后各遮光幼苗的总叶绿素含量开始上升,并在第 15 天后高于日光(见图 3 A)。滤光膜遮光幼苗的叶绿素 a/b 值在处理第 5 天后明显低于日光(见图 3 B)。在整个处理过程中,不同滤光膜下幼苗间的叶绿素含量存在差异,但没有明显的规律。

2.3　滤光膜对喜树幼苗光合作用的影响

在不同的遮光环境下,喜树幼苗的日最大净光合速率表现出差异(图 4)。处理后的第 5 天,黄膜、蓝膜、红膜下幼苗的净光合速率则表现出不同程度地低于日光下的幼苗。第 10 天以后,遮光幼苗的净光合速率显著地低于日光下幼苗。在整个处理过程中,幼苗日最大净光合速率的顺序是:日光>黄膜>红膜>蓝膜。红膜下和蓝膜下幼苗之间净光合速率的差异不显著,而这两者与黄膜下及日光下幼苗在第 10 天后均表现为显著性差异。这种差异不仅与遮光造成的光质环境差异有关,还与光照强度的差异有关。因为黄膜下的光照强度为日光的 60.69%,而红膜和蓝膜下的光照强度分别为日光的 51.76%和 26.96%。

幼苗在不同遮光环境下光响应曲线的差异,进一步表明不同颜色滤光膜对幼苗光合作用的影响。遮光处理后第 20 天时,日光下幼苗的光饱和光合速率(A_{max})为 7.42μmol CO_2/(m^2·s),光饱和点(I_s)为 560.13μmol photons/(m^2·s),光补偿点(I_c)为 45.48μmol photons/(m^2·s),最大表观量子效率(AQY_{max})为 32.7 mmol CO_2/mol photons。不同滤光膜下生长的幼苗的这些指标都不同程度的低于日光。黄膜下幼苗的 A_{max}、I_s、I_c 和 AQY_{max} 分别为日光下幼苗的 80.86%、61.01%、26.66%和 81.35%,表现出显著性差异。红膜下幼苗的 I_c 明显低于黄膜,其它指标两者差异不明显。蓝膜下幼苗的 A_{max}、I_s、AQY_{max} 明显低于黄膜和红

膜，I_c 显著高于这两者，但全部指标都明显低于日光下幼苗(见表 1)。

表 1 滤光膜对喜树幼苗光合作用的影响

Table 1 Effect of films on photosynthesis of *Camptotheca acuminata* seedlings

	光饱和光合速率 ($\mu molCO_2m^{-2}s^{-1}$) Photosynthesis rate of light-saturated point (A_{max})	光饱和点 ($\mu molCO_2m^{-2}s^{-1}$) Light-saturated point (I_s)	光补偿点 ($\mu molCO_2m^{-2}s^{-1}$) Light-compensated point (I_c)	最大表观量子效率 (mmol CO_2mol^{-1}photons) Maximum apparent quantum yield (AQY_{max})
日光 Sunlight	7.42 ± 0.27^a	590.13 ± 35.05^a	45.46 ± 9.84^a	32.7 ± 2.8^a
黄膜 Yellowfilm	6.00 ± 0.42^b	360.06 ± 20.11^b	12.12 ± 1.78^b	26.6 ± 1.9^b
红膜 Redfilm	5.79 ± 0.35^b	361.89 ± 24.73^b	7.78 ± 0.82^c	26.0 ± 1.8^b
蓝膜 Bluefilm	5.23 ± 0.33^c	304.21 ± 18.92^c	14.49 ± 1.87^b	23.9 ± 0.9^c

同一列数据中字母不同者表示差异性显著($p<0.05$) Data with different letters are significantly different($p<0.05$)in the same column

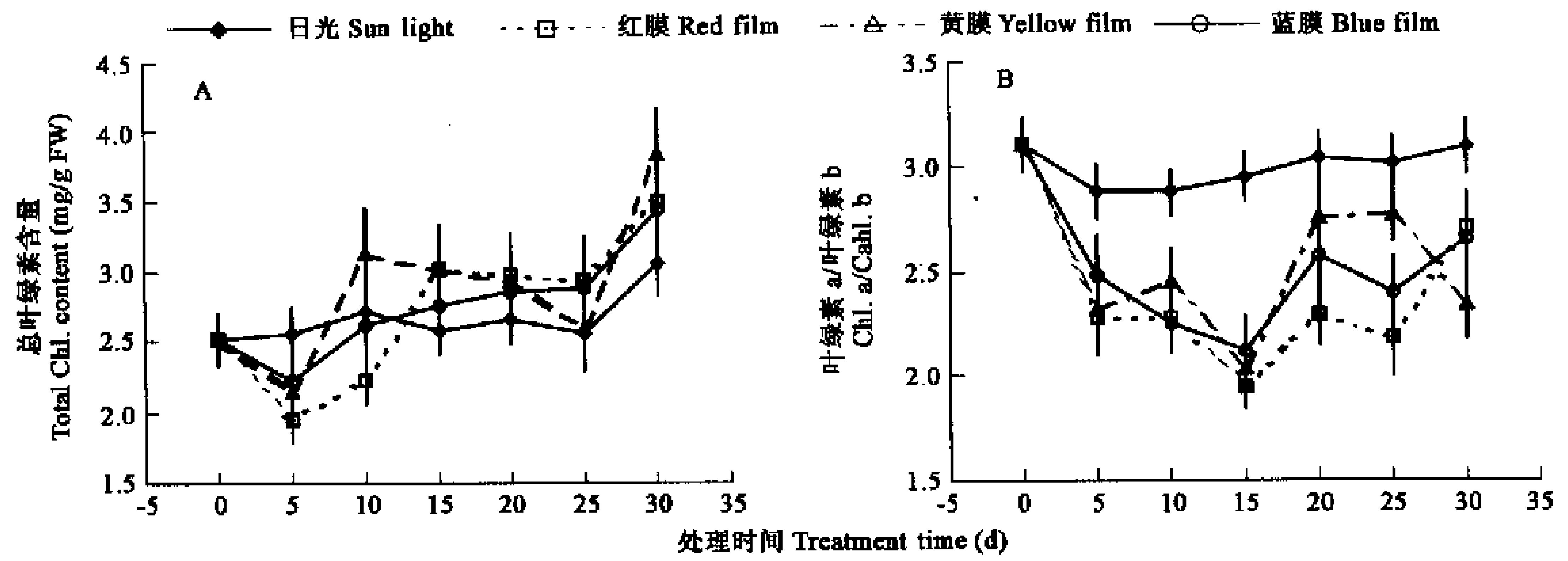

图 3 不同颜色滤光膜下喜树幼苗叶片的叶绿素含量

Fig. 3 The chlorophyll content of whole leaves of *Camptotheca acuminata* seedlings under different color films

2.4 滤光膜对喜树幼苗叶片中喜树碱含量和产量的影响

日光下幼苗叶片的喜树碱含量在第 20 天时最高，然后开始下降。各个时期滤光膜下喜树幼苗叶片喜树碱含量不同程度的高于日光下幼苗(图 5A)。处理后的第 5 天，这种差异不显著，第 10 天以后，各遮光条件下幼苗的喜树碱含量明显高于日光下幼苗。其中，红膜和黄膜下幼苗间的喜树碱含量差异不显著，蓝膜下幼苗的喜树碱含量为各处理中最高，第 10 天至第 30 天的 5 次取样分别为日光下幼苗的 2.40、1.79、1.64、1.76 和 4.23 倍。

对于生产实践而言，人们更加关心叶片中喜树碱的产量(喜树碱含量与叶片干重的乘积)。虽然不同颜色滤光膜下幼苗叶片的喜树碱含量高于日光下幼苗，但是叶片干重却低于日光下幼苗。综合这两个因素，从图 5B 可以看出，蓝膜和黄膜下幼苗的喜树碱产量在处理 10d 后显著高于日光下幼苗，蓝膜下幼苗在处理后的第 30 天的喜树碱产量最高，达到每株 1.486 mg，是日光下幼苗的 2.49 倍。红膜下幼苗的喜树碱产量在第 10 天明显高于日光下幼苗，其它各时期差异不显著。

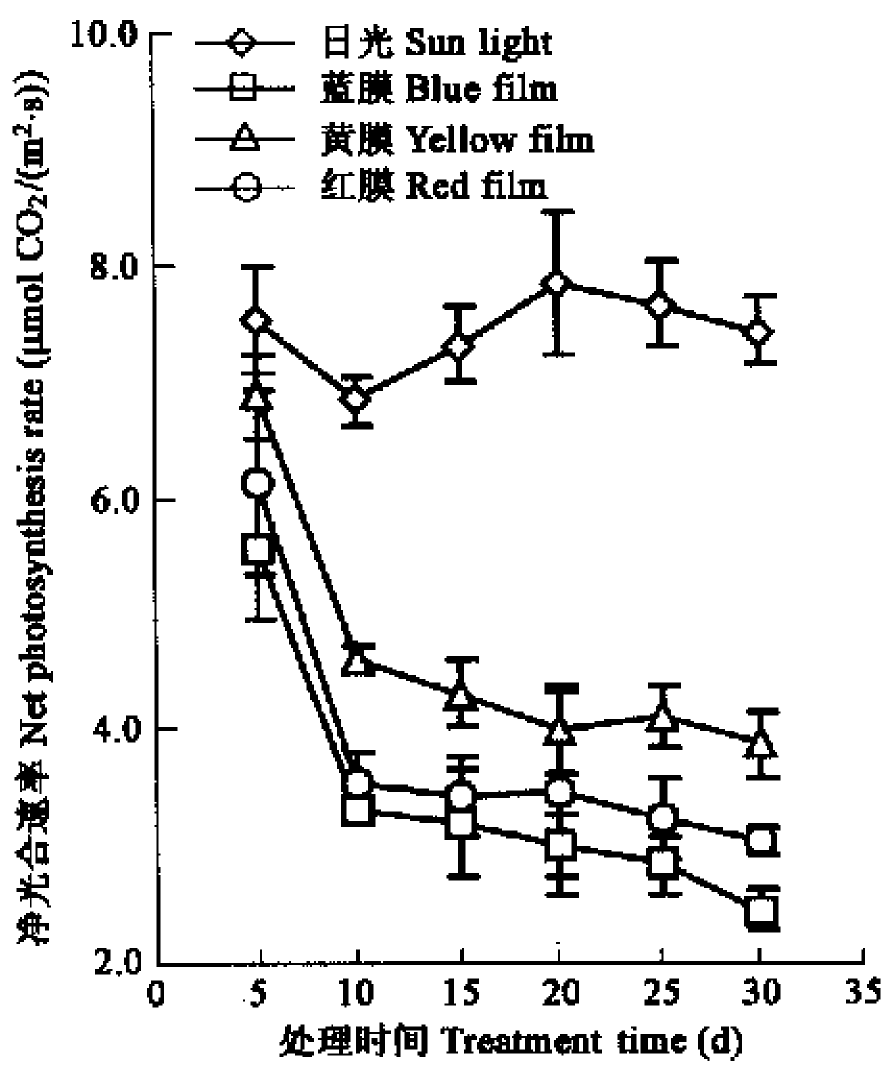

图 4 不同颜色滤光膜下喜树幼苗的净光合速率

Fig. 4 The net photosynthesis rate of *Camptotheca acuminata* seedlings under differnet color films

3 讨论

通过滤光膜遮光来获得不同的光照环境，影响经济作物或药用植物的生长和次生代谢产物的形成，是育苗生产实践中获得高产量的次生代谢物质行之有效的手段[5~8]。白色滤光膜遮光只改变光照强度，而彩色滤光膜遮光在改变光照强度的同时获得不同光质，使生长在特殊的光强和光质环境下的植物在光合能量获得和光形态建成等方面发生改变，从而影响其初生乃至次生代谢机制[6]。

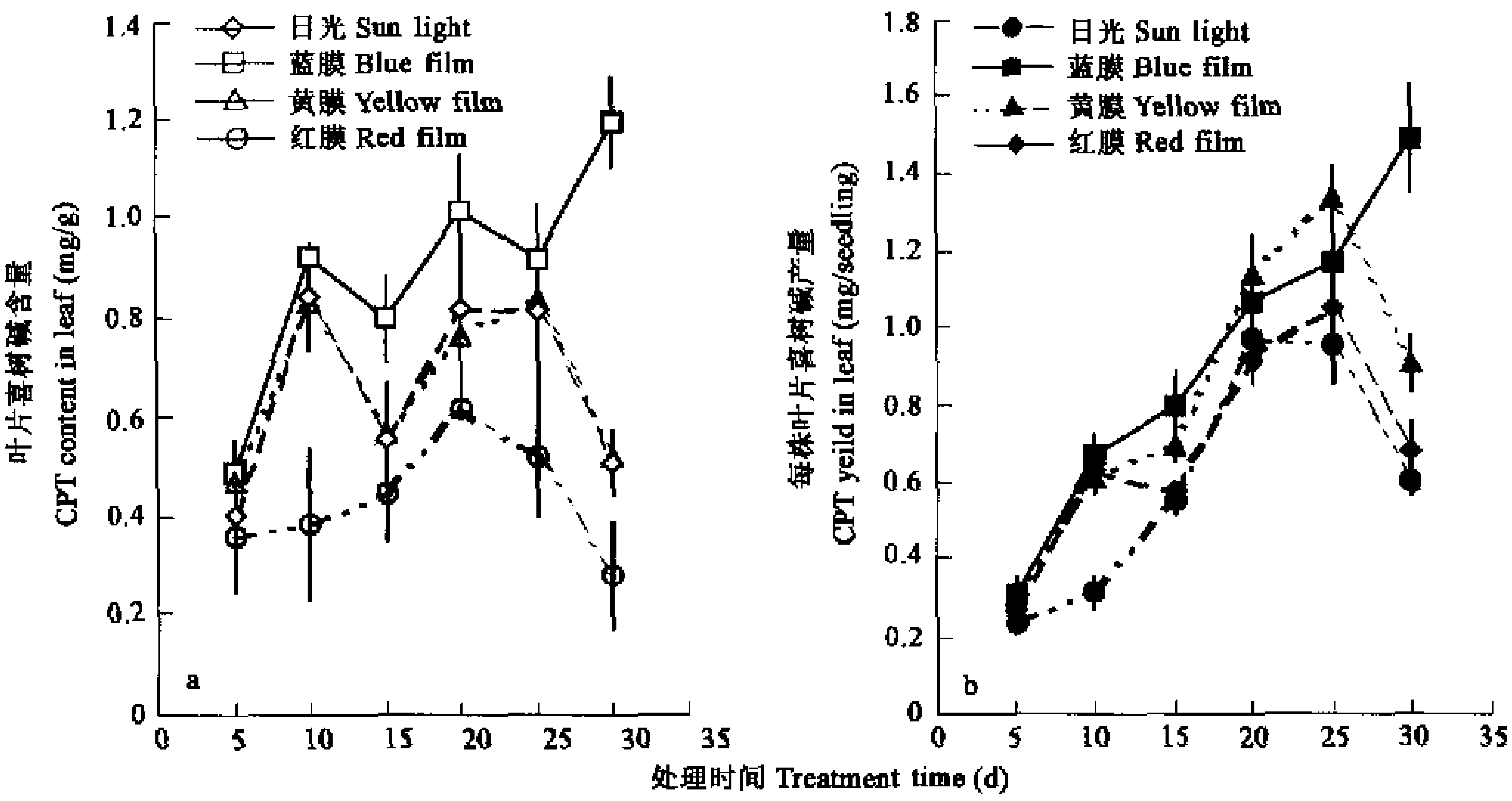

图5　不同颜色滤光膜下喜树幼苗叶片的喜树碱含量和产量

Fig. 5　The camptothecin content and yield in leaf of *Camptotheca acuminata* seedlings under different color films

然而已有的报道多以细胞株系或愈伤组织为材料研究光强和光质对其生长的影响。关于光强对阳生植物的影响有着一致的结论，即随着光强的降低，生长受到抑制，生物量降低[24,25]。而有关光质对植物生长影响的研究结果并不一致[1,3,26~29]。有研究表明蓝光和红光对愈伤组织的诱导、增殖和可溶性蛋白质含量都有促进作用，黄光、蓝光对根、芽的分化有明显的促进作用，而且蓝光对菊花的茎叶生长、侧枝产生也有促进作用[30~32]。在长期(30d)连续用黄、红、蓝3种滤光膜遮光条件下生长的喜树幼苗，叶片生物量都不同程度地低于日光下幼苗。蓝膜和红膜遮光的幼苗叶片生物量在各时期都低于日光，而且随着处理时间的增加，表现得越来越明显。黄膜下幼苗叶片生物量在处理的前20d与日光的差异不显著，但是在后10d也明显低于日光。由此可见，对喜树这一喜光、喜湿的树种而言，在幼苗时期对光强比较敏感，滤光膜遮光导致喜树幼苗叶片生物量的降低主要是由于光强改变所造成的。尤其是红膜和蓝膜下的光强分别只有日光下光强的51.76%和26.96%，显著影响了幼苗生长。遮光幼苗总叶绿素在30d的处理过程中呈现先降低再升高的趋势，叶绿素a/b在整个过程中都低于日光。这表明遮光幼苗叶绿素含量的升高，主要是由于叶绿素b含量升高所造成的，这与蓝光对黄瓜幼苗和菊花叶绿素的影响相近[27,33]。3种颜色滤光膜之间对幼苗叶绿素的影响没有明显的规律，这可能和使用的滤光膜所透过的光谱成分不是单色光光谱有关。各种遮光处理对幼苗光合作用的影响表现出规律性，幼苗各时期的日最大净光合速率、第20天时的光饱和光合速率和光饱和点都表现为：日光＞黄膜＞红膜＞蓝膜，这一顺序与不同滤光膜对幼苗叶片生物量影响的顺序一致。由此可见，在长期遮光的环境下，幼苗叶片为了补偿逆境的影响叶绿素含量升高，然而由于叶绿体光合片层结构的破坏光合效率却明显降低，影响了幼苗的初生代谢过程和碳同化作用，从而导致生物量下降。

与光照对植物初生代谢的影响相比，其对植物次生代谢的影响要复杂得多。植物的次生代谢产物是植物对生长环境长期适应的结果，它们的代谢过程复杂而且种类繁多。很多研究报道了光强和光质对生物碱[4,5,21]、黄酮[9,10]、萜类内酯[10]、挥发性成分[34]、糖苷[6]等次生代谢物质影响。这些研究表明红光强烈抑制黄酮的合成，蓝光则相反[9]；蓝光和黄光促进毛地黄叶组织培养物中强心苷的产生和积累，而红光则抑制[35]；红光较蓝光更有利于悬浮培养细胞中阿玛碱的生成[36]；光质对2年生银杏幼苗中银杏黄酮苷的生物合成与积累影响较小[10]。已有研究表明红色滤光膜下生长的高山红景天(*Rhodiola sachalinensis*)的红景天甙含量显著高于对照，而黄膜对红景天甙含量的抑制作用和蓝膜的促进作用均不明显[6]。在不同滤光膜下生长的喜树幼苗叶片中的喜树碱含量都高于日光下幼苗，而且在蓝膜下最为显著。这说明，滤光膜造成的光质环境有利于促进喜树碱在幼苗叶片中积累，蓝膜的这种促进作用尤为明显。

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