

光强对喜树幼苗叶片次生代谢产物喜树碱的影响

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摘要:喜树碱是我国特有树种——喜树中所含的重要次生代谢产物,在人工控制条件下观察了光强对喜树幼苗叶片喜树碱含量的影响。喜树幼苗叶片的喜树碱含量随着遮荫程度的增加(光照强度降低)而增加,但严重遮荫的(光强为全光照的 20%)在处理后期(75 d)喜树碱含量降低。叶片的喜树碱产量(喜树碱含量与叶片生物量乘积)在处理初期(30 d)随光强减弱而缓慢地略有增加,处理后期(45 d 以后)随光强的减弱而有明显增加,但光强低于全光照的 60%以后喜树碱产量迅速下降。喜树碱的增加可能是喜树幼苗通过次生代谢过程对不良环境(遮荫)的一种适应性反应。

关键词:光强;喜树;喜树碱

Effects of light intensity on secondary metabolite camptothecin production in leaves of *Camptotheca acuminata* seedlings

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Abstract: Alkaloids represent one of the largest groups of natural products. In general, alkaloids serve as defense chemicals against herbivores and to a lesser degree against bacteria, fungi, and viruses or provide a means of interaction with other plants. Camptothecin (CPT) is a monoterpene indole alkaloid originally isolated from *Camptotheca acuminata*, a deciduous tree, and native to south China, that has gained great attention for its significant antitumor activities in experimental studies. CPT is present in all organs of *C. acuminata*, but the contents are usually very low. To enhance CPT yield in plants, some studies on the relationship between CPT production, accumulation and environmental conditions have been performed. In the present work, under controlled conditions, we observed the effects of light intensity on CPT production in leaves of *C. acuminata* seedlings.

Seedlings were grown from seeds in a greenhouse and transported to pots (diameter 15 cm, depth 15 cm). The ventilating windows had been opened to get a similar air temperature and humidity to outdoors in the greenhouse since July 8. On July 15, when the fourth real leaves came out, shading-controlled experiments were conducted with shade-cloth to achieve five different irradiances of 100%, 80%, 60%, 40%, and 20% of full sunlight, respectively. 5 Seedlings were harvested every 15 days from July 30 to October 2 and sampled 5 times. All leaves collected from each seedling were grouped for one sample and the 5 different seedlings harvested at the same time were looked as 5 repetitions.

In full sunlight, leaf biomass gradually increased, and decreased at the end of season of October 2. When the light intensity reduced, the increase of leaf biomass slowed down, but when the irradiance lowered than 40% of full sunlight, leaf biomass tended to decrease after 45 days of treatment.

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In full sunlight, CPT content in leaves of *C. acuminata* seedlings increased during the whole seedling development, and more increment was observed at the end of the growing season, so did in 80% and 60% of full sunlight. When irradiance was 40% of full sunlight, there was little increment of CPT content between the last two samples. On the contrary, under irradiance of 20% of full sunlight, CPT content decreased after 75 days of treatment (Fig. 3 and Fig. 4). Treatment with shading promoted the accumulation of CPT in the leaves of *C. acuminata* seedlings, however long-term treatment (such as 75 d) with heavy shading (irradiance lower to 40% of full sunlight or less) prevented alkaloids content from increasing.

The product of CPT content and leaf biomass results in CPT yield in leaves of *C. acuminata* seedlings. In 100% and 80% of full sunlight, CPT yields increased during seedling development. In 60% of full sunlight, CPT yield increased in the first 60 days under treatment, but then decreased in the samples treated for 75 days. In 40% and 20% of full sunlight, CPT yield increased in the first 30 days of treatment, and decreased after 60 days of treatment (Fig. 5). Under shading treatment, light shading (irradiance of 60% of full sunlight or higher) boosted CPT yield, but with the heavier shading, CPT yield declined during the period of the treatment (Fig. 6).

The enhancement of CPT accumulation in *C. acuminata* seedlings under shading treatment suggests a kind of defensive mechanism. Shading treatment could be selected as a method to enhance CPT content and yield in leaves of *C. acuminata* seedlings.

Key words: light intensity; *Camptotheca acuminata*; camptothecin

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植物的次生代谢是植物在长期进化中与环境(生物的和非生物的)相互作用的结果,次生代谢产物在植物提高自身保护和生存竞争能力、协调与环境关系上充当着重要的角色,其产生和变化比初生代谢产物与环境有着更强的相关性和对应性^[1~4]。生物碱是植物次生代谢产物中较为重要的一类,能对多种环境胁迫做出反应,并可能作为一种防御机制抵御草食动物的危害和病害的侵袭^[5~10]。

喜树碱(camptothecin, CPT)是一种单萜吲哚生物碱,来源于我国特有的落叶阔叶乔木——喜树(*Camptotheca acuminata*),因具有很好的抗肿瘤活性而倍受医学界关注^[11,12]。喜树碱在喜树中的含量通常很低^[13],这导致研究者们关注喜树碱合成、积累与环境条件的关系,并期望找到适宜的培育条件以提高喜树碱的产率。有研究观察了水渍和遮荫处理对喜树幼苗不同器官中喜树碱含量的影响^[14];也有报导增施氮磷钾复合肥后喜树幼苗的喜树碱含量没有升高,甚至出现下降的趋势^[15];还有研究表明干旱条件下喜树幼苗叶片的喜树碱含量明显升高^[16,17]。可能是受喜树分布范围的限制,有关环境因子对喜树中喜树碱含量影响的研究尚不多见,且上述工作多是在美国进行的,而喜树却是我国的特有树种。为此,在人工控制条件下观察了光照、水分等环境因子对喜树幼苗生长及喜树碱代谢的影响,本文报告光强对幼苗叶片喜树碱影响的实验结果。

1 研究方法

1.1 喜树幼苗培养与光照处理

2002年5月初,在温室中由种子获得实生幼苗,栽植于直径15 cm、深15 cm的花盆中,每盆1株。所用土壤的有机质含量为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。温室为自然采光,培养期间的昼间温度主要在23~26℃,夜间温度主要在17~20℃,昼夜温度自然过渡,湿度为50%~60%。7月8日(处理前1周),开启温室两侧的通风窗,使温室内、外的温度、湿度基本上一致,直至实验结束。

7月15日(喜树幼苗长出4对真叶)开始进行遮荫处理。遮荫处理分为4组,组合遮荫网及纱布在幼苗上方做遮荫棚,使棚下的相对光照强度分别为全光照的80%、60%、40%、20%(光强采用LI-6400便携式光合作用测定系统的光量子探头测定,从日出到日落每隔半小时测定一次,由光强-时间曲线积分得全天的总光合有效辐射量进行比较,处理前选择晴好天气连续监测3d)。

1.2 叶片生物量和喜树碱含量测定

处理后大致每隔15 d取样1次,取样日期分别为7月30日、8月14日、8月30日、9月16日和10月2日。每次取样各处理分别取5株喜树幼苗(5重复),摘取每株幼苗的全株叶片,烘干测定干重后粉碎、混匀,用于测定喜树碱含量。喜树碱含量按照阎秀峰等^[18]的方法测定。由喜树碱含量和叶片干重(生物量)的乘积得到幼苗全株叶片的喜树碱产量。所得数据用SPSS软件做统计分析。

2 结果

2.1 光强对喜树幼苗叶片生物量的影响

遮荫处理后光照强度的改变,必然会导致喜树幼苗叶片生物量的变化。考查叶片生物量的变化也是进一步分析叶片中喜树碱含量和产量变化的基础。如图 1 所示,全光照下,喜树幼苗叶片的生物量从 7 月 30 日(15 d)到 9 月 16 日(60 d)是一个逐渐增加的过程,至 10 月 2 日(75 d)叶片生物量的降低,是由于生长季结束、叶内物质向茎、根转移以及少数叶片已经脱落。随着光照强度的减弱,叶片生物量随生育期进程而增加的趋势减缓,光强为 40%和 20%处理的喜树幼苗,叶片生物量在 8 月 30 日(45 d)以后就不再增加,而趋于减少。图 2 更清晰地显示出遮荫处理后不同时间喜树幼苗叶片生物量随光强减弱而降低的趋势。

2.2 光强对喜树幼苗叶片喜树碱含量的影响

全光照下,喜树幼苗叶片中喜树碱的含量随生育期进程而增加,而且越往后期,增加幅度越大。80%和 60%光强下的变化规律与全光照下类似,但在 40%和 20%光强下,最后一次采样时的情况有所不同,40%的喜树碱含量增加幅度明显降低,20%的喜树碱含量反而减少(图 3)。从图 4 可以看出,遮荫(光强减弱)导致喜树幼苗叶片中喜树碱的含量增加,而且随着处理时间的增长,喜树碱含量增加幅度有加大的趋势。不过,在处理的最后期(75 d),严重遮荫的(光强 40%和 20%)已不符合这个规律,40%光强下的喜树碱含量增加幅度减小,20%光强下的喜树碱含量反而降低。

2.3 光强对喜树幼苗叶片喜树碱产量的影响

叶片的喜树碱产量是喜树碱含量与叶片生物量的乘积,是喜树幼苗全部叶片所含喜树碱的总量。从图 5 可以看出,全光照和 80%光强下,喜树碱产量随生育期进程而迅速增加;60%光强下,喜树碱产量也是随生育期进程迅速增加,但在最后时期(75 d)喜树碱产量则减少;40%和 20%光强下,开始时喜树碱产量随生育期进程增加,处理 30 d 后增加速率减缓,60 d 后喜树碱产量下降,光强越弱,下降越严重。从喜树碱产量与光强的关系看,处理后 30 d,随着光强减弱喜树碱产量缓慢地略有增加。处理后 45 d 和 60 d,光强减弱到 60%以前,喜树碱产量增加,而光强进一步减弱后,喜树碱产量降低,处理时间越长,降低幅度越大(图 6)。

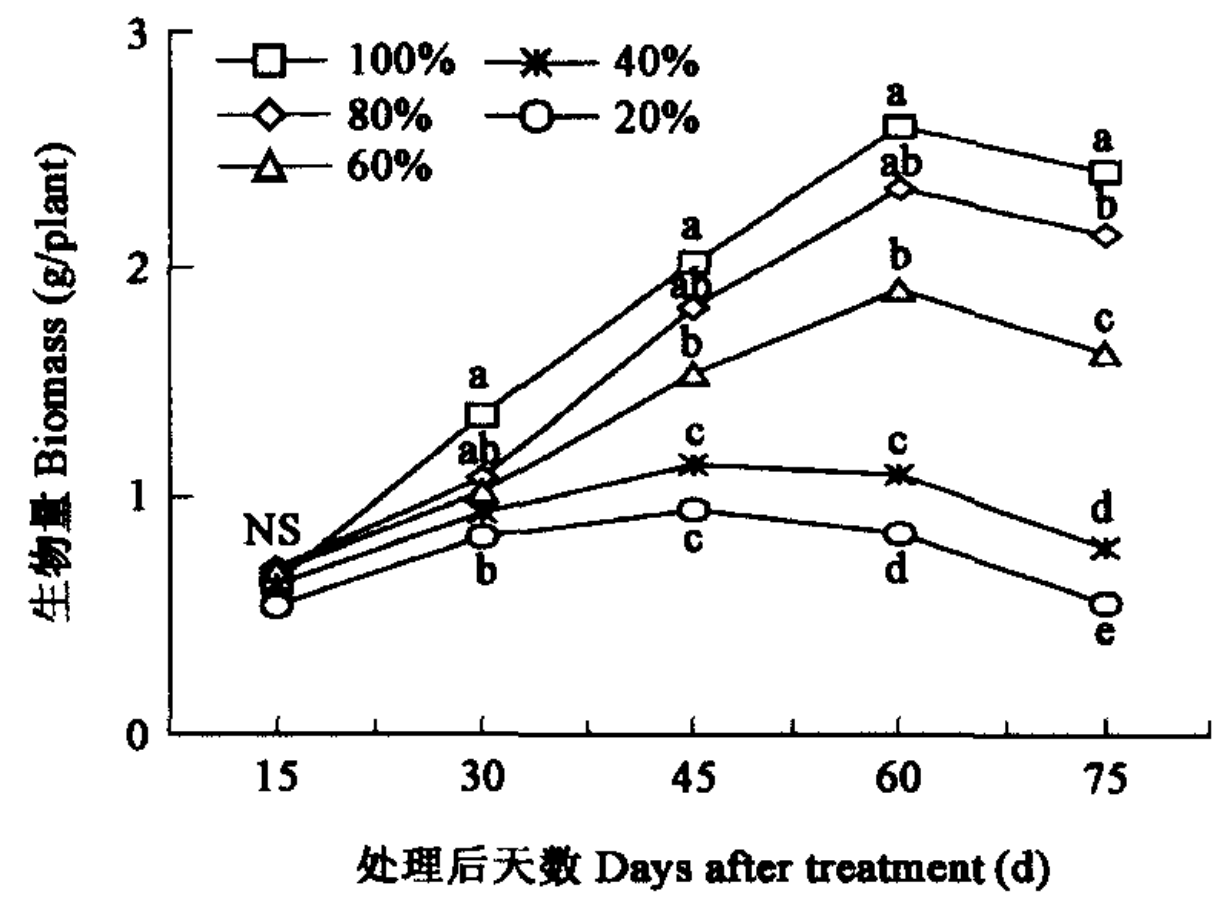


图 1 不同光强处理后喜树幼苗叶片生物量的变化

Fig. 1 Changes of leaf biomass of *Camptotheca acuminata* seedlings after treatment in different light intensity

Different letters adjacent to the date points in a day represent significant difference of the means at $P \leq 0.05$; NS: Not significant; 图 3 与图 5 相同 the same as fig. 3 and fig. 5

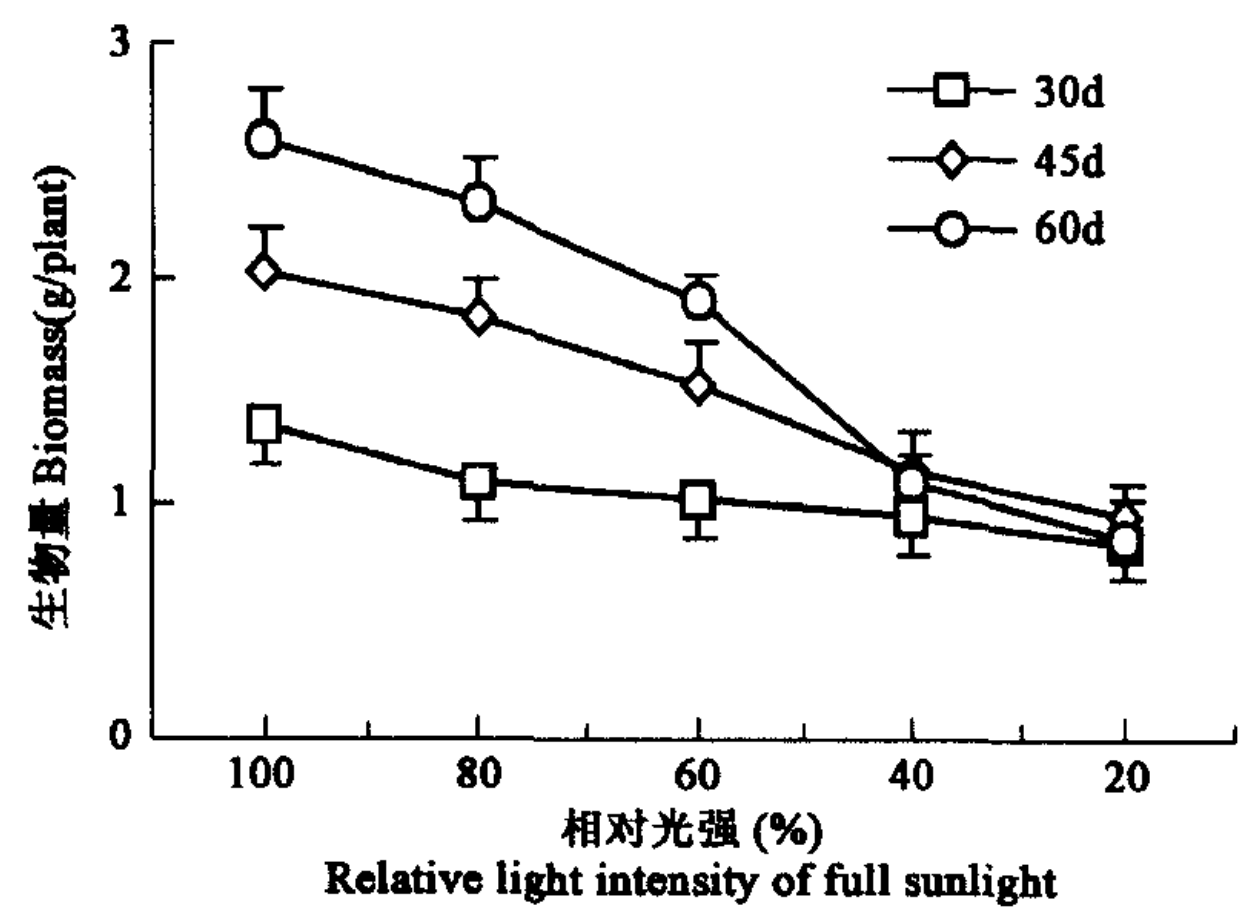


图 2 喜树幼苗叶片生物量与光强的关系

Fig. 2 The relationships between leaf biomass of *Camptotheca acuminata* seedlings and light intensity

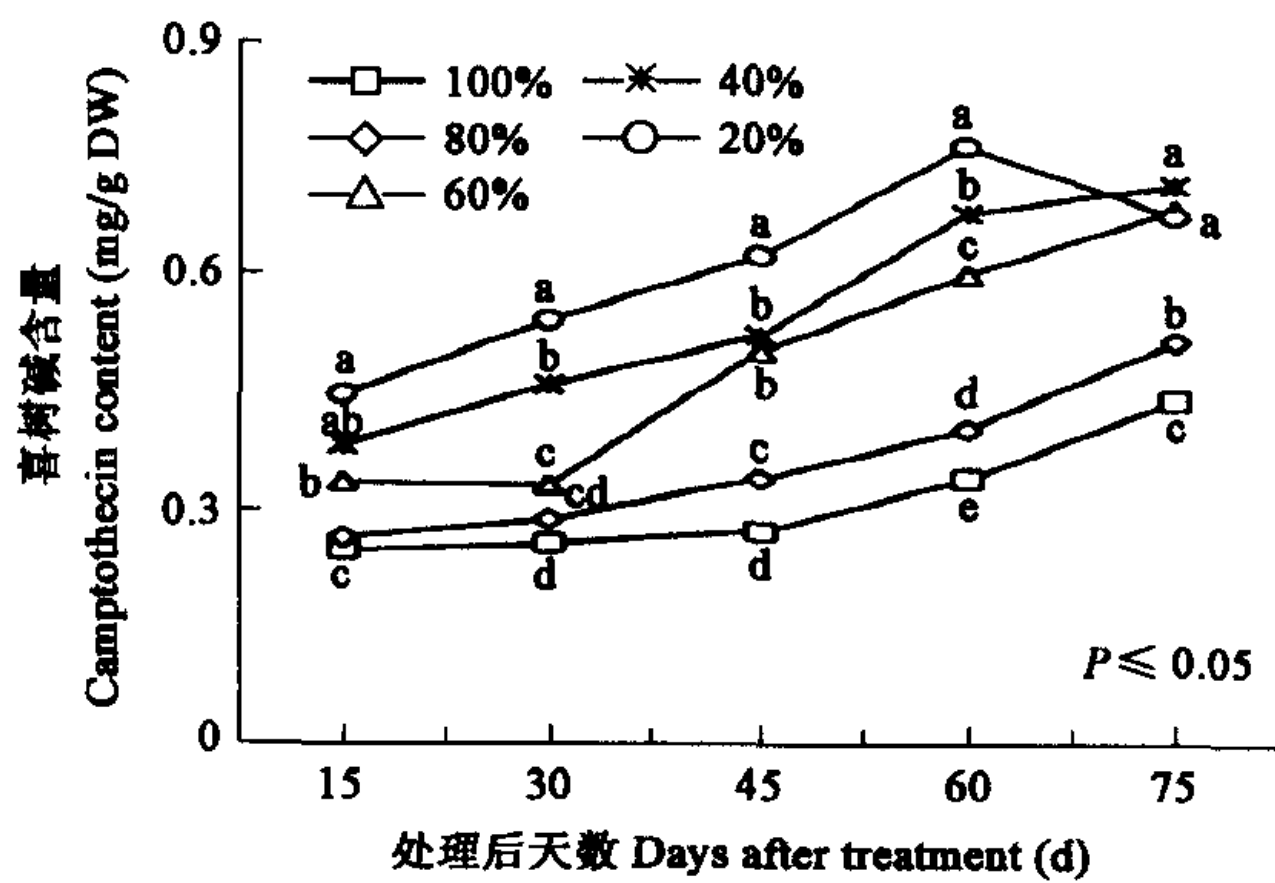


图 3 不同光强处理后喜树幼苗叶片喜树碱含量的变化

Fig. 3 Changes of camptothecin content in leaf of *Camptotheca acuminata* seedlings after treatment in different light intensity

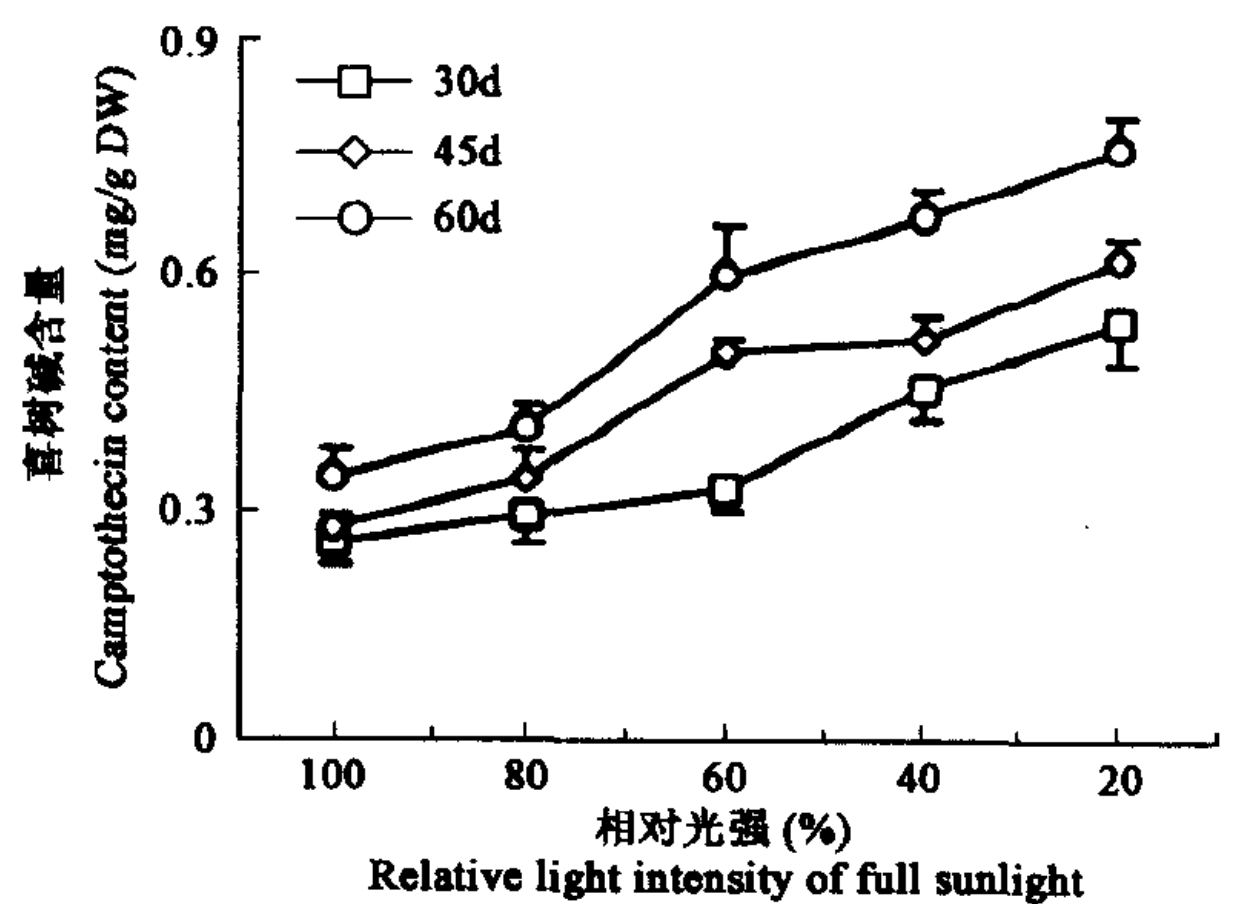


图 4 喜树幼苗叶片中喜树碱含量与光强的关系

Fig. 4 The relationships between camptothecin content in leaf of *Camptotheca acuminata* seedlings and light intensity

3 讨论

植物组织中的生物碱积累与环境的光因子有很密切的关系,这在组织培养材料的研究中已有很多报道^[19]。对于自然生长的植株,关于生物碱含量与光因子关系的研究工作尚很有限。一些研究表明光照强度的减弱诱导生物碱的积累而使植物组织中的生物碱含量增加^[20~22],但也有工作表明光能的增加有利于生物碱的合成^[22]或者光对于生物碱的合成并不重要^[21,24]。Liu 等^[14]以生长 2a 的喜树幼苗为材料,施以 4 周的遮荫处理后,轻度遮荫(全光照的 67%)叶片的喜树碱含量和产量与全光照的相比无显著差异,重度遮荫(全光照的 27%)的喜树碱的含量和产量均显著高于全光照的。而且,如果同时伴以水渍处理还增加了差异程度。本实验所使用的材料是当年生的喜树幼苗,80%光强下,幼苗叶片的喜树碱含量与全光照的相比,在处理的前 30 d 虽有增加的趋势但差异尚不显著,30 d 以后差异显著,而程度更重的遮荫处理下,从处理后的 15 d 开始,叶片喜树碱含量就显著高于全光照的(图 3)。可见,对于喜树幼苗而言,光强的减弱有利于叶片中喜树碱的积累,处理时间长效果也愈明显。由此推断,弱光下叶片喜树碱含量的增加,本质上有可能是幼苗对碳供应不足的反应结果(弱光环境下光合作用效率降低)。植物化学防御理论中的碳/营养平衡(carbon/nutrient balance)假说认为,植物体内以碳为基础的次生代谢物质(如酚类、萜烯类和其他一些仅以碳、氢、氧为主要结构的化合物)与植物体内的 C/N(碳素/营养)比呈正相关,而以氮为基础的次生代谢物质(如生物碱等含氮化合物)与植物体内 C/N 比呈负相关^[25,26]。但是,当碳供应能力进一步降低时,会超过幼苗的调节能力,40%光强下处理后期喜树碱含量增加幅度的降低和 20%光强下处理后期喜树碱含量的下降(图 3)说明了这一点。

喜树嫩叶中的喜树碱含量远高于成熟叶片^[13,27,28],有人推断这是喜树的一种化学防御手段,用以抵御草食动物和病虫害对幼嫩组织的侵害^[13]。从目前的研究结果看,喜树幼苗中喜树碱的代谢可对多种环境胁迫如光照(图 4)、水分^[14,16,17]等产生应答,其反应可能不是特异性的。

Liu 等^[13]曾报告喜树幼树(生长 3a)叶片的喜树碱含量从 4 月到 10 月每月下降 11%,但本实验的结果却是喜树碱含量随时间进程而增加,遮荫处理的也基本上如此(图 3)。另外,对 4 个种源的喜树幼树(生长 4a)的观察结果又与这两者不同,4 个种源的叶片喜树碱含量基本上都是出现两个高峰^[28],也有报告喜树碱的含量在 6 月份呈现一个高峰^[29]。喜树碱含量季节变化的不一致,说明喜树碱代谢可能不是严格地受控于喜树的生育节律,而是更主要地受到季节变化过程中环境因素变化的影响。事实上,自然生长的喜树植株,叶片中喜树碱含量的季节变化与气温、降雨量、蒸发量有很密切的关系^[28],这也说明喜树碱的代谢确实是响应于多种环境因素变化的。

从图 6 可以看出,适当的遮荫能够显著地增加喜树幼苗叶片中喜树碱的产量,这个结果对于以获取喜树碱为目的的喜树栽培生产有一定的指导意义。

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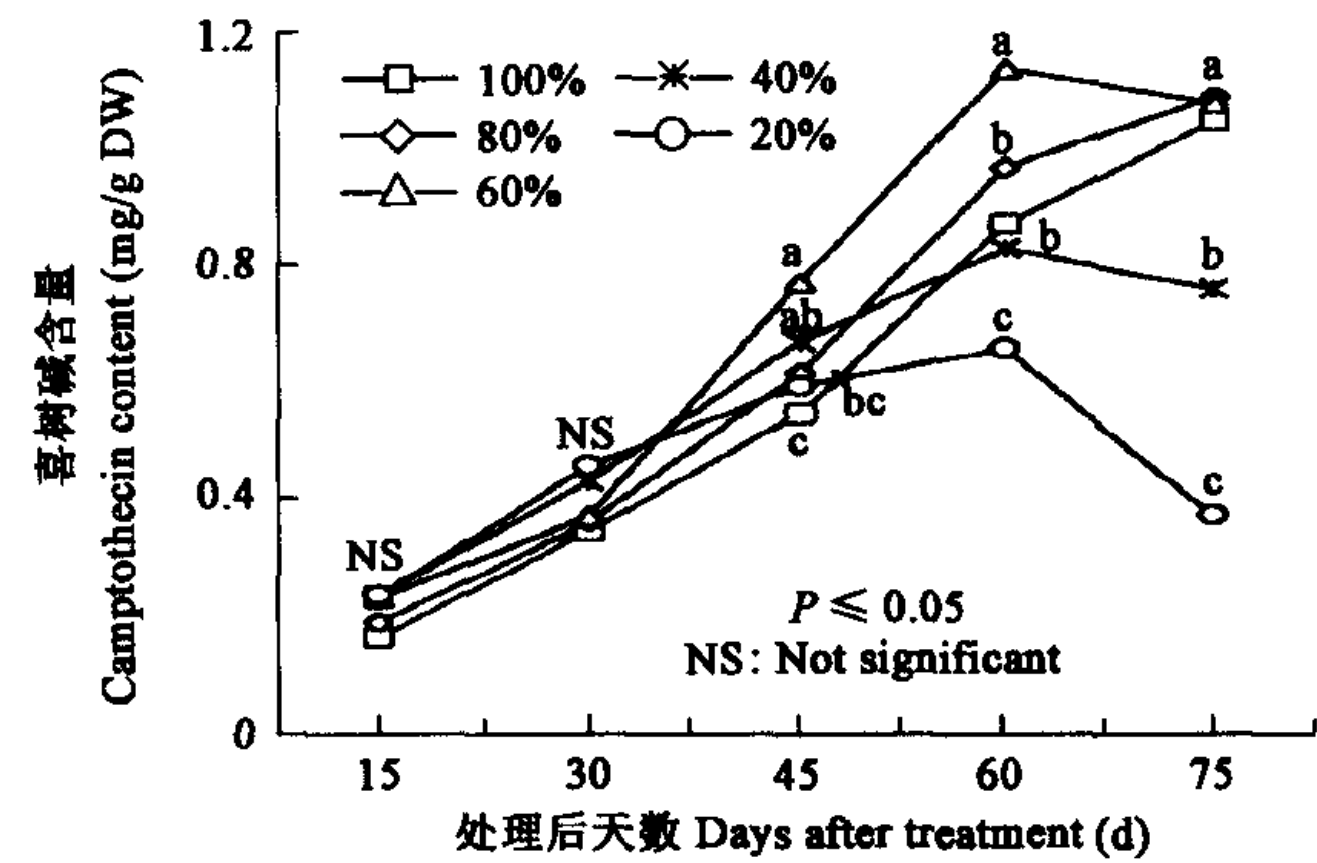


图 5 不同光强处理后喜树幼苗叶片喜树碱产量的变化

Fig. 5 Changes of camptothecin yield in leaf of *Camptotheca acuminata* seedlings after treatment in different light intensity

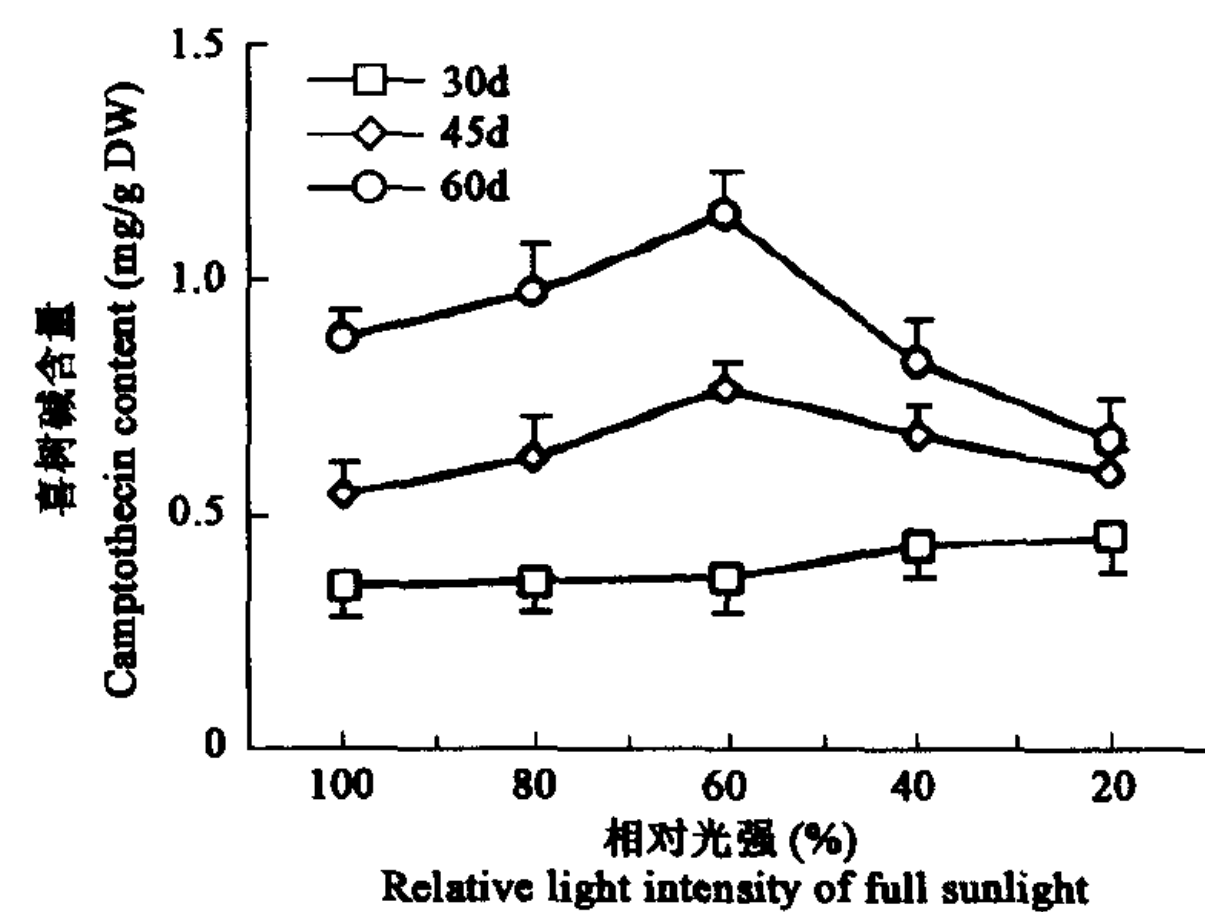


图 6 喜树幼苗叶片的喜树碱产量与光强的关系

Fig. 6 The relationships between camptothecin yield in leaf of *Camptotheca acuminata* seedlings and light intensity

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