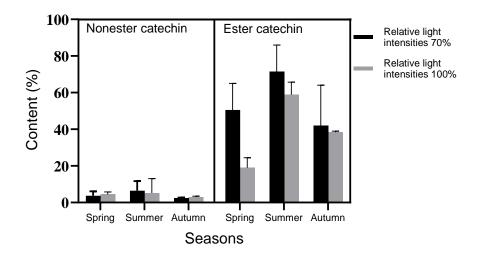
# Influence of Natural Light Intensities on Growth and Content of Catechins and Caffeine in *Camellia* formosensis Leaves and Tea Infusion

Hong-Chyi Jhou, <sup>a</sup> Chung-I Chen, <sup>b</sup> Jia-Yi Shen, <sup>c</sup> Chieh-Ting Wang, <sup>a</sup> and Fang-Chih Chang , <sup>a</sup>,\*

\*Corresponding author: d90541003@ntu.edu.tw

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## **GRAPHICAL ABSTRACT**



## Influence of Natural Light Intensities on Growth and Content of Catechins and Caffeine in *Camellia* formosensis Leaves and Tea Infusion

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Camellia formosensis is a significant under the forest canopy crop in Taiwan. This study investigated the composition and yield variations of native Camellia formosensis tea leaves across different relative light intensities levels and seasons. Increasing the relative intensity of natural light resulted in a corresponding increase in the dry weight of C. formosensis leaves. At a relative light intensity of 70%, the dry weight of the leaves per unit area was 15.8 g. However, the yield of C. formosensis was lower at relative light intensities of 40% or 20%. A positive correlation was observed between relative light intensities and the contents of catechin gallate (CG), epicatechin gallate (ECG), and gallocatechin gallate (GCG) and relative light intensity. At 20% relative light intensity, the mean CG, ECG, and GCG contents were 4.97, 0.57, and 4.43 mg/g, respectively. These values surged to 6.93, 2.23, and 7.53 mg/g, respectively, at 70% relative light intensity. Caffeine content exhibited an inverse relationship with relative light intensity, declining notably after surpassing 70%. Moreover, C. formosensis leaves harvested in summer under 70% relative light intensity boasted higher contents of CG, epicatechin (EC), ECG, epigallocatechin gallate (EGCG), and caffeine than those harvested in spring. Ester catechin content surpassed nonester catechins, with the former consistently higher at 100% relative light intensity, irrespective of the season.

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Keywords: Camellia formosensis; Natural light intensity; Catechin content; Caffeine

Contact information: a: The Experimental Forest, College of Bio-Resources and Agriculture, National Taiwan University; b: Department of Forestry, National Pingtung University of Science and Technology; c: Taiwan Biodiveresity Research Institute; \*Corresponding author: d90541003@ntu.edu.tw

#### INTRODUCTION

Environmental awareness has led to an unfavorable reputation or outright ban on large-scale deforestation in Taiwan for various reasons, such as conserving water sources, protecting national land, and maintaining ecological environments. The under-forest economy emerges as an approach that balances ecological principles and economic needs, aiming to foster sustainable forest development and effective management practices within Experiential Forests. This approach ensures that natural resources are not depleted and forest productivity and biodiversity are maintained.

Under-forest economy entails managing forest byproducts under the forest canopy, utilizing forest resources, understory spaces, and forest ecological environments. The primary objective of under-forest economy is to enable long-term forest farmers to supplement their income through short-term harvests. Under the Satoyama Initiative,

under-forest economic initiatives should be developed to help indigenous communities develop natural resources, conduct research on forest biodiversity within indigenous communities, and sustainably use plants in traditional customs and practices (Satoyama Initiative, 2018).

Camellia formosensis, a member of the Theaceae family and the Camellia genus, primarily thrives in the mid- to low-altitude mountains of Taiwan, ranging from 700 to 1,650 m above sea level. This evergreen small tree species can reach heights of up to 8 m and trunk diameters of 40 cm. C. formosensis was previously considered a variety of Camellia sinensis (L.) Kuntze, but nucleic acid sequencing studies by Su et al. (2009) established it as an independent endemic species native to Taiwan. In 2017, the Red List of Vascular Plants of Taiwan listed C. formosensis as near threatened (The Red List of Vascular Plants of Taiwan 2017).

C. formosensis tea was first mentioned in the Zhuluo County Memorial in 1717, where it was noted for its unique taste and green color resembling Usnea. However, its harvesting was limited due to safety concerns related to dangerous roads and Aboriginal territories (Shen 2018). During Japanese colonial rule, Japanese tea researchers showed keen interest in C. formosensis, leading to its cultivation and the production of black tea. The exceptional quality of the black tea prompted further research and cultivation efforts even after the end of Japanese colonial rule (Chiu 2004). In 1999, Taiwan Tea No. 18 (Red Jade) was created through crossbreeding C. formosensis with Myanmar large leaf Burma (B-729), demonstrating its potential for tea tree breeding. In 1999, Myanmar large leaf Burma (B-729) was used as the female parent to crossbreed with C. formosensis, resulting in the creation of Taiwan Tea No. 18 (Red Jade). Renowned for its ease of cultivation and distinct mint and cinnamon undertones, Taiwan Tea No. 18 was well received by the market, demonstrating that C. formosensis is an excellent candidate for tea tree breeding (Shen et al. 2015). Currently, various C. formosensis products are available on the market. In addition to Liouguei tea and the Yuchi Farmers Association's black tea, the Tea and Beverage Research Station has identified C. formosensis from Yongkang Mountain, Taitung County as a source for breeding a fast-growing and disease- and pest-resistant cultivar with a unique flavor profile. This cultivar was officially named Yongkang tea (Taiwan Tea No. 24) in 2019.

Climate significantly influences tea tree growth, with factors such as temperature, rainfall, humidity, and solar radiation exhibiting specific thresholds. Sunlight and temperature play crucial roles in shaping plant morphology, growth, and physiological functions, affecting the synthesis and metabolism of key compounds such as tea polyphenols, caffeine, and L-theanine (Rodriguez-Lopez et al. 2014; Chen et al. 2015). Adjustments in environmental conditions, such as shading tea trees or modifying sunlight exposure, can impact the biochemical composition and quality of tea leaves, with moderate shade accelerating photosynthesis and improving leaf quality. During the summer and autumn, which are characterized by intense sunlight and elevated temperatures, tea leaves tend to exhibit higher levels of catechins and anthocyanins and lower levels of amino acids. Previous study revealed that the methylated products of catechins not only contribute to the umami of tea infusion but also amplify its sweetness (Li et al. 2024). Changes in the content of catechins can also cause variations in the color of black tea infusion (Wang et al. 2022). Consequently, tea leaves produced during these periods may have inferior quality and reduced commercial value. Moderate shade has been observed to accelerate the rate of photosynthesis in tea trees, thereby contributing to the improvement of tea leaf quality. Tea trees receive carbon through photosynthesis and absorb carbon and energy through nitrogen metabolism. Carbohydrates, serving as a carbon source, also function as signaling molecules that regulate the synthesis and metabolism of flavonoid compounds. Therefore, environmental factors such as sunlight, temperature, and photosynthesis rate play pivotal roles in determining the quality of tea leaves harvested during the summer and autumn seasons (Zhang *et al.* 2018).

This study investigated the composition and yield of *C. formosensis* leaves under various relative light intensities to provide fundamental data for cultivating and managing *C. formosensis* for understory growth. Planting understory *C. formosensis* could generate commercial advantages and contribute to the establishment of a multistratal forest, enhancing forest cover and rehabilitation. Furthermore, exploring the plantation and performance of *C. formosensis* can establish cultivation and management models tailored to this species, benefiting forest farmers and indigenous communities engaged in underforest economic activities. Additionally, it facilitates assessing the variety and management models of under-forest economy.

#### **EXPERIMENTAL**

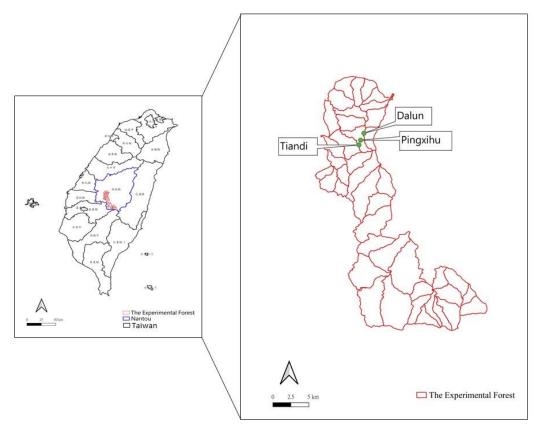
#### **Site Overview**

The wild Taiwan camellia experimental sites considered in this work are located in Tiandi (231858, 2623876), Pingxihu (232092, 2624591), and Dalun (232556, 2625637) in Qingshuigou Tract, National Taiwan University Experimental Forest, as shown in Fig. 1. These sites serve as test sites for wild *C. formosensis* because the majority of these trees are found within this area, situated at an altitude ranging from 800 to 1100 m. Historical records indicate ancestral harvesting and utilization of *C. formosensis* leaves, and presently, *C. formosensis* continues to thrive across these sites. For this study, these sites were selected as experimental sites, with relative light intensity levels set at 100% (control group), 70%, 40%, and 20%. Three plants were selected for experimentation under each light intensity condition. The PG200N spectral PAR meter (UPRtek, Taiwan) was used to measure the relative light intensity. The forest canopy partly blocked incident sunlight was used to adjust different levels of relative light intensity.

The *Camellia formosensis* tree can be classified as an evergreen tree having smooth branchlets, and leaf buds nearly smooth. The leaves are elliptic-lanceolate, 8 to 18 cm long, 2 to 5.5 cm wide, with a sharp apex or a tapering tail shape, and a smooth leaf surface. The diameter at breast height, crown width, and height of *Camellia formosensis* trees selected for the study were 8 to 14 cm, 1.2 to 1.5 m, and 2 to 2.5 m, respectively.

## Tea Leaf Yield under Various Light Intensities

The experimental design allowed tea trees to grow naturally. Throughout each season, weeds and vines were cleared once. In April, June, and September, a designated area measuring  $30 \times 30$  cm<sup>2</sup> was marked around each tea tree, and bud leaves were harvested. The average maximum light intensities in spring, summer, and autumn in the trial area were 1250, 1765, and 1413  $\mu$ mol m<sup>-2</sup>s<sup>-1</sup>. Subsequently, new shoots with one-tip two-leaf were picked and dried in an oven set at 60 °C for 10 hours from the Taiwan *C. formosensis* sample (Fang *et al.* 2014). The picking the buds is an important agricultural method to produce high yielding and high-quality tea (Tan *et al.* 2024).



**Fig. 1.** The location of the wild Taiwan camellia experimental site in Qingshuigou Tract, National Taiwan University Experimental Forest

## **Caffeine and Catechin Analysis**

Fresh bud leaves were first dried at 60 °C. Subsequently, 0.25 g of the dried leaves were ground and combined with 75% ethanol until the total volume reached 10 mL. The mixture was extracted for 1 h and was subjected to ultrasonication for 1 min before and after the extraction process. Following extraction, the resulting extract was diluted with 10 times its volume of pure water and filtered. High-performance liquid chromatography (Shimadzu, Japan) was then employed for analysis of caffeine content and eight types of catechin, namely ((-)-epigallocatechin 3-gallate (EGCG), (-)-epicatechin 3-gallate (ECG), (-)-epigallocatechin (EGC), (+)-catechin, (-)-epicatechin (EC), gallocatechin gallate (GCG), gallocatechin (GC), and (-)-Catechin Gallate (CG)). A C6H5 (phenyl) tube was used as the separation column. A volume of 10 μL of the sample was injected, and the flow rate was set at 0.8 mL/min. Detection of compounds was conducted at a wavelength of 280 nm (Song *et al.* 2012). Standard agents for calibration were purchased from Sigma.

## **Statistical Analysis**

GraphPad Prism 10 was used for the analysis and visualization of tea leaf yield and composition. Analysis of variance and post hoc tests were conducted using SPSS version 26. Specifically, Bonferroni and Tukey's honest significant difference tests were used to compare the significance levels among different experimental groups (p < 0.05).

#### **RESULTS AND DISCUSSION**

## **Effect of Relative Light Intensities and Seasons**

The yield of *C. formosensis* leaves under varying light intensities is depicted in Fig. 2. As the relative light intensity increased, the number of bud leaves per unit area also increased. Notably, the highest yield was achieved at a relative light intensity of 100%, followed by 70%, 40%, and 20%, respectively. Although the mean number of bud leaves was the highest (69) at a relative light intensity of 100%, this yield did not significantly differ from that under other relative light intensities. Conversely, the lowest mean number of bud leaves (13) was observed at a relative light intensity of 20%. Tea trees are understory canopy plants that prefer shady and cool environments. However, increased shade tends to reduce yield due to several factors: thinner leaves, lighter buds, larger leaf surface areas, and reduced dry leaf weight (Cheng and Fan 2005). The results of this study revealed that exposing C. formosensis trees to a relative light intensity of 100% resulted in a higher bud leaf count. However, other studies have suggested that a high light intensity can reduce the number of bud leaves in certain tea tree species. Furthermore, the dry weight of tea leaves increased with the increasing relative light intensity (Wijeratne et al. 2008). At a relative light intensity of 70%, the dry weight of tea leaves was 15.8g, whereas when the relative light intensity was increased to 100%, the dry weight of tea leaves was reduced. The yield of tea leaves did not significantly differ across different relative light intensities. Overall, these findings highlight a positive correlation between C. formosensis yield and increasing relative light intensity.

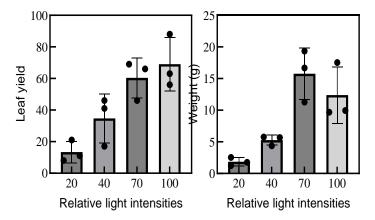


Fig. 2. The yield of *C. formosensis* leaves under varying relative light intensities

At relative light intensities of 20% or 40%, *C. formosensis* exhibited budding only during March and April, with no subsequent bud formation observed. Tsai and Chen (2020) investigated the management of *C. formosensis* at Baoshan and discovered that only spring *C. formosensis* exhibited budding under a low light intensity, whereas summer *C. formosensis* exhibited an extremely low yield that could not be harvested. In addition to the lack of sunlight, Liu and Tsai (2020) argued that monthly rainfall exceeding 100 mm is preferred during tea tree growth and that a rainfall less than 50 mm is unfavorable for its growth. A drought in early 2023 resulted in monthly rainfall from January to April falling below 50 mm, which might have affected tea leaf growth. Summer and autumn budding in *C. formosensis* occurred only at relative light intensities of 70% and 100%. When the relative light intensity was 70% or 100%, the number of bud leaves was the highest in

spring, followed by summer and autumn. *C. formosensis* did not bud during the winter months, possibly because the monthly rainfall since October 2023 was <50 mm, which created an unfavorable environment for the growth of *C. formosensis*. Temperature directly influences various aspects of tea tree physiology, including photosynthesis, bud growth, and bud sprouting (De Costa *et al.* 2007). Tea trees typically thrive in temperatures ranging from 18 to 25 °C. However, in October 2023, the mean monthly temperature was 21.2 °C, gradually decreasing in the following months. This gradual decline in temperature could have impeded bud sprouting during winter, ultimately resulting in the absence of buds. Notably, the dry weight of tea leaves per unit area did not exhibit a significant correlation with the season (as shown in Fig. 3).

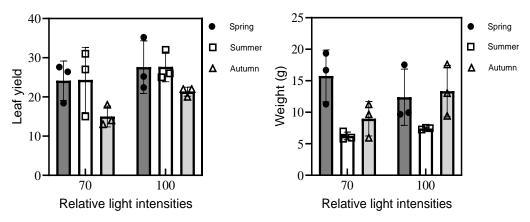


Fig. 3. The yield of C. formosensis leaves under varying relative light intensities and seasons

## **Caffeine and Catechin Analysis**

Tea leaves contain a diverse array of chemicals, each playing a crucial role in determining the quality of the tea produced. Key chemicals closely associated with tea quality include tea polyphenols, alkaloids, pigments, aromatic substances, free amino acids, and carbohydrates. Among these, tea polyphenols are particularly significant, as they contribute to both the bitterness and flavor of the tea. Catechins, which account for 70 to 80% of tea polyphenols, are especially noteworthy. Various factors influence the catechin content, including the tea species, growing season, growth location, cultivation and management practices, climate conditions, sunlight exposure, and tea processing methods. Catechins can be further categorized into ester catechins and nonester catechins, with the majority falling under ester catechins, including epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and gallocatechin gallate (GCG). Ester catechins are known for their potent antioxidant activity, making them pivotal determinants of tea quality.

Shade has a dual impact on tea leaves, reducing both their biomass and catechin content while concurrently increasing the concentration of free amino acids (Fu *et al.* 2017, Chen *et al.* 2017). Table 1 illustrates the effect of various relative light intensities on catechin and caffeine levels. Specifically, catechin gallate (CG), ECG, and GCG contents exhibited a positive correlation with relative light intensity, with mean values reaching 4.97, 0.57, and 4.43 mg/g, respectively, at a relative light intensity of 20%, and peaking at 6.93, 2.23, and 7.53 mg/g, respectively, at the relative light intensity of 70%. Conversely, at a relative light intensity of 100%, CG, ECG, and GCG contents were decreased. The GC and EGCG contents decreased as the relative light intensity decreased, whereas EC content exhibited a negative correlation with relative light intensity increasing as the relative light intensity decreased. The caffeine content similarly decreased with decreasing relative light

intensity, with a notable decline observed after a relative light intensity of 70% was reached. Gu (2017) observed that shade initially led to a decrease in ester catechin content, particularly EGCG and ECG, before ultimately increasing. Conversely, for nonester catechins, the catechin content increased, whereas the EGC and EC contents decreased. Additionally, Yu *et al.* (2020) revealed that covering Yinghong No.9 before harvest resulted in decreased levels of catechin, CG, EC, ECG, EGCG, and GC contents. The findings of the present study were similar to the results of Yu, except the result for EC.

**Table 1.** Catechin and Caffeine Contents of *Camellia formosensis* Across Various Relative Light Intensities

Relative Light	CG	EC	ECG	EGC	EGCG	GC	GCG	Ccaffeine
Intensities (%)	(mg/g)							
20	4.8	5.1	0.4	ND	39.8	35.7	3.3	38.2
	4.3	8.4	ND	ND	22.5	67.3	2.8	44.2
	5.8	7.7	1.3	ND	62.5	45.4	7.2	40.0
40	3.0	2.7	0.4	ND	13.7	32.4	1.0	31.4
	5.6	6.8	ND	ND	27.2	80.7	5.4	55.4
	7.6	9.1	1.7	ND	44.3	66.1	9.9	51.6
70	6.1	2.0	1.4	ND	41.4	19.6	3.7	22.0
	8.3	6.5	4.1	ND	63.2	46.2	11.7	38.4
	6.4	2.6	1.2	ND	40.3	34.6	7.2	27.8
100	5.5	3.5	0.4	ND	22.2	30.8	3.1	28.8
	4.0	3.9	ND	ND	12.6	42.4	1.1	28.5
	5.5	6.0	1.0	ND	20.6	61.6	7.4	38.1

Figures 4 and 5 illustrate the impact of various seasons and relative light intensities on the caffeine and catechin levels. Although no significant differences were observed in the overall catechin and caffeine contents, the levels of CG, EC, ECG, EGCG, and caffeine in summer-harvested C. formosensis were notably higher than those in C. formosensis harvested in spring. Specifically, at a relative light intensity of 70%, the mean CG, EC, ECG, EGCG, and caffeine contents in summer-harvested C. formosensis were 10.4, 4.03, 2.37, 69.07, and 31.97 mg/g, respectively, whereas spring-harvested C. formosensis exhibited mean contents of 6.93, 3.7, 2.23, 48.3, and 29.4 mg/g, respectively. Figure 6 presents the influence of different seasons and relative light intensities on ester catechin and nonester catechin levels. The ester catechin content consistently exceeded that of nonester catechins across all conditions. Irrespective of the season, higher relative light intensity, particularly at 100% compared with 70%, consistently resulted in elevated levels of both ester and nonester catechins. This trend held true across all conditions except for summer-harvested C. formosensis, where the nonester catechin content was higher at a relative light intensity of 70% instead of at 100%. Yao et al. (2005) observed that in different seasons in Australia, tea leaves harvested during warmer seasons exhibited significantly higher ECGC content than those harvested during colder seasons. The combination of high temperatures and intense sunlight during summer months tends to increase the catechin and caffeine contents in tea leaves. This suggests that tea leaves harvested during winter exhibit lower EGCG and ECG contents. Lin et al. (1996) reported that the EGCG, EGC, and caffeine levels in summer Bohea tea and Xianger tea were significantly higher than those in spring Bohea tea and Xianger tea. Consistent with these findings, this study revealed that regardless of the season, the ECG, EGCG, and CGC contents decreased with increasing relative light intensity.

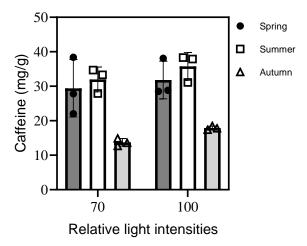


Fig. 4. Caffeine contents of *Camellia formosensis* across various relative light intensities and seasons

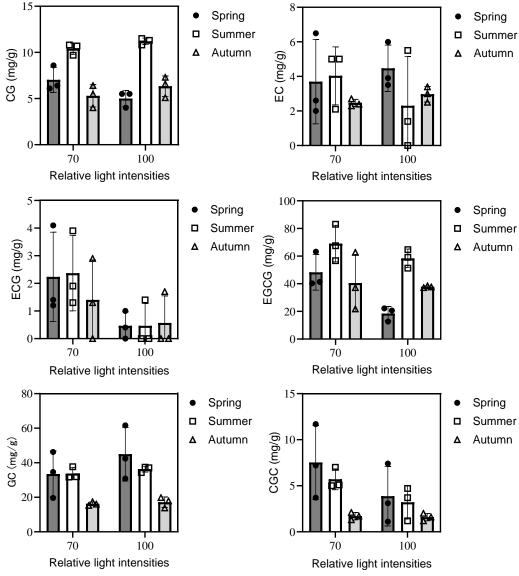
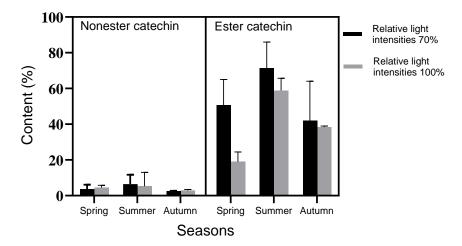


Fig. 5. Catechin a contents of *Camellia formosensis* across various relative light intensities and seasons



**Fig. 6.** Ester catechin and nonester catechin contents of *Camellia formosensis* across various relative light intensities and seasons

Shamala *et al.* (2020) noted that exposure of tea trees to ultraviolet B rays for 30 min led to an increase in catechin content. However, prolonged exposure (2 to 8 h) limited the increase in catechin levels.

#### **CONCLUSIONS**

- 1. *Camellia formosensis* is suitable for understory planting; however, its annual yield drastically diminishes when the relative light intensity drops below 40%, rendering it economically unviable for forest farmers.
- 2. The yield of *C. formosensis* is notably influenced by environmental factors, with higher yields observed in spring than summer and autumn. Consequently, optimizing management practices during January and February and mitigating water shortages could increase annual yields.
- 3. At a relative light intensity of 100%, *C. formosensis* exhibited the highest yield and elevated catechin content. However, the abundance of catechins can impart bitterness to the tea, compromising its quality. Therefore, understory planting should be practiced to ensure the production of high-quality tea, though the forest trees require moderate pruning or thinning to facilitate the growth of *C. formosensis* and maintain its yield.
- 4. The ester catechin content in *C. formosensis* was higher than the nonester catechin content. Because ester catechin can be used in the manufacture of medical and health products, processing *C. formosensis* into tea leaves or medical and health products can enhance its commercial value.

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