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Influence of Filtered and Unfiltered Solar Radiation on the Growth Pattern and Secondary Metabolite Synthesis in *Catharanthus roseus* (L.) G. Don - A Preliminary Study

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JM and GP performed the experimental design and analysed the data. Author KL wrote the manuscript. All authors read and approved the final manuscript.

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Abstract

Catharanthus roseus (L.) G. Don is an important medicinal plant known for the production of biologically active indole alkaloids and other secondary metabolites. This preliminary study evaluated the influence of different solar-radiation regimes on plant growth, epicuticular wax accumulation and vincristine content in *C. roseus*. Seedlings were grown for 50 days under three light environments: ambient high solar radiation, ambient low solar radiation produced by 40-50% light filtration, and UV-B-filtered solar radiation. Growth responses were assessed using shoot length, leaf area, fresh weight and dry weight, while epicuticular waxes and alkaloids were analysed using spectrophotometric, thin-layer chromatographic and high-performance liquid chromatographic methods. Plants grown under filtered radiation showed improved vegetative growth compared with plants exposed to ambient high solar radiation. In contrast, open daylight conditions were associated with earlier flowering. Total epicuticular wax content was highest under ambient high solar radiation, with values of 345.6 +/- 28 ug/g fresh weight, followed by ambient low solar radiation at 309.6 +/- 42 ug/g fresh weight and UV-B-filtered radiation at 291.6 +/- 32 ug/g fresh weight. Wax-class analysis indicated variation in free fatty acids, primary alcohols, secondary alcohols, aldehydes, beta-diketones, wax monoesters and alkanes among treatments. HPLC analysis detected vincristine in leaf extracts from all three light environments. Vincristine content was 820.4 +/- 18 ug/g leaf dry weight under ambient high solar radiation, 830.4 +/- 32 ug/g leaf dry weight under ambient low solar radiation and 316.2 +/- 27 ug/g leaf dry weight under UV-B-filtered radiation. These findings suggest that light quality and quantity influence growth and secondary metabolite accumulation in *C. roseus*, with UV-B exclusion reducing vincristine accumulation under the conditions of this study.

Keywords: Catharanthus roseus; solar radiation; UV-B filtration; ambient low light; plant growth; epicuticular wax; secondary metabolites; alkaloids; vincristine; HPLC; thin-layer chromatography.

1. Introduction

Catharanthus roseus (L.) G. Don (Apocynaceae) is one of the most extensively investigated medicinal plants and is known for its anticancer properties (Van der Heijden et al., 2004). More than 100 phytochemicals can be produced in *C. roseus*, of which vincristine and vinblastine are among the most extensively studied indole alkaloids (Moreno, 1995). Plants produce a wide range of chemical compounds that are effective in defence against infection and environmental factors, including biotic and abiotic stresses (Harborne et al., 1999). According to Hadacek (2002), plants contain more than 100,000 secondary compounds, representing all the main classes of organic compounds, including aliphatic, aromatic, hydroaromatic and heterocyclic compounds. Secondary metabolites play a vital role in plant interactions with the environment, for example as toxins that defend plants against microorganisms or predators, and as messengers, attractants, repellents or camouflage agents (Verpoorte, 1998; Shanks et al., 1999). In many cases, genes coding for secondary metabolites are regulated by stresses such as UV-B and wounding (Constabel, 1999; Long & Jenkins, 1998).

UV-B is known to alter plant growth, development and other physiological processes (Dai et al., 1992; Nogués et al., 1999). UV radiation is strongly absorbed by proteins and nucleic acids and therefore has important photobiological consequences (Caldwell, 1971). Several reports have emphasised the potential effects of UV-B on vegetative growth and photosynthetic activity (Bornman, 1989; Kulandaivelu et al., 1997), and UV-B radiation can alter both flowering time and the number of flowers in certain species (Ziska et al., 1992; Staxén & Bornman, 1994). Light treatment has been reported to reduce plant growth, secondary metabolite production and primary metabolites, including amino acids, sugars and phenolic compounds (Gholizadeh et al., 2023; Harborne, 1989; Shanks et al., 1999).

Environmental factors such as light intensity, photoperiod, humidity, temperature, ultraviolet radiation and other atmospheric conditions are known to influence the synthesis of cuticular waxes (Tevini & Steinmüller, 1987). Martin and Juniper (1970) showed that wax formation on pea leaves was suppressed when the plants were treated with trichloroacetate. Steinmüller and Tevini (1986) reported that the cuticular waxes of cucumber, bean and barley leaves were mainly composed of alkanes, aldehydes, primary alcohols and fatty acids. Wax ester synthesis was also found to occur predominantly in the epidermis by Kolattukudy (1967), and cuticular waxes minimise cuticular transpiration and play an important role as barriers to water diffusion (Hall & Jones, 1961; Martin & Juniper, 1970). Kolattukudy (1980) reported that waxes on the leaf surface protect plants from water

loss and abrasive damage. The regulation of flavonoid biosynthesis is under the control of a UV-B photoreceptor (Schmelzer et al., 1988; Greenberg et al., 1997), and, similar to flavonoids, anthocyanin accumulation is regulated by both phytochrome and a UV-B photoreceptor (Hashimoto et al., 1991). Ramani and Chelliah (2007) reported signalling pathways mediating UV-B-induced catharanthine accumulation in *C. roseus* suspension cultures. Variations in UV and visible-light exposure significantly influenced carotenoid biosynthesis (Badmus et al., 2022) and enhanced alkaloid accumulation in *Catharanthus roseus* (Asano et al., 2010; Rady et al., 2021).

Although the influence of UV-B radiation and light intensity has been examined in several plant systems, information on their combined influence on whole-plant growth, epicuticular wax deposition and vincristine accumulation in *Catharanthus roseus* remains limited. In particular, comparative observations under ambient high solar radiation, partially filtered solar radiation and UV-B-filtered solar radiation are needed to clarify how changes in light quality and quantity are associated with morphological and biochemical responses in this medicinal species.

The present study was undertaken to investigate the effects of ambient solar radiation (high solar flux at Sivakasi), low ambient light (ambient light filtered through a 40% wire mesh) and UV-B-filtered solar radiation (ambient light filtered through polyester Mylar film that transmits visible light but excludes UV-B radiation) on plant growth and the biosynthesis of selected secondary metabolites in *Catharanthus roseus*. We hypothesised that variations in light quality and quantity would significantly affect the synthesis and accumulation of primary and secondary metabolites in *C. roseus*. In addition, we proposed that UV-B exclusion and reduced ambient light conditions would modify plant growth and biochemical responses by altering the plant light environment.

2. Material and Methods

2.1 Cultivation of Plants

Seeds of *Catharanthus roseus* (L.) G. Don were obtained from local seed suppliers in Sivakasi. Prior to sowing, the seeds were surface-sterilised with 0.2% mercuric chloride (HgCl2) solution for 5 min and subsequently rinsed thoroughly with tap water to remove any residual sterilant. Earthen pots were filled with a soil mixture consisting of red soil, sand and farmyard manure (FYM) in a 1:1:1 ratio. The seeds were soaked in running water for 12-18 h and then sown in the prepared pots for germination.

2.2 Growth Characters

After the initiation of the cotyledonary and first primary leaves, the seedlings were grown in the respective light environments. Growth parameters, including shoot length, leaf area, fresh weight and dry weight, were measured immediately after harvest. Only the above-ground plant parts were used for growth analysis. For dry-weight determination, the harvested plant material was dried in a hot-air oven at 60 °C for 48 h or until a constant weight was attained.

2.3 Light Treatment

Six-day-old seedlings were grown in open daylight, low light and UV-B-filtered daylight for up to 50 days. The plants were uprooted 50 days after sowing to estimate the biochemical constituents.

2.4 Ambient High Solar Radiation

Seedlings of *Catharanthus roseus* (L.) G. Don were cultivated in 1.5 dm3 earthen pots containing a mixture of red soil, sand and farmyard manure (FYM) in a 1:1:1 ratio. Plants were watered daily throughout the experimental period. After germination, 6-day-old seedlings were exposed to direct sunlight and maintained under ambient environmental conditions, which served as the open-light treatment.

2.5 UV-B Filtered Solar Radiation

Seedlings of *Catharanthus roseus* (L.) G. Don were grown in 1.5 dm3 pots filled with a soil mixture consisting of red soil, sand and farmyard manure (FYM) in a 1:1:1 ratio. The plants were watered daily throughout the

experimental period. Six-day-old seedlings were transferred outdoors and exposed to ambient solar radiation and UV-B-filtered solar radiation under an 11 h light/13 h dark photoperiod. Solar UV-B radiation was effectively excluded by covering the experimental cage with transparent polyester Mylar film (0.3 mm thickness). The experimental procedure was adopted according to the method described by Lingakumar et al. (1999). Control seedlings were maintained in a similar cage covered with polyethylene film, which allowed the transmission of ambient solar radiation. To ensure adequate ventilation and minimise microclimatic variation, the cages were elevated 20 cm above the ground to permit free air circulation.

2.6 Ambient Low Solar Radiation

To achieve ambient low solar radiation, the seedlings were grown inside a cage covered with green wire mesh, which removed 40-50% of incoming solar radiation. Seedlings of *C. roseus* (L.) G. Don were grown in 1.5 dm3 pots filled with a soil mixture containing red soil, sand and farmyard manure (FYM) in a 1:1:1 ratio and watered daily. Soon after germination, 6-day-old seedlings were transferred to the green shade house for the low-light condition.

2.7 Extraction of Alkaloids

Shade-dried leaves harvested from Catharanthus grown under different light regimes were powdered to obtain alkaloids using a Soxhlet apparatus with ethanol as the solvent. The extracts were concentrated to one-third of their original volume and used for testing the chemical constituents. After completion, the extract was filtered and concentrated to dryness in a hot-air oven at 55 °C. The residue appeared as a dark brown powder.

2.8 Quantification of Alkaloids

The alkaloids separated by the above procedure were further quantified by recording the absorption maxima in the 200-300 nm waveband at room temperature. The alkaloids were then separated on a TLC plate and eluted using 95% ethanol. To confirm the presence of vincristine, standard vincristine purchased from Sigma-Aldrich (USA) was co-run with the samples.

2.9 Preparation of TLC Plates

The TLC method was adopted following Harborne et al. (1999). The glass plates used in TLC were carefully cleaned with ethanol to remove grease. A slurry of silica gel G in water (20 g of silica gel per plate) was shaken vigorously for 90 s and coated on the plates to a thickness of 0.5 mm using a commercial spreader. The plates were activated at 105 °C for 30 min and then used. After spotting, the TLC plates were kept in a chamber containing the solvent, and the chromatogram was developed using the ascending technique. The running solvent used was a tertiary mixture of chloroform, methanol and benzene mixed in a 4:1:5 ratio.

2.10 Extraction and Estimation of Cuticular Waxes

Extraction of cuticular waxes followed the modified procedure of Köhlen and Gülz (1976). Leaf samples of 2 g fresh weight were taken and immediately dipped in 5 ml of distilled chloroform. Care was taken to avoid mechanical injury to the leaf samples during extraction. The wax extract was evaporated to dryness and redissolved in a small volume of chloroform. The extract was spun at 2,500 g for 5 min. The clear supernatant was used to measure absorbance at 275 nm using a Shimadzu Pharmaspec 1700 spectrophotometer. The amount of total cuticular wax was quantified using pure paraffin wax (40-60 °C; Merck, India). An absorbance value of 0.1 at 275 nm was equivalent to 6 ug of wax.

2.11 Separation of Wax Classes

The extracted surface waxes were further separated into individual wax classes by thin-layer chromatography on activated silica gel (silica gel G with binder) plates using benzene as the solvent system. An aliquot of 50-100 ul of cuticular wax was carefully loaded onto the gel surface. The waxes were stained with iodine vapour (resublimed iodine crystals), and each wax class was spotted immediately under UV fluorescence. The Rf values

of the various wax classes at room temperature were compared with the standard values reported by Steinmüller and Tevini (1982). Each wax spot was scraped, eluted in chloroform and quantified at 275 nm.

2.12 Spectral Analysis by UV and HPLC

The ethanolic extract of pure vincristine (Sigma, USA) and vincristine samples obtained from plants grown under high light, low light and UV-B-filtered radiation were injected into a C18 column (7 um; 4 x 250 mm) at a flow rate of 0.7 ml/min. The alkaloid was eluted using ethanol. Vincristine determination was carried out with ethanol as the mobile phase. Optimum HPLC separation was achieved at 30 °C and monitored at 254 nm.

2.13 Test for Total Phenolic Compound

The amount of total phenolics in the extracts was determined with Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965). Briefly, 1 ml of extract solution (5 mg/ml) was added to a 100 ml volumetric flask containing about 60 ml of distilled water. Then, 5 ml of Folin-Ciocalteu reagent was added, and the contents were mixed thoroughly. After 1-8 min, 15 ml of Na2CO3 (20%) was added, and the volume was made up to 100 ml using distilled water.

2.14 Tests for Alkaloids

The extract, amounting to 0.5 g, was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate, 2 ml of dilute ammonia and 5 ml of chloroform were added, and the mixture was shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream precipitate with Mayer's reagent or a reddish-brown precipitate with Dragendorff's reagent was regarded as positive for the presence of alkaloids (Sofowora, 1993; Trease & Evans, 1989).

2.15 Radiation Measurements

Absolute solar irradiance with and without UV-B was measured using a monochromatic spectroradiometer (IL-700, International Lights, USA). The average level of ambient UV-B during the experimental period was 10 KJ m-2 day-1, whereas under the UV-B-filtered condition it was 1.5 KJ m-2 day-1. The daily average PPFD inside the filter cages was 54 mol m-2. The measurement procedures were the same as those reported by Lingakumar et al. (1999).

3. Results and Discussion

The present experiment aimed to study the impact of light quantity and quality on the biosynthesis of selected key metabolites. For this purpose, *Catharanthus roseus* was selected and grown under different light regimes, and secondary metabolites such as cuticular waxes and alkaloids were assessed. *C. roseus* seedlings were grown in open daylight, low light and UV-B-filtered solar radiation. To study the effects of solar radiation without the UV-B component, *C. roseus* seedlings were raised inside a cage covered with UV-B cut-off transparent polyester (0.13 mm) Mylar-type film. The control seedlings received the same amount of visible light along with 85% of ambient UV-B. For low light, green nylon wire mesh was used to cover the seedlings, which removed 40% of ambient light.

3.1 Effect of Different Light Regimes on Plant Morphology

The changes in plant morphology are shown in Fig. 1. Seedlings grown under ambient light showed stunted growth compared with those grown under low light (-40% filtered) and UV-B-filtered solar radiation. Throughout the growth period, a significant increase in growth parameters was observed. The changes in various growth parameters are shown in Figs. 2 and 3.

A maximum increase of 50% in shoot length, 11% in shoot fresh weight, 30% in dry weight and 60% in leaf area was observed in 20-day-old seedlings. The present results are consistent with those reported by Gholizadeh et al. (2023), who observed significant differences in plant height under different light conditions.

Although a positive increase in growth parameters was observed under filtered solar radiation (Fig. 1B1), ambient high solar radiation promoted flowering in *C. roseus* (Fig. 1A1). *C. roseus* seedlings grown under different light environments were assessed for growth parameters such as shoot length, fresh weight, dry weight and leaf area at various time intervals. Throughout the growth period, ambient low light and UV-B-filtered light stimulated growth. The growth response was particularly evident under UV-B-filtered solar radiation, which induced overall growth compared with low light. These results correspond to earlier studies (Lingakumar et al., 1999; Nouchi & Kobayashi, 1995; Ziska et al., 1991).

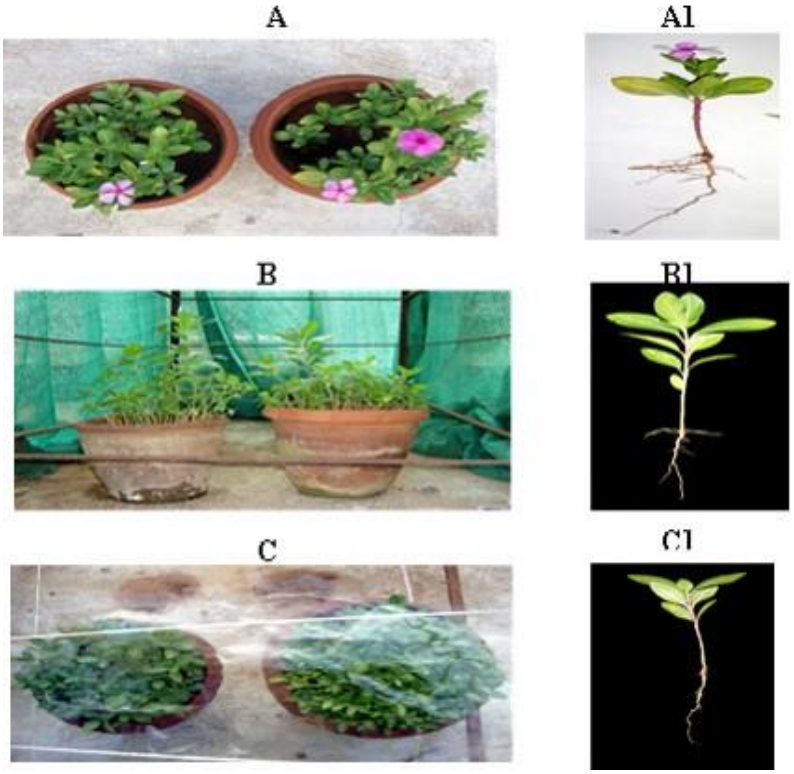

Fig. 1. Typical changes in plant morphology under filtered and unfiltered solar radiation in *Catharanthus roseus* (L.) G. Don.

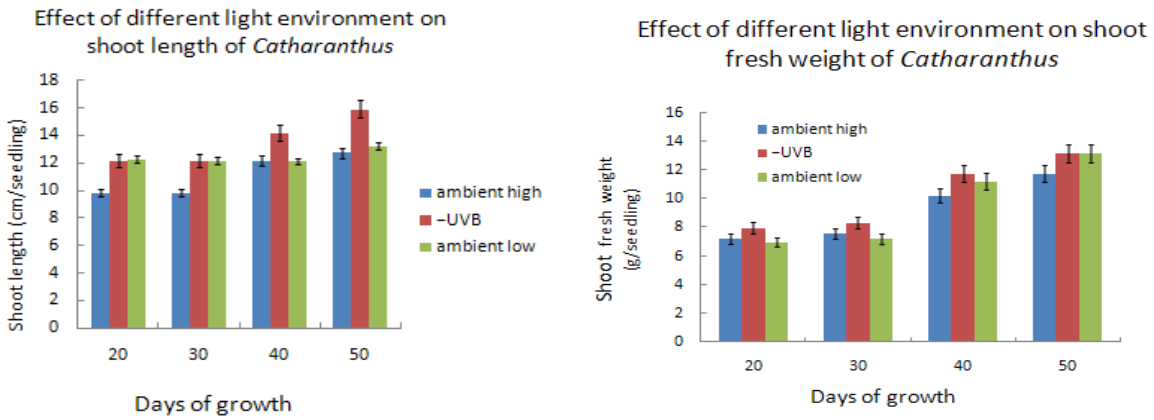

Fig. 2. Typical changes in various growth parameters in Catharanthus seedlings exposed to different light environments. Ambient high light refers to unfiltered daylight, ambient low light refers to 40% filtered daylight, and -UV-B refers to UV-B-filtered solar radiation using polyester film.

Among the parameters studied, leaf expansion was more prominent under filtered radiation. It is well known that UV-B radiation inhibits vegetative growth, which is attributed to the destruction of endogenous IAA (Kulandaivelu et al., 1989; Tevini & Iwanzik, 1986). Thus, the enhanced vegetative growth under UV-B-filtered radiation is assigned to a high level of auxin synthesis or low stimulation of the oxidative enzymes of IAA metabolism (Lingakumar et al., 1999).

Enhancement of secondary metabolites by biotic elicitors, such as bacteria and fungi, and abiotic elicitors, such as UV radiation and salt, has been reviewed by Singh (1999). Exposure of *Catharanthus roseus* cell suspension cultures to a low dose of UV-B irradiation was reported to increase the amount of catharanthine and the transcription of genes encoding tryptophan decarboxylase (Tdc) and strictosidine synthase (Str) (Ramani & Chelliah, 2007). Alterations in the alkaloid content of *Catharanthus roseus* have also been reported in response to abiotic stresses such as drought and salt (Jaleel et al., 2007a; Jaleel et al., 2007b).

Thus, the present study also confirms the differential role of ambient solar radiation in growth parameters and secondary metabolite synthesis. As far as growth is concerned, high solar radiation, including UV-B as a component, inhibits the growth and development of *C. roseus*. This finding is in agreement with Sharma et al. (2023), who reported that high-light stress reduced the photosynthetic rate and caused damage to photosystem I (PSI). Considering secondary metabolites such as epicuticular waxes and leaf alkaloids, the UV-B component of solar radiation seems to be an essential prerequisite. Filtration of high solar flux was beneficial for growth response only.

Although high solar flux inhibited growth parameters such as plant height, leaf area, and fresh and dry weights, the initiation of flowering was observed only under ambient high solar radiation. Low light or UV-B-filtered solar radiation did not induce flowering in *Catharanthus roseus* during the course of the study. There are reports that UV-B alters the time of flowering and the number of flowers (Staxén & Bornman, 1994; Ziska et al., 1992). Thus, exposure to solar UV-B plays a vital role in the initiation of flowering.

3.2 Effect of Different Light Regimes on Secondary Metabolites

The influence of different light regimes was assessed based on biochemical constituents such as epicuticular waxes and leaf alkaloids in *Catharanthus roseus*. To analyse the role of different light environments on key metabolites such as epicuticular waxes and indole alkaloids, leaves were harvested from plants grown under various light environments. Epicuticular wax content and composition revealed some interesting observations. Total epicuticular wax content decreased by 11% and 16% when ambient solar radiation was filtered to 40% using synthetic green wire mesh and to 15% using polyester Mylar film (0.12 mm), respectively. Total wax was separated into various classes using TLC. The TLC plates showed the presence of alkanes, primary and secondary alcohol esters, aldehydes and free fatty acids. Alkanes and esters represented the major constituents of *Catharanthus roseus* leaves. Ambient high solar radiation caused a significant increase in the levels of aldehydes and fatty acids compared with plants grown under filtered radiation. This indicates positive UV-B induction of wax biosynthesis. Increased glaucousness in *Eucalyptus* is primarily due to beta-diketones. Beta-diketones are the major components of epicuticular waxes (Kolattukudy, 1980). Sunlight-grown plants exhibit a high degree of crystalline epicuticular wax compared with greenhouse-grown or growth-chamber-grown plants (Steinmüller & Tevini, 1986).

3.3 Epicuticular Wax Biosynthesis

The effects of ambient solar radiation and filtered solar radiation on epicuticular wax content and composition are shown in Tables 1 and 2, respectively. UV-B-filtered *Catharanthus roseus* plants showed a 20% decrease in total epicuticular wax content. Total wax was further separated into various classes by TLC (Plate 2). The relative mobilities of each wax class corresponding to the Rf values of standard wax classes were calculated, and each fraction was eluted and quantified. Both ambient high solar radiation and ambient low solar radiation induced the synthesis of almost all wax classes compared with UV-B-filtered solar radiation. However, young leaves of *Catharanthus roseus* did not exhibit such an increase in wax content. The biochemical composition of epicuticular wax revealed high synthesis of alkanes and wax esters, followed by aldehydes, secondary alcohols and free fatty acids, in plants grown under ambient high solar radiation.

3.4 Spectral Analysis

The room-temperature absorption spectrum of epicuticular wax extracted in distilled chloroform is shown in Fig. 5. The spectral range was from 260 nm to 340 nm. Samples obtained from leaves grown under ambient light showed high absorption, especially below 280 nm, compared with the UV-B-filtered samples. The room-temperature absorption spectra of epicuticular wax extracted in distilled chloroform showed a gradual increase below 300 nm in all three plant samples. Ambient high solar radiation induced more epicuticular wax synthesis in *Catharanthus roseus* leaves. Tulloch (1976) reported that the 275 nm peak is attributed to beta-diketones. In the present study, no distinct peak at 275 nm was observed; instead, a gradual increase from 340 nm to 300 nm was noted, followed by a steep increase thereafter.

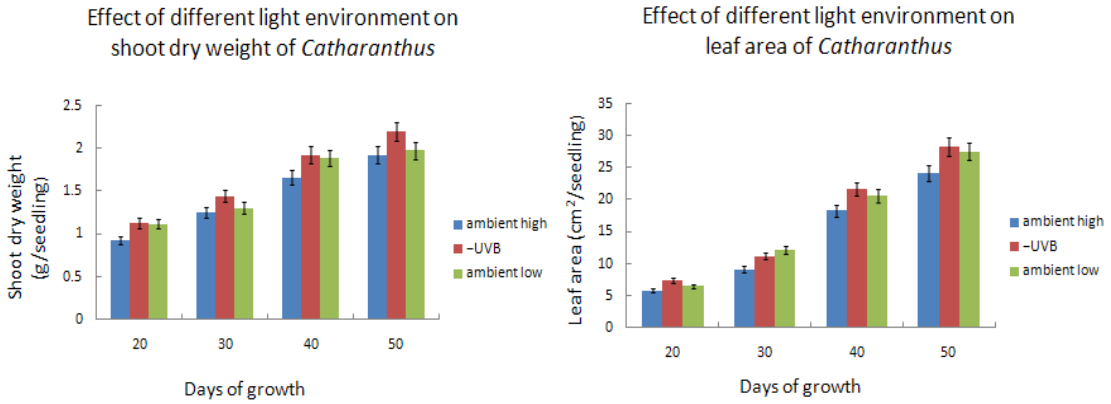

Fig. 3. Typical changes in various growth parameters in Catharanthus seedlings exposed to different light environments. Ambient high light refers to unfiltered daylight, ambient low light refers to 40% filtered daylight, and filtered solar radiation was provided using polyester Mylar-type film.

Fig. 4. TLC of epicuticular wax isolated from *Catharanthus roseus* leaves grown under various environmental light regimes. H: ambient high light; L: ambient low light; -UV-B: UV-B-filtered solar radiation

3.5 Alkaloid Biosynthesis

Catharanthus is known to yield an array of alkaloids, including vincristine and vinblastine, from different parts of the plant. The crude foliar extract of alkaloids from plants grown under different light environments was separated by TLC and subjected to HPLC analysis. The separated vincristine alkaloid is shown in the TLC profile, which was run with the standard (Fig. 6). From the thin-layer chromatogram, it is clear that ambient high solar radiation induced a greater amount of vincristine alkaloid in the leaves compared with ambient low and UV-B-filtered solar radiation. Total alkaloid content was quantified by measuring absorbance at 205 nm.

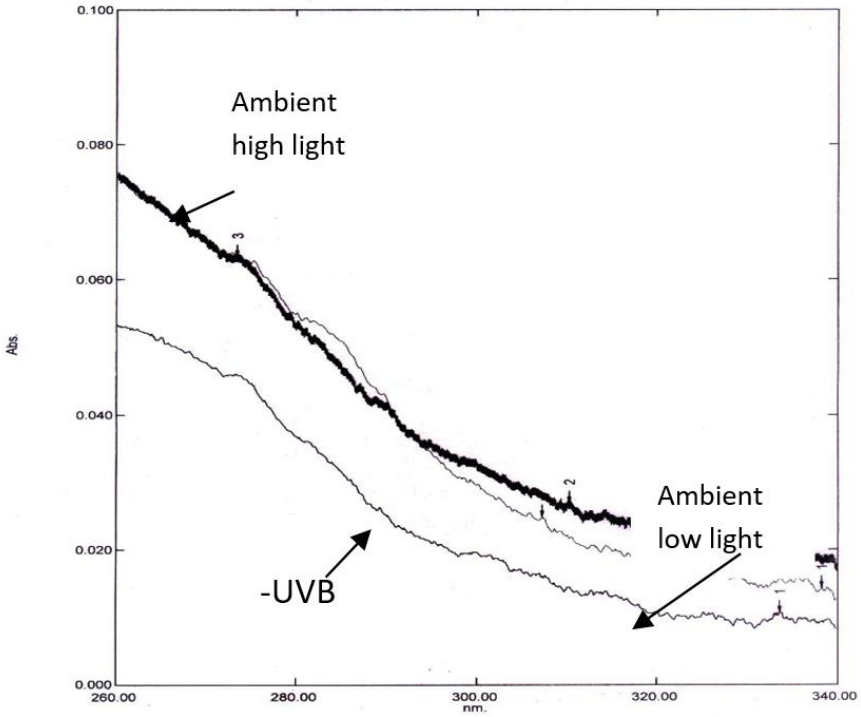

Fig. 5. Absorption spectrum of epicuticular wax extracted in distilled chloroform

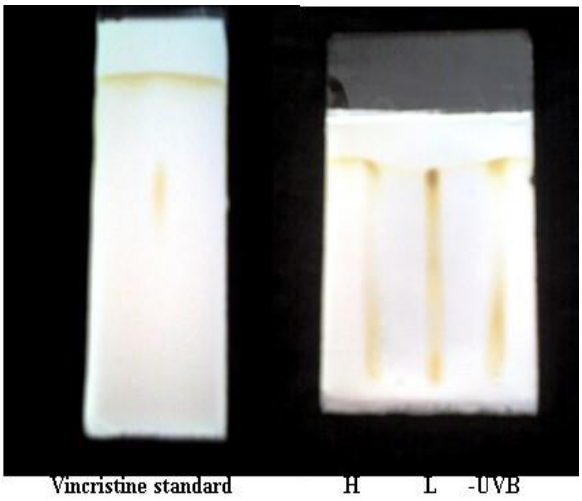

Fig. 6. TLC of alkaloids isolated from *Catharanthus roseus* grown under various environmental light regimes. H: ambient high solar radiation; L: ambient low solar radiation; -UV-B: UV-B-filtered solar radiation.

The separated alkaloid spot was scraped and eluted in methanol. *Catharanthus roseus* is well known for its highly valued leaf anticancer alkaloids, such as vincristine and vinblastine, and the hypertensive root alkaloid ajmalicine. All parts of the plant are rich in alkaloids, with maximum concentrations found in the root bark, especially during flowering. *Catharanthus roseus* is one of the most extensively investigated medicinal plants known for its anticancer properties (Van der Heijden et al., 2004). In the present study, an attempt was made to understand the impacts of various light conditions on growth patterns and secondary metabolite synthesis in Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don). The light environment consisted of natural daylight conditions and UV-B-filtered daylight conditions. To characterise the amount of solar flux on the above parameters, incoming solar radiation during the course of the study was filtered to 40% using a synthetic green wire mesh. The UV-B component of solar radiation was effectively removed using polyester Mylar-type film (0.12 mm). The transmittance spectra were those reported by Lingakumar et al. (1999).

HPLC analysis spectrum of *Catharanthus roseus* (L.) G. Don

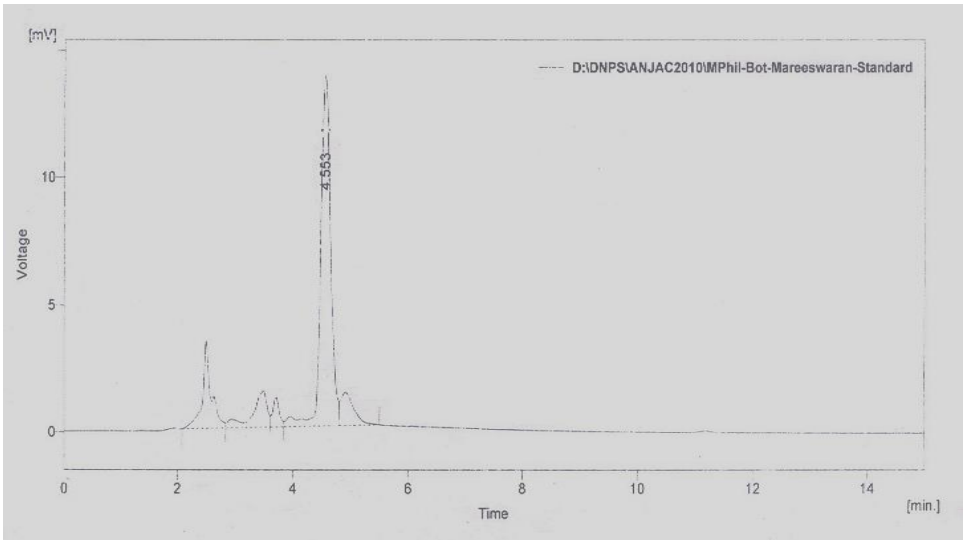

Fig. 7(a). HPLC spectra of vincristine standard

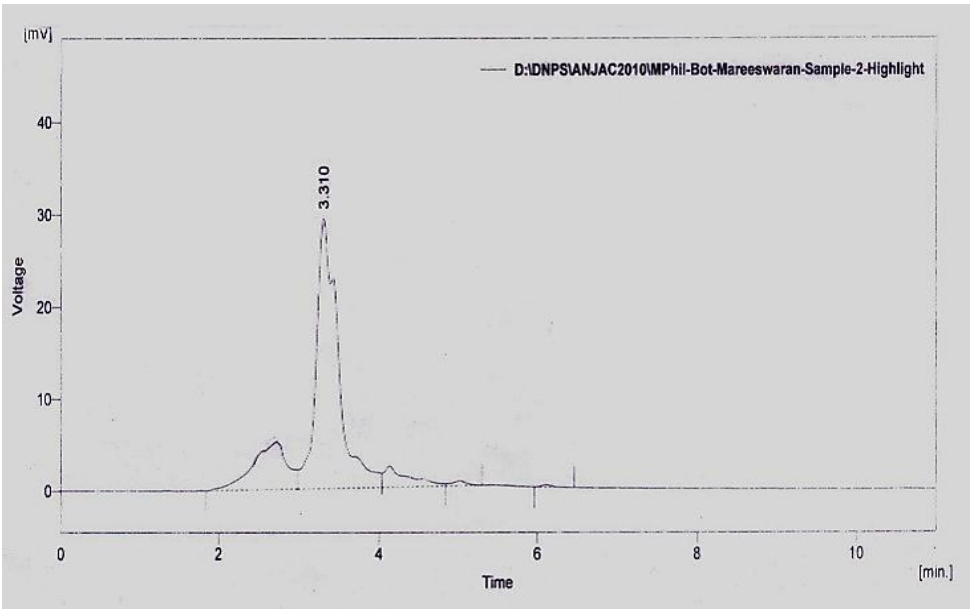

Fig. 7(b). HPLC spectrum of vincristine isolated from *Catharanthus roseus* grown under ambient high solar radiation

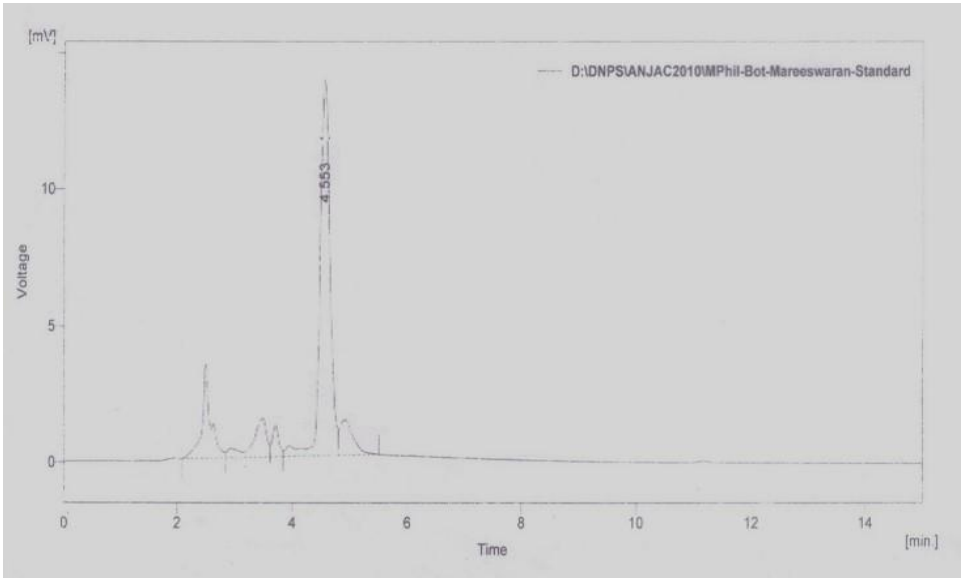

Fig. 8(a). HPLC spectrum of vincristine standard

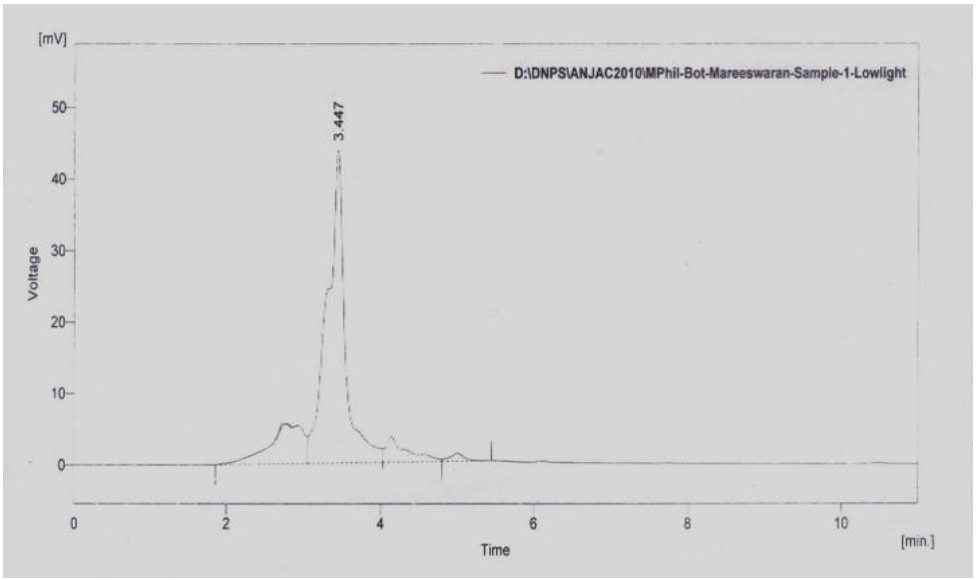

Fig. 8(b). HPLC spectrum of vincristine isolated from *Catharanthus roseus* grown under ambient low solar radiation

High solar radiation induced the accumulation of metabolites such as epicuticular wax, whereas growth parameters were inhibited compared with filtered radiation. Since there was a differential role of solar flux in growth and epicuticular wax composition, the common leaf alkaloid of *Catharanthus roseus*, namely vincristine, was separated and quantified using TLC and HPLC techniques. The content of vincristine estimated on the basis of leaf dry weight showed that UV-B-filtered solar radiation significantly reduced vincristine biosynthesis. Even 40% filtration of solar radiation did not bring about any change in alkaloid content, indicating the role of the UV-B component in solar rays in influencing the biosynthetic pathway of vincristine.

3.6 HPLC Analysis

To confirm the presence of vincristine or any other alkaloids in Catharanthus leaves, the eluted alkaloid spot from the TLC plate was subjected to HPLC analysis. The eluted spot was re-extracted with methanol, and a low-speed spin cleared the silica gel particles. Standard vincristine was run through a C18 column using ethanol as

the carrier solvent. Pure vincristine showed a major peak with a retention time (RT) of 4.553 min and a few secondary peaks with varying RT.

The samples from ambient high light, ambient low light and UV-B-filtered solar radiation (-UV-B) also showed similar peaks with marginal differences in RT. The differences in RT were due to moisture traces or minor contamination in the samples. In the plant samples, a major peak with a minor shoulder appeared in the spectrum, which could be due to alkaloids or other secondary metabolites absorbing at 254 nm. The HPLC spectra of the plant samples are shown in Figs. 7-9. Among the samples, high light induced a greater amount of vincristine compared with low light and UV-B-filtered solar radiation (Table 3). The amount of vincristine in plants grown under ambient daylight conditions was 820 ug/g leaf dry weight compared with 40% filtered solar radiation (830 ug/g LDW) and UV-B-filtered solar radiation (316 ug/g LDW).

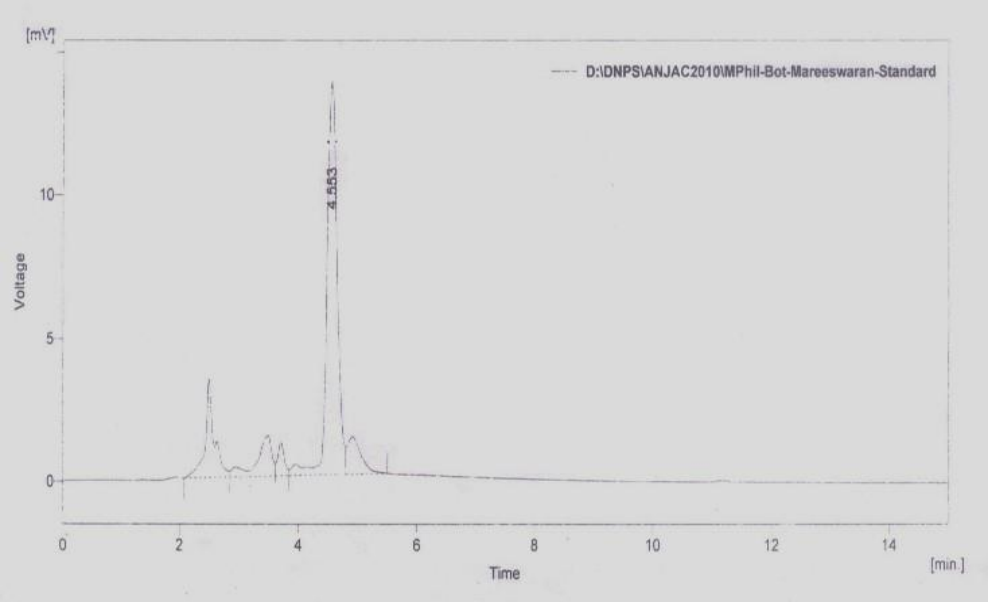

Fig. 9(a). HPLC spectra of vincristine standard

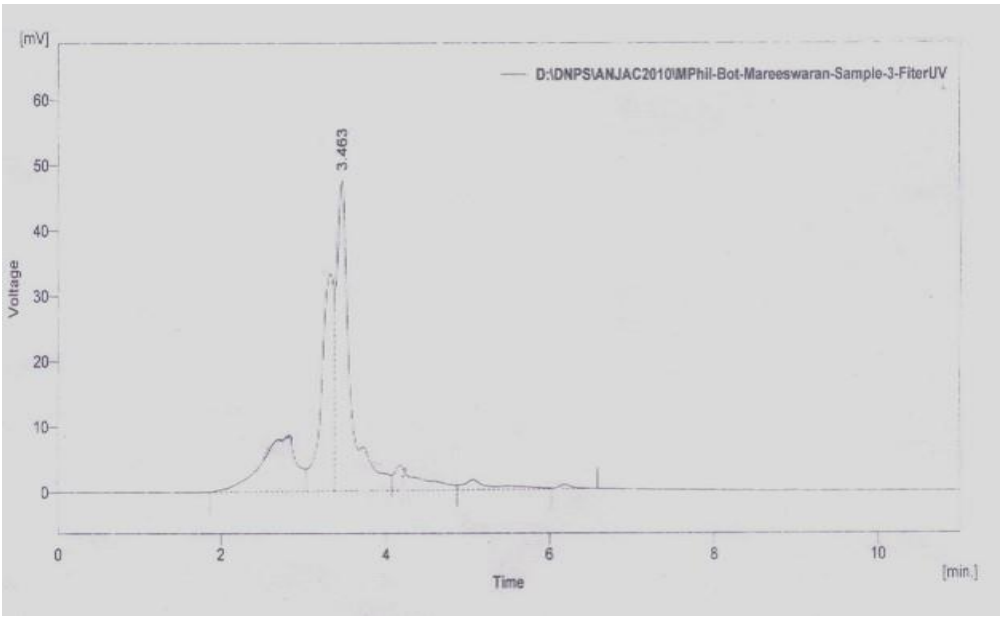

Fig. 9(b). HPLC spectrum of vincristine isolated from *Catharanthus roseus* grown under UV-B-filtered solar radiation

Table 1. Amount of cuticular wax obtained from *Catharanthus roseus* leaves based on unit fresh leaf mass

Sample	Mean value (µg /g FW)
Ambient high solar radiation	345.6 ± 28
-UVB	291.6 ± 32 (84.5)
Ambient low solar radiation	309.6 ± 42 (-10.5)

The values are the averages of three independent measurements. Values in parentheses are percentage changes with reference to high light

Table 2. Amount and composition of cuticular wax obtained from *Catharanthus roseus* plants grown under different light environments for 50 days (ug/100 ml TLC eluate)

Sample	Free fatty acid	Primary alcohol	Secondary alcohol
Ambient high	6.48 ± 0.3	6.84 ± 0.3	7.2 ± 0.6
-UV-B	Not detectable	2.52 ± 0.3	3.24 ± 0.2
Ambient low	7.2 ± 0.6	5.04 ± 0.5	5.4 ± 0.5

The values are the averages of three independent measurements.

Table 2 (Continuation). Amount and composition of cuticular wax obtained from *Catharanthus roseus* plants grown under different light environments for 50 days (ug/100 ml TLC eluate)

Sample	Aldehyde	β-diketones	Wax monoester	Alkanes
Ambient high	11.88±0.9	8.64 ±0.9	9.36 ±0.7	10.44±0.8
-UV-B	4.68 ±0.5	4.32 ±0.3	5.76 ±0.4	6.48 ±0.7
Ambient low	7.2 ±0.8	7.56 ±0.6	8.28 ±0.5	5.64 ±0.4

The values are the averages of three independent measurements.

Table 3. Effect of different light environments on vincristine alkaloid content in the leaves of *Catharanthus roseus* (L.) G. Don calculated by HPLC analysis

Sample	Vincristine content (µg/g leaf dry weight)
Ambient high solar radiation	820.4 ± 18
-UV-B	316.2 ± 27
Ambient low solar radiation	830.4 ± 32

The values are the averages of three independent measurements.

4. Conclusion

The present preliminary study indicates that filtered and unfiltered solar radiation produced distinct effects on growth and secondary metabolite accumulation in *Catharanthus roseus*. Vegetative growth was generally improved under filtered radiation, particularly under UV-B-filtered conditions, whereas plants exposed to ambient high solar radiation showed comparatively reduced growth but earlier flowering. Epicuticular wax accumulation was highest under ambient high solar radiation, suggesting that exposure to full solar radiation may enhance surface wax deposition in leaves. The composition of epicuticular waxes also varied among the light treatments, indicating that wax biosynthesis is responsive to changes in the light environment. HPLC analysis confirmed the presence of vincristine in leaf extracts from all treatments. Vincristine content was markedly lower under UV-B-filtered radiation than under ambient high and ambient low solar radiation. However, the value recorded under ambient low solar radiation was comparable to, and slightly higher than, that recorded under ambient high solar radiation. Therefore, the results should be interpreted as evidence that UV-B exclusion reduced vincristine accumulation under the present experimental conditions, rather than as proof that maximum vincristine synthesis requires unfiltered high solar radiation. Overall, the study supports the role of solar-radiation quality in modulating growth, flowering, epicuticular wax production and alkaloid accumulation in *C. roseus*.

5. Limitations

This preliminary study had several limitations. The experiment was conducted under outdoor filtered-light conditions, where microclimatic variables such as temperature, humidity and airflow may have differed among treatments despite the use of filter cages. The number of independent measurements was limited, and detailed statistical analysis was not presented to confirm the significance of treatment effects. The radiation environment was described, but some methodological details, including filter thickness and light-transmission values, require clarification. Vincristine quantification was based on TLC and HPLC analysis; however, the method would be strengthened by calibration curves, validation parameters, chromatographic-resolution data and recovery studies. The study focused mainly on leaf growth, epicuticular waxes and vincristine content at a single harvest stage. Other alkaloids, developmental stages, longer growth periods and plant organs were not comprehensively analysed. Therefore, further controlled experiments with stronger statistical and analytical validation are required before broader conclusions can be drawn from these preliminary observations.

Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies, such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators, have been used during the writing or editing of this manuscript.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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