GMR
The Original

IN VITRO SPECTRAL INFLUENCE OF LIGHT EMITTING DIODES (LEDS) ON THE MORPHO-PHYSIOLOGICAL DEVELOPMENT AND BIOCHEMICAL PROFILES OF *CELASTRUS PANICULATUS* WILLD

Vandita Parashar[1], Poonam Jaiswal[2], Priyanka Das[3], Alok Kumar Srivastav[4*], Mamta Bhayal[5]

[1]Department of Biotechnology, Medicaps University, Indore, Madhya Pradesh, India
[2]Department of Microbiology, IES University, Bhopal, Madhya Pradesh, India
[3]Department of Health Science, University of the People, Mysuru, Karnataka, India
[4*]Department of Health Science, University of the People, Mysuru, Karnataka, India
[5]Department of Zoology, Government College, Manawar, Indore, Madhya Pradesh, India

ABSTRACT

Celastrus paniculatus Willd. is a high-value, threatened medicinal plant prized for its unique neuroprotective and antioxidant properties. Due to intensive overexploitation and poor natural seed germination, establishing highly efficient micropropagation systems is vital for its long-term conservation and commercial application. This study evaluated the chronic effects (120 days) of different narrow-bandwidth Light Emitting Diode (LED) spectra—White, Yellow, Red, and Blue—on the growth mechanics and biochemical attributes of *C. paniculatus* cultures. Explants were maintained on Murashige and Skoog (MS) basal medium under identical climate parameters. Long-term exposure revealed a profound spectral dependency on morphogenetic pathways.

Blue LED light promoted peak vertical axial development, maximizing shoot elongation 6.2cm and root network development 2.5mm. Conversely, Yellow LED light accelerated biomass accumulation, yielding the highest absolute fresh weight 5.5g per 10 shoots and a multi-fold surge in total soluble proteins 0.609mg/FW. Notably, Yellow LED also exhibited superior retention of Chlorophyll a 3.81mg/50mg FW and total chlorophyll over 120 days compared to traditional monochromatic Red or Blue setups, which displayed marked pigment degradation ("Red light syndrome"). These results provide concrete empirical insights for developing optimized, multi-staged spectral lighting protocols for large-scale micropropagation and secondary metabolite cultivation of *C. paniculatus*.

KEYWORDS: *Celastrus paniculatus*, Micropropagation, Light-emitting diodes, Photomorphogenesis, Chlorophyll kinetics, Biomass optimization.

1. INTRODUCTION

Celastrus paniculatus Willd. (Family: Celastraceae), popularly referred to as Jyotishmati or Malkangni, is an endangered climbing shrub indigenous to the Indian subcontinent. The seeds contain secondary metabolites, primarily sesquiterpene polyol esters and alkaloids, which are extensively used in traditional Ayurvedic medicine to enhance cognitive capacity, treat neurodegenerative disorders, and mitigate oxidative stress. Unfortunately, intense illegal wild harvesting coupled with inherently poor seed viability has drastically depleted its natural populations, categorizing it as a high-priority threatened plant requiring immediate biotechnical intervention.

While *in vitro* micropropagation offers an effective solution for rapid clonal propagation, the success of plant tissue cultures depends heavily on the physical micro-environment within the culture room. Among these variables, light is one of the most critical environmental stimuli, regulating photosynthesis, photomorphogenesis, metabolic partitioning, and organogenesis. Historically, standard cool-white fluorescent lamps have populated growth chambers. However, fluorescent lamps present several operational bottlenecks, including high heat dissipation, excessive energy consumption, a fixed broad spectrum, and rapid spectral degradation over time.

Light Emitting Diodes (LEDs) provide a precise technical alternative. Characterized by high energy efficiency, low heat output, a long operational life, and highly customizable narrow spectral bandwidths, LEDs allow researchers to isolate specific wavelengths to steer plant development. While the influences of Red and Blue spectra have been documented generally across various plant species, the specific long-term morpho-physiological responses of *C. paniculatus* under monochromatic LED illumination remain largely unexplored.

Accordingly, this investigation systematically monitors the 120-day developmental dynamics of *C. paniculatus* under White, Yellow, Red, and Blue LED arrays. The primary objective is to define how spectral variations alter growth mechanics, protein synthesis, photosynthetic pigment degradation, and lipid peroxidation to optimize commercial micropropagation frameworks.

2. MATERIALS AND METHODS

2.1 Plant Material and Surface Sterilization

Healthy nodal explants of *Celastrus paniculatus* were sourced from mature mother plants. Explants were rinsed thoroughly under running tap water for 30 minutes, treated with a 0.1% (w/v) systemic fungicide (Bavistin) for 15 minutes, and surface-sterilized using a 0.1% (w/v) aqueous mercuric chloride ($HgCl_2$) solution for 5 minutes inside a laminar airflow bench. After four successive washes with sterile double-distilled water, the node segments were trimmed cleanly to approximately 1.0-1.5 cm.

2.2 Culture Media and Environmental Conditions

Explants were inoculated onto Murashige and Skoog (MS) basal medium supplemented with 3.0% (w/v) sucrose and solidified with 0.8% (w/v) agar. The medium pH was calibrated precisely to 5.8 prior to autoclaving at 121˚C, 15 psi for 20 minutes. All experimental cultures were incubated inside climate-controlled growth rooms maintained at a steady ambient temperature of 25˚C relative humidity of 60%-65%.

2.3 LED Light Treatment Setup

Cultures were exposed to continuous spectral regimes generated by solid-state LED fixtures under a strict 16h light / 8 h dark photoperiod. The specific light treatments were organized as follows:

- Control / White LED: Broad spectral white emission.
- Yellow LED: Monochromatic narrow emission peaking near 590 nm.
- Red LED: Monochromatic narrow emission peaking near 660 nm.
- Blue LED: Monochromatic narrow emission peaking near 460 nm.

All spectral treatments were maintained at a standard photosynthetic photon flux density (PPFD) of 45\mu\mol\$m^{-2}$\$s^{-1}$.

2.4 Morphological Parameter Assessment

Observations were continuously recorded over an extended 120-day cultivation period. The parameters evaluating structural development included:

- **Shoot Length (cm):** Absolute vertical length measured from the base of the shoot to the terminal apex.
- **Fresh Weight (g):** Total fresh biomass calculated using an average sample size of 10 microshoots per treatment.
- **Root Length (mm):** Total length of newly induced primary roots.

2.5 Biochemical and Pigment Analysis

Fresh leaf and stem tissues harvested at designated time intervals (Day 0 and Day 120) were used for biochemical assays:

- **Photosynthetic Pigments:** Chlorophyll a, Chlorophyll b, and Total Chlorophyll concentrations were extracted in 80% cold acetone, measured spectrophotometrically at wavelengths of 663 nm and 645 nm and quantified as 50mg/ fresh weight (FW).
- **Soluble Proteins:** Protein estimation was determined by analyzing the optical density (O.D.) profiles of fresh tissue homogenates using standard spectrophotometric parameters.
- **Malondialdehyde (MDA) Content:** Lipid peroxidation was estimated to evaluate tissue stress and structural integrity by assessing total MDA accumulation mol/ g^{-1} /FW.

2.6 Experimental Design and Statistical Analysis

The experiment utilized a Completely Randomized Design (CRD). Each individual treatment contained three distinct biological replicates (n=3). Experimental datasets were analyzed using one-way Analysis of Variance (ANOVA). Treatment mean differences were evaluated via Critical Difference (CD) testing, establishing a statistical significance threshold of P\le 0.05.

3. RESULTS

3.1 Structural Growth Dynamics and Morphogenesis

The structural development of *C. paniculatus* microshoots exhibited distinct morphological adaptations over the 120-day developmental timeline (Table 1).

- **Axial Elongation:** Blue LED treatments promoted terminal shoot elongation, with final shoot lengths reaching 6.2cm at 120 days. Conversely, Yellow LED light limited vertical growth, resulting in compact, short shoot architectures 3.6cm.
- **Biomass Production:** Yellow LED illumination maximized fresh biomass production, yielding a total weight of 5.5g per 10 shoots by Day 120. Microshoots under Blue 5.2g and Red 5.1g treatments also accumulated substantial weight, while White LED cultures lagged behind at 3.9g.
- **Rooting Parameters:** Root extension was most pronounced under White and Blue LED setups 2.5mm whereas Yellow LED showed minimal support for primary root extension 1.7mm.

3.2 Biochemical Alterations and Pigment Degradation

The internal biochemical parameters and pigment retention of *C. paniculatus* were heavily influenced by long-term spectral exposure (Table 2).

LED Spectral Source	Chlorophyll a (mg/50mg FW)	Chlorophyll b (mg/50mg FW)	Total Chlorophyll (mg/50mg FW)	Soluble Proteins (mg/50 mg FW)	MDA (µmol/g FW)

Baseline (Day 0)	7.868	5.049	12.918	0.046	0.097
White LED	3.730 ± 0.007	3.946	7.676	0.069 ± 0.002	0.096
Yellow LED	3.806 ± 0.008	2.245	6.051	0.609 ± 0.012	0.245
Red LED	1.873 ± 0.001	1.087 ± 0.002	2.961	0.199 ± 0.005	0.116
Blue LED	1.013 ± 0.003	0.568 ± 0.001	1.582	0.232 ± 0.006	0.100

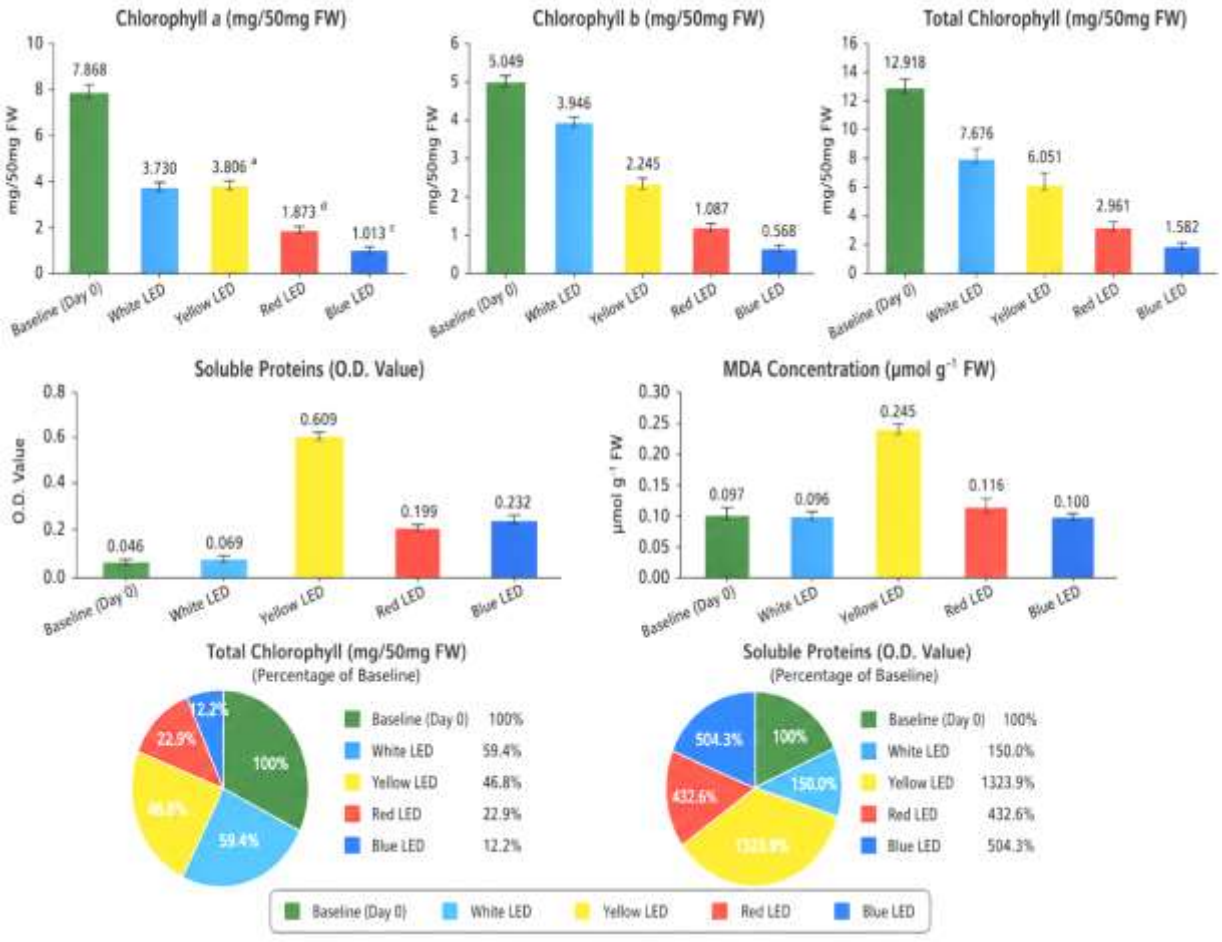
"Data are presented as mean ± SE (n = 3). Superscript letters in the Chlorophyll a column denote significant differences between treatments at P ≤ 0.05 based on critical difference testing."

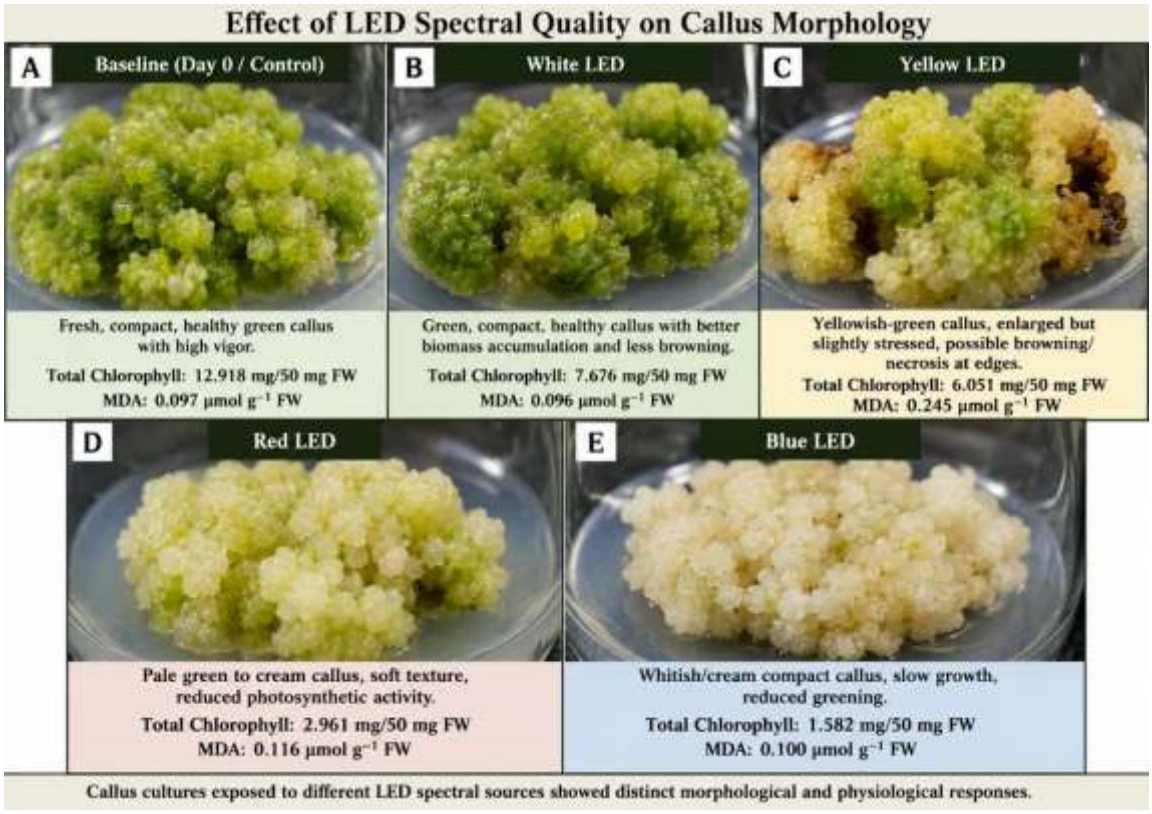
Pigment Depletion Dynamics

Although baseline pigment concentrations were identical across all treatment groups on Day 0, prolonged cultivation for 120 days resulted in a marked reduction in total chlorophyll content. Explants exposed to **Yellow LED** retained the highest chlorophyll *a* concentration (**3.806 mg/50 mg FW**), closely followed by those under **White LED** (**3.730 mg/50 mg FW**). In contrast, explants grown under monochromatic **Red LED** (**1.873 mg/50 mg FW**) and **Blue LED** (**1.013 mg/50 mg FW**) exhibited substantial pigment loss, indicating severe long-term chlorophyll degradation. These findings suggest that yellow and white spectral conditions are comparatively more effective in preserving photosynthetic pigment stability than red and blue wavelengths.

Soluble Protein Accumulation

Total soluble protein content increased markedly in explants subjected to different LED spectral treatments over the 120-day cultivation period. Explants exposed to **Yellow LED** exhibited the highest protein accumulation, rising substantially from the Day 0 baseline of **0.046 mg/50 mg FW** to **0.609 mg/50mg FW** at Day 120. **Blue LED** treatment also promoted noticeable protein synthesis, reaching **0.232mg / 50 mg FW** while **Red LED** showed a moderate increase **0.199mg/50 mg FW**. In contrast, explants maintained under **White LED** displayed only a marginal increase in soluble protein content **0.069mg/ 50 mg FW**. These results suggest that yellow and blue spectral conditions may enhance protein synthesis or stimulate stress-responsive metabolic pathways during prolonged cultivation.





4. DISCUSSION

4.1 Photomorphogenic Response Modulated by Blue and Yellow Spectra

Morphological Adaptations under Different LED Spectra

The morphological observations revealed that distinct LED wavelengths induced specific photomorphogenic responses in *Celastrus paniculatus*. Exposure to **Blue LED** acted as a major stimulus for axial elongation, resulting in the maximum shoot length (**6.2cm**) and root length (**2.5mm**). This enhanced elongation response may be attributed to the activation of photoreceptors such as cryptochromes and phototropins, which regulate apical dominance, directional growth, and cellular expansion during in vitro micropropagation.

In contrast, explants cultivated under **Yellow LED** exhibited a comparatively compact and robust shoot architecture, with reduced shoot elongation (**3.6cm**) but the highest fresh biomass accumulation (**5.5g**). This growth pattern suggests that yellow light suppresses vertical extension while favoring lateral growth, tissue thickening, and overall biomass production. The observed morphological shift is further supported by the substantial increase in soluble protein content under Yellow LED (**0.609 mg/50 mg FW**), indicating enhanced metabolic activity associated with the synthesis of structural proteins, enzymes, and cell wall components. Collectively, these findings demonstrate that spectral quality not only modulates plant architecture but also influences biomass allocation and metabolic reprogramming during culture establishment.

4.2 Pigment Kinetics and "Red Light Syndrome"

A notable biochemical finding was the severe degradation of photosynthetic pigments under monochromatic Red and Blue LED light fields over the 120-day culture cycle. The drop in Chlorophyll a under Red LED light 1.873 mg/50mg FW aligns with the well-documented physiological condition known as "red light syndrome". Monochromatic red light exposure can disrupt the excitation properties of accessory light-harvesting complexes, causing down-regulated pigment biosynthesis and accelerated chlorosis in prolonged *in vitro* environments.

Pigment Stability and Stress Response under Yellow LED

Conversely, the relatively high retention of chlorophyll *a* under **Yellow LED** treatment (**3.806 mg/50 mg FW**) suggests that the yellow spectral region (~**590 nm**) effectively supports long-term pigment stability in *Celastrus paniculatus*. Maintenance of elevated chlorophyll levels likely contributed to sustained photosynthetic efficiency and metabolic activity, thereby promoting enhanced soluble protein synthesis and biomass accumulation even after prolonged cultivation for 120 days. However, the significantly elevated Malondialdehyde (MDA) concentration under Yellow LED (**0.245 µmol g⁻¹ FW**) indicates increased lipid peroxidation and oxidative stress. This suggests that although yellow light supports pigment preservation and biomass production, prolonged exposure to a narrow spectral range may impose metabolic stress. Therefore, Yellow LED may be more effective when applied in combination with complementary wavelengths to balance growth promotion and stress mitigation.

5. CONCLUSION

The present study demonstrates that LED spectral quality significantly influences the morphological, physiological, and biochemical responses of *Celastrus paniculatus* during in vitro culture. Distinct wavelengths elicited specific photomorphogenic adaptations, highlighting the critical role of light spectrum in regulating plant growth and metabolic activity. **Blue LED** effectively promoted shoot and root elongation, indicating its importance in enhancing axial growth and improving explant vigor during the early stages of micropropagation. In contrast, **Yellow LED** supported superior chlorophyll retention, maximum fresh biomass accumulation, and elevated soluble protein synthesis, suggesting enhanced metabolic activity and tissue development under this spectral condition.

However, the increased Malondialdehyde (MDA) levels observed under Yellow LED indicate that prolonged exposure to a narrow spectral range may induce oxidative stress despite improved biomass production. **White LED** maintained comparatively stable chlorophyll content with minimal stress, reflecting its role in preserving physiological balance.

Overall, the findings suggest that no single LED wavelength is optimal for all developmental stages. Instead, a **multi-stage LED lighting strategy**, integrating Blue and White LEDs during the proliferation phase followed by Yellow LED enrichment during biomass accumulation and pre-hardening, offers a promising approach for optimizing micropropagation efficiency. Such stage-specific spectral management can improve large-scale propagation, conservation, and sustainable utilization of the threatened medicinal species *C. paniculatus*.

REFERENCES

1. George, E. F., Hall, M. A., & De Klerk, G. J. (2008). *Plant propagation by tissue culture* (3rd ed.). Springer.
2. Schäfer, E., & Nagy, F. (2006). *Photomorphogenesis in plants and bacteria: Function and signal transduction mechanisms*. Springer.
3. Food and Agriculture Organization (2021). *Micropropagation for sustainable plant conservation*. Rome: FAO.
4. Bantis, F., Smirnakou, S., Ouzounis, T., Koukounaras, A., Ntagkas, N., & Radoglou, K. (2018). Current status and recent achievements in horticulture with the use of LEDs. *Scientia Horticulturae, 235*, 437–451.
5. Billore, V., Sharma, S., & Sharma, P. (2016). Isolation, screening and optimization of cellulase enzyme from local garden soil. *International Journal of Pharmacy and Pharmaceutical Research, 7*(4).
6. Li, H., Xu, Z., & Tang, C. (2010). Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum*) plantlets *in vitro*. *Plant Cell, Tissue and Organ Culture, 103*, 155–163.
7. Nhut, D. T., Takamura, T., Watanabe, H., Okamoto, K., & Tanaka, M. (2003). Responses of strawberry plantlets cultured *in vitro* under superbright red and blue LEDs. *Plant Cell, Tissue and Organ Culture, 73*, 43–52.
8. Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development* (6th ed.). Sinauer Associates.
9. Billore, V., Jain, M., & Suprasanna, P. (2017). Monochromic radiation through light-emitting diode (LED) positively augments *in vitro* shoot regeneration in orchid (*Dendrobium sonia*). *Canadian Journal of Biotechnology, 1*(2), 50–58.
10. Pathak, N., & Srivastav, A. K. (2024). Antimicrobial activity of selected medicinal plants from around Ujjain City, India. *International Journal of Emerging Research in Engineering, Science, and Management, 3*(4), 57–61.
11. Hodges, D. M., DeLong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acid-reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta, 207*, 604–611.
12. Das, P., & Srivastav, A. K. (2015). In-Vitro Micropropagation of the Miracle Plant Aloe vera – A Method of Rapid Production. *International Journal of Pure and Applied Research in Engineering and Technology*, 3(11), 12–27.
13. Ashraf, M., & Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science, 166*, 3–16.
14. Billore, V., Khatediya, L., & Jain, M. (2016). Sink–source system of *in vitro* suspension culture of *Celastrus paniculatus* under regulation of monochromatic lights. *Plant Tissue Culture and Biotechnology, 26*(2), 175–185.
15. Srivastav, A. K., Das, P., Jaiswal, P., & Parashar, V. (2024). CRISPR-Cas systems for targeted therapy in multidrug-resistant infections: A new frontier in medical microbiology. *International Journal of Innovative Research in Science, Engineering and Technology, 13*(11), 18332–18340.
16. Billore, V., & Jain, M. (2017). *In vitro* regeneration through somatic embryogenesis for quality propagation of *Gerbera jamesonii*. *International Journal of Scientific Research and Reviews, 6*(4), 244–251.
17. Das, P., & Srivastav, A. K. (2015). To Study The Effect of Activated Charcoal, Ascorbic Acid and Light Duration on In Vitro Micropropagation of Aloe vera L. *International Journal of Innovative Research in Science, Engineering and Technology*, 4(5), 3131–3138.
18. Billore, V., Mirajkar, S. J., Suprasanna, P., & Jain, M. (2019). Gamma irradiation induced effects on *in vitro* shoot cultures and influence of monochromatic light regimes on irradiated shoot cultures of *Dendrobium sonia* orchid. *Biotechnology Reports, 22*, e00343.
19. Parashar, V., Jaiswal, P., Jaiswal, P., Yadav, S., Bhayal, M., Das, P., & Srivastav, A. K. (2025). Light-emitting diodes in plant tissue culture: Effects on *in vitro* callus, shoot, root, and plantlet development. *Journal of Applied Bioanalysis, 11*(7), 1009–1017.

20. Srivastav, A. K., Das, P., Jaiswal, P., & Parashar, V. (2024). Plant pathology in the age of climate change: Challenges and adaptations. *International Journal of Innovative Research in Science, Engineering and Technology*, 13(10), 17112–17120.
21. Gupta, S. D., & Jatothu, B. (2013). Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. *Plant Biotechnology Reports, 7*, 211–220.
22. Srivastav, A.K., & Das, P. (2025). *Biotechnology and IoT in Agriculture and Food Production: Green Innovation*. Apress, Berkeley, CA.