

**EFFECT OF DIFFERENT LIGHT CONDITIONS AND PLANT GROWTH  
HORMONE ON EARLY FLOWER INDUCTION IN *CHRYSANTHEMUM  
MORIFOLIUM***

**A**

**PROJECT REPORT**

*Submitted in partial fulfillment of the requirements for the award of the degree*

*of*

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**IN**

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*Under the supervision*

*of*

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**MAY, 2025**

## DECLARATION

We, hereby declare that the work represented in the project report entitled “**EFFECT OF DIFFERENT LIGHT CONDITIONS AND PLANT GROWTH HORMONE ON EARLY FLOWER INDUCTION IN *CHRYSANTHEMUM MORIFOLIUM***” submitted towards partial fulfillment of the requirements for the award of the degree in Bachelor of Technology in Biotechnology at the **Jaypee University of Information Technology, Wagnaghat** is an original work carried out by me under the supervision of **Dr. Hemant Sood**. The work has not been submitted elsewhere for the reward of any degree/diploma. We are fully responsible for the contents of the project report.

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## CERTIFICATE

This is to certify that the work being presented in the project report **“EFFECT OF DIFFERENT LIGHT CONDITIONS AND PLANT GROWTH HORMONE ON EARLY FLOWER INDUCTION IN *CHRYSANTHEMUM MORIFOLIUM*”** in partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted to the Department of Biotechnology and Bioinformatics, **Jaypee University of Information Technology, Waknaghat** is an authentic record of work carried out by **Pelden Tshomo (211802)** and **Devanshi (211821)** during a period from August 2024 to May 2025 under my supervision. The above statement is correct to the best of our knowledge.

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## ABSTRACT

*Chrysanthemum morifolium* is a short-day plant that is the second most popular floriculture next to the rose. The plant in ancient China is used as traditional medicine having lots of health benefits. Due to its popularity, the demand for the flower is growing and it is estimated that billions of branches of chrysanthemum are sold every year. As it is a seasonal flower, techniques such as in vitro micropropagation are utilized to mass-produce the flower.

The propagation of chrysanthemum involved utilizing the nodal segment as an explant and supplementing the Murashige and Skoog's (MS) media with growth hormones such as Benzyl aminopurine (BAP), kinetin (KN), and Indole-butyric acid (IBA) for the optimized condition for rapid micropropagation. After 10 weeks, the white chrysanthemum variety exhibited significant shoot formation in MS-4 media, while the yellow variety also thrived in MS-4 media. Other varieties such as pink, orange, and purple responded very slowly to both media.

The *Chrysanthemum morifolium* plants stationed under red, blue, and white light was unresponsive and it was responsive under greenhouse light. The hardened in vitro plants treated with gibberellic acid recorded with maximum shoot length of 16 and 13.5 cm for both 5 and 10 mg/L whereas maximum leave number was obtained for 10 mg/L and the shoot number remained same. And the early flowering for the plantlets is still awaited.

Keywords: *Chrysanthemum morifolium*, Growth hormone regulators, In vitro propagation, LED lights, Plant growth hormone

# CHAPTER 1

## INTRODUCTION

*Chrysanthemum morifolium*, commonly known as 'Autumn Queen,' belongs to the Asteraceae family and is considered the most ancient and medicinal flower cultivated worldwide. Greek words "Chryos," which means "gold," and "Anthemion," which means "flower," are the source of the term "chrysanthemum"[1]. It behaves as an annual and perennial flower and has been used in all ornamental, medicinal, and industrial uses. Medicinal properties include the flower being anti-oxidant, anti-inflammatory, anti-microbial, anti-genotoxic, and anti-cancer. *Chrysanthemum* is used in culinary, and cosmetics, and is a natural source of insecticides where the pyrethrins are extracted[2]. The flower is native to Asia and Northeast Europe. Huge regions in China and Japan are covered in chrysanthemum production. Around 600 chrysanthemum varieties are mentioned in the National Chrysanthemum Society of Britain[3], [4]. Some varieties include Ajay, Aparjita, Kelvin Yellow, White Star, and many more[5]. *Chrysanthemum* is the world's second most economically important floriculture, after rose. Due to its increasing demand, lucrative markets have developed, which are advantageous to both suppliers and cultivators.



Fig 1. (A) bulb yellow chrysanthemum; (B) bulb white chrysanthemum; (C) bulb pink chrysanthemum; (D) bulb purple chrysanthemum; (E) spider orange chrysanthemum

Traditionally, these flowers have been used in medicine. In addition to treating bruises, sprains, snake and centipede bites, rhinitis, diphtheria, cholera, and malaria, it also reduces inflammation. Additionally, it has antipyretic and antihypertensive properties. The petal of the flower cures illnesses like wind-heat syndrome and fever. In addition, the Chinese dry the petals and use them in salad and tea[6]. It is also used as an herbal remedy in Chinese traditional medicine [3].

Plant cells, tissues, or organs can be sterilely cultivated on a nutritional medium in a controlled environment using the tissue culture technique known as "*in vitro* propagation." Chrysanthemums are among the crops that can be mass-produced and cloned using this technology. Chrysanthemums can be propagated *in vitro* primarily using callus induction from different explants, like nodal or pedicel segments, and direct organogenesis. Conventional culture media, like Murashige and Skoog (MS), are frequently used to maximize growth and multiplication[7] [8]The capacity to generate true-to-type offspring, the ability to produce vast numbers of plants in a short period, and the capacity to do mutation breeding to create new kinds with desirable qualities, like changing flower colors, are some of the benefits of *in vitro* propagation[9].

Chrysanthemums are so popular that bulk production of them is accomplished using micropropagation. The main methods of proliferation include callus induction, shoot cutting, and root suckers [5]. The traditional method can be completed *in vitro* and is inexpensive and straightforward. The aforementioned procedure has several limitations that are addressed using an efficient propagation method like micropropagation[10]. While there are many benefits to *in vitro* propagation, there are drawbacks as well. For example, contamination and the requirement for exact control over growth conditions can negatively affect the success rates of plant regeneration. The process of rapidly growing plant material to generate a large number of offspring plants is known as micropropagation[10]. The growth of the progeny frequently does not provide all the nutrients required. As a result, growth media, including vermiculite and coco peat, are employed. By doing this, it hopes to propagate chrysanthemums *in vitro* under ideal conditions and demonstrate how altering these parameters affects the plant's ability to thrive[11].

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 BRIEF BACKGROUND ON LEDS**

Before the introduction of LEDs, other lighting such as enclosed gaseous lamp, incandescent lamp, CFLs, and high-pressure sodium lamp were utilized. The challenges associated with these lights were firstly their efficiency, secondly their lives, and lastly the ability to give broad-spectrum light. It was also spectrally and electrically not efficient concerning various photoperiods to ornamental plants. So, the LED was approached in terms of reducing the electricity consumption. LED is described as a unique kind of semiconductor, which is a light capable of spectral control and wavelength that can match the photoreceptors [12]. The discovery of the LED goes back to the early days, and although white light was utilized, the brightness became a major issue. Nick Holonyk Jr. was the first person to create the visible spectrum LED light, where his work was later carried out by his students, named NH and M. George Cardford, with yellow and red LEDs. LED is advantageous to use as it is durable (15 years life span), energy efficient (monochromatic and single direction), has high spectral quality with non-toxic elements (no mercury content), and does not harm the environment[13].

#### **2.2 EFFECT OF LEDS ON SHORT-DAY PLANT**

The response of the plant and its flowering majorly depend on change of light, wavelength of light, and photoperiod. Usually for the short-day plant it only blooms when exposed to the light period that are less than specific threshold length. As chrysanthemum is a short-day plant, it requires extra light for their growth. When blue and red light was utilized as lighting for short-day plant, it inhibited the flowering, whereas the far-red light delayed the flowering. When the LEDs were used on chrysanthemum morifolium cv. Zembla, the shortest bud formation took place in 28 days under blue light induction and there was delayed flowering with far-red LEDs[14]. But when treated with intense blue light with long photoperiod, it resulted in delayed flowering in short-day plant and promotion of flowering in long-day plants. According to Sharat et al. when short-day plant was exposed with 4-hour extension of blue/red light, it was insufficient to affect the flowering initiation [24].

## 2.3 CHEMICALS INDUCING EARLY FLOWERING IN THE PLANTS

For the early flowering in many of the plants, the chemicals such as gibberellic acid and ethephon is utilized. Gibberellic acid (GA) is a tetracyclic diterpenoid plant growth hormone that plays a key role in promoting the growth and development of plants. Mostly, gibberellic acid helps plant in germination, elongation of shoots, and induces vegetative flowering. GA influences the expression of key genes involved in floral development[15]. For instance, in *Arabidopsis*, gibberellic acid promotes the expression of **LEAFY (LFY)** and **SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)**, both of which are essential for the transition from vegetative to reproductive growth. Additionally, GA can upregulate **APETALA1 (AP1)**, a gene that determines floral meristem identity, thereby initiating flower formation[16].

Furthermore, the chemical ethephon releases ethylene, a gaseous plant hormone that play an important role in flowering. In species like *Ananas comosus* (pineapple), ethephon application induces synchronized flowering by promoting ethylene production, leading to uniform floral induction. The effectiveness of ethephon in inducing flowering is influenced by several factors, including application timing, concentration, and environmental conditions such as temperature and humidity. Optimal conditions enhance the absorption and subsequent ethylene release, thereby improving the consistency and uniformity of flowering induction [10].

## 2.4 TECHNIQUES INCORPORATED

Callus regeneration and direct shooting are two methods used most frequently for chrysanthemum micropropagation. The process of inducing shoots straight from the explant without the requirement for callus induction is known as direct shooting. With this method, shoots can regenerate from a variety of plant tissues. The primary benefit of this method is that it produces faster regeneration than callus production, allowing the plantlets to heal more quickly. Furthermore, it can result in genetically stable and homogeneous plants, lowering the possibility of soma-clonal variation. The process of forming a mass of undifferentiated cells from an explant by exposing it to a suitable media is referred to as callus induction. The explant utilized and the growth regulators applied during the procedure determine how well the callus regenerates[17].





## 2.5 PATHOGENESIS OF CHRYSANTHEMUM

There are now two to nine viruses that are known to affect the chrysanthemum. Plant diseases known as viroid's possess circular single-stranded RNA that lacks protein-encoding. Posiviroidae and Avsunviroidae are two families that comprise the thirty species of viroid. Two viruses, such as Chrysanthemum stunt viroid (CSVd) and Chrysanthemum chlorotic mottle viroid (CChMVd), frequently host chrysanthemum. Young, light-green leaves with CSVd stunt lose some of their ability to root. The infectious material was separated from the stunted chrysanthemum, nuclear acid extraction was performed, the mixture was centrifuged in a sucrose density gradient, and polyacrylamide gel electrophoresis was carried out to examine the presence of the CSVd in the plant. Grafting one chrysanthemum with another spreads the illness known as Chrysanthemum chlorotic mottle. Chlorosis, dwarf symptoms, and yellow-green molting are the signs. Moreover, compared to CSVd, CChMVd is less common in plants. The main source of the illness was the transcript of the CChMVd, which caused the chlorotic mottle disease.






Certain viruses, such as CSVd and CChMVd, can infect chrysanthemum plants and can spread to *Gynura aurantiaca* plants when injected. Many plants, such as *Petunia hybrida*, *Ageratum*, *Dahlia*, *Senecio*, *Vinca major*, and *Argyranthemum frutescens*, contain CSVd. Tools used on infected chrysanthemums and healthy material for grafting and flower cutting can mechanically spread CSVd. Although CSVd cannot spread by infected soil or insects, it can spread through seeds [18].

Numerous serious infections that cause a range of illnesses damage *Chrysanthemum morifolium*, affecting both its aesthetic and financial worth. Important pathogens include *Fusarium oxysporum*, which produces root diseases that impede nutrition absorption, *Alternaria alternata*, which causes leaf blight and causes rapid tissue deterioration, *Nigrospora sphaerica* and *Nigrospora oryzae*, which cause leaf blight and leaf spot diseases, respectively[4]. Additionally, *Golovinomyces spp.* create powdery mildew, which can seriously harm plants and flower quality, while *Puccinia horiana* causes chrysanthemum white rust. To lessen the negative effects of these infections on the production of chrysanthemums, resistant cultivars and efficient management techniques must be developed [19].



**Table 1: The pathogens that cause the disease along with the symptoms and pictures.**

Disease	Symptoms	Pathogens	Picture
Ascohyta Ray Blight	The flower developed retarded on one side of the bud, with brown discoloration in petals and a drooping stem. Brown to black spots develop on leaves.	<i>Ascochyta</i> ( <i>Mycosphaerella</i> )	
Alternaria or Stemphylium Ray Speck	Dead spots developed on the petal.	<i>Alternaria</i> or <i>Stemphylium</i>	
Bacterial Blight	The cutting turns brown, with surviving cuttings and established plants being infected	<i>Erwinia</i> <i>chrysanthemi</i>	
Bacterial Leaf Spot	Dark brown to black spots are spotted in lower leaves and become irregularly shaped, which can eventually spread to the whole plant.	<i>Pseudomonas</i> <i>cichorii</i>	



Botrytis Blight	Light brown spots were observed on the petals, which spread to other petals.	<i>Botrytis cinerea</i>	
Chlorotic Mottle	The plant's leaves turn yellow initially, but it doesn't show symptoms when grown under low light conditions and temperatures below 20°C.	<i>Chrysanthemum chlorotic mottle viroid</i>	
Fusarium Wilt	Yellowing of the leaves, wilting, and discoloration.	<i>Fusarium oxysporum</i>	
Powdery Mildew	Appearance of white and dry fungal growth on the leaf surface.	<i>Golovinomyces cichoracearum</i> (formerly <i>Erysiphe</i> )	
Rhizoctonia Stem Rot	A reddish-brown dead area developed above the soil line. The infected plant wilts during the day and recovers during the night.	<i>Rhizoctonia solani</i>	



Rust, Brown	Dark brown masses of spores formed on the leaves.	<i>Puccinia chrysanthemi</i>	
Stunt	Young, light green leaves are stunted and upright, while infected plants prematurely flower, reducing size and displaying small dead spots or dots.	<i>Chrysanthemum stunt viroid</i>	

## 2.6 LITERATURE REVIEW ON THE EFFECT OF DIFFERENT LED LIGHTS ON THE GROWTH AND FLOWER INDUCTION IN CHRYSANTHEMUM

Levi et al. (2019) described *C. morifolium* as a short-day plant. Also known as a long-night plant, as it needs long periods of darkness to flower. The most effective inhibition was found with the light of wavelength 596nm (amber light), which caused a reduction in the expression of genes related to flower induction. The white light and green light inhibit the flowering of the chrysanthemum. In addition to the flowering induction, it also affects the shoot architecture. When far-red light was used as the lighting condition, the growth was affected, and an extended stem length was observed. When the far was removed, it resulted in reduced height and shorter internodes. The overnight illumination of blue light inhibited the flowering of the chrysanthemum, but the efficiency depended on the duration of the lighting[20].

Kemelia L. et al. (2018) found that each light has a different effect on the plant. Blue light has a wavelength of 400-580nm and usually affects the formation of the flower bud. The red light slows down the flowering and produces the flower with the smallest diameter. As for the green light, it

increases the propagation of the chrysanthemum. Blue light resulted in the shortest segments and stems whereas red light showed a smaller number of leaves and weight. However, the white light showed the highest content of chlorophyll. Due to the spectrum difference, it results in different morphogenetic and photosynthetic responses. The treatment with red light resulted in the fastest flowering age averaging to 57 days whereas white light resulted in longest flowering age averaging to 59 days after plantation. Which also became equivalent to red light giving largest flower diameter with a difference of 0.3cm to 0.5cm[21] [22].

Zakrzewska A. et al. (2021) studied to investigate the effect of different light colors on the rooting cutting and growth of chrysanthemums. The lighting can be categorized into long-day phase and short-day phase. Long-day phase aims to improve the vegetative growth, including stem length, width, and increasing number of leaves. Short-day phase aims to promote vegetative growth which includes flower bud formation. During the long-day phase, the plant was exposed for 12 hours per day for 10-15 days. The plant was exposed for less than 12 hours per day for 6-11 days in the short-day plants. A variety of light affected the growth and flowering of the plants. Different intensities of the lighting also manipulated some morphological features such as flower development and leaf elongation. Blue and white lighting accelerates the flowering whereas the red light delays the flowering. LED are not used in conventional farming but Japan made use of the LEDs in urban agriculture. The reason was that there were limited cultivation fields and insufficient sun exposure and LED also generates a low amount of heat. Exposure to blue and white light increased the plant's weight, whereas the white + blue and red + blue light combination gave an average weight. The results indicated that the combination of red and blue light (RB) significantly enhanced the development of flower buds and overall biomass compared to other treatments. The plants that are grown under the white + blue light produced nearly double the number of flowers compared to other treatments[23].

Sharath Kumar et al. (2021) investigate the effects of photoperiod and light spectrum on the flowering responses of *Chrysanthemum morifolium*, a short-day plant. The study aims to understand how different light conditions influence floral initiation and development, revealing that extending short-day conditions with blue light leads to complete flowering, while red light only initiates flowering without further development. The findings highlight the critical role of light quality in flowering and suggest that manipulating light conditions can optimize

chrysanthemum production in controlled environments. Additionally, the research underscores the need for further exploration of the molecular mechanisms involved in photoperiodic flowering, particularly the roles of key regulatory genes. Plants under the RB, LD treatment (15 hours of red and blue light) showed earlier floral initiation compared to those under the RB, SD treatment (11 hours of mixed light). The percentage of flowering plants and the number of flower buds per plant were higher in the long-day treatment compared to the short-day treatment. The RB and LD treatments had greater flower buds than the RB and SD treatments, which indicates that longer photoperiod enhances biomass accumulation[24].

Jeong et al. (2013) studied the influence of four different colored LEDs on the flowering of the chrysanthemum and determined the level of the polyphenol using the HPLC. The colored LEDs, such as blue, green, red, and white, were utilized where the blue light regulates the phototropism, chloroplast migration, stomatal opening, leaf expansion, and photoprotection. The green light induces leaf growth and stem elongation, with red light helping in the development of the photosynthetic apparatus. While the white light was kept in control. Polyphenols are the secondary metabolites that defend the plant against a broad spectrum of environmental stressors, such as light. From the findings, it was noted that polyphenols played a role in short-day plants such as chrysanthemum. They studied the response of chrysanthemums to different lights to find the role of polyphenols. The chrysanthemum plant was given a photoperiod of 16 hours per day. For the white light, it was 12 hours, and for blue, red, green, and white light, it was 4 hours. Under the blue light, the flower bud developed in 20.3 days, whereas no bud formation was observed in red, green, or white light. The flowering occurred when the plant was given a photoperiod of 13.5 hours per day, where a longer photoperiod prevented the flowering. Moreover, Polyphenols were extracted from each leaf with 80% methanol, and the isolated component was identified by the HPLC method and compared with literature data. A total of nine polyphenols were recorded in the absorbance segment of the chromatogram at 254nm[25].

Singh et al. (2018) investigated the influence on the flowering of chrysanthemums by using incandescent bulbs, compact fluorescent lights, and light-emitting diodes as a night break treatment. As chrysanthemum is known as a short-day plant, flower initiation is affected by the photoperiod. With the various uses of light, they evaluate how different lights with photoperiod can delay flowering, which proportions to increased flower production. Through the night break

treatment, LED showed a delay in the flowering nuts with increased vegetative properties. The plants stationed under LED light resulted in increased heights of 66.85 and 66.76cm compared to incandescent and CFCs. Following the night break treatment, a delay in the flowering was observed with LED, with a flowering time recorded at 143.34 days. The results indicated that flowers produced under LED lights were larger, with some genotypes like "Reagan White" and "Yellow Delight" showing impressive sizes (up to 8.31 cm). LED was more effective for photoperiodic manipulation in chrysanthemum production than incandescent and CFCs[26].

Keheir et al. (2019) Chrysanthemum is treated with various lights and its effects are investigated. The main focus is on the blue light and far-red light in regulation with floral induction. The flowering of the chrysanthemum plant is sensitive to the light quality and duration. The main lights utilized are blue light, green, red, and far-red light to influence the flowering of chrysanthemums. When blue light was used as a day extension treatment, there was inhibition in flowering. However, the flowering inhibition depended on the photoperiod of the light source. If a short-day photoperiod is used, there is no effect. For red light acted as the light period to determine the flowering response to blue light. Far-red light combined with blue light decreased the chrysanthemum plant's flowering. During the time blue light was used as the day-extension treatment, there was inhibition in the flowering, but the effectiveness varied depending on the photoperiod light source. The far-red light used was critical in determining the flowering response to blue light during the main light period. So, when it was used with blue light as the day-extension treatment, a decrease in the flowering was observed. Depending on the condition in which the plant was kept, the response was different [27].

Liao et al. (2014) studied the night break effect on chrysanthemums with several different wavelengths of LED lamps. Six different wavelengths of LED were used, such as LED-630, LED-660, and 12h LED-670, LED-735, LED-630 + 735, and INC. For each of them, the night break treatment given was 6h daily and 12h for photoperiod (short-day) conditions without treatment. Three chambers were utilized for this study. After six weeks, in LED-735, FBD (floral bud differentiation) was at the beginning of the third week, and for other LEDs, the plant did not produce FBD Jimba). As for 'Iwa no hakusen', the FBD was observed in LED-735, LED-630, and LED-660 in the sixth week. However, other treatments resulted in a strong inhibitory effect on FBD [28].

Singh et al. (2015) studied the effect of LED to induce flowering under an artificial long day, using LEDs such as blue and red wavelengths. In addition, the growth and flowering response of chrysanthemum is also investigated. The chrysanthemum induces flowering and requires an uninterrupted dark period. Under artificial conditions, flowering can be induced by shortening the day length to 22 h. Plants were grown under four different light treatments, such as 80% Red, 20% blue (SD for 11 hrs.), long days for 15 hrs, and SD + blue for 12 hrs.

Park et al. (2020) investigated the effects of low-intensity blue light flowering process and morphogenesis in chrysanthemum. The blue light was supplied during night interruption to examine the influence flowering of chrysanthemums. In this, chrysanthemum *Dendranthema grandiflorum* (Gaya fellow) was used. The plant was treated with different photoperiods such as SD9 +4B, LD13+ NI- 4B, providing NI after 10 hrs. SD (SD10-NI-4B). Plants that were exposed to NI blue light had increased height, dry mass, leaf number, and chlorophyll content, whereas these were found to be less when exposed to blue light. Flowering was observed in all of the treatments, but SD10 induced the fastest flowering [29].

Taweesak et al. (2025) studied the impact of the different LEDs on the growth and flowering of the chrysanthemum. The researchers took two cultivars, namely, Sweet Pink and Polaris, and were treated under five different light conditions, such as fluorescent lamps, grow light LED, white light, blue light, and red light. To the plants, the photoperiodism was given from 7 pm to 11 am. Every two weeks, growth parameters were observed and checked. From the recorded results, it was observed that the highest height was obtained in plants that were exposed to white LED light, whereas the blue LED light showed the shortest flowering time, with 56.25 days from the white light, which took 71 days [30].

Sajid et al. (2018) investigated how gibberellic acid, a plant growth hormone, affects the flowering time of the *Chrysanthemum morifolium* using different concentrations of gibberellic acid. Six different dosages of gibberellic acid, such as 50, 100, 150, 200, and 250mg/L, were applied to the flowers to induce flowering. The gibberellic acid was sprayed during morning hours on the plant after 15 days from incubation and continued week after week. The result obtained contained maximum height, a greater number of branches, and leaves for the plant which was treated with 250mg/L. Also, shorter flowering times and higher blooming periods were obtained when chrysanthemum was treated with 250 mg/L of gibberellic acid [31].

Vieira et al. (2011) studied the effect of gibberellic acid to check the quality of the chrysanthemum (*Dendratheuma grandiflora*). The experiment included 40 plants for each of the treatments. To each of 40 plants, the dosages given were 0, 15, 30, and 45 mg/L of gibberellic acid. From the above experiment, it was observed that there were no significant changes in the phenotypic characteristics of the chrysanthemum cultivar. In conclusion, it can be noted that a small concentration of gibberellic acid does not affect the quality of the chrysanthemum [32].

Aparna et al. (2018) studied the effect of gibberellic acid on plant growth and flowering, and its role substituting the artificial light conditions chrysanthemum cv, Thai Chen Queen. The chrysanthemums were sprayed in three different concentrations such as 200, 300, and 400 mg/L of gibberellic acid at 7 days, 14 days, and so on. The observation was made around 30, 45, and 60 days. The treatment of the gibberellic was done on a month-old self-rooted cutting chrysanthemum Thai Chen Queen which was planted on well-prepared beds. Maximum height was recorded at 45.09 cm with 400 mg/L of gibberellic treatment. Flower bud initiation was observed after 35 days, and flowering was hastened in the chrysanthemums that were treated with gibberellic acid [33].

## **2.7 RESEARCH GAP**

Due to in vitro propagation, the mass production of chrysanthemum varieties has been much more convenient, as all the parameters required for growth are already optimized. However, the literature mentions only the parameters such as light. Other environmental parameters, such as temperature, humidity, and the interaction of light, are not taken into consideration. Furthermore, it lacks proper or detailed guidelines for cultivators to grow chrysanthemum in vitro conditions. And there is no recommendation of what specific optimal light spectra to be used or for how much duration the plant should be exposed to the LED lights. No reports were mentioned on the effect of LED lights during the development phases of in vitro regenerated plantlets. Although the literature provided information about the effect of LEDs on the chrysanthemum, it does not consider the other environmental conditions, lacks a detailed protocol, and has no specification on optimal light spectra and their duration. With the utilization of the gibberellic acid, there was a lack of a protocol on how gibberellic acid was prepared, and there was no mention of how much the plant growth hormone should be sprayed on a plant or a group of plants.

## **CHAPTER 3**

### **OBJECTIVE**

#### **3.1 OBJECTIVES OF THE PROJECT**

- ❖ To optimize the culture medium to carry out rapid micropropagation of *Chrysanthemum morifolium*
- ❖ To induce early flowering by using plant growth hormone and LEDs.

## CHAPTER 4

### MATERIALS AND METHODOLOGY

#### 4.1 MATERIALS REQUIRED

Chrysanthemum's mother plant (procured from the nursery of Uttarakhand named Satyam nursery, Reagents and chemicals required, MS media, Growth hormones (BAP, KN, and IBA), Bavistin, Mercuric chloride, Scalpel and forceps, Petri plate, Culturing jars, Beakers.



Fig 2: Materials required (1) Chrysanthemum's mother plant (2) Culturing jar (3) Beaker (4) Petro plate, forceps and scalpel (5) MS media (6) Plant growth hormone (7) Sucrose and agar agar (8) Bavistin and mercuric chloride

#### 4.2 MEDIA PREPARATION

Different growth regulators were used to create two distinct MS media. Two different combinations of media were used: one had MS media plus BAP (2 mg/L) and kinetin (1.5 mg/L), while the other contained MS media plus BAP (1.5 mg/L), kinetin (0.5 mg/L), and IBA (0.5 mg/L).



Agar was melted through boiling, and pH was maintained between 5.6 and 5.7. The prepared medium-filled jar was autoclaved and kept in a cold room for further use.

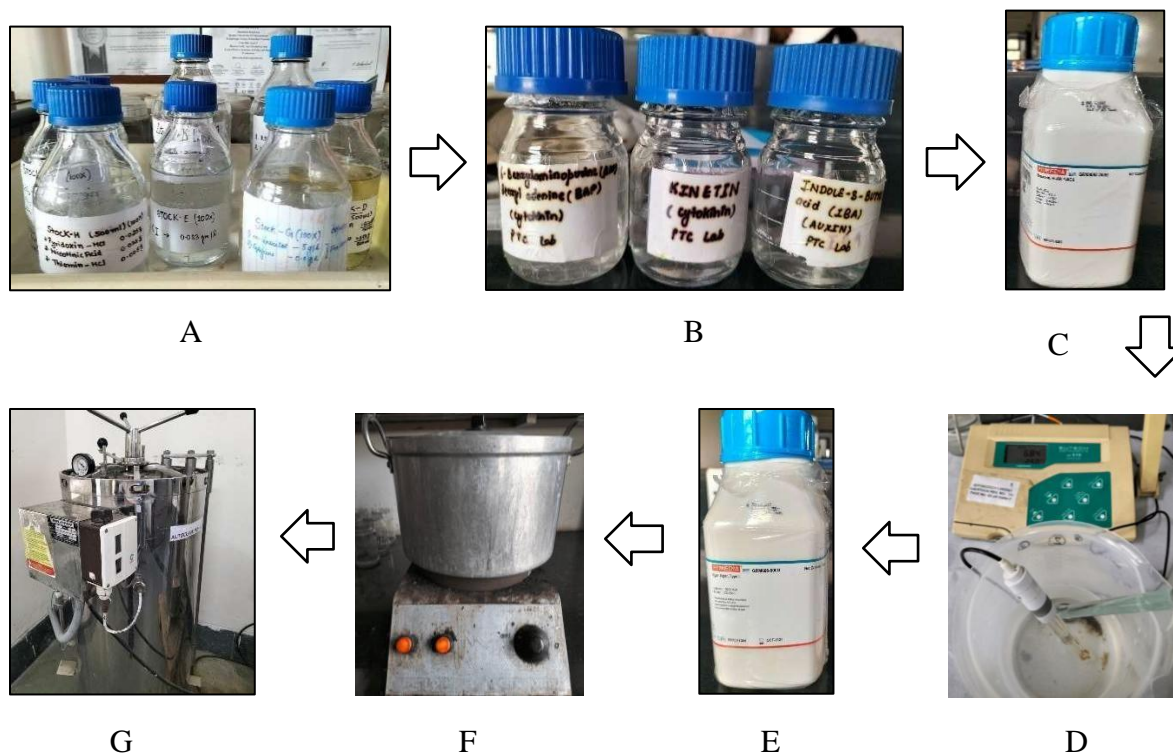


Fig 3: (A) MS media (B) Plant growth hormone regulator (C) Add sucrose to media (D) pH measuring (E) Agar agar addition (F) Boiling of the media (G) Media autoclaving

### 4.3 ESTABLISHMENT OF CULTURE

0.5% bavistin and 0.1% mercuric chloride were prepared in a clean jar. The LAF was used for 15 to 20 minutes to sterilize the reagent, media, Petri plate, autoclaved distilled water, and beaker. Using a scalpel, the explant was removed from the purchased chrysanthemum mother plant and was cleaned four or five times with labolene. The explant was further cleaned under LAF using bavistin, mercuric chloride, and distilled water. With the help of forceps, the explant was cultured in front of the flame in the jar containing MS media. Following the culturing process, the jars were labeled and placed in a culture room with a 24-hour photoperiod, a humidity level and a temperature of  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

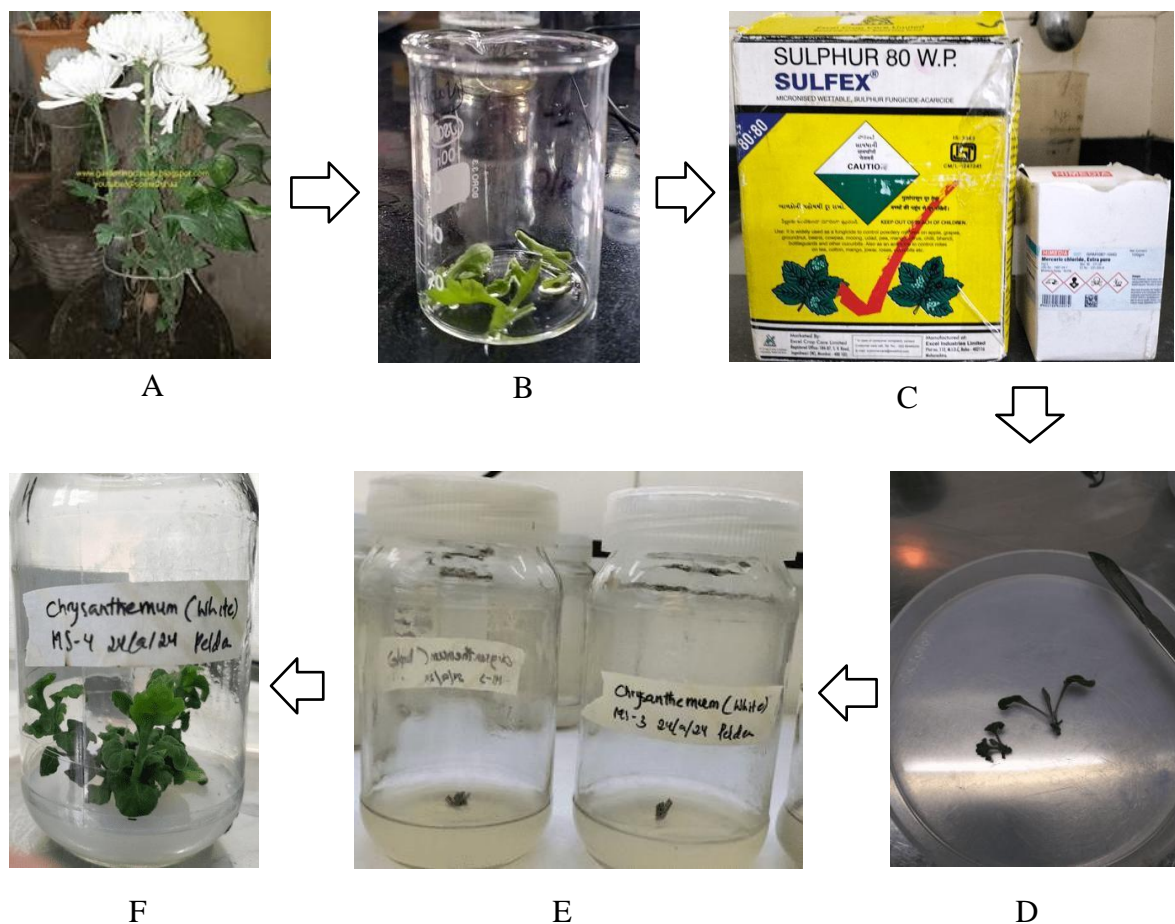


Fig 4: Establishment of culture (A) Chrysanthemum's mother plant (B) Explant washed with labolene (C) Explant washed with bavistin and mercuric chloride in LAF (D) Placed on a petri plate and cultured using forceps and scalpel (E) Cultured in vitro plants (F) Grown in vitro plants under optimized condition

#### 4.4 MULTIPLICATION OF CHRYSANTHEMUM SHOOTS

The required material is sterilized under LAF for 15-10 minutes. After sterilization, the grown chrysanthemum is brought from the culture room and placed in the LAF. Grown shoots are taken out and cut for their nodal segment with a scalpel. The nodal segment is cultured in the media using forceps and placed in the culture room.

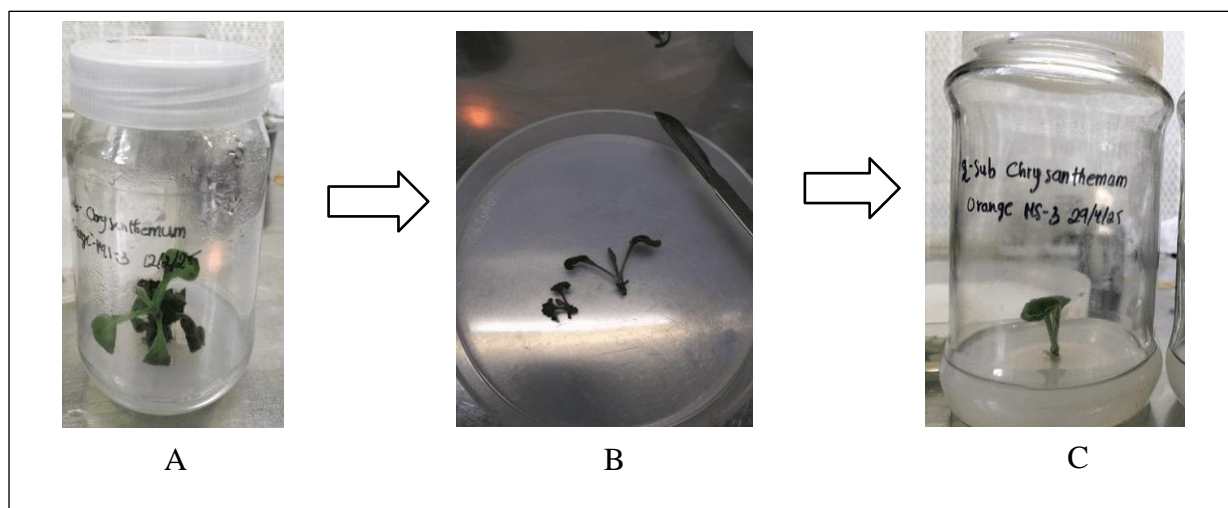


Fig 5: Multiplication (A) In vitro grown chrysanthemum plant (B) Nodal section taken (C) Cultured in agar media and kept under optimized condition

#### 4.5 HARDENING OF THE PLANT CULTIVARS

A potting mixture of vermiculite and cocopeat is utilized to harden the culture. The plant is dipped in 0.5% bavistin to remove the residual media, followed by rinsing with running water. A potting mixture is prepared, and water is added to make the soil moist. The cultured plant is placed in the soil and dapped softly, and the plant is covered with a jar.

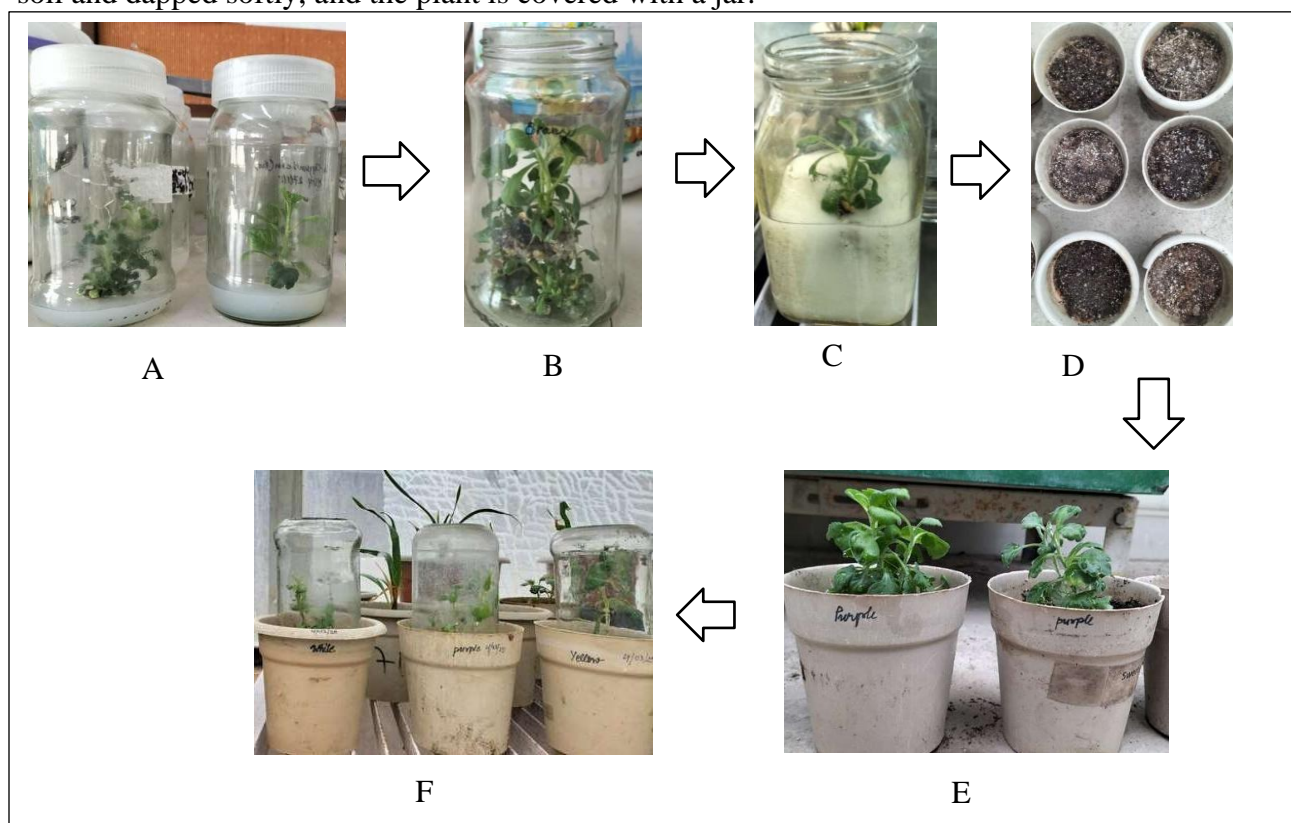


Fig: Hardening of in vitro grown plant (A) Grown in vitro culture; (B) Separation of the plant; (C) Immersing in the bavistin solution; (D) Potting mixture; (E) Hardened plant; (F) Covering with the jar;

#### 4.6 TREATMENT OF PLANT CULTURE WITH DIFFERENT LIGHT CONDITIONS

Different colors of chrysanthemum potted plants are placed under different light conditions. Under each light condition, five of the chrysanthemum plants were stationed to induce early flowering, and observation was made every three days.

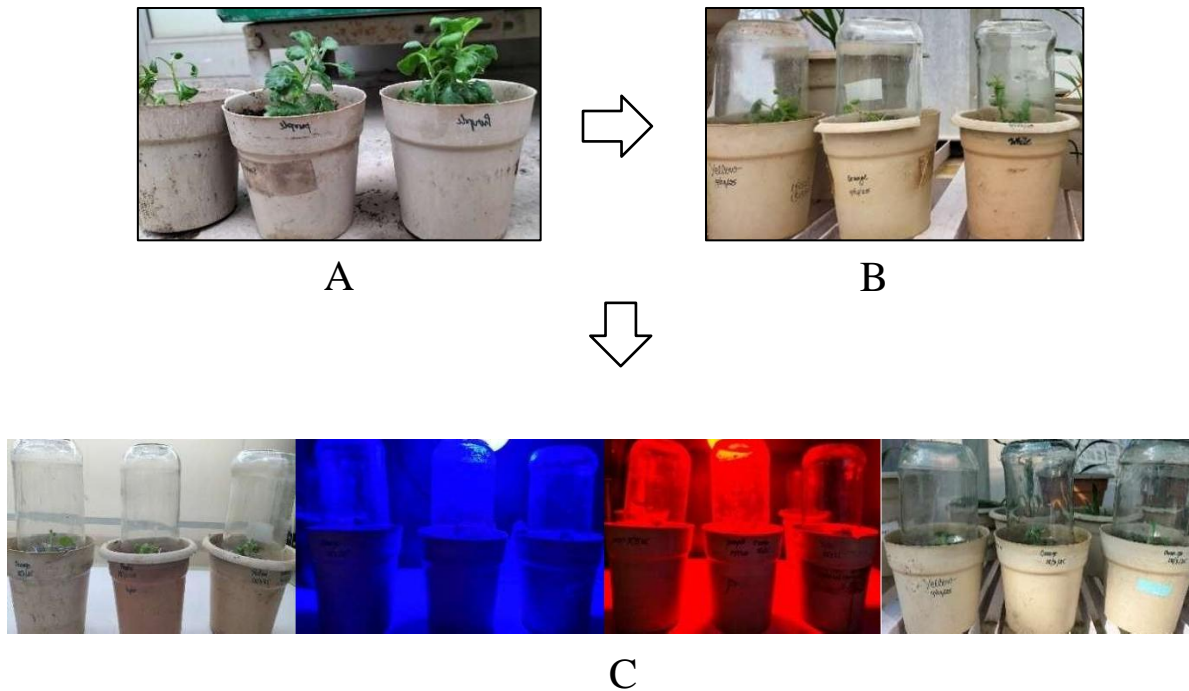


Fig 6: Incubation under different light conditions (A) Hardened culture; (B) Hardened culture covered with jar; (D) Incubation of hardened plant under different conditions of lights (white, blue, red, and greenhouse)



## 4.7 TREATMENT WITH GIBBERELLIC ACID WITH DIFFERENT DOSAGE

After 7-10 days of incubation of the in vitro-grown plant under different light conditions, it was treated with the plant growth hormone gibberellic acid. Two different concentrations, 10 mg/L and 5 mg/L, were used. The observations were made every week.

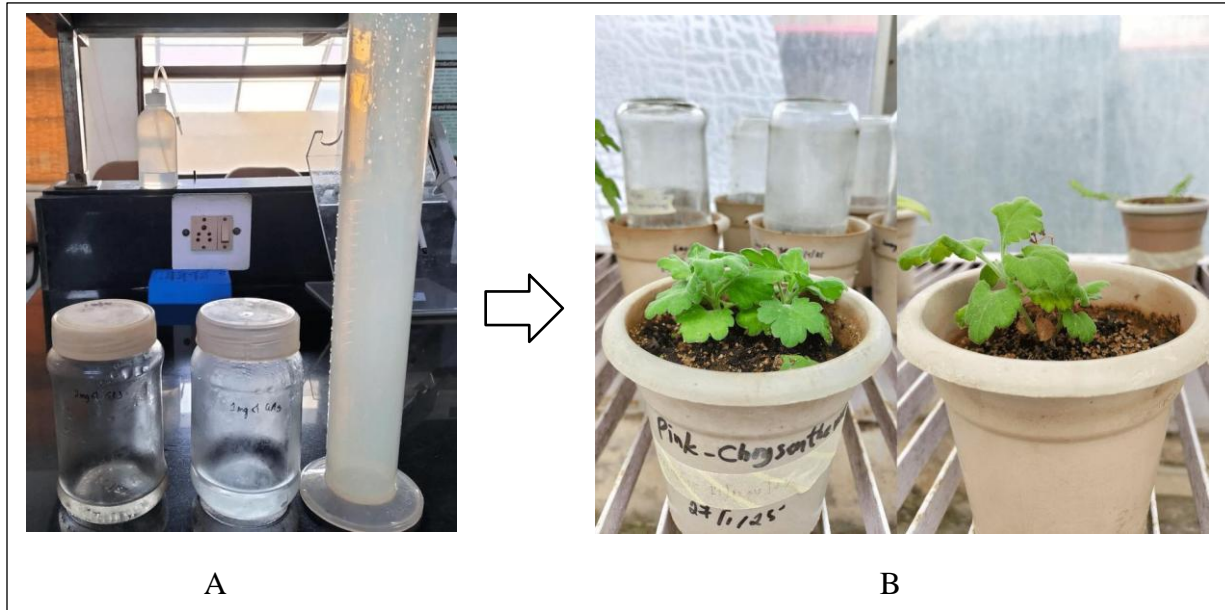


Fig 7: Treatment with gibberellic acid (A) Gibberellic acid with two different concentrations (B) Added to the hardened plants



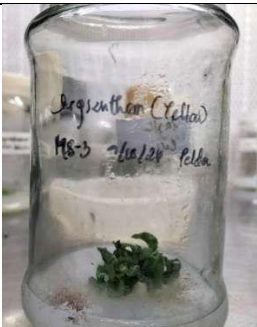

## CHAPTER 5

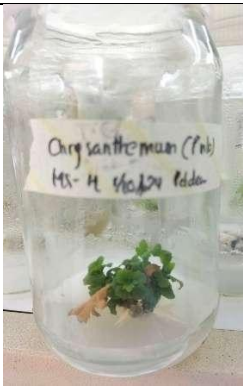



### RESULTS AND DISCUSSION

#### 5.1 EXPERIMENT 1

















Under the optimized conditions in the culture room, the growth of *Chrysanthemum morifolium* occurred gradually, with some varieties, such as the white and yellow varieties, having multiple shoots.

**Table 1: Response of chrysanthemum variety to both MS-3 and MS-4 media**

Chrysanthemum variety	MS media + composition		Inferences
	MS-3 (BAP + KN)	MS-4 (BAP + KN + IBA)	
White color			Multiple shoots grown per jar of explant in 10 weeks of duration.
Yellow color			Long shoots were observed at a length of 1.5cm to 7.5 cm in 10 weeks.

Pink color			Shoot growth only occurred in MS-4 media.
Purple color			Moderate shoot length with several shoots was observed.
Orange color			Growth of shoots only occurred in MS-3 media, whereas chrysanthemum explants for MS-4 media mostly died.

**Table 2: Established culture and the multiplication (sub-culturing)**

Chrysanthemum variety	MS-media + composition							
	Culture		1 <sup>st</sup> subculture		2 <sup>nd</sup> subculture		3 <sup>rd</sup> subculture	
	MS-3	MS-4	MS-3	MS-4	MS-3	MS-4	MS-3	MS-4
White								
Yellow								



Purple								
Orange								
Pink								

For the established culture, the chrysanthemum plant responded well to MS-4 media, and the growth was very slow. Maximum shoot length was obtained in the MS-4 media plant. Following each subculture, the in vitro chrysanthemum plant grew fast and responded well to both of the MS media. The contamination also reduced significantly as more subcultures were done.

**Table 3: Growth parameters observed for in vitro growth shooting of *Chrysanthemum morifolium***

Explant color	Label media composition	Shoot growth	Time period	Shoot length (cm)
Yellow	MS-3 MS-4	10-11 shoots	10 weeks	1.5-7.5
White	MS-3 MS-4	13-14 shoots	10 weeks	2.5-6
Purple	MS-3 MS-4	4-5 shoots	10 weeks	2-2.5
Pink	MS-4	4-5 shoots	10 weeks	1-3
Orange	MS-3 MS-4	7-8 shoots	10 weeks	1.5-3

After 10 weeks, multiple shoot growth was seen per jar of both MS media. most shoot growth was observed in the chrysanthemum white variety with 13-14 shoots followed by yellow. The least shoot growth was shown in both pink and purple with 4-5 shoots each. As for the shot length, the longest shoot was recorded in yellow, followed by white, pink, and orange whereas purple recorded the shortest shoot length.

**Table 4: Growth parameters compared between two MS-medias in in vitro grown plantlet**






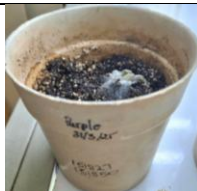


Media	Culture		1 <sup>st</sup> subculture		2 <sup>nd</sup> subculture		3 <sup>rd</sup> subculture	
	Shoot number	Shoot length (cm)	Shoot number	Shoot length (cm)	Shoot number	Shoot length (cm)	Shoot number	Shoot length (cm)
MS-3	4	7.5	3	10.5	4	6	5	5
MS-4	5	9	6	7	5	6.5	4	3

The in vitro grown plant chrysanthemum plant was compared between the two MS media and with two different growth parameters. Maximum shoot length of 10.5 cm was recorded for MS-3 media and for the shoot number, MS-4 recorded highest with 6 numbers.

## 5.2 EXPERIMENT 2

The hardened in vitro grown chrysanthemum plant is incubated under light conditions.

**Table 5: Response of hardened in vitro-grown chrysanthemum plants under different light conditions:**

	Different light conditions				Inferences
	Red LED light	Blue LED light	White light	Green house	
Before					The hardened plants were stationed under different light conditions
After					The plant under red, blue, and white light died after 3 weeks, whereas the plant grew in greenhouse conditions









The hardened in vitro chrysanthemum plant placed under the different light conditions showed different changes. For the plants that were stationed under red and blue led light, not much of the changes was observed and it only survived for 3 weeks. Within those weeks, there were no phenotypical changes and died afterwards. The plant placed under white light survived longer than the red and blue led lights and there were some phenotypical changes such as new shoot appearance





and increase in leave number. But the plants that were placed under greenhouse conditions survived longer and showed many phenotypical changes. The changes include the increase in shoot length, leaf number, and shoot appearance. The size of the leave also increased from before. So, the most effective growth was recorded in the plant that was stationed under greenhouse conditions.

### 5.3 EXPERIMENT 3

After hardening of the in vitro grown chrysanthemum plant, the plats were treated with different concentration of the gibberellic acid and observed weekly.

**Table 4: Growth parameters observed in hardened in vitro plants before and after treatment with gibberellic acid.**

Variety	Concentration (mg/L)	Shoot length (cm)		Shoot number		Leave number		Picture	
		Before	After	Before	After	Before	After	Before	After
Yellow (control)	0	6	7	2	2	9	12		
Yellow	5	10.5	16	3	7	6	12		
White	10	8.5	6.5	2	2	8	12		
Yellow	5	8	8	2	2	8	13		

Yellow	10	8	13.5	4	4	8	22		
Pink	10	3.5	5	3	3	10	19		

The hardened in vitro-grown plant treated with 5 and 10 mg/L of gibberellic acid showed significant phenotypical changes. The phenotypical changes observed and recorded were shoot length, shoot number, and leaf number. Maximum shoot length was observed in both the concentration with yellow of 5 mg/L of gibberellic acid with 13 cm and yellow and pink of 10 mg/L of gibberellic acid with 12 cm and 5 cm. the shoot number remained almost same for both the concentration except for 5 mg/L of yellow with shoot number of increased 3 times from before. There was an increase in the number of leaves for both concentrations, with the highest number observed in 10 mg/L of the yellow variety and the lowest observed in 5 mg/L of the white and yellow varieties. The early flowering was not observed when gibberellic was used because of the time constraint.

## 5.4 DISCUSSION

The establishment of the cultures of the *Chrysanthemum morifolium* demonstrated significant growth responses under optimized conditions, particularly with the utilization of different media compositions. The results indicate that both the white and yellow varieties of chrysanthemum exhibited distinct growth patterns, which can be attributed to the specific hormonal compositions in the media used.

The use of MS-3 media, which contained a combination of BAP (Benzyl aminopurine) and KN (Kinetin), resulted in the proliferation of multiple shoots in the white variety. This suggests that the hormonal balance in the MS-3 media is conducive to shoot multiplication, which is critical for mass propagation in commercial horticulture. The observation of multiple shoots per jar within a

10-week duration highlights the effectiveness of this media formulation in promoting vegetative growth [1].

Conversely, the yellow variety showed a preference for the MS-4 media, which included an additional component of IBA (Indole-3-butyric acid). The presence of IBA enhanced the elongation of shoots, with lengths ranging from 1.5 cm to 7.5 cm observed over the same period. This finding underscores the role of auxins in promoting cell elongation and suggests that the yellow variety may have a higher requirement for auxin to achieve optimal growth.

When the culture of in vitro was subjected to multiplication, there were many changes observed. One of the many changes was that the plant grew fast with each subculturing, and the variety of the chrysanthemums responded well to both of the MS media. The maximum shoot length observed was 10.5 cm for MS media, and the maximum number of shoots was observed for MS-3 media. The contamination for the first, second, and third subcultures of the plant was reduced from the initial culture establishment.

The in vitro hardened chrysanthemum plants stationed under different light conditions, such as red, blue, and white, survived for 3-4 weeks and died after that week. Sharat et al. mention in their paper that red light and white light induce flowering in addition to an increase in shoot length, number, and leaf number [24]. But during this project run, most of the plants stationed under the red, blue, and white lights died, which differed from their observation. Most of the plants wilted, decayed, and died. According to Srivastava et al. (2023), the plants stationed under different light conditions died due to excessive heat produced by the lights, improper duration of light given, lack of nutrients, or the incorrect placement of the lights. But in case of greenhouse light conditions, the plant survived, and there was an increase in the number of leaves, shoots, and shoot length. The reason may be due to the natural sunlight that the plant is exposed to.

To induce flowering, chemicals such as gibberellic acid, which is a plant growth hormone, are utilized with different concentrations. In 4 weeks, the plants showed a significant change in terms of shoot length, shoot number, and leaf number. The highest shoot length was observed for both 5 and 10 mg/L of gibberellic acid, with a length of 13 cm and 12 cm. Shoot number remained the same for both concentrations, whereas the maximum number of leaves was recorded for 10 mg/L. So, the plantlets are already present in the greenhouse to observe early flowering time. As it has

been reported by Aparna et al. (2018), it was shown that higher concentration of gibberellic acid gives higher numbers of shoot length, shoot number, leaf number, bud number, and early flowering. The flowering time reported was 35 days from the day of treatment of gibberellic acid. So, high concentration of gibberellic acid recorded a high number of shoot length and leaf number for the chrysanthemum plant [33].

Overall, the results contributed valuable insights into the in vitro propagation of *Chrysanthemum morifolium*, emphasizing the importance of selecting appropriate growth media and hormonal combinations to achieve desired growth outcomes [5]. It also gave insight into inducing early flowering in the plants with the utilization of LEDs and chemicals such as gibberellic acid.

## CHAPTER 6

### CONCLUSION AND FUTURE SCOPE

Chrysanthemum is commonly known as ‘Autumn Queen’, and it is the second most popular floriculture following rose. As it is a seasonal flower that flowers during autumn, and pests and diseases affect the flower, methods such as in vitro micropropagation are utilized for its mass production. In vitro propagation of *Chrysanthemum morifolium* has yielded significant insights into the effects of various growth hormone compositions on shoot proliferation and flowering induction. Furthermore, different light conditions and chemicals such as gibberellic acid are used to induce early flowering. LEDs are semiconductors that have become well-known for their long life and energy efficiency. So, the red and blue LED lights induced early flowering. The chemical gibberellic acid is mostly utilized for inducing early flowering. The higher concentration of gibberellic acid gives a higher number of shoots, number, leaves, and buds.

The findings revealed that the white variety of chrysanthemum responded favorably to the MS-4 media, resulting in multiple shoot formation, where the yellow variety also thrived in the MS-4 media, which included IBA, leading to enhanced shoot elongation. The plant treated with gibberellic acid, a plant growth hormone, recorded high shoot length, shoot number, and leaf number within 4 weeks. But early flowering was not observed when gibberellic acid was utilized. As for the chrysanthemum plants stationed under the red, blue, and white light, all died after a 3-week duration. The reason for death is excessive heat, lack of nutrients, and improper duration of light given to the plant. But the plant stationed under the greenhouse condition responded well, and increased shoot length, shoot number, and leaf number were observed.

Future studies should focus on optimizing environmental parameters, such as light quality, temperature, and humidity, to enhance growth and flowering responses. Additionally, exploring the effects of other growth regulators beyond BAP, KN, and IBA could provide a more comprehensive understanding of plant development. Integrating biotechnological approaches, such as genetic engineering, could lead to the development of new chrysanthemum varieties with desirable traits. Collectively, these directions will enhance the understanding and cultivation of *Chrysanthemum morifolium*, benefiting both the floriculture industry and the field of plant biotechnology.



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