EFFECT OF LIGHT QUALITY IN THE REGULATION OF MORPHOLOGY AND FLOWERING OF PETUNIA (*Petunia hybrida*)

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Effect of light quality in the regulation of morphology and flowering of petunia (*Petunia hybrida*)

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ABSTRACT

Manipulation of plant morphology is greatly important for commercial use of ornamental plants. Use of chemical is an effective option for growth manipulation but of environment concern. Temperature manipulation is also an option but is not found practical in all greenhouse systems or in all seasons. Manipulation in light quality may be a practical and effective option in petunia. Therefore, the main aim of the study was to investigate the effect of red and blue light and far red absorbing film (solatrol), alone and in combination, in the manipulation of morphology of two cultivars of petunia 'Tidal Wave' and 'Mambo Formula Mixture'. Two experiments were conducted, the first one was conducted in early spring (March-April) and the second was conducted in summer (May-June). Three types of treatments i.e., blue-LED, red-LED (50 µmol $m^{-2}s^{-1}$) and control were executed under solatrol and under shade of white plastic, compared with control (no additional light). Cultivar 'Tidal Wave' was found to be more responsive than 'Mambo Formula Mixture' to light quality manipulation by using solatrol or by using red or blue LED. However, in case of parameters - days to visible bud and days to flowering, 'Mambo Formula Mixture' was more responsive. In the cultivar 'Tidal Wave', plant height was found to be 70% shorter in solatrol + red-LED compared to shade + blue-LED, which showed the tallest plants and it was found to be 40 % shorter compared to shade + red-LED. In the cultivar 'Tidal Wave', the earliest visible bud was seen under shade + red-LED and the latest was found after 3 days under solatrol + red-LED in the spring and solatrol-control in summer. In the cultivar 'Mambo Formula Mixture', the earliest visible buds and flowers were seen under solatrol + blue LED and latest under solatrol – control with the difference of 9 and 5 days respectively. Under solatrol, chlorophyll content was found to be 60%, 9% and 21% greater than under shade with red-LED, control and blue-LED treatment respectively. Solatrol and Red LED were found effective in reducing height alone and in combination. Solatrol delayed flowering with less number of flower whereas blue-LED enhances flowering earlier with highest number of flowers. The results from this study clearly show that light quality can be an effective tool in controlling morphology and flowering in petunia but the responses will vary with cultivars.

Key words: LEDs, Petunia, Solatrol, Light Quality, plant height, visible buds, open flower, chlorophyll.

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ABBREVIATIONS

%	Percent
^{0}C	Degree Celsius
ADT	Average daily temperature
CM	Covering Material
C-PE	Common Poly-ethylene
CM	Centimeter
DIF	Difference between day and night temperatures
DT	Day temperature
EOD	End of the day
FR	Far red
HPS	High pressure sodium
LED	Light emitting diodes
LQ	Light quality
NT	Night temperature
PE	Poly-ethylene
PPFD	Photosynthetic photon flux density
R	Red
UV	Ultraviolet Rays

1 INTRODUCTION

Plant morphology is an important consideration in the production of ornamentals especially in bedding plants and pot plants. So, manipulation with respect to compactness, stem length, branching and shoot orientation of ornamental plants of commercial use is of great importance, either it is due to genetic modification or the manual (physical, chemical, mechanical) modification. There are different methods of growth manipulation, such as manipulation of light intensity, manipulation of light quality, manipulation of temperature (DIF and DROP treatment), use of plant growth retardants (PGR) and use of screening materials also called selective films. Use of plant growth retardants (PGR) for the control of plant height is common in pot plants and bedding plants industry but it is of environmental concern (Lund et al., 2007). Increasing concern over the impact of these chemical compounds on human health and environment has led to increased focus on alternative methods to control plant morphology. Further, one of the most important tools is to manipulate the light climate (Barro et al.,, 1989; Mortensen & Strømme 1987; Pearson et al.,, 1993; Smith 1982). Light quality in greenhouses can be manipulated in different ways, either by different cladding material of the greenhouse (Khattak & Pearson 1996; Rajapakse et al., 1993; Rajapakse & Kelly 1995), different types of selective films (Cerny et al., 2003; Clifford et al., 2004) or use of lamp types with different spectral distribution (Appelgren 1991; Bula et al., 1991).

Until now the most common lamp type in greenhouse is high-pressure sodium lamp (HPS) before which the fluorescent light, incandescent light, metal halide were also in use. But recently new lighting technology with the use of light emitting diodes (LEDs) have become of huge interest in the greenhouse industry. Light emitting diodes (LED), which have pronounced peak at narrow bandwidth wavelength (at 10 nm); combined to their operational advantages such as small size, low mass, long functional life and high electrical energy conversion efficiency when cooled properly (Morrow 2008) are potential irradiation source for intensive plant growing systems (Ménard et al., 2006). Moreover LED lighting system which have relatively cool emitting surface (Massa et al., 2008) can be operated digitally as they are solid-state devices and they also have a good ability to control spectral composition allowing wave lengths to be matched to plant photoreceptors in order to provide more optimal production and finally influence plant morphology and composition (Morrow 2008). Therefore, manipulating light quality by using such LEDs can be a good substitute for chemical growth retardants as well as other means for controlling height of the plants, which is so far not only economical but also environmental friendly.

Petunias are popular and according to the Alabama cooperative extension it is one of the mostly used bedding plants among five (Impatiens, Petunias, Geraniums, Marigolds and Pansies) in the United States of America. The importance and the rising market of petunias (Bedding plants) during summer and mild winter in whole Europe cannot also be shadowed. Artificial light provided by using LEDs or photo selective plastic films to maintain R/FR ratio may be the effective light quality manipulation method to obtain desired plant morphology.

Thus, the main aim of this study was to investigate the effect of red (R) and blue (B) light, by using LEDs on morphology of the important bedding plant *Petunia* x *hybrida*. Further, a selective plastic film which alters the ratio of red to far red light reaching the crop by absorbing light in the far red wavelength (Solatrol®) was used to see if changes in the R/FR ratio can be used as a tool alone or in combination with red or blue LEDs.

2 LITERATURE REVIEW

2.1 Petunia as a bedding plant

Petunias, popular bedding plants, are herbaceous perennials, usually grown as annuals and belong to the family Solanaceae. Petunia has become one of most popular garden plants. Its origin is considered to be in South America. The name ‘petunia’ is derived from French, where, *petun* means “tobacco”. Most of the varieties grown today are hybrids (*Petunia* x *hybrida*). Its origin was thought to be by hybridization between *P. axillaris* (the large white or night scented petunia) and *P. integrifolia* (violet flowered petunia). Most of the petunias are diploid with 14 chromosomes and are infertile with other petunia species. Petunias are largely used in flower beds. They are perennial in warm climates (roughly zone 9 or warmer) although generally grown as annuals (at least in temperate areas). They are also great container plants. They can be found in every color of the rainbow in solids, contrasting veins or edges, and star patterns. Petunia hybrids are now popular garden and container plants all over the world.

2.2 Photomorphogenesis

Photomorphogenesis is the regulatory effect of light on plant growth, development, and differentiation of cells, tissues, and organs. Photoperiod, light quality and light intensity directly impact plant growth from germination to flowering (Runkle & Heins 2006). The more sunlight a plant receives, to a degree, the higher the photosynthetic rate will be. Light duration refers to the amount of time that a plant is exposed to sunlight. Light quality refers to the color or wavelength reaching the plant's surface. Flowering and subsequent growth can be controlled with the manipulation of light quality and photoperiod by using artificial lights (Heo et al., 2003).

2.2.1 Light Intensity

Light intensity can be described by instantaneous or cumulative (daily light integral, or DLI) measurements and it influences photosynthesis and thus plant growth parameters such as branching, stem thickness, flower number, and flower size(Runkle & Heins 2006).

Higher light intensities and longer durations of supplemental lighting are used during the winter in more northerly climates (e.g., lat >50 N), particularly in greenhouse production (Moe 1997). Plant quality and mass of increase and flowering time decreases as DLI increases in *Catharanthus roseus(Pietsch et al., 1995)* and *Campanula carpatica (Niu et al., 2001)*. DLI can be effective in inducing flower earlier by reducing number of node below the first flower (Armitage & Tsujita 1979) (Erwin & Warner 2000). Similar effect was found by Faust et al., in 2005 by studying effect of DLI on eight bedding plants i.e., ageratum, begonia, impatiens, marigold, petunia, salvia, vincia and zinnia. They reported that total dry mass increased for all the species except begonia and impatiens as DLI increased from 5 to 19 mol.$m^{-2}d^{-1}$.Impatiens and begonia total dry mass increased as DLI increased from 5 to 19 mol. $m^{-2}d^{-1}$. Similarly time to flower is decreased for all the species except begonia and impatiensas DLI increased from 43 mol. $m^{-2}d^{-1}$.

Many studies showed that light intensity also influences the time of flower initiation. As DLI increased from 5 to 25 mol.$m^{-2}.d^{-1}$, dry mass and flower number of *Impatiens wallerana* and *Celosia argentea* var. plumosa at first flowering increased (Pramuk & Runkle 2005). Similarly, in a study of *Eustoma grandiflorum*, plants grown under a high DLI was found with greater shoot dry weight, stem diameter, and flower number as compared to plants grown under lower one (Islam et al., 2005). (Carpenter & Beck 1973) reported that time of flower in petunia is decreased up to 12 days by using high intensities of fluorescent lights (287 μmol·s -1·m-2) to supplement natural light compared to plants receiving low-intensity incandescent lighting to extend the photoperiod.

2.2.2 Photoperiod

In response to photoperiod, bedding plants may be either long-day, short-day, or day neutral. Some long-day species are obligate (or qualitative) long-day plants, require day lengths longer than a certain critical length in order to flower whereas other long-day species are facultative (quantitative) long-day plants, initiate flowers under any day length, but flower earlier with long-days (Cox 2009). In long day plants as petunia, flowering is faster under long days although they can flower in any photoperiod. However, in addition to photoperiod, the total amount of light a plant is exposed to each day, or Daily Light Integral (DLI), impacts the flowering for several important bedding plant crops (Warner 2006), as described above.

Most growers use photoperiod control to shorten crop time by inducing earlier flowering and to save some energy by being able to delay seeding/planting (Cox 2009; Warner 2006). In greenhouse, long days can be provided either by extending the day with supplemental lighting or by breaking the dark period by turning lights (Warner 2006).

2.2.3 Light Quality

Light quality can influence plant morphogenesis as stem elongation and branching and it also regulate flower initiation and development, Heo et al., (2003); Runkle & Heins (2006) reported that blooming period of flowers are influenced by light quality and photoperiod treatments. Light quality is mainly sensed by the different photoreceptors specific for different wavelengths, i.e., phytochrome, cryptochrome and phototropin. Phytochrome receives red and far red light, cryptochrome receives blue, green and UV (Ultra Violet) light and the phototropins are blue light receptors.

2.2.3.1 Phytochrome

The red/far (R/Fr) red photoreceptors are called phytochrome. Phytochrome exists in two photo convertible states called Pfr and Pr with different absorbtion spectra. Pfr has its maximum absorbance at 730 nm and Pr at 660 nm (Khattak et al., 2004). However, both forms can absorb in the UV-area (300-400 nm) and the blue area (400-500 nm). Absorption of red light converts Pr to Pfr while absorbtion of far-red converts Pfr to Pr. Phytochrome responses are classically defined by their red/far red reversibility. Phytochrome can measure light quality because if light contains more red than far red light (as is the case in daytime sunlight), most phytochrome will be in the Pfr form, which is believed to be the active form. Light quality detected by phytochrome receptors affect plant growth and morphogenesis in different ways but is very important in controlling stem elongation and flowering (Smith 1982).

2.2.3.2 Cryptochrome

Cryptochrome recognizes blue, green and UV-A light. It is a flavin protein with 2 chromophores attached, one for green, and one for blue. There are 2 cryptochrome genes in Arabidopsis, CRY1 and 2. Cryptochrome action requires the presence of phytochrome because some phytochrome mutants are non-photomorphogenic in blue or green light. However the cryptochrome mutant is photomorphogenic in red light (with far red reversibility) indicating that phytochrome action does not require cryptochrome. By absorbing blue light cryptochromes participates in inhibition of stem elongation (Runkle & Heins 2006). Different studies reported the suppressing effect of blue light in extension growth as in *Hyoscyamus niger* L. (Stolwijk & Zeevaart 1955) and *Arabidopsis thaliana* Heynh (Mozley & Thomas 1995). Similarly, blue light is deficient in the plant growing environment; it can promote stem extension (Brown et al., 1995). The cryptochrome is also believed to be involved in control of flowering (Fukuda et al., 2011a).

2.2.4 Light and flowering in petunia

The important requirement for growing petunias successfully is a location with plenty of light. Petunias need at least five or six hours of good sunlight; they can perform even better when located in full sun all day (Brown 2009). High intensity of light is helpful in decreasing flowering time in petunia (Carpenter & Beck 1973). Flowering time in petunia decreases with increased plant quality and mass as Day light Integral (DLI) increases and DLI can be effective in inducing flower earlier (Faust et al., 2005). Petunias are quantitative long day plants, flowering faster under long days although they can flower in any photoperiod (Kessler 1998). High R/Fr ratio increases the plant height in petunia (Rajapakse et al., 1999). However, petunia seems to need far red light to flower early (Haliapas et al., 2008b). Red light inhibits increase in height of petunia (Haliapas et al., 2008b). Blue light induce flowering whereas low intensity of red light inhibits flower bud formation but red light of higher intensity can induce flowering (Fukuda et al., 2011a). Fukuda et al., (2011a) proposed a model of effect of red and blue light in petunia in which they describe the stimulation of cryptochrome by which trinscript level of flowering induction gene increases to activate flower bud differentiation and development. Red light stimulate phytochrome, which either inhibits or not sufficient to activate the expression of floral induction genes.

2.3 Methods for the manipulation of morphology of ornamentals in greenhouse production

Attention towards non chemical methods to alter the morphological characters of plants is increasing because of the increased concern on the effect of chemical growth retardants (Bachman & McMahon 2006). Plant morphology can be manipulated by different abiotic factors such as drought, mechanical stress, temperature and light (Patil et al., 2001). However, it is important to find tools that do not affect branching or flowering in a negative way.

2.3.1 DIF and Drop Effect

Temperature manipulation to regulate plant morphology is in practice for a wide range of plant species. Average daily temperature (ADT) influences internode elongation in some ornamental plants, such as petunia (Ludolph 1992) and chrysanthemum (Langton 1998). In chrysanthemum ADT influences stem elongation which is the result of both increase in internode number and length (Langton & Cockshull 1997; Langton 1998).

DIF is the difference in day (DT) and night temperature (NT). Negative DIF is a condition when there is high night temperature and low day temperature where reverse is called positive DIF. Increasing the DT increases plant height whereas increasing the NT decreases the plant height. So, negative DIF may be responsible for shortening the plant height (Bielenin & Joustra 2002; Frąszczak et al., 2011). DIF treatment mainly affects the length of the internodes to control the plant height (Erwin et al., 1989). Erwin et al.,, (1991) studied the temperature effects on morphology of *Fuchsia* x *hybrida* and reported that internode length and leaf area increased linearly as DIF increased from –15 to +15^{o}C with DT and NT between 10 and 25^{o}C.

An alternative to the DIF treatment is DROP treatment i.e., a short term temperature reduction in the morning, also called morning drop. Several studies found that the effectiveness of DROP treatment in reducing stem length is related with magnitude duration and timing (Bertram 1992;Cockshull et al., 1995; Hansen et al.,1996; Sach 1995).

Many authors reported that the efficiency of DROP treatment is lower than that of DIF treatment in reducing stem length Bertram (1992); Bielenin & Joustra (2002); Hendriks & Ueber (1995) studied effect of DIF and Drop treatments in *Cotinus coggygria*, *Photinia fraserii*, *Pieris japonica* and *Viburnum tinus*, and showed that negative DIF reduced growth of all species except *Viburnum* and internode length was decreased only in *Photinia*. But in this study Drop treatment was found ineffective in controlling plant growth. The effect of DIF and Drop effect in shoot length is not persistent when plants are transferred to the normal environment. Changes in plant morphology by DIF and Drop treatments were reported insufficient for practical employment in greenhouse (Bielenin & Joustra 2002). Petunia is responsive to DIF and more compact plants have been found under negative DIF compared to positive DIF (Kubota et al., 2000; Patil et al., 2001). However, to establish negative in a greenhouse is difficult most of the year. Also, it will require high-energy consumption because the greenhouse has then to be heated in the night. Negative DIF is therefore not so relevant in commercial production of petunia.

2.3.2 Manipulation of light quality

Mortensen & Strømme (1987) were first to study the potential of manipulating light quality to control the growth of ornamental plants by growing a range of species in growth cabinets. Light is primary energy source for plants and also act as a signal for their morphogenesis (Ubukawa et al., 2004). Red (R), far-red (FR), and blue (B) are the most effective components of the spectrum of light (Tsegay et al., 2005). To control the plants growth, spectral distribution of the incoming light can be manipulated. But the possibility of using artificial lighting with high level of red and blue light for reducing stem elongation is only beneficial when natural light levels are low otherwise sunlight would dominate and dilute the effect of supplementary light (Grindal et al., 2000). In a greenhouse system there are several possibilities to manipulate the light quality and the different methods will be described below.

2.3.2.1 History of using supplement lighting

The use of electric light in greenhouses to regulate plant growth and development was introduced in the middle of the 19th century. Mangon (1861) had done some of the first attempts to study effect of electric light on plan source by using carbon lamps as light source (Petterson 2008).

These lamps are not practical because of their short lifetime. In 1878 Thomas Edison started his research in developing a practical incandescent lamp and with its development its production and availability increased. Researchers became interested in using electric incandescent light in the study of photoperiodic response. After this photoperiod control tool for floricultural crops, many fluorescent lamps were installed in greenhouse but sometimes this may cause problem of failure or delay flowering in long day plants. After Second World War, many types of effective lamp were developed with affordable price as high-pressure mercury lamps, high-pressure metal halide lamps, high-pressure sodium lamp. Different researchers carried out comparison among the different lamp types as: Kristoffersen (1952) studied comparison between fluorescent lamps and fluorescent lamps, (Grimstad 1981) studied among fluorescent lamp, high pressure metal halide lamp and the high pressure sodium lamp. Fluorescent lamps were found highly efficient however it was difficult to use those in large scale. So, the nearest alternative high-pressure sodium lamps were adopted for large scale.

Afterwards, the use of LEDs (light emitting diodes) as radiation sources has become attractive more than others because of their vast potential for commercial application. LEDs emit narrow spectral range around the specific wavelength and this feature croad spectral range emitted from fluorescent lamps. Hence there is possibility of precise manipulation of light quality and light intensity by controlling independently each spectral range with the use of LEDs (Folta et al., 2005). Additionally they are promising light source because of their small mass and volume, solid-state construction, safety, and longevity (Bula et al., 1991; Nhut, D.T. et al., 2003). Moon et al., (2006) reported that plant growth can be controlled by using LEDs most effectively, compared with the performance observed when conventional fluorescent lamps are utilized.

2.3.2.2 Using Light Emitting Diodes (LEDs)

The effect of light quality on plant morphology has been documented in many studies in different plant species. According to the different studies, use of red light and blue light inhibits elongation and far red light increases elongation. Similarly, high R/Fr ratio enhances axillary branching and reduces stem elongation and low R/Fr ratio will stimulate apical dominancy and hence increase stem elongation. Although responses of bedding plants are different from different plant species, light quality influences the characteristics of qualitative and quantitative growth and morphogenesis (Heo et al., 2006). Heo et al., (2006)found while experimenting on the growth and flowering of ageratum, marigold, and salvia bedding plants, that the combination of blue plus red radiation with fluorescent lighting system (control) enhanced the dry weights, but plants exposing to blue plus red light had the shortest shoots compared to either red or blue plus far red treatments. In another study, it was found that the red light significantly increased the stem elongation while blue light strongly inhibited the stem elongation in *Pelargonium* (Appelgren 1991).

Many studies showed that LED light is more suitable for plant growth than that of a fluorescent lamp. Such studies were done in different plants as grape (Poudel et al., 2008) strawberry (Nhut, D.T. et al., 2003), banana (Nhut, D. T. et al., 2003) , maize (Felker et al., 1995), Lilium (Lian et al., 2002), Chrysanthemum (Kim et al., 2004; Kurilčik et al., 2008), Euphorbia milii (Hahn et al., 2007), and orchids (Wongnok et al., 2008).

In chrysanthemum plants, blue light inhibited shoot elongation (Shimizu et al., 2006) whereas greatest stem length of chrysanthemum plants was observed under red LEDs and far-red LEDs when the plants were grown under six different light qualities: fluorescent, blue LEDs, red LEDs, red and blue LEDs, red and far-red LEDs, and blue and far-red LEDs (Kim et al., 2004). It was found that the blue light deficient environment promoted stem extension (by 10% to 100%) in five long day plant species (Runkle & Heins 2001). Blue light enhancement by blue LED lamp reduced internode lengths in cucumber and tomato (Ménard et al., 2006). Poudel et al., (2008) found that grape plants cultured under red light emiting diods produced the longest shoots and internodes while testing three cultivars of grapes under red and blue light emitting diods. In a study of upland cotton plantlets grown under different light six different lights, it was found that stem length and second internode length is higher in 1:1 proportion of blue and red light supplied by LEDs followed by blue light and it was lowest in plantlets cultured under a fluorescent lamp (Li et al., 2010). In a study, Hoenecke et al., (1992) investigated the effect of ‘Blue’ photon levels for lettuce seedlings. Blue photon level significantly affects the elongation of the hypocotyl. In the study it was reported that hypocotyl elongation decreased from 30 to 2 mm as seedlings were exposed to increasing flux of blue photons from 0 to 60 $\mu molm^{-2}s^{-1}$.

In vitro study on growth and development of Doritaenopsis plants showed that blue LED promoted Carbohydrate accumulation and chlorophyll biosynthesis whereas, the red LED induced leaf elongation or expansion (Shin et al., 2008). Leaf thickness and leaf number were highest under blue light in comparison with red light and different mixture of red and blue light in upland cotton plantlets (Li et al., 2010). In a study of Alternanthera brasiliana, it was reported that number of leaves/plant, thickness and area of the leaf blade, were significantly increased in plants grown under blue fluorescent light as compared with the other fluorescent-light and dark treatments(Macedo et al., 2011).

No flower buds were formed in salvia when monochromic B or R was used, fluorescent with far red light (FlFr) was also found to be inhibitory to flower bud formation in marigold (Heo et al., 2002). Heo et al., (2003), found that the number flower buds and open flowers being highest in the plants grown under blue plus red LED (10 h per day) as compared with monochromatic blue or red LED in Cyclamen plants.

2.3.2.3 Using screening materials

The 'fluid roof system filter' method (with copper sulphate solutions) is efficient in controlling height of different bedding plants but difficult to apply in green house scale (Hendriks & Ueber 1995; Oyaert et al., 1999). Light weighted plastic films are engineered to selectively reflect the significant amount of Fr light without practical limitation of fluid roof system (Clifford et al., 2004). Khattak & Pearson (1997) studied on a range of solid spectral filters giving different phytochrome photoequilibria and transmission to blue light and developed a simple model predicting that a spectral filter which exclusively removes all light between 700 and 800 nm could reduce height by up to 29% in chrysanthemum. Similarly, the removal of far red radiation by spectral filters led to a reduction in final height at flowering by 19% and this effect was most marked between internodes 6 and 11(Khattak et al., 2004).

Frequency and amount of chemical growth regulators can be decrease by using photo selective films (Wilson, S. & Rajapakse, N. 2001). Wilson, S. B. & Rajapakse, N. C. (2001) reported that YXE- 10 FR-absorbing film reduced the height of different varieties of lisianthus by 10 to 19 percent without affecting the development of flowers or flower quality whereas SXE-4 Red light absorbing film did not significantly affect plant height, bud number, flowering time, or plant dry weight. Oyaert et al., (1999) showed that the inhibition of stem elongation in chrysanthemum increased with increasing pigment concentration under the blue polyethylene (PE) films, where maximum of 22% growth reduction was observed as compared to the control. In a study of bell peppers , it was reported that FR-absorbing films reduced height of bell peppers and the effects are pronounced as time progressed (Cerny et al., 2000). Red light absorbing films lower the R/Fr ratios which increases plant height in snapdragon and petunia (Rajapakse et al., 1999). Murakami et al., (1994) reported that plant height of sunflower increased by 21% in red light absorbing films. It has been reported that blue light inhibits internode's extension only if it is combined with low R/Fr ratio (CASAL & SMITH 1989; Gautier et al., 1997). Cerny et al., (2003) studied the influence of using Far red absorbing films on stem growth and flowering of six plant species and found that anthesis of short day plants (cosmos, chrysanthemum, zinnia) and day neutral plant (miniature rose) was not significantly affected whereas anthesis in long day plants as in petunia was affected during weakly inductive photoperiods by delaying overall growth and flowering (Cerny et al., 2003). This may be useful in increasing the production time and also increase the scope of off season petunia production.

Plastic films may also serve as shading material therefore and can be an alternative methods for growth control in periods with high temperature and excess of light when temperature strategies are difficult to apply and the effect of chemical growth retardants may be weak (Patil et al., 2001). In spite of potentiality of these plastic films for commercial use, they may have negative consequences for total plant dry mass (Grindal et al., 2000), for plant quality (Clifford et al., 2004) and for flower initiation (Runkle & Heins 2001) because of restricted light transmission. Photoselective polyethylene (P-PE) films reduce photosynthetic photon flux density (PPFD) affecting the photosynthetic rate of the plants (Wilson, S. & Rajapakse, N. 2001). Lykas et al., (2008) studied the possibility of producing compact plants of *Gardenia jasminoides* and found that leaves differentiated and developed under P-PE films are smaller compared to common polyethylene (C-PE) films. The inhibition in shoot elongation is increased with the increasing concentration of pigments. So, the plastic films can be used for the specific cultivation periods, such as, when elongation rate is higher or only during specific moments of the day when the R/Fr ratio is naturally low (Clifford et al., 2004). Hence modifying commercial greenhouse by using photoselective films may be an additional effect for flower production schedule and flower quality. Under far red deficient light treatment by using filters suppresses the height from 11 to 37 % and delay the flowering from 7 to 13 days depending upon cultivars in *Petunia * hybrida* (Kim et al., 2002). Kubota et al., (2000) reported inhibition in stem elongation by far red deficient treatment but they did not found any delay in flowering in *Petunia * hybrida* ‘Fulcon Red and White’. Hence, Response to modified light environment is different for different species (Cerny et al., 2003) and different cultivars as well (Kim et al., 2002, Kubota et al., 2000).

3 MATERIAL AND METHODS

3.1 Site description

The experiment was carried out in a greenhouse compartment (with glass on the top and inner wall and the acryl in the side) at SKP (SENTER FOR KLIMAREGULERT PLANTEFORSKNING), Ås, Norway from March 05 to April 04 in winter and again from May 06 to June 15 in 2009. The mean daily light integral (DLI) during the experiment period at Ås was 19.30 mol/m^2/day in early spring and 42.2 mol/m^2/day in the summer experiment, Fig. 1 and 2.

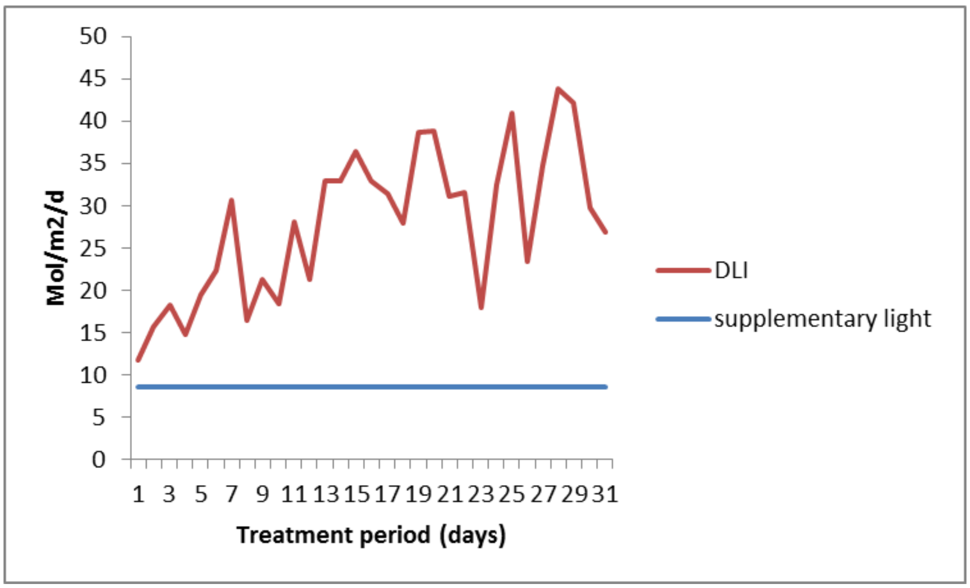

Figure 1. Graph showing the additional natural irradiance to the constant supplementary light (HPS and LEDS) during early spring experiment.

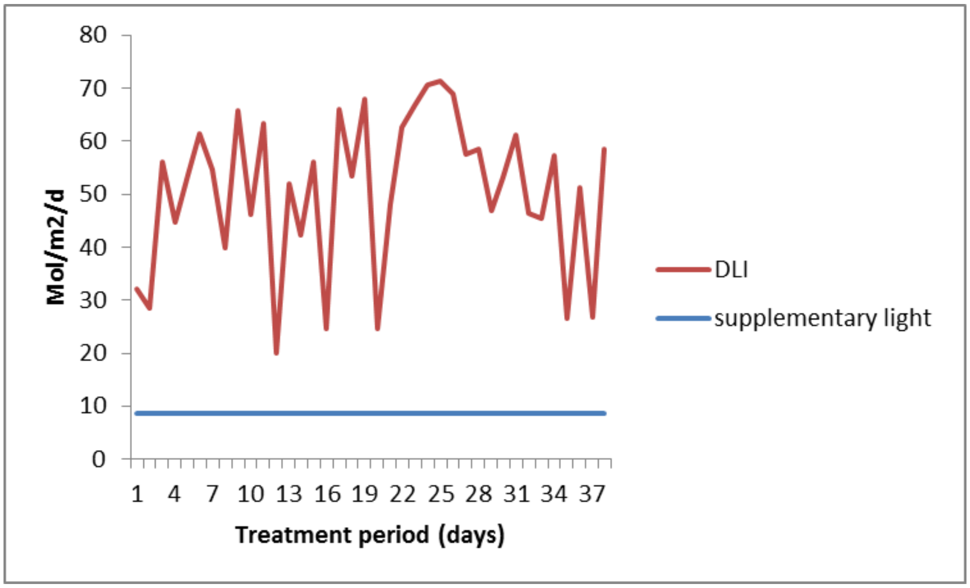

Figure 2. Graph showing the additional natural irradiance to the constant supplementary light (HPS and LEDS) during summer experiment.

3.2 Plant material and pre cultivation:

Seeds of petunia were seeded in a tray containing peat mix (spaghnum Ullensaker Allmenning, Norway).Two cultivars of petunia, viz. Tidal wave and Mambo Formula Mixture were used for the whole research. After 15 days, two leaf stage plant of petunia (both the cultivar) were transplanted in the 11 cm pot (containing standard peat mix (sphagnum). Twelve plants were selected from each cultivar for each treatment and they were grown in the greenhouse until marketing stage (4 complete weeks).

3.3 Experimental set up

The plants were placed on the table for experiment. The HPS (high pressure sodium) (LUCALOX Type LU400/XO/T/40 X-tra output. Product code 93269, Watt 400) light were placed as a regular source of light with 16 hours of lighting. The plants were grown under two different coverings (solatrol & white plastic) which both reduce the irradiance with 30% and the spectrum of light under solatrol and without solatrol is shown (Fig.3). Under the coverings two different light qualities was used , red light emitting diodes (Red-LED), blue light emitting diodes (Blue-LED) (Fig.4) and one with control inside the covering materials (i.e. without LED) lights (described in

Table 1). The shade was used on one table to compare the light quality in a far-red deficient environment with natural light and the light levels were adjusted to be at a similar level. The spectral distribution of the used LED and the HPS is shown in (Fig.4). The ventilation in the greenhouse opened at 23°C automatically and the relative air humidity was 70%. LED lights were installed to provide 50 µmol/m^2/s light. The light intensity was fixed by regulation of HPS light. Temperature inside the plastics was measured before the plants were placed and fixed to 21°c and the night temperature was fixed at 13°c Plants were harvested in fifth week when the maximum plants were fully bloomed. Experiment was done two times. First experiment was done in March and it was repeated in June. The light intensity was measured by using the Photometer (LI-COR, LI-250LIGHT METER, USA) AND UNDER SOLATROL we also used sky instrument (660/730 µmol/m^2/s, IRELAND) to measure R/Fr ratio. The red far ratio under solatrol is 10.2 and the shade 1.2.

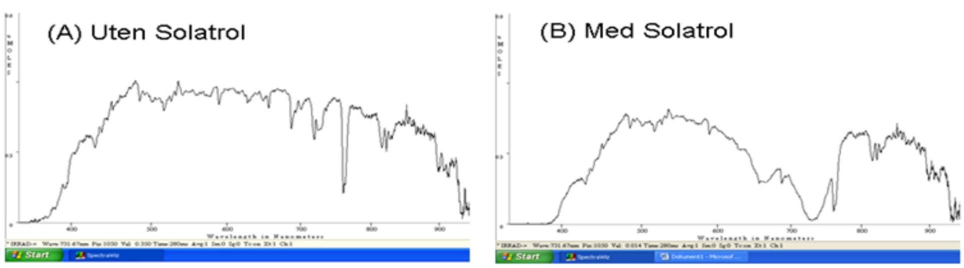

Figure 3. Graph showing the spectrum of light under solatrol and without solatrol

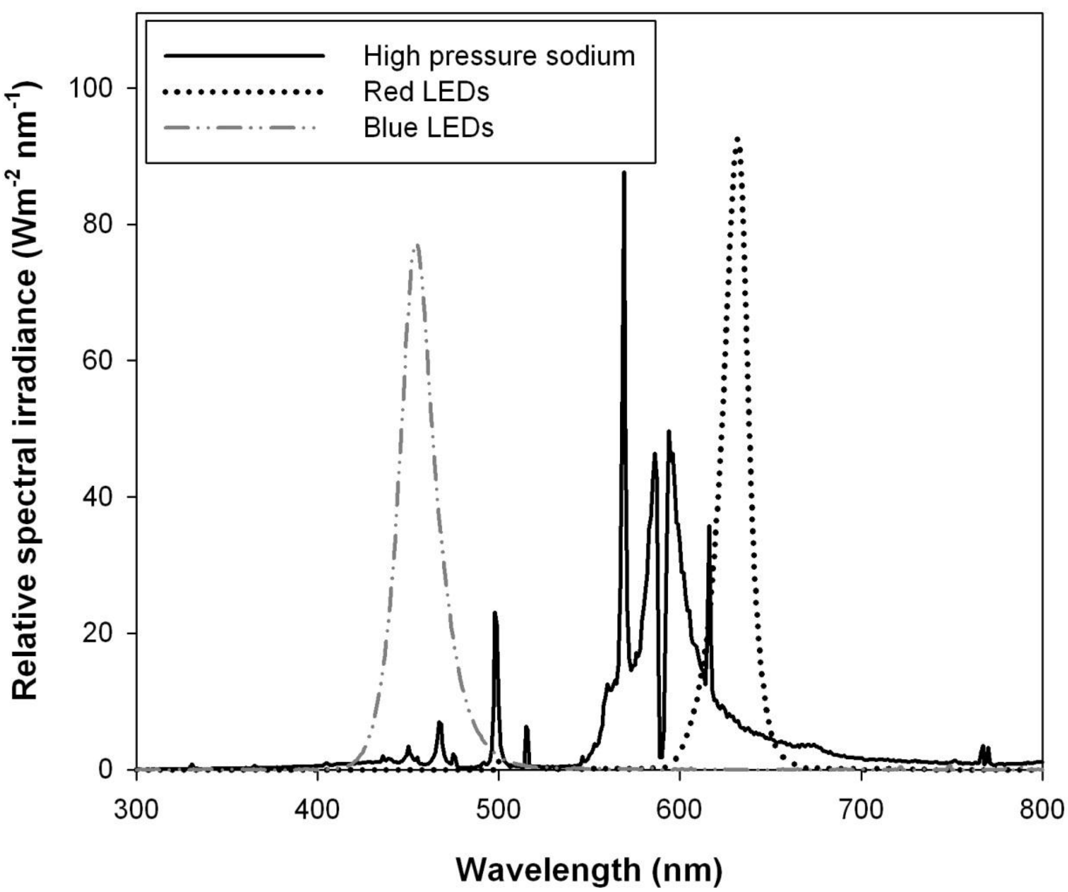

Figure 4. Spectral distribution of LED and HPS light used in the experiment

Table 1. Experimental Setup

SOLATROL + RED-LED (100+50) µmol/m^2/s 21^{O} C (12 plants from each cultivar) 'Tidal Wave' & 'Mambo Formula Mixture'	SOLATROL 150 µmol/m^2/s 21^{O} C (12 plants from each cultivar) 'Tidal Wave' & 'Mambo Formula Mixture'	SOLATROL + BLUE-LED (100+50) µmol/m^2/s 21^{O} C (12 plants from each cultivar) 'Tidal Wave' & 'Mambo Formula Mixture'

SHADE + RED-LED (100+50) µmol/m^2/s 21^{O} C (12 plants from each cultivar) 'Tidal Wave' & 'Mambo Formula Mixture'	SHADE 150 µmol/m^2/s 21^{O} C (12 plants from each cultivar) 'Tidal Wave' & 'Mambo Formula Mixture'	SHADE + BLUE-LED (100+50) µmol/m^2/s 21^{O} C (12 plants from each cultivar) 'Tidal Wave' & 'Mambo Formula Mixture'

3.4 Cultural practices

Watering along with the fertilizer with EC 1, 5, and pH 5, 6 – 6, 5, was done daily.

3.5 Data Collection (Measurement of growth parameters)

Final data was taken from 10 plants of each cultivar in each treatment.

3.5.1 Plant height and canopy diameter

Plant height was measured from root collar to the tip of the apical shoot weekly. Height measurement was starting from two days after transplanting and its increment was calculated up to fifth week. Plant canopy diameter was measured at the day of harvesting. The diameter was measured by making two perpendicular lines on the top of the plants touching its boundary.

3.5.2 Number of side shoots and length of dominant shoot

The number of side shoots on each plant was counted and recorded after harvesting. The length of dominant side-shoots (marked by thread) of each plant was also taken weekly. It was measured from the main stem to the apical bud.

3.5.3 Internode number

Internodes number was recoded after harvesting of the plants only in the second experiment. Three longest shoots of each plant were selected and the number of internodes of those shoots measured and recorded.

3.5.4 Number of true leaves and leaf area

Truly developed leaves of each plants were counted and recorded weekly only in first experiment. Leaf area of all the leaves of randomly selected three plants was measured in first experiment whereas in the repeat experiment, leaf area of 10 largest leaves of randomly selected three plants was measured. Leaf area meter LI-COR 3100 (LI-COR INC. LINCOLN, NE,USA) was used to measure the leaf area.

3.5.5 Visible buds and open flower

Time of appearance of first visible bud was noted. Total number of visible buds of each plant was counted after harvesting. Time to open the complete flower was also noted. Total number of flower was counted and recorded after harvesting.

3.5.6 Fresh weight and dry weight

Fresh weight of the plants, excluding roots, was taken after harvesting. For the measurement of dry weight, plants were put on the paper bags and placed inside the oven at 70°c and heated until they reached constant mass.

3.5.7 Chlorophyll content

The chlorophyll content was measured using the digital chlorophyll content meter (Hansatech instrument, Model **CL-01**, England) from the sample twice within the whole experiment period.

3.6 Data analysis

The results were analyzed by one way ANOVA and GLM to compare the means of the treatment (MINITAB16) where significance level was $P \geq 0.05$. Graphical representation was done using the Sigma plot 10 by using the means and sum of the error mean (S.E.) and Microsoft Excel was also used.

4 RESULTS

4.1 Weekly growth performance

4.1.1 Side shoot length

Experiment 1: Early Spring (March-April)

In the cultivar ‘Tidal Wave’, side shoot length was found shortest in the plants grown in solatrol + red-LED compared to solatrol + blue-LED and solatrol - control from the first week to fourth week (Fig 5). Similarly while comparing three treatments under shade, again the shortest side shoot length was found in shade + red-LED compared to shade - control and shade + blue-LED. (Fig 6). In cultivar 'Mambo Formula Mixture', only small differences in shoot length were found between the treatments both under Solatrol and under shade (Fig. 7 and 8).

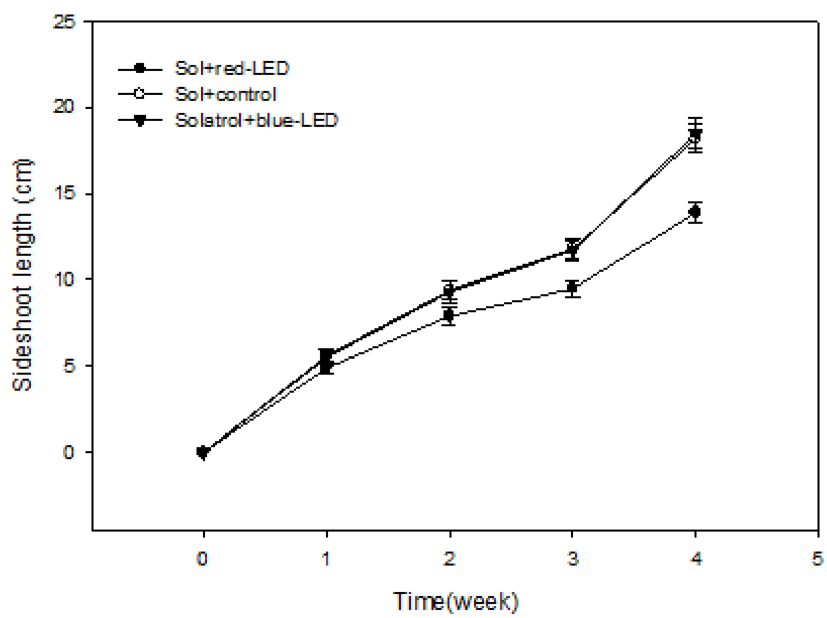

Figure 5. Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED on side-shoot length of petunia ‘Tidal Wave’. The measurement were done on the longest side-shoot and the measurements ended at the time of harvest (stage of marketing)

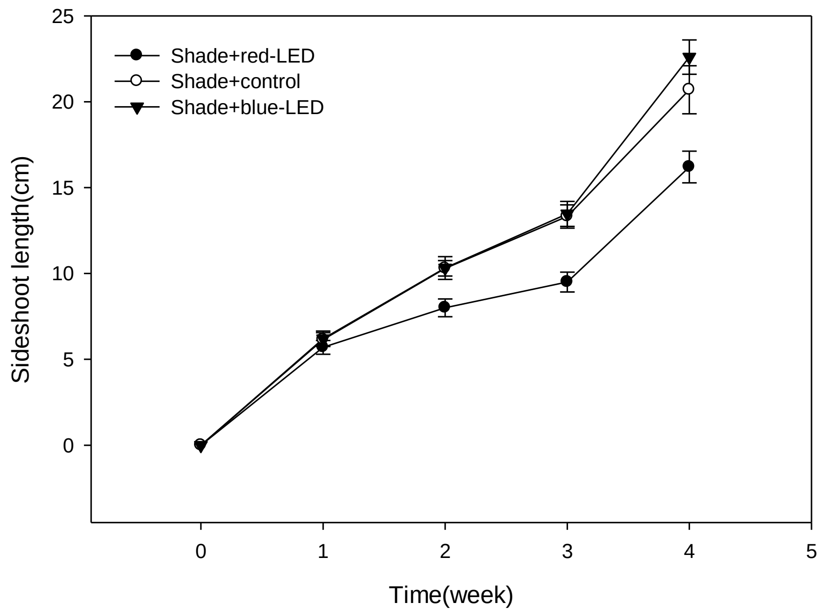

Figure 6. Effect of shade-control, shade + red-LED, shade + blue-LED on side-shoot length of petunia 'Tidal Wave'. The measurement were done on the longest side-shoot and the measurements ended at the time of harvest (stage of marketing)

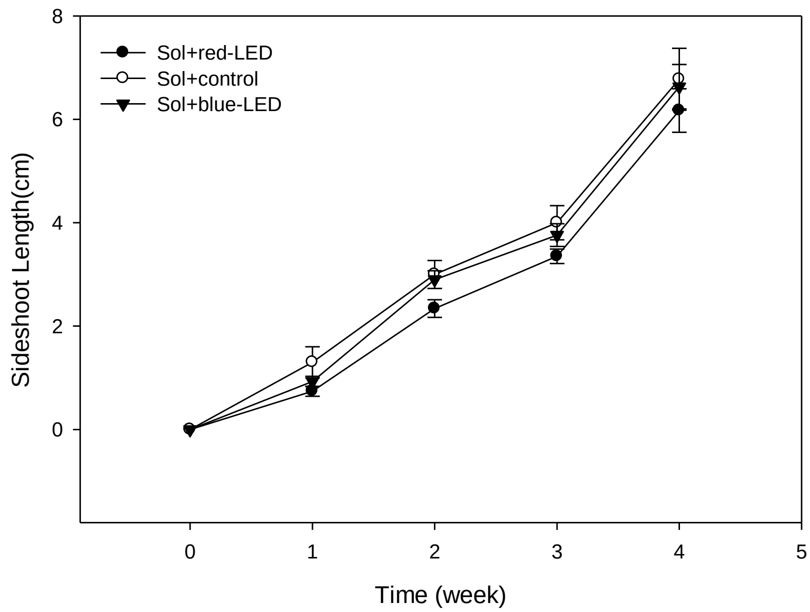

Figure 7. Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED on side-shoot length of petunia ‘Mambo Formula Mixture’. The measurement were done on the longest side-shoot and the measurement ended at the time of harvest (stage of marketing)

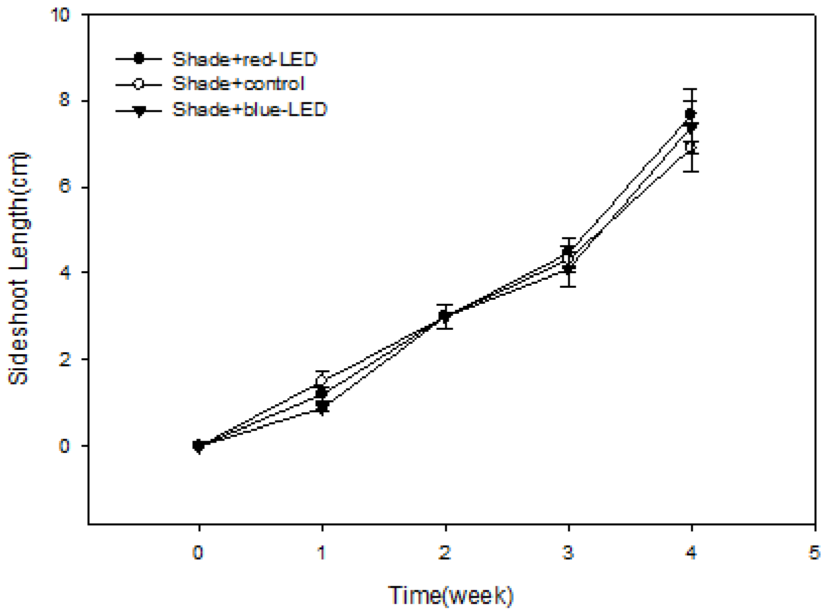

Figure 8. Effect of shade-control, shade + red-LED, shade + blue-LED on side-shoot length of petunia 'Mambo Formula Mixture'. The measurement were done on the longest side-shoot and the measurements ended at the time of harvest (stage of marketing)

Experiment 2: Summer (May-June)

In the cultivar 'Tidal Wave', side shoot length was found shortest in the plants grown in red LED under solatrol compared to blue-LED and control under solatrol (Fig 9). It was followed by blue-LED under solatrol. Similarly, while comparing three treatments under shade, again the shortest side shoot length was found in red-LED under shade compared to shade with control and blue-LED, followed by blue-LED with shade (Fig 10).

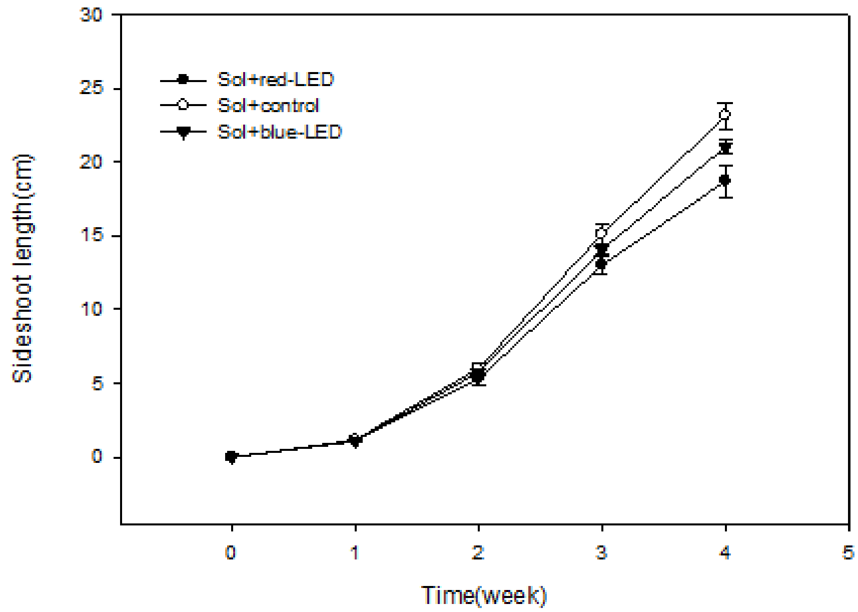

Figure 9.Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED on side-shoot length of petunia ‘Tidal Wave’. The measurement were done on the longest side-shoot and the measurements ended at the time of harvest (stage of marketing)

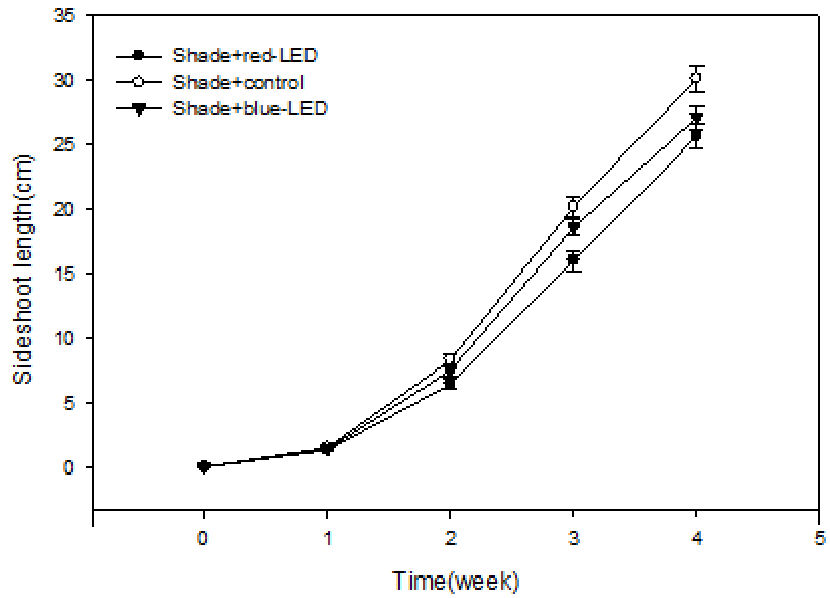

Figure 10. Effect of shade-control, shade + red-LED, shade + blue-LED on side-shoot length of petunia 'Tidal Wave'. The measurement were done on the longest side-shoot and the measurements ended at the time of harvest (stage of marketing)

4.1.2 Plant height

In the cultivar 'Mambo Formula mixture' small differences were seen between the treatments under both Solatrol and shade (Fig. 11 and 12). However, under Solatrol, red-LED gave the shortest plants (Fig. 11).

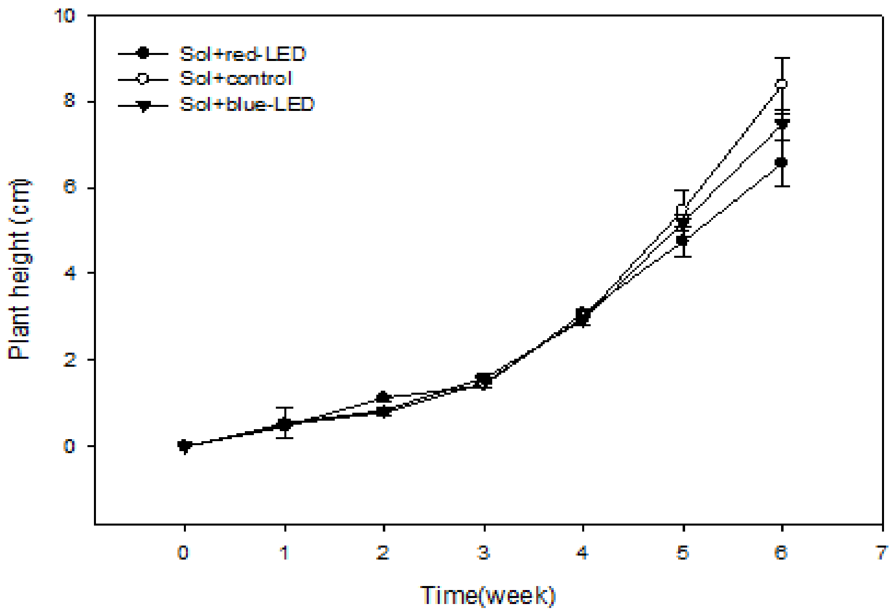

Figure 11. Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED on plant height of petunia 'Mambo Formula Mixture'. The measurement were done on the main shoot and ended at the time of harvest (stage of marketing)

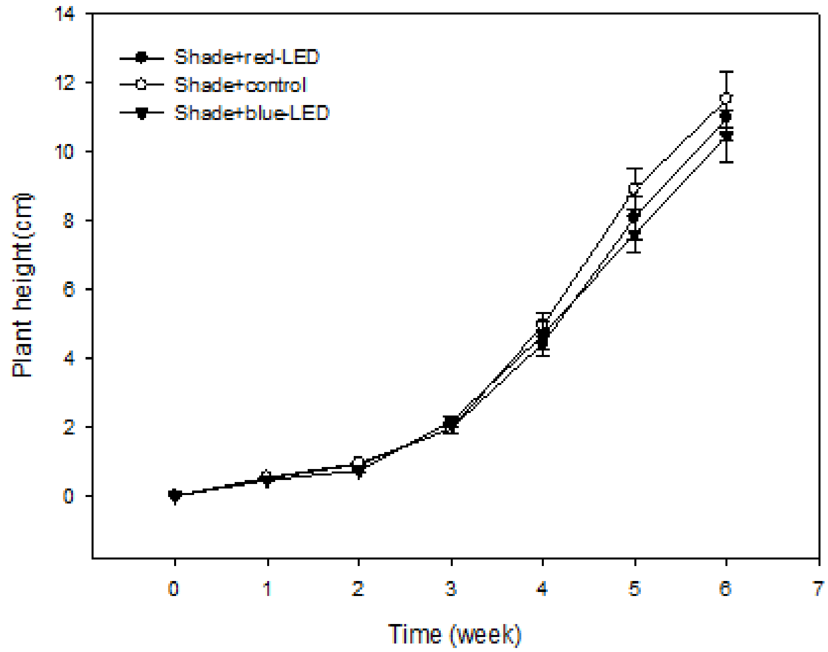

Figure 12.Effect of shade-control, shade + red-LED, shade + blue-LED on plant height of petunia 'Mambo Formula Mixture'. The measurement were done on the main shoot and ended at the time of harvest (stage of marketing)

4.2 Effect of covering material and light quality on different growth parameters

4.2.1 Plant height

Experiment 1: Early Spring (March-April)

In the cultivar 'Tidal Wave' a significant effect of covering material and light quality was found on total plant height. The shortest plants were found under solatrol. However, a significant interaction was found between covering material and light quality. Under solatrol, no significant difference was found between control and red-LED but both treatments gave the shortest plants (Fig. 13). Under shade, the red-LED gave the shortest plants and significantly shorter than control (Fig. 13). Blue-LED enhanced stem extension significantly under both solatrol and shade (Fig. 13).

In the cultivar ‘Mambo formula Mixture’ a different response on plant height came out compared to ‘Tidal Wave’. No significant effect was found on light quality and no interaction between covering material and light quality was found. The Mambo formula Mixture’ responded only to covering material and significantly shorter plants were found under solatrol compared to shade (Fig. 14).

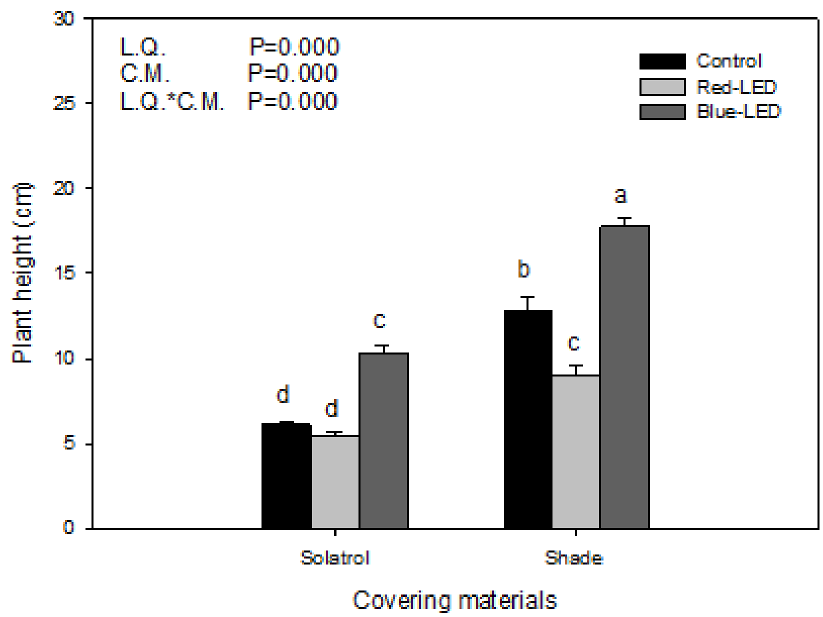

Figure 13.Effect of solatrol–control, solatrol + red-LED, solatrol + blue-LED, shade - control, shade + red LED, and shade + blue LED on the final height of petunia ‘Tidal Wave’. Different letters on the bars represents significant differences according to Fisher test (p< 0,05). N = 10.

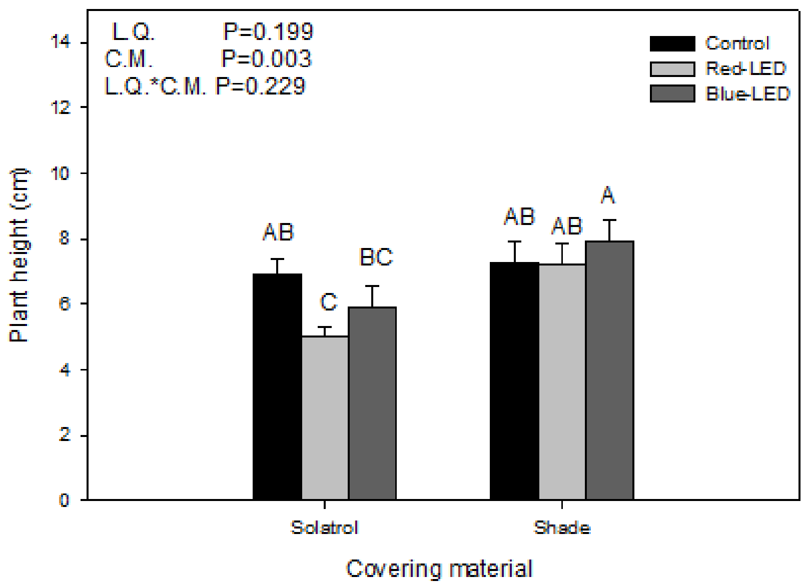

Figure 14. Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the final height of the petunia 'Mambo Formula Mixture'. Different letters on the bars represents significant differences according to Fisher tests (p<0,05). N=10

Experiment 2: Summer (May-June)

In the summer experiment done with 'Tidal Wave' a significant effect of covering material and light quality was found but no significant interaction between covering material and light quality was found. The plants were shortest in the red LED under solatrol followed by control under solatrol and the red LED under shade the tallest plants were found under shade with control and supplementary blue LED (Fig. 15). All the treatments under solatrol showed shorter plant height comparing all the treatments under shade (Fig. 16).

In the summer experiment the cultivar 'Mambo Formula Mixture' responded similarly as the spring experiment. No significant effect of light quality was found (Fig. 17) and no interaction between light quality and covering material was found. The shortest plant height was observed under solatrol compared to shade in all light qualities in the cultivar 'Mambo Formula Mixture' (Fig. 18).

Figure 15. Petunia grown in the greenhouse with six different treatments

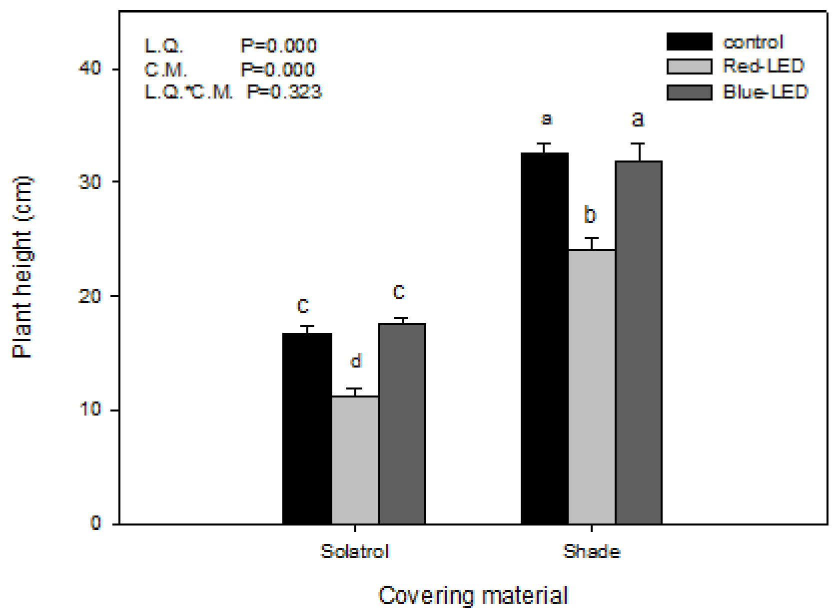

Figure 16.Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the final height of petunia 'Tidal Wave'. Different letters on the bars represents significant differences according to Fisher tests ($p<0.05$). N=10

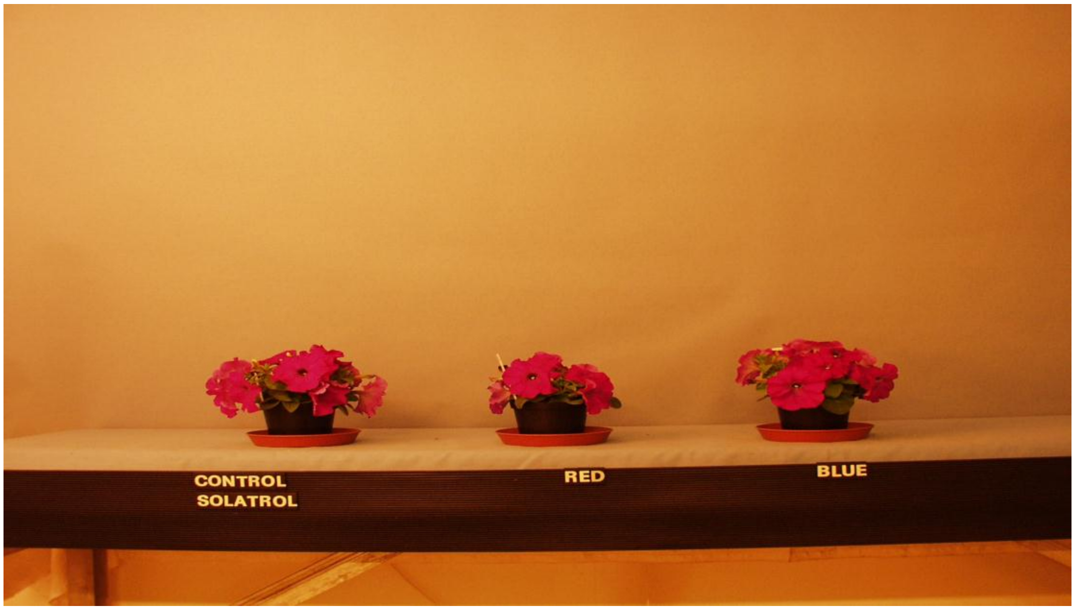

Figure 17. Petunia ' Mambo Formula Mixture' grown under solatoral , solatoral with blue and red-LED light

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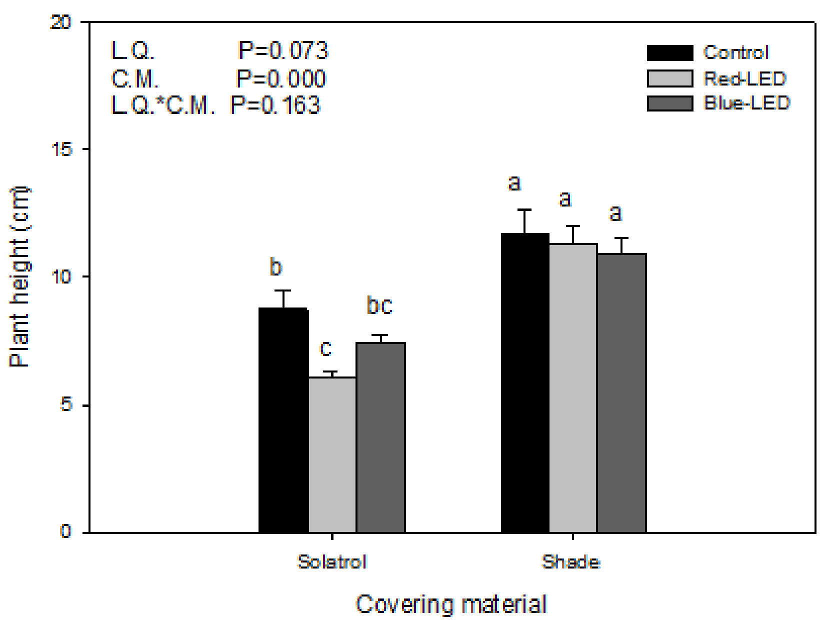

Figure 18. Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the final height of the petunia ‘Mambo Formula Mixture’. Different letters on the bars represents significant differences acco according to Fisher tests (p<0,05). N=10

4.2.2 Number of visible Buds

Experiment 1: Early spring (March-April)

The lowest numbers of visible buds were found in the cultivar 'Tidal Wave' under solatrol compared to shade. Also, under both solatrol and shade, the red-LED was inhibiting the bud number (Fig. 19). All the other treatments showed no remarkable difference. In the cultivar 'Mambo Formula Mixture', no significant effect of covering material or light quality was found on the number of visible buds (Fig. 20).

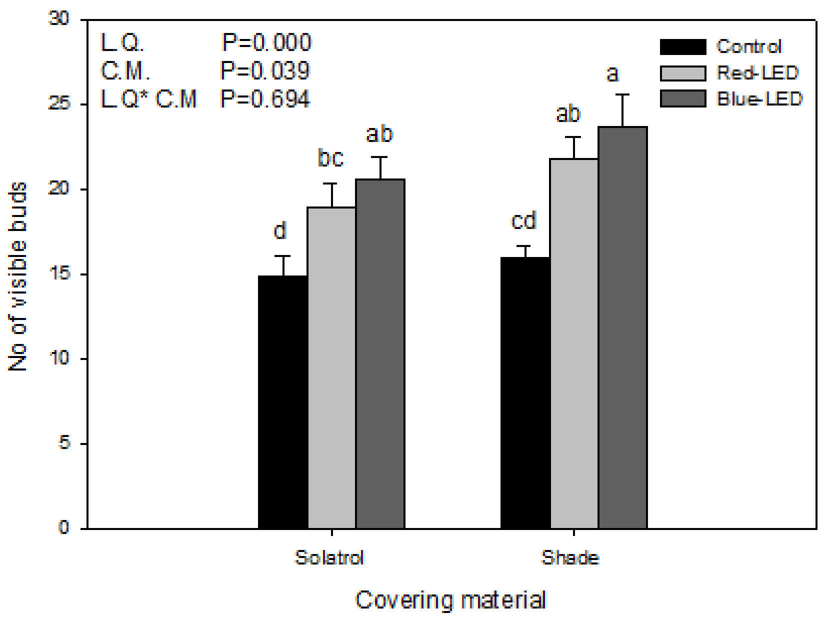

Figure 19.Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of visible buds of the petunia ‘Tidal Wave’. Different letters on the bars represents significant differences according to Fisher tests (p<0,05). N=10.

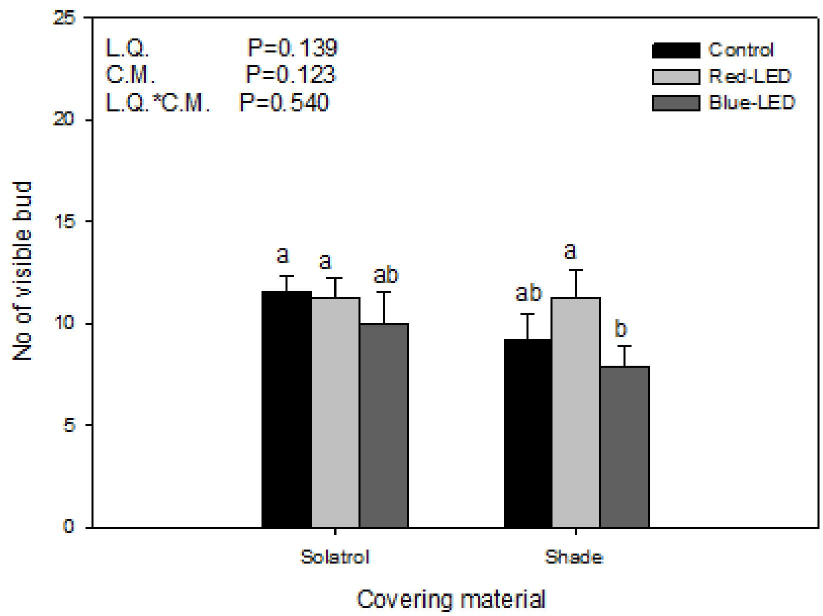

Figure 20.Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of visible buds of the petunia 'Mambo Formula Mixture'. Different letters on the bars represents significant differences according to Fisher tests (p<0,05). N=10.

Experiment 2: Summer (May-June)

In the summer experiment a different trend appeared compared to the spring experiment. A significant interaction between covering material and light quality was found. The lowest number of visible bud was found in solatrol+control whereas remarkably highest number of visible buds was found in shade+control in the cultivar 'Tidal wave' (Fig. 21). Only small differences were found between red-LED and blue-LED under both solatrol and shade (Fig. 21). Similarly, in the cultivar 'Mambo Formula Mixture' a significant interaction between covering material and shade appeared. The lowest number of visible bud was found in solatrol+control but highest number was found under shade-control (Fig.22).

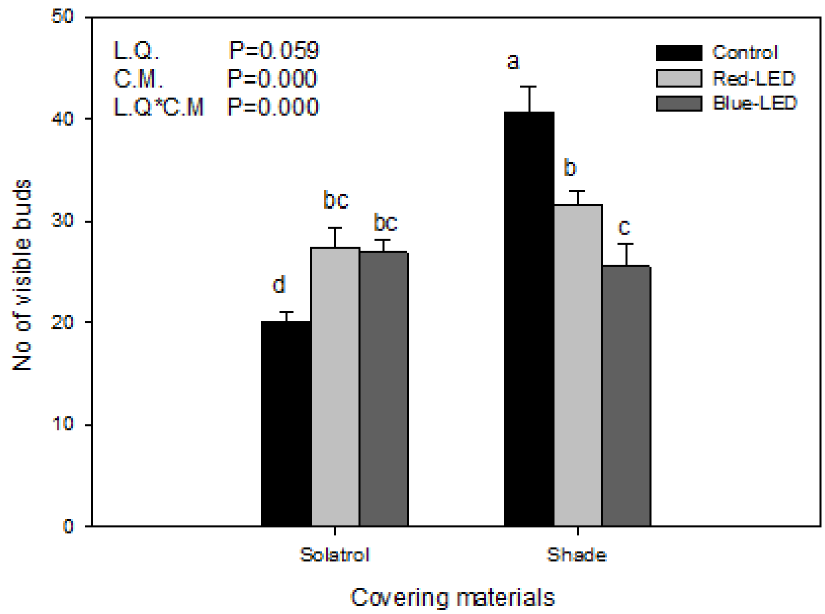

Figure 21. Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of visible buds of the petunia 'Tidal Wave'. Different letters on the bars represents significant differences according to Fisher tests (p<0,05). N=10.

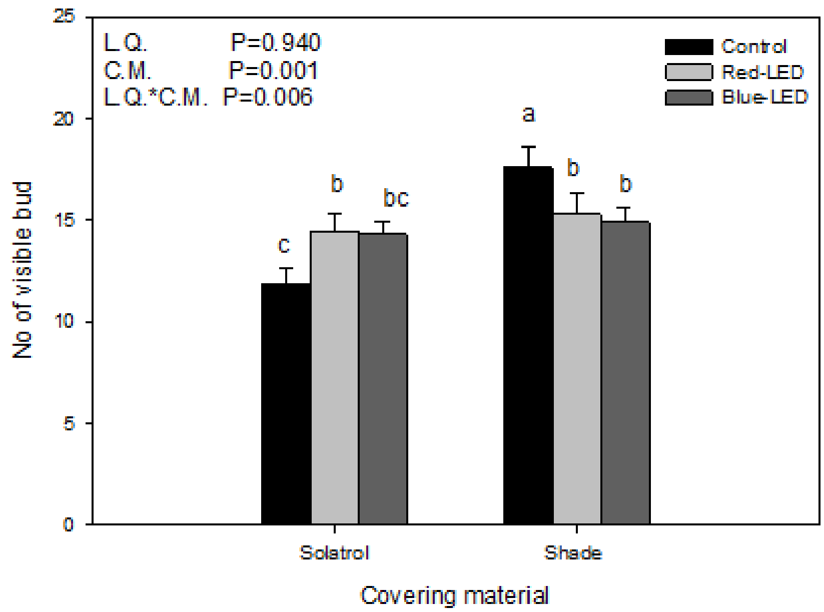

Figure 22. Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of visible buds of the petunia ‘Mambo Formula Mixture’. Different letters on the bars represents significant differences according to Fisher tests (p<0.05). N=10.

4.2.3 Number of Open Flower

Experiment 1: Early Spring (March-April)

A significant effect of covering material and light quality was found on the number of open flowers in ‘Tidal Wave’ but no significant interaction between covering material and light quality was found (Fig. 23). In the cultivar 'Tidal Wave’ the solatrol induced significantly lower number of open flowers compared to control. The highest number of open flowers was found under blue-LED both under solatrol and shade (Fig. 23).

The cultivar ‘Mambo Formula Mixture’ showed the similar results as the ‘Tidal Wave’. The number of open flower was found highest in shade+blue-LED and solatrol + blue-LED (Fig.24).

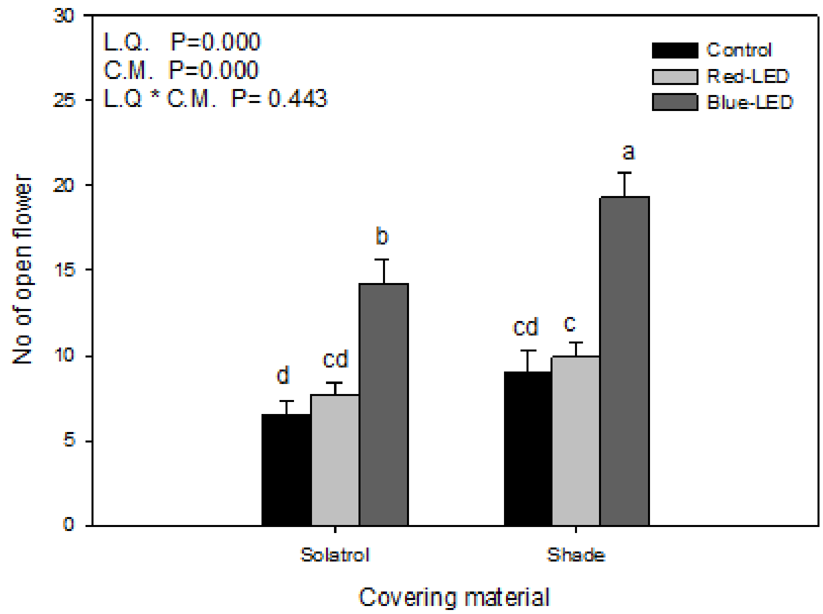

Figure 23.Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of open flower of the petunia ‘Tidal Wave’. Different letters on the bars represents significant differences according to Fisher tests (p<0,05). N=10.

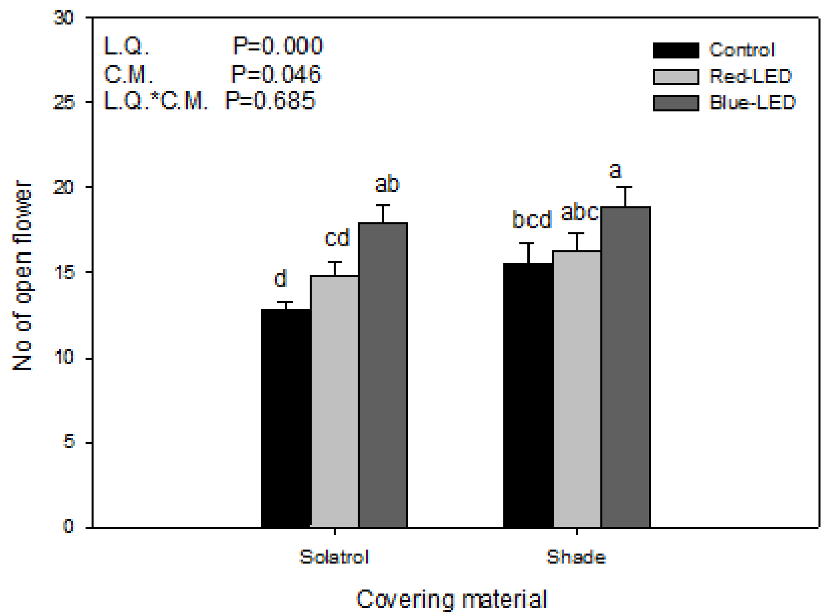

Figure 24.Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of open flower of the petunia 'Mambo Formula Mixture'. Different letters on the bars represents significant differences according to Turkey tests ($p<0.05$). N=10.

Experiment 2: Summer (May-June)

In the summer experiment a significant effect of covering material and light quality was found on the number of open flowers (Fig. 25). The plants under solatrol had a lower number of open flowers than shade. Further, the red LED under solatrol showed lowestt bud number. The blue LED had a clear promotive effect on the bud number under both covering materials (Fig. 25). In the cultivar 'Mambo formula Mixture' a strong effect of the covering material was seen and plants under solatrol had significantly lower number of open flowers compared to shade (Fig. 26). However, a significant interaction was found between covering material and light quality. The solatrol control showed very low number of open flowers and significantly lower than solatrol+ red LED and solatrol +blue LED. Under shade, no such effect was seen and the number of open flowers was the same in shade-control and shade-red LED. The highest open flower number was observed in blue LED for both covering materials (Fig.26).

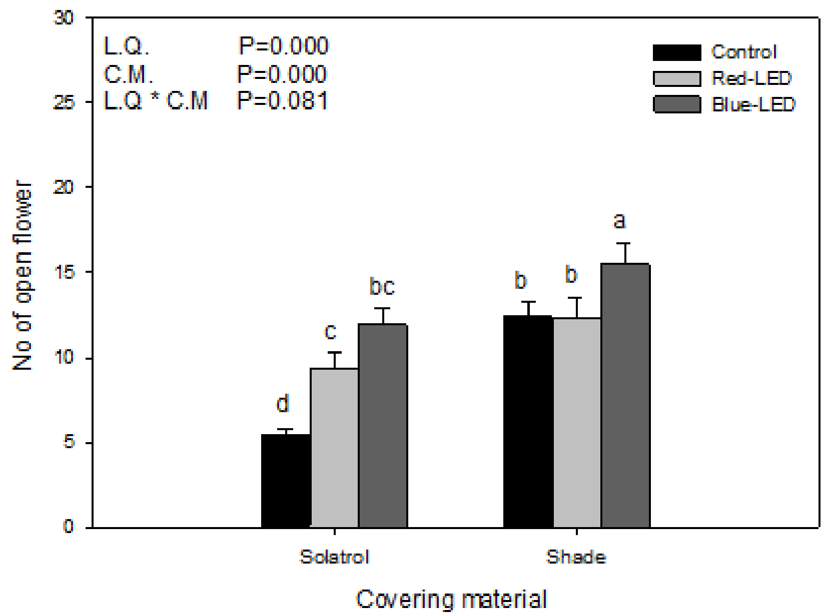

Figure 25.Effect of solatrol.control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of open flower of the petunia 'Tidal Wave'. Different letters on the bars represents significant differences according to Turkey tests (p<0,05). N=10.

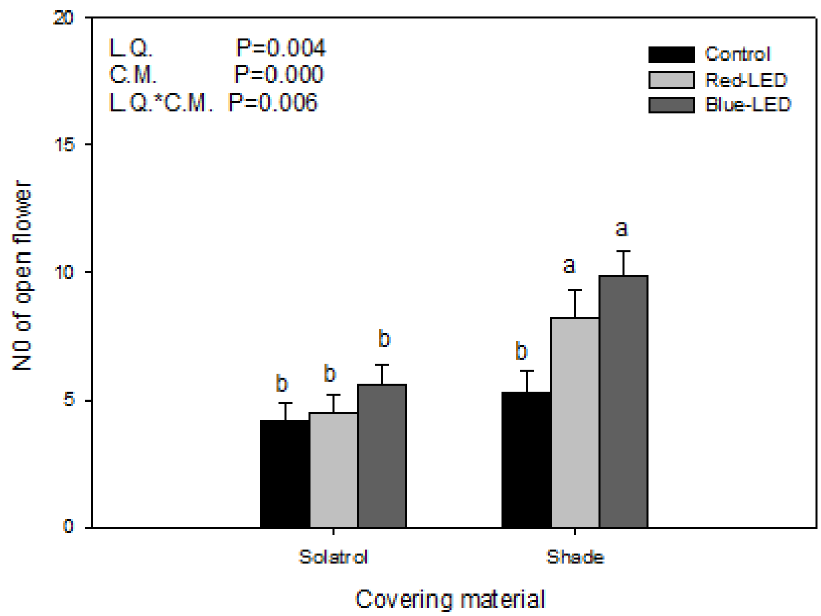

Figure 26.Effect of solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, and shade + blue-LED on the no of open flower of the petunia 'Mambo Formula Mixture'. Different letters on the bars represents significant differences ccording to Turkey tests (p<0,05). N=10.

4.2.4 Plant diameter

Experiment 1: Early Spring (March-April)

The diameter in cultivar 'Tidal Wave' was found significantly smaller in red-LED under solatrol compared to all the other treatments (Table 2). Similarly, in the cultivar 'Mambo Formula Mixture', the smallest plant diameter was found under solatrol + red-LED (Table 4).

Experiment 2: Summer (May-June)

In the summer experiment the diameter in cultivar 'Tidal Wave' was also smallest under solatrol + red-LED. The diameter was found to be significantly higher in blue-LED under shade compared to all the treatments except control+shade (Table 3).

In the cultivar 'Mambo Formula Mixture', there was no significant difference in diameter of the plants grown in red-LED, control and blue-LED under shade. But the diameter of plants grown in red and blue LED under solatrol was significantly smaller compared to all other treatments (Table 5).

4.2.5 Number of side shoots

Experiment 1: Early Spring (March-April)

In the cultivar 'Tidal Wave', no significant difference was found among the treatments solatrol + control, solatrol + blue, shade + red, shade + control and control + control. However, the number of side shoots was significantly higher in solatrol + red LED and solatrol + blue LED (Table 2).

The numbers of side shoots in cultivar 'Mambo Formula Mixture' were not significantly different among all the treatments under both solatrol and shade (Table 4).

Experiment 2: Summer (May-June)

In cultivar 'Tidal Wave' no significant difference was found among the treatments solatrol + control, solatrol + blue-LED and shade + red-LED. However, the number of side shoots was found significantly higher in solatrol + red-LED. The lowest shoot number was found in shade - control and shade + blue LED compared to all other treatments (Table 3).

4.2.6 Average number of internode

Experiment 2: Summer (May-June)

In the cultivar 'Tidal Wave', the average number of internodes were found significantly lower in solatrol + control and solatrol + blue-LED compared to shade red-LED and shade control (Table 3). No remarkable effect of light quality was found in number of internodes.

4.2.7 Fresh weight

Experiment 1: Early Spring (March-April)

The Fresh weight in the cultivar 'Tidal Wave' was found to be highest in blue-LED under shade compared to all the treatments followed by blue-LED under solatrol without any remarkable difference with all the treatment in control conditions (Table 2). Significantly lowest fresh weight was found to be in red-LED under solatrol compared to blue-LED under both solatrol and shade.

In the cultivar 'Mambo Formula Mixture', the fresh weight was lower in solatrol + red-LED compared to control whereas it was not significantly different among all other treatments. Similarly fresh weight was significantly higher in sunlight treatment compared with red + solatrol only and insignificant with all the other treatments (Table 4).

Experiment 2: Summer (May-June)

The fresh weight in the cultivar 'Tidal Wave' was found to be higher in all the treatments under shade with no significant difference in the cultivar ‘Tidal Wave’. A significantly lower fresh weight was found in all the treatments under solatrol (Table 3).

In the cultivar 'Mambo Formula Mixture', the fresh weight was lower in all the treatments under solatrol compared to all other treatments (Table 5).

4.2.8 Dry weight

Experiment 1: Early Spring (March-April)

A significantly higher dry weight of cultivar 'Tidal Wave' was found in shade + blue-LED compared to all the treatments in shade and solatrol + control (Table 2).

No significant difference was found among any treatments in dry weight of cultivar 'Mambo Formula Mixture' (Table 4).

Experiment 2: Summer (May-June)

The dry weight in the cultivar 'Tidal Wave' was found to be high in all the treatments under shade (Table 3). Significantly lower dry weight was found to be in all the treatments under solatrol.

No significant difference was found among any treatments in dry weight of the cultivar 'Mambo Formula Mixture' under solatrol (Table 5). Similarly there was no significant difference among any of the treatments under shade (Table 5).

Table 2. Comparison of three treatments of light quality on diameter, no of side shoot, leaf area, fresh wt., dry wt. under solatrol - control , solatrol + red-LED, solatrol + blue-LED, shade - control, shade + red-LED, shade + blue-LED in petunia 'Tidal Wave'. N=10. The mean values within the one row followed by same letter are not significantly different at p =0.05. Experiment was done in march-April. The measurements were done at the plant marketing stage.

Parameter	Treatments					
	Red-LED		Blue-LED		Control	
	Solatrol	Shade	Solatrol	Shade	Solatrol	Shade
Diameter	41.82 B	40.02 B	46.61 A	48.45 A	49.10A	48.05 A
Number of side shoots	11.4 A	9.7 B	10.4 AB	8.3 C	10.1 B	9.5 B
Leaf Area	58.47 C	64.1 BC	67.81 ABC	71.22 ABC	76.31 AB	80.17 A
Fresh Weight	46.9 C	47.3 C	56.2 AB	63.0 A	49.71 BC	53.81 BC
Dry Weight	9.45 B	9.92 B	10.35 AB	11.56 A	9.22 B	10.22 AB

Table 3. Comparison of diameter, no. of side shoot, average no of internode (of 3 side shoot), fresh wt., dry wt.under solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, shade + blue-LED in petunia 'Tidal Wave'. N=10. The mean values within the one row followed by same letter are not significantly different at p =0.05. Experiment was done in May- June. The measurements were done at the plant marketing stage.

Parameter	Treatments					
	Red-LED		Blue-LED		Control	
	Solatrol	Shade	Solatrol	Shade	Solatrol	Shade
Diameter	39.72 D	43.65 BCD	39.97 CD	49.3 A	43.8 BC	46.20 AB
Number of side shoots	11.70 A	10.40 B	9.90 B	8.10 C	9.80 B	8.10 C
Average number of internode	14.28 AB	14.46 A	13.83 B	14.19 AB	13.91 AB	14.46 A
Fresh Weight	78.61 B	89.11 A	76.36 B	86.50 A	76.53 B	90.99 A
Dry Weight	12.64 B	14.27 A	12.07 B	14.39 A	12.42 B	15.02 A

Table 4. Comparison of diameter, no. of side shoot, fresh wt., dry wt. under solatrol-control, solatrol + red-LED, solatrol + blue-LED, shade-control, shade + red-LED, shade + blue-LED in petunia 'Mambo Formula Mixture'. N=10. The mean values within the one row followed by same letter are not significantly different at p =0.05.Experiment was done in March-April. The measurements were done at the plant marketing stage.

Parameter	Treatments					
	Red-LED		Blue-LED		Control	
	Solatrol	Shade	Solatrol	Shade	Solatrol	Shade
Diameter	24.25 C	28.90 A	25.55 BC	28.85 A	26.40 B	28.90 A
Number of side shoots	8.70 A	7.80 A	8.80 A	8.30 A	8.00 A	8.80 A
Fresh Weight	31.05 B	34.36 AB	34.67 AB	34.09 AB	34.55 AB	38.59 A
Dry Weight	7.26 A	7.64 A	7.41 A	7.58 A	7.37 A	7.48 A

Table 5. Comparison of diameter, fresh wt., dry wt. under solatrol - control, solatrol + red-LED, solatrol + blue-LED, shade - control, shade + red-LED, shade + blue-LED in petunia 'Mambo Formula Mixture'. N=10. The mean values within the one row followed by same letter are not significantly different at p =0.05. Experiment was done in May- June. The measurements were done at the plant marketing stage.

Parameter	Treatments					
	Red-LED		Blue-LED		Control	
	Solatrol	Shade	Solatrol	Shade	Solatrol	Shade
Diameter	16.82 B	19.70 A	18.12 B	20.55 A	20.50 A	20.67 A
Fresh Weight	40.24 C	50.02 A	43.08 BC	47.77 AB	46.52 AB	48.04 AB
Dry Weight	7.03 B	8.09 A	7.03 B	8.09 A	7.14 B	8.02A

4.3 Effect of covering material and light quality on days to visible buds and days to open flower

Experiment 1: Early Spring (March-April)

In the first experiment in the greenhouse, it was observed that the first visible bud appeared in ‘Tidal Wave’ in the treatment shade + red-LED with an avearge of 19.92 (±1.50) days. The latest visible bus was observed in the treatment solatrol + red-LED with an average of 22.75 (±3.52) days. The earliest time to open flower was noticed in shade + blue-LED with an average of 25.75(±0.96) days and the latest time to open flower was found in solatrol–control with an average of 29.50 (±1.83) days. The comparison between the treatments was highly significant in with P=0.008 on no. of visible buds and with P=0.000 on no of open flower.

In ‘Mambo Formula Mixture’ there is a significant difference between the treatment (P=0.000) in both days to visible bud and open flower in the cultivar ‘Mambo Formula Mixture’ during early spring.

While in case of ‘Mambo Formula Mixture’, the earliest time to visible bud was noticed on solatrol + blue-LED (9.25±0.45days) and the latest was observed on solatrol+ red-LED (18.58±1.68) days which seems highly significant(P=0.000).

Blue-LED under solatrol (17.83±1.7days) was found to open the flower first and as same as in other case solatrol+control was latest to open the flower (22.41±1.93) (P=0.000).

Experiment 2: Summer (May-June)

In 'Tidal Wave' the earliest visible bud was found under shade + blue-LED with a mean of 22.33 (±0.65) days. The latest bud was seen under solatrol+control treatment with the mean of 25.17± 1.9 days. The earliest time to open flower was noticed in shade + blue-LED with an average of 29±1.48) days and the latest time to open flower was found in solatrol–control with an average of 32.66 (±1.77) days which was highly significant among the treatments (P=0.000).

The same trend was found in both visible bud and open flower in the 'Mambo Formula Mixture' with earliest visible bud in shade + blue-LED (21.83±1.03) and the latest was with solatrol+control (23.58±1.68) but with no significant differences among the treatments(P=0.076) in visible buds and 0.460) in the number of open flower.

4.4 Effect of covering material and light quality on Chlorophyll content

Experiment 2: Summer (May-June)

A remarkable difference in chlorophyll content was found between the treatments in both cultivars (Table 6). Under shade, the chlorophyll content was found lower than all the treatments under solatrol. No significant difference among three treatments under shade was observed. The highest chlorophyll content was found under solatrol with supplementary red LED in both the cultivars.

Table 6.Chlorophyll content under different treatments (P=0.05)

Cultivars	Tidal Wave		Mambo Formula Mixture	
Treatments	Solatrol	Shade	Solatrol	Shade
Red LED	20.75(3.89)A	12.92(2.56)C	16.24(4.48)A	15.22(3.50)AB
Control	14.17(2.10)BC	12.97(2.03)C	10.67(2.50)C	11.02(2.46)C
Blue	15.60(3.84)B	12.92(1.5)C	11.51(1.64)C	12.76(2.81)BC

5 DISCUSSION

Plants use light as an environmental signal with responses to the intensity, wavelength and direction in photosynthesis as energy. Light quality has a profound effect on growth and development of the plants and can thus be a strong tool to control different processes. Red light is important for photosynthesis and photosynthates translocation (Saebo et al., 1995). However, altering red and far red light ratios can stimulate growth and development, morphology and flowering of the plants (Sage 1992). Solatrol is covering material known to alter the light quality under its environment by absorbing far red light and thus increasing the R/Fr ratio. Blue light is very much important for chlorophyll formation, enzyme synthesis and photomorphogenesis as well (Ménard et al., 2006). Light quality and covering materials greatly affects growth parameters, flowering parameters and chlorophyll content of the petunia in this study also.

5.1 Effect of covering material and light quality on growth parameters

5.1.1 Plant height

The shorter plants were found under solatrol compared to shade in both the cultivars 'Tidal Wave and 'Mambo Formula Mixture'. As a far-red absorbing film, solatrol reduced the far red light under its environment and altering R/Fr (red/far red) ratio. Under natural light conditions the R/Fr ratio is 1.1-1, 2 but under solatrol the R/Fr ratio is increased to 10.2. This high R/Fr ratio will put the phtyocrome into the active form Pmr and suppress stem extension (Fukuda et al., 2011a).

Moe et al., (1991) also reported similar result in different bedding plants, i.e., far red light strongly enhanced stem elongation. Similar results were also reported Patil et al., (2001) and plastic film with increased R/Fr ratio reduced the main shoot length of petunia. (Lykas et al., 2006) reported similar results and showed remarkable reduction in the height of potted plants (59%) under light filtering plastic films with high R:Fr and B:Fr ratio compared to common plastic films. Similarly, many other results showed that stem elongation can be reduced by light quality with high R: Fr ratio using plastic filters (Cerny et al., 2000; Murakami et al., 1994). (Cerny et al., (2003) reported that far red light absorbing films did not reduce plant height of snapdragon whereas in the same research it was reported that zinnia, cosmos and chrysanthemum grown under far red light absorbing films significantly reduced the height of main shoot. Similarly 19 percent reduction was found in stem length of chrysanthemum under far red filter (Khattak et al., 2004). Kubota et al., (2000) also found inhibition in stem elongation in hybrid seed of petunia 'Fulcon Red and White' grown under far red light. Thus, most plants become shorter under far red light absorbing films. However, it can vary with season. The effect of the solatrol was somehow stronger in the summer experiment compared to the spring experiment. The reason for this observation is probably connected to the natural irradiance in those two periods. In spring, the natural light is low and when the plants are covered with solatrol the irradiance will be even lower. Solatrol does not only reduce the far red light but also a large part of the red light and will reduce the total irradiance up to 30%. This reduction in irradiance will enhance stem extension and abolish the effect of the high R/Fr ratio.

Red LED was very effective in suppressing the stem extension, both under solatrol and shade. The result obtained by Haliapas et al., (2008a) also reported that red light treated plants were shorter and far-red light treated plants were found taller compared to control treatment. In grapes also, red light was found highly significant to reduce plant height (Poudel et al., 2008). But sometimes it may be varied depending upon the plant species.

In the cultivar ‘Tidal Wave’, the presence of blue light enhanced stem velongation whereas the presence of red light inhibited stem elongation. Blue light are known to play equal or a greater role than R:Fr in long day plants. Similar to high far red, low blue light also produced tall plants in soybean and sorghum (Britz & Sager 1990). In contrast, Novičkovas et al., (2012) reported that blue light from blue LED did not significantly affect the height of cucumber plants. In the cultivar ‘Mambo Formula mixture’, there was also no significant difference among blue LED, red LED and control. Thus, it looks like blue light rather has a promotive effect of stem elongation in petunia. Similar result was observed by Fakuda and Olsen, (2009) where the highest petunia plants were found in the treatment with additional blue light.

Depending upon the species, different synergistic interactions between blue and red light receptors promote the stem elongation (Kim et al., 2004). Many studies reported that blue deficient or red biased environment promote stem length (Brown et al., 1995; Sager et al., 1991).

In Salvia Fluorescent light with Far red treatment showed highest seedling height whereas in marigold, plant height was found higher in florescent light with blue and florescent light with far red treatment while comparing four different treatments, i.e, florescent light and red, blue and far red with florescent light (Heo et al., 2000). Thus the background light can have an effect of the response to different qualities like red or blue light. However, in the presented experiment the responses to red and blue light were the same under both covering materials. Under solatrol, the differences in plant height among three treatments were seen only after four weeks whereas under shade, it was seen after three weeks. Stem extension was inhibited in red- LED treatments compared to blue under solatrol and under shade

5.1.2 Number of internode

In the variety 'Tidal Wave', no remarkable difference was found in average numbers of internodes between the treatments. So, it can be said that the increase in height and stem elongation was because of increase in length of internodes rather than number of internodes however internodes length was not measured in this study. Several other reports showed similar results. Appelgren (1991) reported no difference in internodes number was due to the effect of light quality. Kim et al., (2004) found stem elongation in chrysanthemum was due to internodes length rather than their number and no effect of light quality in number of internodes was reported. Similar results were reported by da Silva & Debergh (1997) by suggesting that the light quality affects internodes length without affecting the number of internodes.

5.1.3 Side shoots

In the variety ‘Tidal Wave’, the number of side shoots was found higher under solatrol than under shade for all the treatments. This can be supported by the research of Moe & Heins (1990) which showed that lateral branching in pot and bedding plants are inhibited by far red light. A high number of side shoots is often considered as a good quality plant, especially if the side shoots are short. Also, the number of side shoots can also lead to a higher number of flowers. But in the variety 'Mambo Formula Mixture', no significant differents among the treatments was found in number of side shoots Thus, depending upon the cultivars, petunia respond to solatrol in increasing the number of side shoots. However, red and blue LED did not show any remarkable effect on the number of side shoots.

5.2 Dry weight

The total dry weight was found to be higher in blue LED treatment compared to red LED treatment both under shade and solatrol in the cultivar 'Tidal Wave'. The result is consistent with Ménard et al., (2006) , they reported that supplementary blue light increased plant dry weight of cucumber and tomato. Similarly, many other studies showed that red light biased and blue light deficient sources reduced the dry mass of plants (Brown et al., 1995; Smith 1982). In contrast to this, dry weight of marigold seedlings was found to be significantly increased in monochromatic red light and reduced in monochromatic blue light (Heo et al., 2002). But dry weight of Ageratum plug seedlings was not different among blue and red light treatment in combination with florescent light but it was found three times greater in far red light treatment in combination with florescent light (Heo et al., 2002). No significant differences among the treatments in the variety 'Mambo Formula Mixture' was found. Significant differences in dry weight by the effect of covering materials was not found. Thus, blue LED was most effective to increase dry weight in petunia depending upon cultivars. The higher dry weight of plant can be due to higher photosynthesis, reduced respiration, or both, but we did not measure these parameters in our experiment.

5.3 Effect of covering material and light quality on flowering parameters

Plants under solatrol showed delayed floral initiation with less number compared to shade. From this it can be said that environment with reduced far-red light is favourable for delay flower induction and will give a lower number of flowers. This can be supported by the findings of Runkle and Heins, they found that deficiency in far-red light with high R/Fr ratio can be responsible for delaying the flower initiation in some species (Mata & Botto 2009; Runkle & Heins 2001). Similarly anthesis in snapdragon and petunia was found to be delayed by 7 and 13 days respectively under far-red absorbing films but it did not affect flowering of miniature rose (Cerny et al., 2003). Far-red deficient environment delayed flowering by 1 to 2 weeks depending on the cultivars (Kim et al., 2002). In contrast to all these reportings, Kubota et al., (2000) reported that flowering of petunia was not delayed by the treatment of far-red light.

For flowering, far-red light seems to play positive role because plants under solatrol showed delay flowering. Even in the case of early spring when irradiance was low, cultivar ‘Tidal Wave’ showed early flowering with additional red light but if there was no far-red light i.e., under solatrol again flowering was found delay in red light. This can be supported by the findings of Kim et al; they reported that number of lateral visible buds are greater in the plants with far-red light treatments in the ‘Blue Vein’ variety of petunia (Kim et al, 2002). In contrast Heo et al., (2002) reported that the number of flowers is four times greater in florescent, florescent with red and blue than in florescent with far- red light.

Flowering is a complex phenomenon in which light reacts through photoreceptors as red light via phytochromes and blue light via cryptochromes (Hopkins & Huner 2004) and also involving several flowering genes (Suárez-López et al., 2001). Transcript levels of flowering induction gene get increased in petunia when blue light stimulate cryptochrome signal, and leads to flowering whereas under red LED of 100 μmol·m-2·s-1, phytochrome is stimulated, which either not sufficient to express flowering induction or inhibits expression of these genes. So lack of blue light means no stimulation of cryptochrome and hence inhibits floral induction in petunia but red light can induce floral development at higher irradiance (Fukuda et al., 2011b). In consistent with this, both the cultivars ‘Tidal Wave’ and ‘Mambo Formula Mixture’ showed a high number of open flowers under blue LED irrespective of the covering material. But in the case of number of visible buds, no remarkable difference was found between red LED and blue LED. But Flower bud and open flower were both seen earliest under shade with blue light and the latest was under solatrol with control treatment in all the case except in 'Tidal Wave' cultivar during early spring experiment in which red LED under shade showed early flowering and red LED under solatrol showed latest flowering. In Arabidopsis, plants, which is also a long day plant, under red LED was delayed in flowering (Heo et al., 2003). In contrast to which Mockler et al., (1999) reported that monochromatic blue light delayed flowering in Arabidopsis.

The number of open flowers and visible buds were found remarkably less under solatrol than under shade whereas number of open flower was found more in blue LED treatment than red LED treatment under both solatrol and shade. Heo et al., (2003) also reported that highest number of flower buds and open flowers in the plants under mix treatment of blue and red LED whereas blue and red LED alone reduced the flowering response which does not match with the result of this study where blue LED induce flowering.Visible buds as well as open flowers were earliest in the plants with additional blue light with high number of open flowers. This may be because of the stimulation of cryptochrome which in turn activate flowering inducing genes to induce flowering. Similar effect may be with number of open flowers.

5.4 Effect of covering material and light quality on chlorophyll content

The chlorophyll content was found to be higher in plants grown under solatrol compared to shade in all treatments 'Tidal Wave and 'Mambo Formula Mixture' cultivars of petunia. The highest numbers were found under solatrol with supplementary red light by red LED. Far red light seems to decrease the chlorophyll content because solatrol led to higher xhlorophyll content. High R/Fr ratio is also inductive for cholorophyll content as solatrol with supplementay red light led to highest cholophyll content. Similarly, leaves treated with far red light led to lower chlorophyll content in Birch (Caesar 1989), Acer species and other deciduous trees (Hanba et al., 2002; Oguchi et al., 2005).

Red LED treatment induced higher chlorophyll content compared to the blue LED treatment in both the experimented cultivars. However, the difference was only prominent under solatrol. Chlorophyll content in lettuce leaves was also found to decrease with the treatment of blue light (Brazaityte et al., 2006). In contrast, (Poudel et al., 2008) reported that blue LED treatments led to the highest and red LED treatments led to lowest chlorophyll content in grapes. Blue LEDs led to highest chlorophyll content also in upland cotton plantlets (Li et al., 2010). Whereas Shin et al., (2008) reported that chlorophyll content was found to be highest in the plants grown under mixed blue plus red LED, followed by blue LED and florescent treatment and the red LED treatment showed remarkable reduction in chlorophyll content.

Compared to blue light, red light was found to be much efficient to increase chlorophyll content in both the experimented cultivars.libird LED can show its effect in higher chlorophyll content but remarkable difference was found only under the solatrol. Thus, presence of red light may be important to increase chlorophyll content in the absence of far-red light. Presence of far-red light minimizes the effect of LEDs in increasing or decreasing chlorophyll. Red light led to phytochrome stimulation, and then signals from phytochrome because of this stimulation may increase the expression of gene to increase chlorophyll content. So phytochrome stimulation in absence of far-red light seems to be important for higher chlorophyll content.

6 CONCLUSIONS AND RECOMMENDATION

- The response to light quality is dependent on cultivar, and 'Mambo Formula Mixture' is found less sensitive to light quality than that of cultivar 'Tidal Wave'.
- The covering material solatrol, which does not transmit far-red light, can reduce stem extension but the effect was more prominent under high irradiance (summer experiment) than low irradiance (early spring experiment). The main reason is that Solatrol reduces the total light transmittance with 30% which again enhances stem extension.
- Red LED is very effective in reducing stem extension in petunia and can be an alternative in controlling plant height but combined with solatrol it can reduce the number of flowers and flower buds prominently.
- Additional blue light will not reduce stem extension of petunia. Rather higher plants were found when blue LED was added under both covering materials.
- Earlier flowering and more flowers were found with additional blue light quality.
- The light quality treatment did not affect the number of internodes but mainly the length of the internodes.
- Also Chlorophyll content of both the cultivars of petunia was higher under solatrol compared to shade.
- In summary, petunia is very sensitive to light quality and it can be used as a strong tool to control morphology and flowering. Red light is effective in reducing the stem extension growth (plant height) but blue light, as well as far red light, is the most effective in promoting flowering.

From the study we can recommend the following observation to the growers

- ➢ The red LED light can be used in the early spring in order to reduce the height of the petunia plants.
- ➢ The solatrol can be of importance during summer when the irradiance is high and will then reduce the height of petunia.

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