Interactive Effects of Spectral Lights and Salt Stress on Production of Secondary Metabolites and Growth Behavior in Economically Important Brassica Rapa

Iftikhar Muhammad Khan
NIBGE https://orcid.org/0000-0001-8412-5641
Javeed Iqbal
University of Malakand
Nisar Ahmad (ahmadn@uswat.edu.pk)
https://orcid.org/0000-0003-3299-3409
Waqar Ali
University of Malakand
Hina Rehman
Quaid-i-Azam University
Syed Shujait Ali
University of Swat
Hina Fazal
Pakistan Council of Scientific and Industrial Research Lahore Laboratories Complex
Abbas Khan
Shanghai Jiao Tong University
Dong Qing Wei
Shanghai Jiao Tong University School of Life Sciences and Biotechnology

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Abstract

Background

Environmental factors like, temperature, humidity, light intensity, the supply of water, minerals, and CO_2 influence the growth of a plant and biomass accumulation and production of secondary metabolites. Plant cell culture technologies have been effective tools for both studying and producing plant secondary metabolites under in vitro conditions and for plant improvement. The main objective of the current study was to investigate the biomass production and accumulation of secondary metabolites in *Brassica rapa* in response to synergism of abiotic factors including salt and colored lights.

Methods

Brassica rapa sterilized seed were germinated and exposed to different colored lights (white as a control, yellow, blue, green and red) in combination with different concentrations of salt. The length of shoot, root and biomass (fresh and dry) were monitored during the developmental stages. Total phenolics and flavonoids content of the samples were also investigated. Data was analyzed using ANOVA-single factorial.

Results

Results revealed that plants exposed to the synergism of salt stress and spectral lights have shown negative effects on the seed germination. Shoot length (75 mm) increased, while root length (55 mm) was inhibited in plants exposed to synergism of spectral lights and different concentrations of salt when compared with controlled conditions (48 mm for shoot and 100 mm for root). However, the fresh weight of the plants was increased at lower concentration of salt than controlled. Lowest concentration of salt in synergism with green light showed the maximum response. Total phenolic content increased, while total flavonoid decreases with increasing salt concentration. Maximum total phenolic and flavonoids content were recorded in dark incubation with a high and medium concentration of salt respectively.

Conclusion

Based on these results, it was concluded that synergism of low salt concentration and colored lights are effective but high concentrations of salt in combination with colored lights inhibited the growth of *Brassica rapa*.

Background

Plants growing in the normal conditions are exposed to many of the stresses from the environmental conditions. These environmental conditions containing light, heat stress and salt stress. Passing variation in these stresses changes the biochemical pathways for the production of the important

compounds in plants [1]. The products quality and quantities are related to the fluctuation of these stresses [2] This fluctuation either increase or decrease the quantity of the desired compounds [3, 4]. Light is one of the important sources for the growth of the plants. Light is necessary to produce food in the plants. Light plays a key role in the metabolic pathways which affect the production biologically active constituents of plants [5]. Fluctuating the intensity of light can lead to change the biochemical pathways of secondary metabolites [6]. It is also reported that any changes in the wavelength of light color bring changes in the production metabolic compound [7, 8]. Some major components of these medicinal compounds are light sensitive, and these compounds change the quality of the secondary metabolites by the fluctuation of the light quality in these plants. The quality and quantity of light have a direct effect on the chlorophyll to produce food in the plant's body. By changing the light quality/quantity increases or decreases many pharmaceutical products formations in many economically important plants (Ahmad and Abbasi, 2014). These pharmaceutical products either increases or decreases in quantity by making changes in the visible range wavelength and the intensity of that light color

Salt concentration in soil is one of the main abiotic stress to the plants. Salt quantity in the soil either effect positively or negatively the production of important medicinal compounds [9]. Salt concentration in the soil affects the uptake of water by the roots [10]. The saline environment does not allow the seed to germinate and brought changes in the biochemical pathways which may lead to a dormant stage [11, 12]. It is necessary to cultivate the important plants in the saline environment to check their effect for increasing important metabolites. The soil is one of the important factors for the germination of seeds. Seeds present under the soil is in complete darkness. Light does not penetrate the soil. Seeds present deep in the soil experiences physiological changes for the growth [13]. Light quantity and quality reaching the seed depend upon the soil [14]. Many crops are sensitive to the salinity in the soil particularly at the stage of seed germination. It is more important to keep the salinity of the soil below the level of tolerance in the crop plant [15]. By optimizing the salt quantity in the soil, it may change in the pathways to produce useful metabolites.

Brassica rapa is a member of *Brassica* family which is like *Brassica compistris.* The general classification of the species is Kingdom Plantae, Phylum Angiosperms, Sub-phylum Eudicots, Class Rosides, Order Brassicales, Family *Brassicacea*, Genus *Brassica*, Specie *Rapa)* (adapted from Wikipedia). This plant species is found to be evolved from the mountainous regions where the temperature is usually low [16]. *Brassica* is the diverse group of crops, which have much economic importance in the world. These plants produce vegetables food of human beings, containing pickles leafy vegetables and salad [17]. The *Brassica* in known in United stated for the production of some of the important vegetables, e.g. cabbages [18]. Rapeseed oil of the *Brassica* family is one the important source of vegetable oil in the world, which contains about 40% of the oil in the seed and containing the proteins for the animals feed [19]. *Brassica* species contain the anticancer drugs [20]. For increasing the quantity of these drugs, the plants must be grown in saline and fluctuated light environments. The family of *Brassica* has antioxidant activity which prevents the human from the toxic effects of free radicles [21]. Among various plant fine chemicals, polyphenolics play a major role in controlling growth in plants and play a key role in the prevention of human diseases including cardiovascular diseases. Among the flavonoids, flavones are

responsible for plants for the interaction with other organisms such as plants, insects, and microbes. These flavonoids help in the prevention of the inflammations because they inhibit some major enzymes system in inflammatory response such as cyclooxygenases and activating some other enzymes like nitric oxide synthase [21].

Therefore, the overall objective of the current study was to investigate the synergistic/antagonistic effect of colored lights and salt stress on growth parameters of *Brassica rapa*. During synergism, the plant biomass accumulation and production was gradually investigated. Furthermore, sample was also tested for the biosynthesis of polyphenolics (phenol and flavonoid) contents. This study will help in the understanding the effect of salt stress and colored light on the biochemical pathways of hormones depended on variation in plumule and radicle growth of *Brassica rapa*.

Methods

Selection of plant and seeds

The plants from the *Brassicacea* family (*Brassica rapa*) were selected because of their medicinal importance. The seeds of *Brassica rapa* were collected from the PCSIR and were brought to the Lab of the Center for Biotechnology and Microbiology University of Swat.

Surface Sterilization of seeds

The seeds were sterilized with 70% ethanol and Mercuric chloride (0.2%) according to the protocol of Ahmad et al. [22]. Seeds were washed two to three times with distilled water after treating with chemicals to remove the chemicals and dried with filter paper to remove water droplets completely.

Seeds germination

The seed was germinated on the cotton provided as a medium for the growth of the plants. The cotton was taken in the Petri plates along on their surface with autoclaved filter paper. The sterilized seeds were inoculated on the surface of the autoclaved filter paper and were given time to germinate in a growth room. The seeds were germinated, and the plantlets were used for the calculation of results.

Synergistic combination of salt stress and different spectral lights

The normal salt (NaCl) in a concentration of 0.5gram, 1 gram, and 1.5 gram was dissolved in 200 ml of distilled water and poured in the Petri plates on cotton for the germination of the seeds respectively. The salt in different concentration was added to different Petri plates for the germination of the plants at a different rate and different value of bioactive compounds. The sterilized seeds were placed in the Petri plates under different spectral light for germination. These seeds were placed in three plates without salt under white light energy saver (25Watt) as a control. Triplicate of Petri plates of salt concentrations 0.5 g,1 g and 1.5 g per 200 ml of distilled water were placed under yellow, blue, green and red LED lights.

Biomass determination

The plants from each Petri plate were selected and its mean radicle and plumule length were calculated to get the biomass gain in the plants each day. The plant's fresh weight was calculated by sotorius analytical balance. Average weight was taken after seven readings in triplicates.

Extracts preparation

After the calculation of fresh weight, plants were then placed on the surface of the filter paper to remove water droplets. The plantlets were dried in an oven at 50 °C. After drying the plants were collected and converted into powder form by using mortar and pestle. The powder form was collected in the small test tubes labeled with the different color and salt concentration. In each tube, 5 ml of absolute ethanol was added, and the solution was placed for two weeks in refrigerator.

Analytical analysis

The total phenolics and flavonoids content of the samples were determined using Ahmad et al. [6] protocol in which two hundred microliters solution of each sample were added to Folin–Ciocalteu's reagent (1:10) followed by 7 ml of sodium carbonate (0.115 mg/mL) to the mixture. The mixture was incubated for two hours, and at 765 nm absorbance was taken. For the quantification of TFC, [6] 250 µl of sample extract was mixed with sterile water (1.25 ml) and $AlCl_3$ (0.075 ml; 5% w/v). Further 500 µl of sodium hydroxide was added to the sample mixture. The mixture was dark incubated for 30 mints and then centrifuged at 10,000 rpm for 15 min. Absorbance was observed at wavelength of 510 nm. For the calibration of the curve, Gallic acid and Rutin (1.0–10 mg/mL) were used for phenolics and flavonoids comparison. The results were then expressed in mg/g-DW.

Statistical Analysis

All the experiments were done triplicated form. The resulted data of seed germination, biomass accumulation, and polyphenolics contents were subjected to ANOVA for the mean values determination. The mean values were uploaded to the statistical software (origin 8.5) for making of graphs. Mean values are non-significant at $P \leq 0.05$.

Results

Regulation of growth behavior using yellow lights and abiotic salt stress in B. rapa

This research focuses on the synergistic/antagonistic effect of spectral lights and salt stress on growth parameters and secondary polyphenolics production in *Brassica rapa*. Here, the combination exhibited antagonistic effect on seed germination as well as on mean radicle length but showed synergistic effect on mean plumule length. After 12 days of seed incubation, 65% seeds were germinated as compared to control cultures (100%) (Fig. 1 (d & a). However, the radicle length (40 mm) of control cultures was lower than yellow lights and lower salt-induced cultures (118 mm) (Fig. 1 (b & e). Net biomass accumulation (98 mg), and mean plumule length (118 mm) was greater than control cultures (88 mg and 48 mm) as

shown in Fig. 1 (f & c) and Fig. 2. A double increase in biomass accumulation (167 mg) was observed as compared to control conditions (88 mg) and clarifies that salt lower concentration (0.5 g/200 ml) with the synergistic combination of spectral light shows the positive result in the biomass accumulation (98 mg) than higher salt concentration. As the concentration of salt was increased (1 g/200 ml), it reduces the germination rate from 81 mg to 53 mg.

Regulation of growth behavior using green lights and abiotic salt stress in B. rapa

The growth behavior in terms of seed germination, mean radicle and plumule length was investigated under the combined effect of abiotic salt stress with green lights. The seeds grown in three different salt concentrations (0.5 g/200 ml 1.0 g/200 ml and 1.5 g/200 ml) exposed to green lights. The maximum germination responses were observed on day 12th of seed inoculation. Here, the lower salt concentration exhibited 60% seed germination as compared to higher concentrations of salt (50% and 55%) under green lights. Effect of green lights synergistically enhanced the fresh biomass (167 mg) as compared to control cultures (88 m) (Fig. 2). Higher salt concentration along with green lights exhibited 50% seed germination, mean radicle length (80 mm), mean plumule length (58 mm) and the fresh weight (91 mg) of the plantlets was comparatively lower than 0.5 g/200 ml salt concentration (Fig. 1g, h, i & Fig. 2). A random growth behavior was observed when the salt concentration was increased from 0.5 to 1.0 g/200 ml. These results suggest that green lights and lower salt concentration is effective for biomass accumulation but antagonistically reduced radicle and plumule growth.

Regulation of growth behavior using blue lights and abiotic salt stress in B. rapa

The growth regulation was investigated under the blue light and abiotic salt stress, the same three concentrations (0.5 g/200 ml, 1 g/200 ml and 1.5 g/200 ml) of the abiotic salt stress was applied to the test the plant growth behavior in term of mean seed germination radicle, plumule length and mean biomass accumulation. Like yellow and green lights, seed germination started after third day of seed inoculation and maximum germination was recorded on the 12th day. Experiment results reveal an antagonistic effect in term of seed germination (50%, 75% and 55%), and radicle length (104, 65, and 80 mm) as elaborated in Fig. 1 (j & k). However, the fresh biomass (110, 105 and 57 mg) and the plumule length (65 and 40 mm) show antagonistic effect with abiotic stress and blue lights as given in Fig. 1(l) & Fig. 2.

Regulation of growth behavior using red lights and abiotic salt stress in B. rapa

The above said developmental parameter were tested for the combination of abiotic salt stress with red light. The growth condition applied were the same. The incubation time was 12 days when maximum germination was recorded. Seed germination in Salt concentrations (0.5 g/200 ml & 1.5 g/200 ml) was the same 40%. The radicle length 96 mm and 46 mm, Plumule length (80 mm & 75 mm) and fresh biomass (117 mg & 66 mg) showing the antagonistic effect (Fig. 1m, n, o & Fig. 2). 117 mg increase in biomass was recorded having salt concentration 0.5 g/200 ml under red light while in 1 g/200 ml and 1.5 g/200 ml, it was decrease to 73 mg and 66 mg which as compared to control 88 mg. The salt

concentration of 1 g/200 ml in combination with red light randomized plant behavior, germination rate 60% was enhanced, radicle length reduced from 96 mm to 88 mm, while the plumule length 58 mm and fresh biomass 73 mg was reduced.

Collaborative effect of different spectral lights and salt stress on accumulation of secondary metabolites

Plants supplemented with various concentrations of salt were grown in six different spectral lights. The overall results indicated that production of TPC increases and TFC decreases with respect to increases salt concentration. Highest phenolic (366 mg/g-DW) and flavonoid (97.13 mg/g-DW) contents were noted in the extract of dry plant grown under dark and blue lights having high and medium salt concentration respectively as compared to the rest of light regimes as describe in Fig. 3. Among all the treatments, 1.5 mg/200 mL salt concentration showed highest TPC (356 mg/g-DW) and TFC (59 mg/g-DW) under dark and blue light, followed by TPC (266 mg/g-DW) and TFC (54 mg/g-DW) under blue and green light. At medium salt concentration (1 g/200 ml) showed highest TPC (264 mg/g-DW) and TFC (21 mg/g-DW) in the Influence of darkness, followed by TPC (266 mg/g-DW) and TFC (54 mg/g-DW) under blue light. Similarly, lower salt concentration (0. 5 mg/200 ml) indicated high TPC (236 mg/g-DW) and TFC (43 mg/g-DW) in the dark, followed by TPC (136 mg/g-DW) and TFC (5 mg/g-DW) under green light. As for the dark treated sample, direct and inverse relation of salt concentration was observed with respect to secondary metabolites (TPC and TFC) production i.e. TPC accumulation was increases and TFC accumulation was retarded with increase in salt concentration. These results showed that high stress compels the plants to produce secondary metabolites.

Discussions

Khan and Ungar [23] suggested that the halophytic plant's germination is depended upon the condition of the environment. Khan and Gulzar [24] reported work supported our results in which seed germination rate in the grass was greater in the control and germination of seeds were inhibited with the increase in the concentration of salt along with spectral light. Gulzar and Khan, and Lombardi et al. [25, 26] reported that plant can tolerate 250 to 350 mmol/L of common salt but beyond this limit plant growth started signs of welting. Our experimental work results showed some variation. we use the maximum concentration of 1.5 g/200 ml of salt with spectral lights in the media showed negative affect on seed germination but showed inhibited germination at high salt concentrations which showing non-halophytic nature of *Brassica rapa.* It means that *Brassica* can tolerate salt up to some extent, but extended levels inhibited germination along with spectral lights. Gulzar and Khan, Andrews et al. and De Villiers et al. [25, 27, 28] reported in their experiments that salt-tolerant plants need varying degree of lights for their seed germination. Some need the high percentage of light some need moderate and some do not need light for their germination. In our experiment, the plant showed germination at low salt concentration. Baskin and Baskin [29] reported the germination of 23 different species treated with light and dark conditions in which seven species have shown maximum growth rate in light, eight have shown greater growth rate in

the dark while two species showing the same percentage in dark and light condition. But here, the combined effect of spectral lights and salt stress inhibited seed germination. In contrast, the seed incubated under white light without salt stress showed 100% seed germination. The possible reason is that white light is present everywhere and the plants are adapted to the white light.

The present study showing similarity with Tariq and Ali [30] reported work that the callus of *A. absinthium.* significantly increases to (90%) under white light and shows an increase to (82%) under green while the callus under dark increase to (70%). Ahmad and Rab [22] studied showed disagreement with our present results in which that white light showed increase in the biomass while in our experiment, twofold greater biomass was produced under the green light with the synergistic effect of the salt concentration (0.5 g/200 ml). Reason for the contrast is the presence of table salt and the plant species are also different. Green light with the salt of (0.5 g/200 ml) show a maximum response in biomass production and become double of the control 167 mg in (0.5 g/200 ml) salt while (1 g/200 ml) show a little increase in the biomass and become 91 mg while the high concentration shows 69 mg which is less than the control 88 mg. These results confirmed that green monochromatic light shows a maximum increase in biomass production. H. Ali and M.A. Khan [31] reported that in the vitro grown the culture of *A. bracteosa* under the dark shows the maximum biomass accumulation which is contrasted to our experiment in which maximum biomass was recorded in the green lights. In our previous study Nawaz et al. [32] reported study showed contrast results to our study that the fresh biomass was increased under white light while all other light treatment shows a decrease in the biomass.

Recorded maximum biomass in salt stress from low to high concentrations along with blue light were 110 mg, 105 mg and 57 mg respectively. The two said salt lower concentrations (0.5 g/200 ml, 1 g/200 ml) with combination of blue light enhances biomass but further increment (1.5 g/200 ml) reduced the biomass accumulation when compared with control 88 mg. H. Senger [33] reported that blue light having an effect on the development of the chloroplast in the plants, furthermore reported that blue light also affects the plants to grow the stomatal pores in the plants.

Study of Nawaz et al,. [32] investigated increase radicle and plumule length, maximum seed germination in white light and red lights in contrast to our experiment in which white and yellow light shows maximum seed germination and maximum response on the radicle and plumule length. The reason for contrast is that we applied salt stress environment.

Plants have indigenous defense mechanism encompassing huge range of molecules that benefit them to live and grow in response to different ecological environments factors including abiotic stresses (floods, radiation, salinity, drought, temperature, heavy metals) and biotic stresses various pathogens i.e bacteria, fungi, oomycetes and nematodes attack). These phytochemicals key compounds are phenols and flavonoids, which are released in unfavorable environments [34, 35]. In this research work, the effect of different light regimes and salt concentrations on biosynthesis of these metabolites was also examined.

Conclusion

We conclude from the above research work that *Brassica rapa* produce more biomass when grown under the stress condition of common salt in lower quantity. The spectral analysis indicates that green light showing their effect in the production of biomass in the *Brassica rapa.* The effect of spectral light was observed on the growth of the root and shoot length of the plant. The root length was inhibited to grow under the salt stress spectral light treatment (55 mm) and the shoot length increases up to a small limit (75 mm) when compared with the controlled conditions (110 mm for root and 48 mm for the shoot). Thus, we conclude that using spectral light and salt at room temperature have a negative effect on the seed germination of *Brassica rapa.* However, the secondary metabolites (Phenolics and Flavonoids) under salt and spectral light stress increases from normal growth.

Fazal et al [36] reported work exhibited variation in comparison to our work. They reported that the total phenolics and flavonoids content was maximum when the callus was treated with blue spectral light. Our experimental results exhibited maximum phenolics content in dark followed by blue light. The possible reason for the contrast is the differences of plant species and callus and plants from seeds. Ali & Abbasi [37] reported work supported our experimental work as applying the continuous light and dark shows a maximum production of phenolics content. Sengul et al, [38] reported that red light has the effect on the accumulation of secondary metabolites of *Artemisinin* in the *Artemisia annua*. Which contrasts with our results. The red light in our experiment has the negative effect on the accumulation of the TPC and TFC.

M. Younas et al, [39] reported that the maximum Peroxide (POD) and Superoxide (SOD) activity was recorded our experiment shows strongly relation with them. In our experiment total phenolics and flavonoids was increased when compared with the controlled white color light conditions. In our previous study T. Nawaz et al,.[32] we have reported that the total phenolics increased in all color lights. This experiment is strictly correlated with it all the light and dark show maximum response of phenolics accumulation.

List Of Abbreviations

DW Dry Weight

g gram

LED Light Emitting Diodes

mg milligram

mL milli liter

mm millimeter

PCSIR Pakistan Council of Scientific and Industrial Research

TFC Total Flavonoids Content

TPC Total Phenolics Content

W/V Weight/Volume

µl Micron liter

Declarations

Availability of data and materials

Data related to this manuscript will be available on request to the corresponding authors

Ethics approval and consent to participate

Not Applicable

Consent for publication

All the authors approved this submission to BMC Plant Biology

Competing of interest

All authors declare that they have no potential conflict of interest.

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Dong-Qing Wei

Department of Bioinformatics and Biostatistics College of Life Sciences and Biotechnology, The State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, Minhang District, China

Contact: dqwei@sjtu.edu.cn

Author's contribution

IMK and JI conducted the research experiments. NA designs the experiment and review the manuscript, WA, HR, and SSA helped in writing the manuscript, HF provide lab facilities for polyphenolics determination, AK help in data interpretation and DQW and NA critically reviewed this manuscript.

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Figures

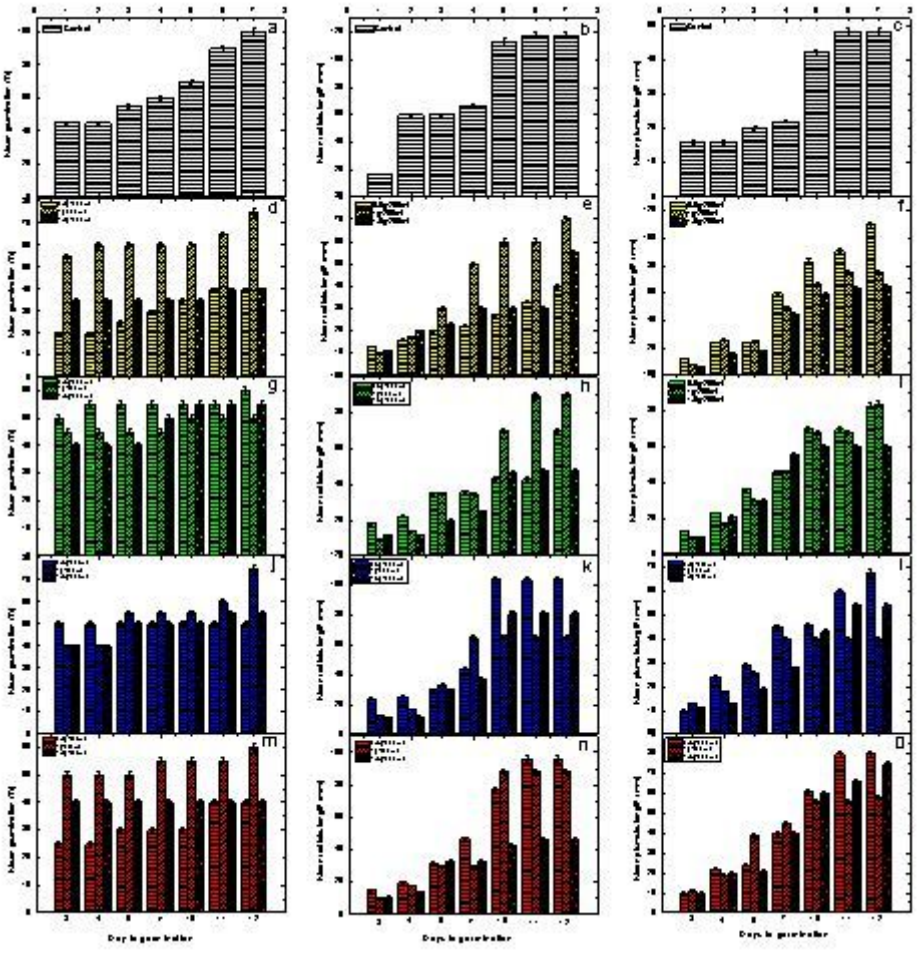

Figure 1

Showing mean germination (%) radicle and plumule length in (mm) in spectral light and salt stress.

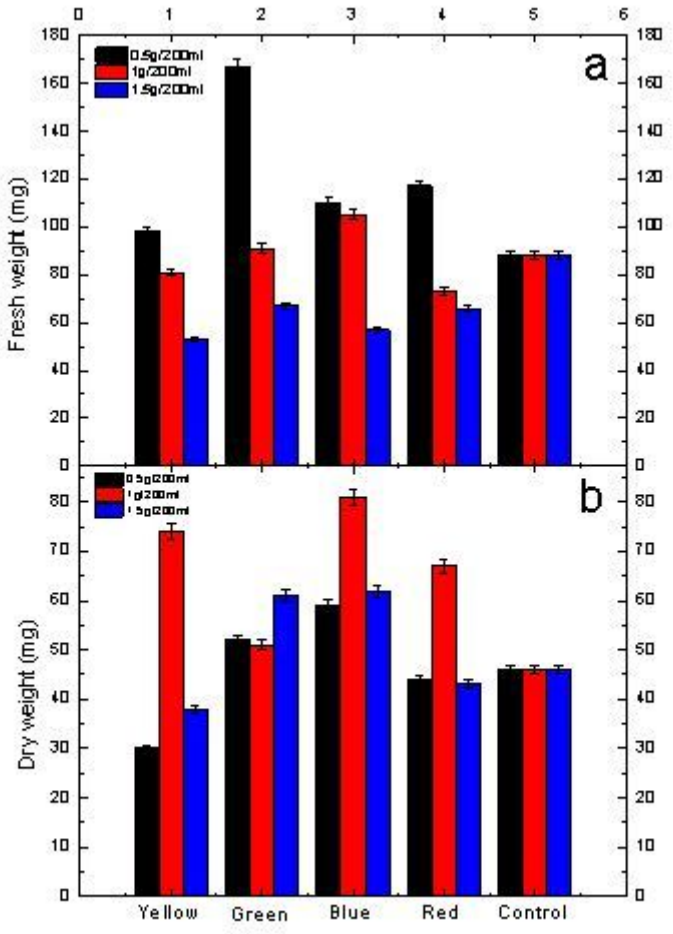

Figure 2

Showing fresh and dry weight in (mg) of the plants grown under salt and light stress.

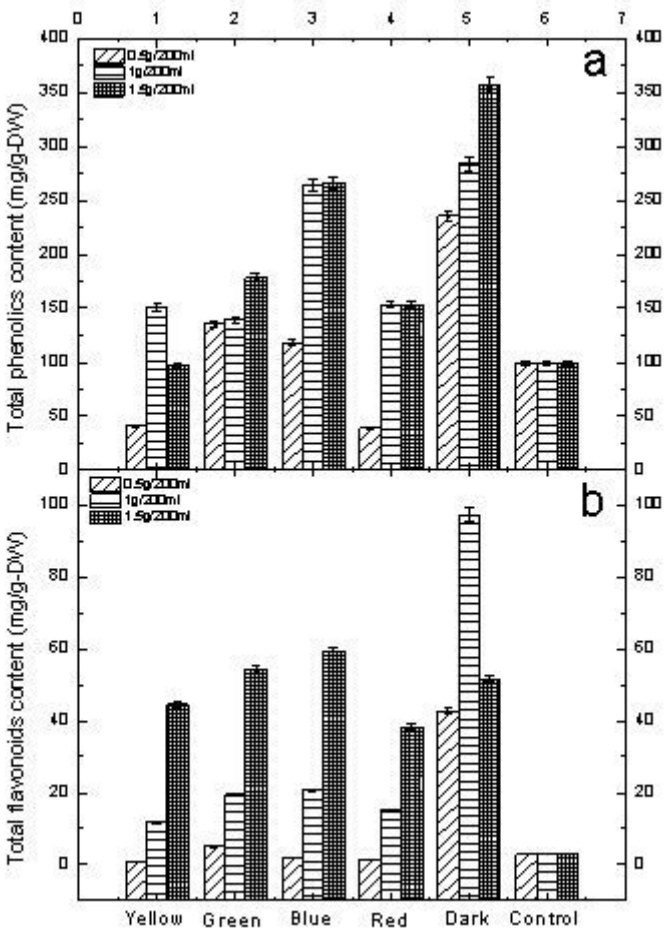

Figure 3

Showing the total phenolics and total flavonoids content in mg/g-DW.