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Effect of LED Lighting on Horticultural Traits and Pathogenicity in Citrus Nurseries

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Abstract: Day-length extension, can significantly enhance the growth and profitability of rootstock seedlings in citrus nursery. An experiment was conducted on potted citrus rootstocks seedlings in greenhouse at Horticulture Research Institute, Giza governorate, Egypt during 2021/2022-2022/2023 to determine the efficacy of extending day length at the period from May to end of January by supplemental illumination using Red -Blue light-emitting diodes (LED) at photon flux density (PPFD) range 5-8 µmol•m^{-2}•s^{-1}, on seedling growth rate, characters and leaf chemical composition, of Volkamer lemon, sour orange, Cleopatra's mandarin, and Carrizo citrange rootstocks, obtained results demonstrated that, day length extension treatment resulted in an increment in stem length and diameter, for all studied rootstock seedlings, Cleopatra's mandarin shows the highest percentage of increment for stem length and diameter, extending day length significantly increase number of leaves / plant, leaf area, seedling dry matter percentage, leaf pigment, carbohydrates and Indoles content, for all studied rootstock compared with normal lightning, Volkamer lemon rootstock record the highest significant values for all studied characters. Carrizo citrange rootstock record the highest percentage of increment in average leaf area, root dry matter, Cleopatra mandarin rootstock shows the highest percentage for total leaf carbohydrate content, for the reduction percentage of citrus leafminers larva and pupa population Volkamer lemon rootstock, recorded the highest reduction percentage for both rarval and Pupal stages, followed by sour orange root stock whereas, Carrizo citrange root stock shows the lowest reduction percentage.

Key words: Citrus・Rootstock・(LED) Light-emitting diodes・Normal lightning・Seedlings・Vegetative growth・Dry matter・Leaf pigment・Citrus Leafminers

INTRODUCTION

Global citrus production depends primarily on nursery propagation, which mainly involves producing grafted combinations of the desired scion cultivar on an appropriate rootstock. In most countries, current citrus nursery production requires potted-tree propagation in greenhouses to reduce serious risks from pest and disease, In these circumstances, there is considerable commercial pressure to maximize growth, preserve disease-free, healthy trees, and minimize the time needed to produce field-ready citrus seedling, propagation in greenhouses, Growing citrus seedling in greenhouses may resulted in significantly higher production expenses and restricted space for propagation Grower observations show that during the winter months, citrus budded trees on all rootstocks develop more slowly, but this issue was considerable for Carrizo citranges rootstocks which is account one of the important citrus rootstocks that is tolerant for tristeza virus and Phytophthora root rot. also, resistant to certain citrus nematode biotypes and Cleopatra mandarin which represent important of citrus rootstocks as it is tolerant to phytophthora foot rot, Tristeza and gummosis, but tends to have slow growth rate during nursery propagation [1, 2].It has been observed that the response of citrus species to photoperiod was typical of tropical plants, where short days usually do not cause dormancy also grown citrus plants showed that the longer the photoperiod, the greater growth occurred in a particular period of time [3]. For one-year-old satsumas Mandarin budded on trifoliate orange rootstock long day length (16-hour photoperiods) during 2 or 3 months starting from late October, increase shoot growth and tree fresh weight and decreased flower buds on spring shoots [4]. Vegetative growth of Carrizo citrange rootstock improved by transfer more reserve from roots to aboveground portions when extending day length 4hours using LED lightning with low light intensity, that activated phytochrome, causing a long day effect [5].

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The main purpose of light in plants is photosynthesis, which makes use of PAR (photosynthetically active radiation), which is composed of light wavelengths ranging from 400 to 700 nm. However, because of the absorption capacity of the light-absorbing pigments chlorophyll a and b, plants do not react to all PAR wavelengths equally. Red (600-700 nm) and blue (420-460 nm) wavelengths are the most effective for promoting photosynthesis [6]. Plant photoreceptors monitor quantity, quality, direction and period of light incident and mediate responses to light signals, photoreceptors include; phototropins cryptochromes, the Zeitlupe family, absorbing ultraviolet UV-A/blue wavelengths, and phytochromes that absorb red [7]. Light Emitting diode (LED) lighting technologies have developed quickly, which has increased their use for illumination in enclosed horticultural systems. Numerous plant subjects showed varied effects of LED light on morphology and development. Plant species express diverse responses to light quality through the photoreceptors. Plant growth and development require a combination of red and blue light in the right proportion, according to numerous studies, rather than only red or blue LED light alone [8, 9]. Plants respond differently at different wavelengths as, red light stimulate phytochrome photoreceptors that affect stem elongation and Blue light are absorbed by cryptochromes which affect the optimization of light harvesting and also absorbed by phytotropins' which affect the avoidance of photo inhibition [7]. Light Emitting Diode (LED) lights are broadly used in agriculture particularly horticulture crops, due to their higher luminous efficiency and lower power consumption.

Citrus leafminer (CLM), *Phyllocnistis citrella*, is considered one of the main pest affecting citrus industry, it invaded almost all citrus cultivars, worldwide [10]. It is considered one of the key pests of citrus nurseries, and newly planted citrus trees. Leaf miner larvae cause damage in the form of mines on immature foliage; generally, the first noticeable symptoms are twisted and curled, in cases of severe infestation, plant growth is retarded, causing serious economic effects especially in citrus nurseries industry. Seasonal incidence revealed the prevalence of citrus leaf miner activity around the year [11, 12]. The effective control of CLM is quite difficult because of its high migration ability from outside of orchards or surrounding uncontrolled fields, and the high fertility of CLM [13].

The study objective was to investigate the possibilities of using LED technology in citrus nursery, for increasing day length and to study the effect day length extension using LED illumination on the vegetative growth parameter and leaf pigment content and leaf chemical composition and on citrus leaf miner infestation for Volkamer lemon, Sour orange, Cleopatra mandarin and Carrizo citrange citrus rootstocks

MATERIALS AND METHODS

This experiment was conducted in a greenhouse in the citrus nursery of Horticulture Research Institute, Giza, Egypt during two consecutive seasons 2021/22-2022/23, on seedling for four citrus rootstocks Volkamer lemon (*Citrus volkameriana*), Sour orange (*Citrus aurantium*), Cleopatra mandarin (*Citrus reshni*) and Carrizo citrange (*Citrus sinensis* x *Poncirus trifoliata*). Seedling were exposed to two light treatments: normal lightning (NL) "no supplementary light" and day length extension using light emitting diode (LED), for light emitting diode (LED) treatment, Blue -red LED with photon flux density (PPFD, 400-700 nm) range from 5-8 $\mu mol \bullet m^{-2} \bullet s^{-1}$, wavelength 470 nm for blue and 660 nm for red, were used, illumination was during the period from sunset to sun rise. the experimental period of 9 months starts at May till the end of January, Complete randomized block design with three replicates was employed, each replicate was represented by ten seedlings thus, total number of seedlings used in the experiment was estimated as follows: 4 citrus rootstocks × 2ligth treatments ×3 replicates×10 seedlings / replicate = 240 seedlings, so 30 seedlings from each rootstock were exposed LED illumination. Seeds for rootstocks were obtained from mature homogenate fruits of adult trees. In mid-February for each season, seeds were extracted, washed and air dried, and preserved until sowing, in 4 kg plastic bags containing mix of compost and sand in ratio 1:3 by volume, all bags were irrigated immediately after sowing, and then irrigation was carried out once every two days with tap water. Agricultural practices, i.e., irrigation, fertilization, pest control, etc., were conducted as Citrus Res. Dept. - Ministry of Agriculture and land reclamation recommendations. during the two successive seasons 2021/22-2022/23. at the beginning of May,30 seedlings from each rootstock were selected carefully to be nearly homogenous and healthy and free from any infection, and placed in a greenhouse to be exposed to light treatments. For vegetative growth characters' samples were collected at the end of the following four periods:

- First period: May and June.
- Second period: July, August and September.
- Third period: October and November.
- Fourth period: December and January.

Average Day Length: Average day length was collected monthly during the experimental period in Table A, according to data of https://www.timeanddate.com [14] and Egyptian Meteorological Authority www.ema.gov.eg.

Table A: Average day length (Hours) during experimental period.

	Average day length (Hours)		Average LED illumination (hr./day)	
Months	2021	2022	2021	2022
May	13:38	13:38	10:22	10:22
June	13:56	13:59	10:04	10:01
July	13:48	13:48	10:12	10:12
August	13:10	13:09	10:50	10:51
September	12:19	12:18	11:41	11:42
October	11:26	11:25	12:34	12:35
November	10:40	10:40	13:20	13:20
December	10:18	10:18	13:42	13:42
January	10:29	10:28	13:31	13:32

Vegetative Growth Characters: At the end of each of the 4 periods of study, stem length (cm) and stem diameter (mm), were measured and values were expressed as percentage of increase in both seedling stem length and diameter by using the following formulae:

$$\text{Increase in seedling stem lenth (\%)} = \frac{\text{Length at period end - Length at period beginning}}{\text{Length at period beginning}} \times 100$$

$$\text{Increase in seedling stem diameter (\%)} = \frac{\text{Diameter at period end - Diameter at period beginning}}{\text{Diameter at period beginning}} \times 100$$

Five leaves per plant from each replicate were picked from the middle part of plant for leaf area (cm^2) measurements and number of leaves per plant for each replicate were determined at the end of the two experimental seasons.

Leaf Chemical Composition: Plant pigments Chlorophyll a, b, total chlorophyll and total carotenoids were measured by spectrophotometer in fresh leaves according to Nornai [15]. Total carbohydrates in dry matter of leaves were determined by using 3,5-dinitrosalicylic acid according to Miller [16]. Total indoles were determined in fresh leaves according to Larsen [17]. Total phenols were determined in fresh leaves according to Swain and Hillis [18]. N, P and K contents (%) were determinate in dried leaves samples. Total N% was determined by semi-micro Kjeldahl method described by Plummer [19]. Phosphorus was estimated colorimeterically using method described by King [20]. Potassium concentration was determined by using the flame photometer by Piper [21].

Grafting Success Percentage: Grafting was performed during 15th of February for each season of study, for rootstock seedling with stem diameter between are 1/4 to 3/8 inch, which represent suitable diameter for budding citrus rootstocks [22]. The number of days until seedlings are suitable for grafting was recorded by calculating the number of days from the beginning of May until seedlings reach the suitable stem thickness using monthly data recorded for seedling stem diameter for both seasons.

Grafting success percent, was recorded 30 days after Grafting using the following equation [23]:

$$\text{Grafting Success (\%)} = \frac{\text{Number of sprouted grafts}}{\text{Total Number of grafts}} \times 100$$

The total number of grafts represented the number of citrus rootstock seedlings that attained the stem thickness appropriate for grafting on February 15 from both normal lighting and LED treatments for both seasons.

Citrus Leafminer (CLM), Invasion Parameter: Leaf samples were collected at the end of the experimental period for both study seasons. Samples were kept in polyethylene bags for inspection in the laboratory by means of a stereoscope microscope. live individuals (larvae and pupae) in each sample were counted and categorized, the reduction percentage of the alive larva and pupa population was estimated according to the Henderson and Tilton equation [24].

Statistical Analysis: The experiment was organized as a complete randomized blocks design and the collected data were statistically analyzed according to Lane *et al*. [25]. Means of treatments were compared using New L.S.D at 5% level of probability by Steel and Torrie [26].

RESULTS AND DISCUSSION

Seedling Growth Rate: Rate of seedling growth was determined by measuring the percentage of increment in both seedling stem length and diameter at the end of each of the four studied period. Obtained results indicate that, all studied rootstock seedlings follow a specific trend of growth, as rate of seedling stem length increased

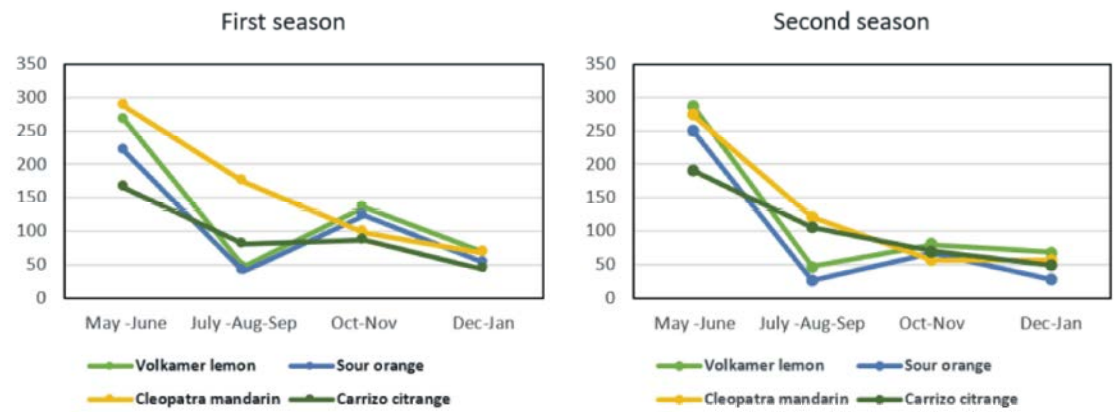

Fig. 1: Effect of LED illumination on percentage of increase in stem length.

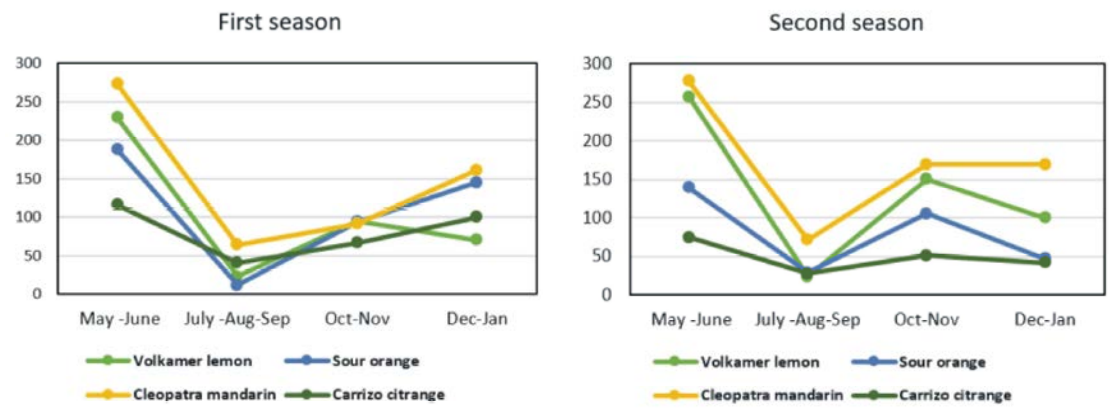

Fig. 2: Effect of LED illumination on percentage of increase in stem diameter.

expressively during the months of May and June and then the increment rate decreased sharply in in the period from July until January, the percentage of increment in seedling stem diameter follows the same pattern as for stem length. Concerning the percentage of increase in stem length, Fig. (2) and (3) reveals that, LED treatment cause expressive increase in the percentage of stem length and diameter increment as compared with normal day light. The highest percentage of increment in stem length was recorded for Cleopatra mandarin rootstock, during the first two periods of study from May till end of September whereas, for months from October till end of January, Volkamer lemon rootstock show the highest value, for both seasons of study. The observed decline in growth for Carrizo rootstock during winter months is possibly related to the concept that trifoliate-type rootstocks respond to photoperiod and grow slowly on short days (photoperiods <12 h) due to their deciduous habit [27].

Obtained results is reinforced by the finding that, under short days (10 h photoperiod) at 28/21°C day/night, over the course of 14 weeks' growth Swingle and Carrizo, rootstocks' decreased, whereas, for exposing plants to long days (14 h photoperiod) using combination of incandescent and fluorescent lamps, average growth was significantly higher by about 170% [3].

The decline in seedling growth rate in the period from July to September may be related to the great increase in temperature during July, August and September months, which reach inside the green house an average of 47°C -51°C and 45°C-48°C respectively, for both seasons for about 5 to 6 hours /day, which represent unfavorable temperature for seedling growth, as high temperatures may subject photosynthesis process to excessive stress, as photosystem II (PSII) activity rapidly decreases, causing gradual and reversible decline in photosynthetic efficiency, results in a partial loss of the photosynthetic ability, Conserving leaf exposure to excessive radiation result in photooxidation, which happens after photoinhibition, causing destruction of photosynthetic pigments (photosystem II (PSII)), which can lead to discoloration and even cell death [28, 29] . In the same line Studies, in growth chambers, show that,

Table 1: Effect of LED illumination on number of leaves / seedling and leaf area during two successive seasons.

	Rootstocks									
Treatment	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
	1st season					2nd season				
	Number of leaves / seedling									
NL	19.8	11.3	12.8	18.4	15.6	20.4	17.8	16.1	15.0	17.3
LED	34.1	21.0	22.4	31.0	27.1	37.5	27.7	27.9	24.9	29.5
AV.	27.0	16.2	17.6	24.7		29.0	22.8	22.0	20.0	
new LSD 0.05	A=1.57	B=1.11	AXB=2.22			new LSD 0.05	A=1.78	B=1.26	AXB=2.53	
	Leaf area (cm^2)									
NL	15.7	21.1	9.6	7.9	13.6	17.4	19.8	11.3	9.5	14.5
LED	23.8	30.2	13.3	12.3	19.9	26.4	31.7	17.5	15.5	22.8
AV.	19.8	25.7	11.5	10.1		21.9	25.8	14.4	12.5	
new LSD 0.05	A=2.01	B=1.42	AXB =2.84			new LSD 0.05	A=2.03	B=1.44	AXB=2.87	

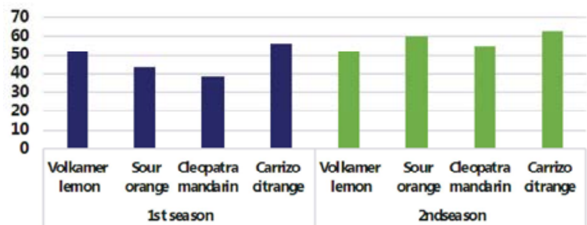

Fig. 3: Effect of LED illumination on percentage of increase in leaf area.

exposure of, Carrizo citrange, Citrus aurantium, Citrus reticulata rootstocks seedling to sublethal high temperature 38° day/28°C night, during 2.5 months' cause, aberrant of seedling growth, decreased shoot elongation and production of smaller and, broader leaves [26]. However, reduction in growth during November, December and January may be due to the decrease in temperature and shortening in day length, in reaction to lower temperatures and a shorter photoperiod (length of days), plants often grow more slowly throughout the winter. A reduction in the number of hours of sunshine that plants require to photosynthesize and produce the carbohydrates necessary for survival is one way that day length might impact plant growth [5]. The results are hardened by documentation that the highest photosynthetic rates were at 25°C. while minimum values were observed at 40°C, increase leaf temperature from 25 to 40°C cause considerable reduction in CO_2 assimilation. The highest CO_2 assimilation values were reached in the 35/20°C [30]. The role of LED illumination in reducing the decline in growth rate during November, December and January may be related to that applying low-intensity supplemental illumination stimulates phytochrome to override growth termination during short days leading seedling to start utilizing assimilates for vegetative growth instead of storing them [31].

Number of Leaves / Seedling and Leaf Area: It is clear from data presented in Table (1) and Fig. (3) that, exposure of rootstock seedling to LED light significantly increased number of leaves per seedling and leaf area for all studied rootstocks, however, Volkamer lemon rootstock holds the greatest significant number of leaves per seedling, whereas Sour orange rootstocks, shows the highest significant increase in leaf area, for both seasons. As to percentage of increment in number of leaves per seedling, sour orange rootstock records the greatest values for first season while Volkamer lemon rootstock shows the greatest percentage for second season. Concerning percentage of increase in average leaf area for LED exposure treatment Carrizo citrange rootstock shows the highest percentage of about a 56%, 63% respectively for both seasons. Obtained results is reinforced by previous studies on Carrizo citrange and Swingle citrumelo reporting that expansion of day length by LED illumination overcome the effect of short-day stimulating vegetative growth consequently increased number of leaves [27]. In the same line, for lettuce plant it was observed that, at low growth temperatures, plant LED lightning as supplementary light during winter at different intensities resulted in increasing effective quantum yield of photosystem II, net photosynthetic rate, transpiration rate and stomatal conductance, which mainly increase vegetative growth consequently increase plant leaf area and leaf number [32]. Whereas for pea seedlings red light (625-630 nm) illumination exhibited significant increment in leaf area and stem length, while blue light (465-470 nm) illumination increased significantly stem length and seedling weight [33].

Leaf, Stem and Root Dry Matter Percentage: Data in Table (2) showed that, for all studied rootstocks exposure to LED light significantly increase leaf, stem and root dry matter percentage, and the highest significant values were

Table 2: Effect of LED illumination on leaf, stem and root dry matter percentage during two successive seasons.

Rootstocks											
Treatment	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Rootstocks Treatments	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
	1st season					2nd season					
Leaf dry matter (%)											
NL	28.4	24.7	15.9	10.9	19.9	NL	25.7	21.3	13.9	11.1	18
LED	45.9	41.8	26.1	18.1	32.9	LED	35.6	37.2	23.2	18.91	28.7
AV.	27.7	33.7	22.4	13.7		AV.	30.65	29.25	18.55	15.005	
new LSD 0.05	A=1.25	B=0.88	AXB =1.77			new LSD 0.05	A=1.61	B=1.14	AXB =2.28		
Stem dry matter (%)											
NL	37.6	33.3	28.1	20.8	30	NL	42.3	29.7	23	18.9	28
LED	61.1	57.6	48.1	35.3	50.5	LED	63.5	52.7	40.1	32.2	47
AV.	49.35	45.45	38.1	28.05		AV.	52.9	41.2	31.55	25.55	
new LSD 0.05	A=1.57	B=1.11	AXB = 2.2			new LSD 0.05		A=1.48	B=1.05	AXB=2.1	
Root dry matter (%)											
NL	26.4	21.3	16.2	10	18.4	NL	23.9	25.1	12.6	9.9	17.8
LED	38.4	34.8	25.9	17.3	29.1	LED	33.6	42.1	20.7	16.8	26.5
AV.	32.4	28.05	21.05	13.65		AV.	28.75	33.6	16.65	13.35	
new LSD 0.05	A=1.34	B=0.95	AXB = 1.89		new LSD 0.05		A=1.68	B=1.19	AXB =2.38		

recorded for Volkamer lemon rootstock in both seasons. Concerning percentage of increase in leaf and stem dry matter, as result to LED illumination, sour orange rootstock shows the highest percentage of increment for both seasons, whereas, the lowest increment percentage recorded for Volkamer lemon. For root dry matter increment percentage, the highest increment percentage were recorded for Carrizo citrange (73% and 70%), for both seasons of study. That stated increment in dry matter percentage may be linked to the relationship between light and nitrogen and carbon metabolism pathway gene expression, as alterations in light conditions quality and photoperiod might induce the regulation of photosensitive pigments on enzymes metabolizing sucrose, increase the activities of sucrose metabolizing enzymes, and increase plantlet photosynthate accumulation [34, 35]. Obtained results are in agreement with experimental result of extending of day length by 4 hours using LED at PPFD of 10 $\mu mol•m^{-2}•s^{-1}$ cause increase in leaves and shoot dry weight of budded Carrizo citrange plants [5]. In the same concern, for lettuce plants, mix of red and blue LED spectra with ratio red/blue (3:1) (Red: Blue, wave length peak at 656 nm), improve shoot and root dry mass and fresh weights of plants [36]. *Cunninghamia lanceolate,* timber species, seedling exposed to LED 20 $\mu mol•m^{-2}•s^{-1}$ irradiance 16 h photoperiod had higher seedling height, extended roots, more number of roots [37]. Light treatment increase root length for apple seedlings, which may be due to the increasing in seedlings shoot height and leaf number, as root growth and shoot growth of a plant are related, balancing source: sink ratio of a plant is influenced by root growth, nutrient uptake, and leaf carbohydrate accumulation [38, 39]. Pepper plant grown in a greenhouse during winter season, under supplementary lighting using LED 65 $\mu mol•m^{-2}•s^{-1}$, blue LEDs with peak wavelength 460 nm and red LEDs with peak wavelength 660 nm and R:B ratio of 6:3 shows increase in plant dry mass production, and encourage in fruit dry mass partitioning, an increment about 303.3 % in Pepper fruits fresh weight of and 501.3 % increase in dry mass, demonstrating that blue light plays an important role in helping to assimilate products to the fruit and facilitate leaf expansion, and red light promotes elongation of the plant stem and dry matter accumulation [40].

Leaf Chemical Composition:

Leaf Pigment Content: Data in Table (3) and Fig. (4 and 5) indicated that, for all rootstocks under study, exposure to LED illumination significantly increase leaf chlorophyll a (chl.a), chlorophyll b (Chl b), total carotenoids and total chlorophyll content compared to normal daylight,, for both seasons, Volkamer lemon rootstock showed the highest significant increment for both chl.a , Chl b content, total carotenoids and total chlorophyll content while Carrizo citrange rootstocks showed the lowest significant values, moreover, Volkamer lemon rootstock shows the highest percentage of increment in leaf chl.a content (51% and66%) whereas, for leaf chl.b content Cleopatra mandarin rootstock shows the highest percentage of about,(60% and 61%) respectively for both seasons, whereas for both total carotenoids and total chlorophyll content Cleopatra mandarin rootstock shows

Table 3: Effect of LED illumination on chlorophyll a and chlorophyll b content in leaves during two successive seasons.

	Rootstocks									
Treatment	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
	1st season					2nd season				
	Chlorophyll a content (mg/g fresh weight)									
NL	0.91	0.93	0.73	0.61	0.8	0.84	0.9	0.66	0.63	0.76
LED	1.37	1.07	0.98	0.83	1.06	1.41	1.15	0.91	0.85	1.08
AV.	1.14	1	0.86	0.72		1.13	1.03	0.79	0.74	
new LSD 0.05	A=0.04	B=0.03	AXB=0.05	new LSD 0.05		A=0.02	B=0.01	AXB=0.01		
	Leaf area (cm^2)									
NL	0.43	0.38	0.3	0.33	0.36	0.47	0.4	0.31	0.34	0.38
LED	0.63	0.59	0.48	0.43	0.53	0.66	0.62	0.5	0.44	0.56
AV.	0.53	0.49	0.39	0.38		0.57	0.51	0.41	0.39	
new LSD 0.05	A=0.05	B=0.03	AXB =0.07			new LSD 0.05	A=0.02	B=0.03	AXB=0.03	

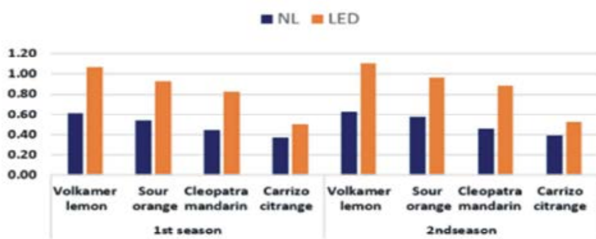

Fig. 4: Effect of LED illumination on leaf total carotenoids content (mg/g F.W.).

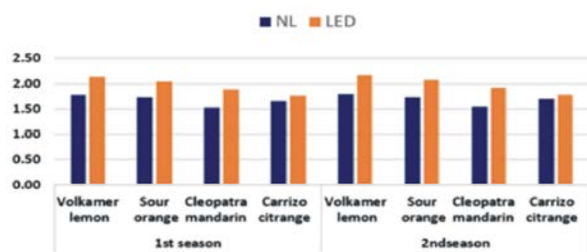

Fig. 5: Effect of LED illumination on leaf total chlorophyll content (mg/g F.W.).

the highest percentage of increment followed by Volkamer lemon rootstock .previous studies, stated that β-carotene content in stems and leaves of pea seedlings was higher at treatment with red light than blue light [33]. In the same concern irradiation of Satsuma mandarin with red at 660 nm LED lights at an intensity of 50 µmol•m^{-2}•s^{-1} for 6 days at 20°C increase flavedo β-carotene content, that response may be attributed to the simultaneous increases in the expression genes encoding enzymes for the main steps of carotenoid metabolism [41]. Exposing *Cunninghamia lanceolate*, timber species, seedling to LED 20 µmol•m^{-2}•s^{-1} irradiance 16 h photoperiod resulted in highest chlorophyll a, chlorophyll b, total chlorophyll leaf content [38]. Results demonstration that low intensity, LED blue lights 462 (nm)with photosynthetic photon flux density 50 µmol•m^{-2}•s^{-1} increase the genes expression encoding chlorophyll and anthocyanin biosynthetic pathways in sweet potato, Moreover, by exposing sweet potato leaves to red and blue LEDs, a total of 615 genes were differentially expressed. Among these, 510 genes are differentially expressed in leaves exposed to blue light [42].

Leaf Total Carbohydrates: It is obvious from data in Table (4) that, exposure rootstock seedlings to LED light treatment significantly increased leaf total carbohydrates for all studied rootstocks, and the highest significant value was recorded for Volkamer lemon rootstock and the lowest significant value was reported for Carrizo citrange for both seasons. Moreover, concerning the percentage of increase in leaf total carbohydrates Cleopatra mandarin rootstock shows the highest percentage of increment in leaves total carbohydrates for lightning extension compared with normal lightning 47%, 48% for both seasons, respectively. Trees grown under LD conditions had the highest soluble sugar concentration [88.78 mg g^{-1} dry weight (DW)] previous studies reported that for Carrizo citrange rootstock grown at 10 h LED illumination at PPFD of 500 µmol•m^{-2}•s^{-1} extension of day length by 4 h using LED illumination at 10 µmol•m^{-2}•s^{-1} improve seedling vegetative growth, that increment may be due to the effect of day light extension which activated phytochrome by low light intensity, which consequently induced a long day effect and promote translocation of carbohydrate reserves from roots to aboveground portions [5]. In the same concern, in apple seedlings, light quality affects the actions of carbon metabolism key enzymes and influences photosynthetic product accumulation, as apple seedling exposed to red light with 664 nm or blue light with 446 nm maximum emissions

Table 4: Effect of LED illumination on leaf total carbohydrates (g/100g D.W.).

Rootstocks										
Treatment	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
	1st season					2nd season				
	Leaf total carbohydrates (g/100g D.W.)									
NL	15.4	14.5	8.9	6.6	11.3	18.7	16.3	10.3	9.5	13.7
LED	21.8	18.1	13.0	9.7	15.7	25.9	21.9	15.3	13.9	19.3
AV.	18.6	16.3	10.9	8.2		22.3	19.1	12.8	11.7	
new LSD 0.05	A=1.57	B=1.11	AXB=2.22			new LSD 0.05	A=1.78	B=1.26	AXB=2.53	

Table 5: Effect of LED illumination on leaf Indoles (mg/100g F.Wt)and phenols content (mg/g F.Wt)

Rootstocks										
Treatment	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
	1st season					2nd season				
	Indols (mg/100g F.Wt)									
NL	0.4	0.3	0.2	0.3	0.3	0.4	0.3	0.2	0.3	0.3
LED	0.7	0.5	0.4	0.5	0.5	0.7	0.5	0.5	0.5	0.5
AV.	0.5	0.4	0.3	0.4		0.5	0.4	0.3	0.4	
new LSD 0.05	A=0.04	B=0.03	AXB = 0.05	new LSD 0.05		A=0.03	B=0.02	AXB = 0.05		
	Phenols (mg/g F.Wt)									
NL	0.8	1.0	1.4	1.5	1.2	0.8	0.9	1.3	1.1	1.1
LED	0.5	0.5	0.7	0.8	0.6	0.4	0.5	0.6	0.8	0.6
AV.	0.6	0.7	1.0	1.2		0.6	0.7	1.0	0.9	
new LSD 0.05	A=0.27	B=0.19	AXB = 0.38			new LSD 0.05	A=2.03	B=1.44	AXB=2.87	

treatments, results show that blue light positively affects the accumulation of soluble sugars and photosynthetic products, whereas exposure to red light promotes sucrose phosphate synthase enzyme activity. More over in this study in order to analyze the (DEGs) differentially expressed genes correlated to the red and blue light treatments, RNA-seq analysis profiles were conducted to leaves exposed to blue light and red light, analysis indicated that both red light and blue light can considerably up-regulate the expression of genes associated to nitrogen and carbon metabolism [43].

Leaf Indoles and Phenols Content: Concerning Indoles and phenols leaf content, achieved results, in Table (5), demonstrate that, total Indoles increased significantly by LED illumination treatment, for all studied rootstock, at the two successive seasons, whereas Volkamer lemon rootstock showed the highest significant values, followed by sour orange rootstock, for total phenols obtained results reveal that LED illumination treatment significantly reduce total phenol concentration in leaves and Volkamer lemon rootstock showed the lowest significant values, followed by sour orange rootstock, obtained results is in agreement with the findings that Growing soybean under LED blue light (470 nm) produced higher levels of phenol, total isoflavones, and antioxidant capacity than under LED green (530 nm) or fluorescent light [44]. evaluating the way various light-emitting diodes (LEDs) influence the build-up of phenolic compounds in tartary buckwheat sprouts. According to the gene expression results The usage of LED lighting increased the amount of phenolic compounds in Tartary Buckwheat sprouts, highest content was detected with Red + Blue and white lights [45].

Leaf Mineral Concentration: Data in Table (6) revealed that LED illumination treatment increased significantly nitrogen, potassium and phosphorus concentration in leaf for all studied rootstock, at the two successive seasons, in this concern there has been a new focus on investigating the mechanisms for nutrient absorption and use in crop plants under various light conditions. there are diversities of mobile signaling molecules from the shoot to the root that activate the nutrient use-associated genes expression or may altering the distribution of roots in the soil and increasing the possibility of absorbing nutrients. The explanation for the increase in macronutrients under the effect of diverse light treatments is related to the fact that the biosynthesized carbohydrates are transmitted to the sink tissues, which include the leaves and roots, which provides the roots and lateral roots with the energy they need to absorb

Table 6: Effect of LED illumination on leaf mineral concentration

N %											
1st season						2nd season					
Rootstocks Treatments	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Rootstocks Treatments	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
NL	1.78	1.65	1.46	1.54	1.61	NL	1.80	1.68	1.48	1.56	1.63
LED	2.51	2.44	2.41	2.35	2.43	LED	2.54	2.46	2.43	2.37	2.45
AV.	2.15	2.04	1.94	1.95		AV.	2.17	2.07	1.96	1.97	
New LSD 0.05	A=0.04	B=0.03	AXB=0.06			New LSD 0.05	A=0.05	B=0.04	AXB=0.08		
P %											
1st season						2nd season					
Rootstocks Treatments	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Rootstocks Treatments	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
NL	0.10	0.08	0.06	0.05	0.07	NL	0.12	0.09	0.07	0.06	0.09
LED	0.18	0.16	0.14	0.10	0.15	LED	0.20	0.17	0.15	0.11	0.16
AV.	0.14	0.12	0.10	0.08		AV.	0.16	0.13	0.11	0.09	
New LSD 0.05	A=0.02	B=0.02	AXB =0.03			new LSD 0.05	A=0.01	B=0.01	AXB = 0.02		
K%											
1st season						2nd season					
Rootstocks Treatments	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.	Rootstocks Treatments	Volkamer lemon	Sour orange	Cleopatra mandarin	Carrizo citrange	AV.
NL	0.91	0.82	0.73	0.60	0.77	NL	0.92	0.84	0.75	0.62	0.78
LED	1.25	1.20	1.12	1.00	1.14	LED	1.26	1.21	1.15	1.01	1.16
AV.	1.08	1.01	0.93	0.80		AV.	1.09	1.03	0.95	0.82	
New LSD 0.05	A=0.04	B=0.03	AXB = 0.06			A=1.68	A=0.05	B=0.04			
NL Normal lightning						LED light-emitting diode					

water and nutrients. The uptake of nutrients by plants is directly impacted by light indicators, such as light wavelength, intensity, and duration. This increases the amount and efficiency of nutrients that are utilized by plants [46]. Confirming previous documentation, for tomato seedlings, low irradiance-long photoperiod treatments resulted in the maximum uptake of main ions, followed by natural light and high irradiance-short photoperiod treatments, and for lettuce grown udder LED with different spectral ranges, combination of blue and red LED illumination showed significant increment in nitrogen, potassium, phosphorus and calcium content in lettuce plants [47, 48].

The observed effect of supplementing LED illumination on citrus rootstock seedling growth rate, dry matter, and leaf carbohydrate content may be primarily related to an increase in photosynthetic activity due to the extension of day length by LED illumination during a period of unfavorable environmental conditions for an efficient photosynthesis process during the winter and high temperature periods. Besides, it may possibly be related to the influence of LEDs on gene expression of different plant metabolic pathways, recently regulation of gene expression by LED has been focused, influence of LED on gene expression has been described in many respects, as photoreceptors and auxin-receptive factors, Specifically, red, blue, and white LEDs, in combination and/or individually, control the expression of vital regulatory genes for numerous plants metabolic pathways [49]. Distinct response in growth and photosynthesis under different LED treatments may contribute to the fact that gene expression under different lights was closely associated with pathways such as high-light response and light stimulus, leaf development, root growth, synthesis and degradation of carbohydrate, photosynthesis, photosynthesis-antenna proteins, plant hormones, secondary metabolism, and antioxidant capacity [50].

The Number of Days until Seedlings Are Suitable for Grafting: According to Data Recorded for Seedling Stem Diameter, it could be noticed that, for normal lightning (NL) treatment, both Sour orange and Cleopatra mandarin did not attain the suitable stem thickness for grafting on February 15th. Also, about 20% and 18% of Volkamer lemon seedlings and 15% and 13% of Carrizo citrange

Table 7: Days for seedlings suitability to graft for LED treatment.

Rootstocks	Days until suitability for grafting
Volkamer lemon	150
Sour orange	180
Cleopatra mandarin	180
Carrizo citrange	150

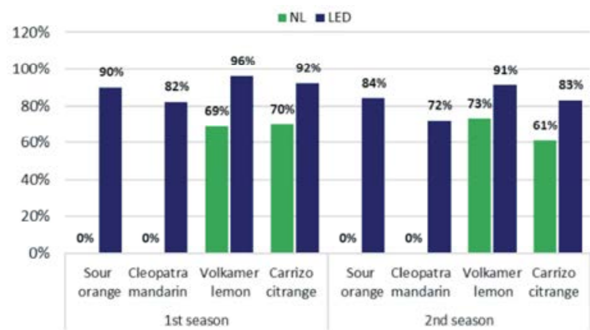

Fig. 6: Effect of LED illumination on Percentage of grafting success.

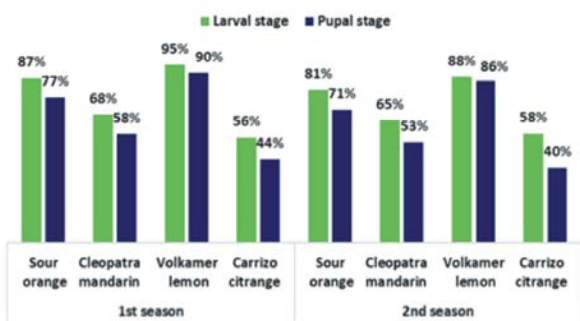

Fig. 7: Effect of LED illumination on the reduction percentage of larva and pupa population.

seedlings respectively for both seasons, attained a suitable stem diameter at the grafting date. Whereas, LED treatment result in decreasing number of days needed by seedling to reach suitable stem diameter as shown in Table (7) Volkamer lemon and Carrizo citrange seedling attained grafting stem thickness after 150 days from transplanting to illuminated green house at beginning of May, while Sour orange and Cleopatra mandarin seedling needs about 180 days to achieved suitable grafting thickness, this reduction in days to grafting suitability is mainly related to the effect of LED illumination on enhancement of seedling growth and consequently, increase the stem thickness by allowing seedlings to perform the photosynthesis process during periods of high temperature, as the temperature measured in the green house may reach more than 45°C. High temperatures decrease photosystem II (PSII) activity, and if continued for a long period, it may lead to the destruction of the photosystem II (PSII) structure, which severely affects seedling photosynthesis process efficiency [29, 30]. Moreover, the effect of LED illumination on increasing seedling vegetative growth may be related to what has been reported in other studies: that in young citrus trees grown in containers, carbohydrates are stored in the roots during short-day photoperiods, causing vegetative growth restriction and low-intensity (10 $\mu mol \bullet m^{-2} \bullet sec^{-1}$) illumination during the short-day period triggered phytochrome signaling, which led to the transition of the red-light absorbing form of phytochrome to the far-red lighting absorbing form, reversing the short-day vegetative growth's termination [5, 51].

From an economical point of view, LED treatment reduces mainly the days until suitability for grafting, thus lessening the period of time seedlings spend in nursery, consequently reducing the cost of citrus seedling production. This reduction markedly appears for a Cleopatra mandarin seedling, which attains grafting after about 180 days under LED treatment, Table (7), whereas under normal conditions, it takes three to four times this period to be suitable for grafting, this raises the expenses associated with producing Cleopatra mandarin seedlings, including those related to fertilizer, irrigation, and pest control, in addition to labour expenses within that period of time, keeping in consideration the low cost of establishing and using an LED lighting system in a greenhouse during that period.

Percentage of Grafting Success: For grafting success, reported date in Fig. (6) reveal that, extending daytime lighting by LED illumination treatment increased significantly the percentage of grafting success for all studied rootstocks for both seasons, and the highest significant percentage of success was recorded for Volkamer lemon rootstock at 96% and 91%, followed by Sour orange rootstock at about 90% and 84% for both seasons, respectively. However, for normal lightning treatment, 20% of Volkamer lemon seedlings and 15% of Carrizo citrange seedlings attained a suitable stem diameter at the grafting date, also results reported in Fig. 7 show that for Volkamer lemon rootstock, the percentage of grafting success was 69% and 73% out of the Volkamer lemon seedlings, which are suitable for grafting at February 15th, respectively, for both seasons, and for Carrizo citrange seedlings, the percentage of grafting success was 70% and 61% out of the Carrizo citrange seedlings, which are suitable for grafting at 15th February, respectively for both seasons and Carrizo citrange seedlings percentage of grafting success were

70% and 61% out of the Carrizo citrange seedling which are suitable for grafting at 15thFebruary for both seasons respectively.

The Reduction Percentage in Citrus Leafminer Larva Stage and Pupa Stages Population: Reported data reveal that, Volkamer lemon rootstock, recorded the highest reduction percentage for both rarval and Pupal stages (95%, 90% and 88%, 86%) respectively for both seasons, followed by sour orange root stock whereas, Carrizo citrange root stock shows the lowest reduction percentage (65%, 44% and 58%, 40%) respectively for both seasons followed by Cleopatra mandarin root stock which recorded (68%, 58% and 65%, 53%) respectively for both seasons. Table (8) studding the correlation between CLM infestation percentage and different rootstocks disclose that, Volkamer rootstock highest infested rootstock followed by Cleopatra mandarin then Troyer citrange and Sour orange rootstocks [52]. The reduction in percentage leaf miner population due to LED treatment confirmed by published review assuming that, lighting is used during night in nurseries to deter adult citrus leaf miners [53]. However, the reason of that respond may be related to that, citrus leaf miner adults appear around dawn and are active in early morning and at night, also females lay eggs at night [54] .Night lightning disturbed the insect Circadian rhythms, as in insects timing of several behaviors and insect physiological status are determined by the day and night, light dark cycle, in the insect nervous system a biological clock cells that manage these behaviors and lightning during night reset the biological clock, causing confusion in the timing of activities [55].

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