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Contents

Chitosan coating for extending postharvest quality of tomatoes (Lycopersicon esculentum Mill.) maintained at different torage temperatures

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ABSTRACT

The growing consumer demand for produces without chemical residues has focused efforts on the assessment of innovative natural antimicrobials. In this context, chitosan, derived from abundantly available chitin sources such as crab, shrimp and insects, has been reported to possess an excellent film-forming ability and inherent antimicrobial properties suitable for development of edible antimicrobial films. Thus, the present study was established to study the effect of chitosan coating on extending postharvest quality of fresh tomatoes (Lycopersicon esculentum Mill. cv. 'Diamentino') maintained at two different storage temperatures (5 °C with 90% relative humidity and 21 °C with 65% relative humidity). Coating the tomatoes with chitosan solutions reduced the weight loss, with greater effect at 1% than 0.5 or 2% concentrations. Chitosan-coated tomatoes were firmer, higher in titratable acidity, and exhibited less biochemical changes than the control fruit at the end of storage. The loss in visual quality was significantly reduced by coating the fruits with chitosan solutions of 0.5, 1.0 and 2.0% as compared to the control. Among the applied concentrations, chitosan at 1% can be recommended as it was pioneering for most of the parameters analyzed during cold storage at both 5 °C for 20 d and at 21 °C for 10 d. Due to its lower cost and convenience to human health, chitosan may be one of the attractive and effective biopolymers for achieving adequate conservation of fresh tomatoes.

Keywords: *chitosan; edible coating; Lycopersicon esculentum Mill.; storage*

1. Introduction

Tomatoes are frequently consumed as they represent the predominant source of antioxidants which possess pivotal role in inhibiting oxidative stress, improving vascular function, and preventing cardiovascular disease in humans [1]. Carotenoids (lycopene, β-carotene, and lutein) and flavonoids in tomatoes have been confirmed as essential polyphenols in conferring antioxidant benefits [2,3]. However, relatively short shelf life of tomatoes limits the long distance commercial transport and availability of this produce around the year. As stated by Benhabiles et al. [4], postharvest losses of tomatoes may drastically reach up to 50% of total production in countries where harvest amount peaks in short period.

The prevalent method of maintaining postharvest quality of horticultural commodities is the use of moderately low temperatures around 0–1 °C. For certain horticultural produces such as tomatoes, however, low temperatures induce chilling injury [5]. Therefore, such produces are inevitably stored at higher temperatures which, on the other hand, accelerates the senescence and postharvest quality loss.General results of previous studies revealed that optimum temperatures for storage of red and mature green tomatoes are 5 °C [6] and 13 °C [7], respectively. Besides low temperature, packaging materials and edible coatings provide a means to protect and distribute foods. They play a significant

role in how these products reach the consumers in a safe and wholesome form without compromising quality. There is a worldwide trend to explore innovative alternatives that control postharvest quality loses, giving priority to methods that reduce decay incidence and avoid side effects on human health resulting from excessive application of synthetic fungicides. Recent studies focused on biodegradable feature of natural compounds derived from plants and animals. Chitosan, as high molecular polymer, nontoxic, bioactive agent, has become a useful appreciated compound due to its fungicidal effects and elicitation of defense mechanisms in plant tissues [8]. Chitosan-based edible coating has been studied for efficacy in inhibiting decay and extending shelf life of perishable produces such as strawberry [9], cucumber [10], plum [11], peach [12] and fresh cut melon [13]. A chitosan coating retarded the decrease in ascorbic acid content of sweet cherry [14]) and strawberry fruits [9] during cold storage. Previous studies also have shown that chitosan reduces decay incidence, mainly caused by Botrytis cinerea in tomato fruit [15], and is effective for controlling P. expansum in apple fruit during storage [16]. These reports indicate that chitosan offers a great potential as a biodegradable substance that has anti-microbial and eliciting activities.

The objective of the present study was to evaluate the effect of different concentrations of chitosan coatings (0.5%, 1.0% and 2.0%) and storage temperatures (21 and 5 °C) on extending the postharvest quality attributes of tomatoes (Lycopersicon esculentum Mill.) during storage.

2. Materials and method

2.2. Plant material and postharvest treatments

Tomatoes (Lycopersicon esculentum Mill.) cv. 'Diamentino' were harvested from commercial field in Cumra, Turkey at light red stage using the tomato ripeness color classification chart of United States Department of Agriculture [17] and immediately transferred to the laboratory of the Department of Horticulture at Selcuk University. The tomatoes at a mean 50.17 ± 1.8 Hue angle value were selected according to their uniformity in color, size and absence of damages. They were randomly divided into four equal groups, in which three were assigned to different concentrations of chitosan (0.5%, 1.0% and 2.0%) treatments while the fourth group was non-treated control. Each group further divided into two lots for different storage temperatures (5 and 21 °C). Sixty tomatoes per treatment were used considering four analysis dates with three replications consisted five tomatoes each.

The chitosan solutions were prepared by dissolving 5.0, 10.0 and 20.0 g chitosan [low molecular weight (50.000–190.000 Da), 75–85% deacetylated and viscosity 20–300 cP, 1 wt. % in 1% acetic acid (25 °C, Brookfield) Sigma-Aldrich] in 1000 ml distilled water containing 10 ml (v/v) acetic acid [18]. Fruits of three of the groups were dipped into different concentrations of chitosan for 5 min while control fruits immersed into distilled water contain 10 ml acetic acid for same duration. After treatments, fruits were dried for 2 hours at room temperature (22 °C). Treated and untreated fruits were stored at 21 ± 1 °C (ambient temperature with 65% relative humidity) for 10 d or 5 °C (cold storage with 90% relative humidity) for 20 d in open boxes. Fruit quality attributes was evaluated after 0, 3, 5, 7 or 10 d at ambient temperature and 0, 5, 10, 15 or 20 d at cold storage by measuring weight loss, fruit firmness, ascorbic acid, total phenol, antioxidant capacity (FRAP), lycopene content and visual quality (9-1 scale).

2.2. Determination of postharvest fruit quality changes

Weight loss was determined as percent loss from initial weight. Five fruits in each replication for each treatment were weighed before storage and at each analysis date. Fruit firmness was evaluated by using digital penetrometer (Fruit pressure tester, model 53205; TR, Forlì, Italy) with 8 mm probe on three different regions of samples and expressed as Newton (N). Visual quality was assessed as described by Azadanlou [19]. Briefly, semi-trained panelists evaluated fruit quality feature such as firmness, color, juiciness and overall visual appreciation using a 1–9 hedonic scale (1 = extremely bad, unusable; 3 = unsalable; 5 = fair; 7 = good; 9 = extremely good). The number of fruits receiving a rating of 5 and above was evaluated as marketable fruits.

2.3. Determination of total lycopene

Total lycopene was determined as previously described by Sharma and Maguer [20] and Rao et al. [21] with slight modifications. For lycopene analysis, pericarp tissue of tomatoes was blended with a warring blender for 1 min. One gram homogeneous tissue and 50 mL hexane: ethanol: acetone (2:1:1, v/v) mixture were shaken in a 100-mL flask wrapped with aluminum foil on an orbital shaker at 150 rpm for 30 min. After shaking, 10 mL of distilled water were added and shaken for 5 min again. The solution was then placed to a separatory funnel and after phase separation, the lower phase was discarded. Extract was filtered via Whatman 42 (Sigma-Aldrich Co., St. Louis, MO) and lycopene concent was determined by measuring the absorbance of solution in UV-vis spectrophotometer (U-5100, Hitachi, Tokyo, Japan) at 503 nm against hexane:ethanol:acetone blank. Results were expressed as mg kg-1 fresh weight.

2.4. Determination of ascorbic acid

Tomatoes were ground with a warring blender and 5 g sample was mixed with 45 mL 0.4% oxalic acid and then filtered via filter paper. One milliliter filtrate and 9 mL 2,6-dichlorophenolindophenol sodium salt solution (C12H6Cl2NO2-Na) was mixed and then read transmittance values at 520 nm in a spectrophotometer. Blank were prepared in the same way but using 1 ml filtrate and 9 ml distilled water. Results were expressed as mg 100 g-1 [22].

2.5. Extraction and determination of total phenol and antioxidant activity

Fruit extracts for total phenol and antioxidant activity were prepared using method described by Thaipong et al. [23], with some modifications. Five grams tomato tissue was homogenized in methanol using the Ultra-Turrax homogenizer (IKA, T18 digital, Staufen, Germany) for 5 min. The homogenates were kept at 4 °C for 14–16 h and then centrifuged at 8000 x g for 15 min at 5 °C. The supernatants were recovered and stored at –20 °C in dark color bottles until analysis.

Total phenols were determined according to the method of Singleton et al. [24] with slight modifications. The 0.1 mL extract, 6.0 ml distilled water and 0.5 ml Folin-Coiocalteu were mixed and then were vortexed. The mixture were incubate 3 min and then 1.5 ml 20% sodium carbonate solution supplemented and volume was made up 10 ml distilled water. The solution was incubated at 25 °C for 2 h and the absorbance was measured at 760 nm. The content of total phenols was calculated on the basis of the calibration curve of gallic acid and was expressed as mg gallic acid 100 g−1 FW.

Antioxidant activity was determined by ferric reducing ability antioxidant power (FRAP) according to the procedure described by Benzie and Strain [25]. 150 µL of extract and 2.85 mL of the FRAP reagent [0.3 M acetate buffer (pH 3.6) containing 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 40 mM

FeCl3.6H2O] was incubated at 30 °C for 30 min. after incubation, reaction mixture was measured at 593 nm on a UV-vis spectrophotometer. Standard curve was prepared using different concentrations of 1 mM trolox and expressed as μmol kg−1.

2.6. Statistical analysis

The experiment was carried out in a completely randomized design with three replications. For each storage temperature, data from analyzed parameters were subjected to analysis of variance separately. Sources of variation were treatment, storage time and their interaction. Means were compared by Student's t-test at $P \leq 0.05$, using JMP statistical software version 5.1 (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Weight loss

As illustrated in Figure 1A, weight loss progressively increased during the cold storage of tomatoes and the magnitude of such increment was more pronounced after 10th d. In cold stored products, treatment effects were quite little up to 5th d, although significant effects of the treatment become apparent later. After 5 d storage at 5 °C, all the treatments significantly delayed the loss in weight with varying degrees with a persistent maximum effects observed in 1.0% chitosan coating during the prolonged cold storage. At the end of the storage, the greatest loss in weight occurred in non-treated control tomatoes (7.4%), while the lowest value was obtained from 1.0% chitosan (3.7%).

As for the tomatoes stored at 21 °C (Figure 1B), similar weight loss course was seen with that of the cold storage findings. Accordingly, all the treatments significantly restricted the loss in weight during the storage at 21 °C, with more pronounced effect following 5th d. Among them, chitosan coating at 1.0% resulted in the lowest loss in weight with the value 4.5%, which was followed by 0.5% chitosan (5.2%). On the other hand, the weight loss in control tomatoes was as high as 9.3%, resulting from a progressive increment in moisture loss from produces along with the storage at 21 °C. The weight loss is known to be the major determinant of storage life and quality of fresh commodities [26]. The slower rate of moisture loss from the chitosan coated tomatoes in both of two storage temperatures may be attributed to the additional barrier against diffusion through stomata as previously indicated by Paull and Chen [27]. It is evident from the present and previous studies that coating tomatoes with chitosan reduced the loss in weight compared with the control fruit, probably as a result of covering the cuticles with chitosan on the fruit surfaces. These findings are in well concordance with those of Pérez-Gago et al. [28], where effectiveness of covering with a plastic film or coating on water loss was emphasized. Further, El-Eleryan [29] demonstrated that dipping Washington Navel orange fruits in chitosan alone was markedly effective in decreasing weight loss percentage. The mentioned researchers indicated that the chitosan formed a film on the fruit skin, reducing the weight loss.

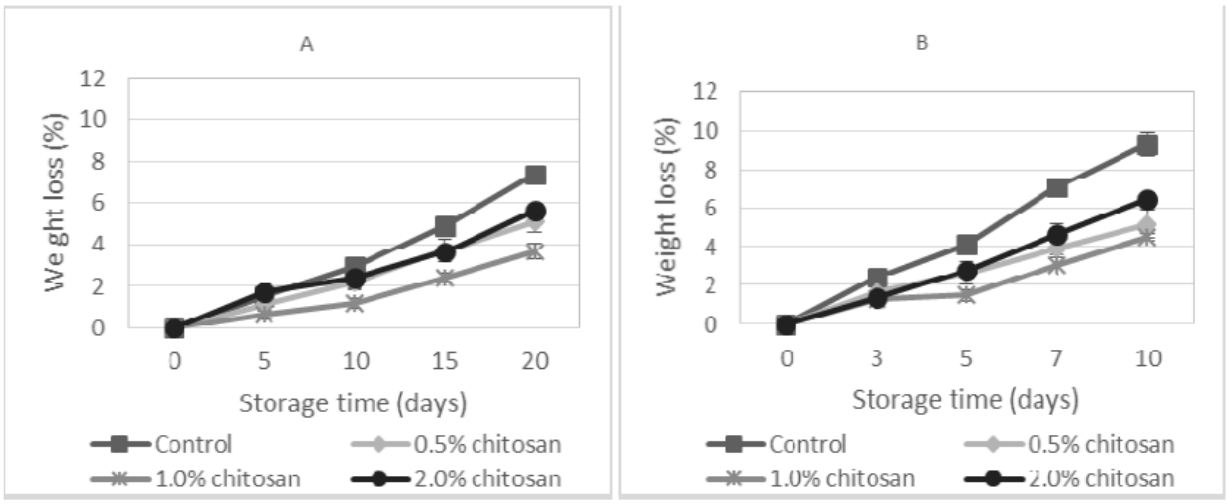

Figure 1. Effects of chitosan coating on weight loss (%) of light red tomato during cold storage at 5 °C (A) and ambient temperature storage at 21 °C (B). Each bar represents the mean of three replicates of 5 fruits each. Vertical bars represent the standard deviation of that mean.

3.2. Firmness

Changes in firmness of the tomatoes during the cold storage at 5 °C is shown in Figure 2A. The lowest firmness values were always determined in control while treatments significantly maintained the fruit firmness along with the 20 d storage duration. The greatest diminish in firmness was observed at 20th d with the lowest value of 6.3 N for control, while the highest value was obtained from 1.0% chitosan coating (12.6 N). At 15th d, the firmness value of tomatoes subjected to 1.0% chitosan was almost the same as that of its 20th d value, indicating its good protective effect. Chitosan coatings at 0.5% (9.0 N) and 2.0% (8.8 N) concentrations had also significantly positive effects on firmness in comparison with the control (6.3 N). Firmness of the tomatoes also underwent a significant decrease during their storage at 21 °C (Figure 2B). A sharp decrease in firmness of tomatoes occurred just before 3rd d of storage at 21 °C and the effects of chitosan treatments at lower doses were insignificant, while its higher dose (2.0%) was significantly higher. Afterwards, control fruits displayed significant decreases during the storage at 21 °C up to 10 d, reaching the lowest value of 6.5 N. After 10 d storage, all doses of chitosan had significantly positive effects compared with control and the highest firmness value was obtained from 1.0% chitosan, followed by its 0.5 and 2.0% doses with similar effects. Firmness is a major attribute that dictates the postharvest life and quality of tomatoes [30]. In the current study, some loss of firmness was observed during the storage of tomatoes, most probably due to the action of endogenous enzymes linked to cell wall degradation [31], since no microbial growth was observed during storage at 5 or 21 °C (data not shown). The retention of firmness with chitosan coating in the present study is in agreement with the results of Benhabiles et al. [4], where tomatoes treated with chitosan coating were firmer than the control during 29 days storage at ambient temperature. Mango fruits have also been reported to be firmer when coated with chitosan [32]. The control fruit lost their textural integrity faster than the higher concentration coatings, particularly 1% which largely maintained the fruit appearance and quality until the end of storage. When the effects of treatments on weight loss and firmness are considered together, it is clear that moisture loss is the main cause of firmness change because the change course of fruit firmness is just opposite of that of weight loss as already revealed by Paniagua et al. [33].

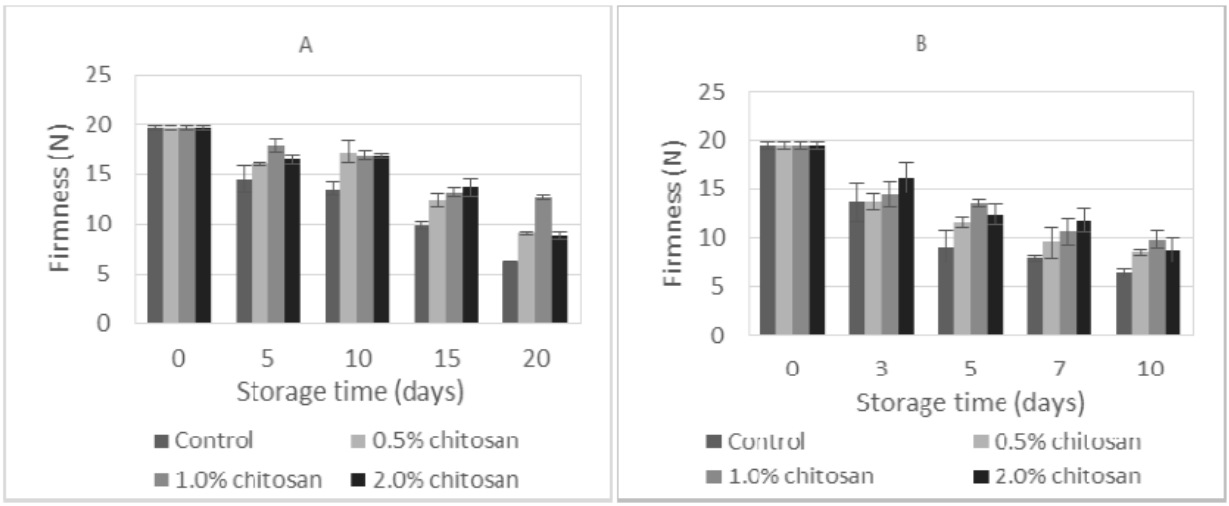

Figure 2. Effects of chitosan coating on firmness (N) of light red tomato during cold storage at 5 °C (A) and ambient temperature storage at 21 °C (B). Each data point represents the mean of three replicate samples. Vertical bars represent the standard deviation of that mean.

3.3. Visual quality

As can be seen in Figure 3A, visual quality of tomatoes determined with 1–9 scale, in cold storage at 5 °C displayed gradual decrease during the storage. Changes in visual quality become more apparent after 10 d storage. According to the investigations performed at 20th d, chitosan coatings at 1% concentrations with panelist score 6.5 had significant positive effects on the maintenance of visual quality of the products. The greatest decrease with a statistical significance in visual quality occurred in control fruits (4.6) that received the lowest panelist score below the acceptability level in markets. Visual qualities of tomatoes during the storage at 21 °C displayed no significant change up to 10th d (Figure 3B). At the end of the storage at 21 °C, the greatest decrease with a statistical significance in visual quality occurred in control fruits that received the lowest panelist score (6.7). Among the chitosan treatments, the highest value was obtained from 1.0% doses (8.2), followed by 2.0% (7.8) and 0.5% (7.7). Similar to our findings, previous studies demonstrated that the external appearance or visual quality of fruits and vegetables is generally improved by chitosan coating. This is most probably due to the fact that anthocyanin degradation on chitosan-treated fruit is generally retarded. Such beneficial impacts have been reported by [34] studying on strawberries and raspberries, although there was certain contradictory knowledge on synthesis of anthocyanins on strawberries treated with chitosan which possibly be associated with cultivar, source of chitosan and doses applied [35].

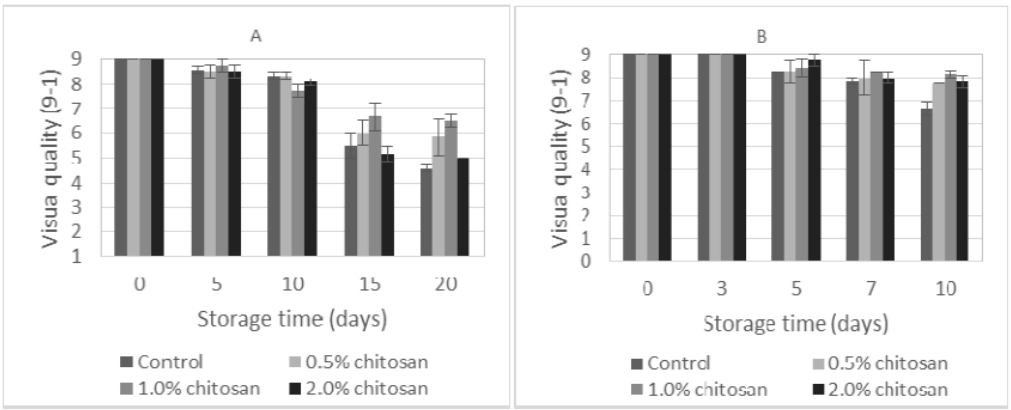

Figure 3. Effects of chitosan coating on visual quality of light red tomato during cold storage at 5 °C (A) and ambient temperature storage at 21 °C (B). Each bar represents the mean of three replicates of 5 fruits each. Vertical bars represent the standard deviation of that mean.

3.4. Total lycopene

Changes in lycopene content of tomatoes during the cold storage at 5 °C were presented in Table 1. Initial lycopene content of tomatoes was 18.96 mg kg-1 and underwent a remarkable increase due to ripening advancement, with the greatest change in control along with the prolonged storage. Chitosan coatings significantly maintained the postharvest lycopene content of the commodities with almost similar effects between the applied concentrations. At the end of the storage, the greatest lycopene content was found in control fruits (32.7 mg kg-1), while the chitosan coatings had remarkable lower values, ranging from 23.26 (1% dose) to 24.98 mg kg-1 (0.5%). As can be seen in Table 2, lycopene content of the tomatoes markedly increased along with the storage at 21 °C similar to cold storage findings. But, the effects of the treatments were insignificant though the chitosan coatings slightly delayed the lycopene changes. Overall lycopene content of tomatoes stored at 21 °C was markedly greater than those stored at 5 °C. Such an inducing effect of high temperature storage on lycopene biosynthesis was also determined by Javanmardi and Kuboto [36] studying on variation

Table 1. Effects of chitosan coating on lycopene (mg kg-1), ascorbic acid (mg 100 g-1), total phenol (mg 100 g-1) and antioxidant activity (μmol kg−1) of light red tomato during cold storage (5 °C).

Chitosan concentration	Storage time (days)				
(%)	0	5	10	15	20
	Lycopene				
Control	$18.96 \pm 2.64^{f-i}$	$19.66 \pm 0.71^{d-i}$	$22.29 \pm 1.79^{b-e}$	31.62 ± 1.76^{a}	32.70 ± 1.42^{a}
0.5		$19.93 \pm 1.27^{d-i}$	$20.20 \pm 0.58^{d-h}$	22.51 ± 2.69^{bcd}	24.98 ± 2.59^{b}
1.0		17.27 ± 0.43^{i}	18.14 ± 0.71^{ghi}	$21.63 \pm 2.06^{c-f}$	23.26 ± 0.51^{bc}
2.0		17.45 ± 0.76^{hi}	$19.45 \pm 0.18^{e-i}$	$20.94 \pm 1.04^{c-g}$	23.72 ± 1.48^{bc}
	Ascorbic acid				
Control	11.26 ± 1.34^{a}	9.15 ± 0.88^{a}	6.03 ± 0.58^{a}	5.28 ± 0.77^{a}	5.98 ± 1.29^{a}
0.5		10.33 ± 1.14^{a}	10.13 ± 0.50^{a}	9.17 ± 0.88^{a}	8.19 ± 0.40^{a}
1.0		10.24 ± 0.20^{a}	9.02 ± 0.22^{a}	7.99 ± 0.69^{a}	7.85 ± 0.26^{a}
2.0		10.31 ± 0.63^{a}	9.99 ± 0.98^{a}	6.69 ± 0.36^{a}	7.44 ± 1.10^{a}
	Total phenol				
Control	41.85 ± 2.67^{f}	47.04 ± 1.61^{de}	47.65 ± 2.78^{cde}	52.10 ± 0.77^{c}	68.27 ± 1.30^{a}
0.5		45.31 ± 1.67^{def}	44.32 ± 0.57^{ef}	47.41 ± 2.25^{cde}	59.01 ± 1.30^{b}
1.0		47.53 ± 5.69^{cde}	47.28 ± 5.67^{de}	46.05 ± 2.73^{def}	59.14 ± 0.77^{b}
2.0		43.21 ± 1.19^{ef}	36.42 ± 3.78^{g}	49.88 ± 3.19^{cd}	60.74 ± 3.87^{b}
	Antioxidant activity				
Control	0.83 ± 0.11^{i}	1.70 ± 0.34^{hi}	3.45 ± 0.84^{def}	4.16 ± 1.20^{bcd}	6.64 ± 0.44^{a}
0.5		1.48 ± 0.21^{hi}	2.85 ± 1.02^{fg}	3.76 ± 0.38^{cde}	4.65 ± 0.20^{b}
1.0		1.13 ± 0.72^{hi}	2.70 ± 0.12^{fg}	3.29 ± 0.91^{def}	4.07 ± 0.49^{bcd}
2.0		2.00 ± 0.49^{gh}	2.91 ± 0.32^{ef}	3.93 ± 0.09^{bcd}	4.54 ± 0.14^{bc}

*Note: For each quality feature, the values significantly different at $P \leq 0.05$ are indicated by different letters according to Student's t-test.

3.5. Ascorbic acid

In contrast to lycopene, ascorbic acid underwent a constant decrease during the cold storage period (Table 1). But the differences between the treatments were statistically insignificant although the lowest and the highest ascorbic acidcontents were always determined in control and 0.5% chitosan treatment. Ascorbic acid decreased with significant differences in response the chitosan doses along with the prolonged storage time at 21 °C. The greatest decrease, from 11.26 to 7.59, was determined in control fruits, while the lowest change was found in the tomatoes treated with 1.0% chitosan (Table 2). Previous studies revealed that the coating with chitosan inhibited ascorbic acid synthesis in strawberries and promotes vitamin C synthesis in cherries [37]. A 0.5% chitosan coating delayed the changes in ascorbic acid content of three sweet cherry cultivars [14] and strawberries [38]. It has been also reported that ascorbic acid content decreased during storage particularly in coated with chitosan carrot sticks [39].

Total phenol and antioxidant activity of the tomatoes increased gradually during the cold storage (Table 1). There were significant differences between the treatments for all the sampling dates regarding total phenol and antioxidant activity. At the end of storage period, the highest values for both parameters were found in control fruits. Chitosan coating markedly delayed phenol changes regardless of application dose. Treatments also restricted the increase in antioxidant activity with maximum effect of 1.0% concentration. Total phenol content of the tomatoes slightly increased during storage at 21 °C. Prolonged storage also led to gradual increases in antioxidant activity with significant differences resulting from the treatments. From the beginning of the storage to the final date, chitosan coatings help to maintain the antioxidant activity with the greatest effect of 1.0% dose. Antioxidative activities of chitosan in food have also been reported in a number of reviews [40,41]. To illustrate, Petriccione et al. [42], evaluated changes in certain biochemical content and antioxidant activity of three strawberry cultivars stored at 2 °C after coating with 1% and 2% chitosan. In accordance with our results, they detected different effect of chitosan doses on antioxidant response of three strawberry cultivars.

Table 2. Effects of chitosan coating on lycopene (mg kg-1), ascorbic acid (mg 100 g-1), total phenol (mg 100 g-1) and antioxidant activity (μmol kg-1) of light red tomato during ambient temperature storage (21 °C).

Chitosan concentration (%)	Storage time (days)				
	0	3	5	7	10
	Lycopene				
Control	18.96 ± 2.64^{a}	26.44 ± 4.95^{a}	34.36 ± 5.23^{a}	34.20 ± 4.29^{a}	37.36 ± 2.40^{a}
0.5		22.37 ± 1.45^{a}	26.00 ± 2.44^{a}	27.11 ± 4.19^{a}	33.95 ± 2.58^{a}
1.0		16.50 ± 5.39^{a}	23.20 ± 4.88^{a}	26.16 ± 4.29^{a}	32.36 ± 2.73^{a}
2.0		19.63 ± 1.82^{a}	22.83 ± 3.69^{a}	27.96 ± 4.62^{a}	33.39 ± 1.19^{a}
	Ascorbic acid				
Control	11.26 ± 1.34^{ab}	10.86 ± 0.40^{abc}	10.15 ± 0.13^{bcd}	9.90 ± 0.51^{bcd}	7.59 ± 1.24^{e}
0.5		10.86 ± 1.02^{abc}	11.93 ± 0.87^{a}	10.28 ± 0.21^{bcd}	10.78 ± 0.62^{abc}
1.0		11.04 ± 0.97^{abc}	11.94 ± 0.65^{a}	9.71 ± 0.20^{cd}	9.27 ± 0.60^{d}
2.0		10.10 ± 0.51^{bcd}	10.21 ± 0.21^{bcd}	10.37 ± 0.10^{bcd}	10.42 ± 0.72^{bcd}
	Total phenol				
Control	41.85 ± 0.74^{a}	48.77 ± 0.77^{a}	49.75 ± 7.46^{a}	48.15 ± 2.67^{a}	51.11 ± 0.98^{a}
0.5		46.30 ± 1.70^{a}	48.52 ± 3.53^{a}	50.74 ± 1.11^{a}	49.63 ± 1.96^{a}
1.0		43.33 ± 1.85^{a}	42.72 ± 3.60^{a}	46.79 ± 2.68^{a}	43.95 ± 2.60^{a}
2.0		47.78 ± 4.17^{a}	44.44 ± 0.00^{a}	48.77 ± 1.07^{a}	44.20 ± 0.21^{a}
	Antioxidant activity				
Control	0.83 ± 0.11^{g}	2.71 ± 0.39^{d}	2.94 ± 0.38^{d}	3.72 ± 0.22^{ab}	4.11 ± 0.18^{a}
0.5		1.32 ± 0.16^{f}	1.69 ± 0.24^{ef}	2.81 ± 0.35^{d}	3.43 ± 0.06^{bc}
1.0		0.88 ± 0.05^{g}	1.67 ± 0.34^{ef}	2.64 ± 0.16^{d}	2.81 ± 0.50^{d}
2.0		1.89 ± 0.40^{e}	2.05 ± 0.19^{e}	2.78 ± 0.02^{d}	3.04 ± 0.20^{cd}

*Note: For each quality feature, the values significantly different at $P \leq 0.05$ are indicated by different letters according to Student's t-test.

4. Conclusion

Overall investigations indicated that coating with chitosan extended the postharvest quality of tomatoes during storage at by reducing weight loss, retaining fruit firmness, maintaining visual quality and delaying the changes in biochemical compounds such as lycopene, ascorbic acid, phenols and antioxidant activity. Among the application doses, chitosan concentration at 1% can be recommended as it was pioneering for most of the parameters analyzed during cold storage at 5 °C for 20 d or at 21 °C for 10 d. Low temperature storage at 5 °C in comparison to 21 °C inhibited weight loss and certain changes regarding ripening process such as lycopene accumulation and loss in fimness. Finally, due to its lower cost and convenience to human health, chitosan may probably be one of the most attractive and effective biopolymer for achieving conservation of fresh tomatoes.

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Conflict of interest

The authors declare no conflicts of interest in this paper.

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Antioxidant capacity and pigment synthesis of marigold (Calendula officinalis L.) as influenced by benzyladenine and epibrassinolide

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A B S T R A C T

he impact of various rates of benzyladenine (BA) and epibrassinolide (EPL) was explored on antioxidant capacity and pigment biosynthesis of marigold in a factorial experiment on the basis of a Randomized Complete Block Design with two factors including benzyladenine (BA) and epibrassinolide (EPL), both at four rates of 0, 1, 5 and 10 mg/L with three replications. Means comparison revealed that the highest antioxidant capacity was devoted to the treatment of "0 mg/L BA × 5 mg/L EPL", the highest flavonoid concentration at three wavelengths of 270, 300 and 330 nm was observed in the treatment of "10 mg/L BA × 10 mg/L EPL", and the highest anthocyanin was obtained from the treatment of "1 mg/L BA × 0 mg/L EPL". According to results, it can be said that the application of BA and EPL significantly influenced catalase, peroxidase, phenol, chlorophyll a, anthocyanin, and carotenoid in marigold plants.

Keywords: *antioxidant capacity; epibrassinolide; flavonoid; marigold; pigment*

1. Introduction

Marigold (Calendula officinalis L.) is one of the widely used medicinal herbs and a flower commonly employed in the urban landscapes and as a pot flower [1]. At present, medications with plant origins compose one-third of all medications consumed by people and this amount is undoubtedly increasing [2]. It has been shown that cytokinin can improve the production and growth of suckers [3]. Foliar application of cytokinin on Hemerocallis itrina showed that this group of regulators increase the size of suckers by postponing cell division and improve their number by stimulating the growth of lateral buds [4]. Exogenous application of cytokinin entailed the strongest impact on carnation longevity. Zeatin, trans-zeatin and isopentenyladenine all retarded the aging of roses [5]. The treatment of marigold with benzyladenine was associated with the increase in plant height,the number of branches, plant fresh weight, and leaf fresh and dry weight as compared to control [6].

Brassinosteroids are a class of polyhydroxylated steroidal plant hormones [7]. They are the derivates of 5-alpha-cholestane and are synthesized in plants through mevalonate pathway. These compounds occur almost in all parts of the plants, but they are mostly observed in reproductive parts [8,9]. These compounds exert diverse physiological influences on the growth and development of plants. They induce growth and cell division, impact seed germination, and influence electrical characteristics, permeability, structure, stability, and activity of membrane enzymes [7,9]. In addition, brassinosteroidschange gene expression and the metabolism and biosynthesis of nucleic acids and proteins at the molecular level [10]. They improve drought tolerance in plants by enhancing the amount of proline and protein in the affected plants [8]. As steroid plant hormones, brassinosteroids play a crucial role in plant growth and development, e.g. pollen tube growth, stem elongation, leaf epinasty, ethylene biosynthesis, and fruit development and maturity [11,12]. They increase photosynthesis and photosynthesizing pigments production in plants [13]. Swamy and Rao [14] showed that the improved

growth of geranium (Pelargonium graveolens) due to the application of 24-epibrassinolide resulted in higher leaf photosynthesis rate and consequently, higher biomass accumulation in the shoot. Zhang et al. [15] reported that 24-epibrassinolide treatment influenced some antioxidant enzymes resulting in the mitigation of oxidative effects. Given the medicinal and ornamental importance of marigold, the present study aimed to explore the impact of benzyladenine and epibrassinolide on the biosynthesis of pigments in marigolds and the increase in the compounds of marigolds.

2. Materials and methods

The present study was a factorial experiment with two factors arranged in an RCBD with 16 treatments, three replications, and two samples per plot. Both benzyladenine (BA) and epibrassinolide (EPL) were applied at four levels (0, 1, 5 and 10 mg/L). The experimental pots amounted to 96 pots. The plants were planted in a substrate composed of 50% cocopeat, 25% garden soil, and 25% perlite. Hormones were applied at three stages once two weeks. The recorded traits included chlorophyll a and b, total chlorophyll, anthocyanin, phenol, flavonoid, catalase and peroxidase activity, antioxidant property, and carotenoid content.

To measure antioxidant capacity, 1 g of the plant was folded in a piece of foil and was kept in liquefied nitrogen for 2–3 minutes. Then, it was ground with 10 mL 85% methanol. The samples were placed at room temperature for one hour. They were infiltrated and centrifuged for five minutes. Then, 150 mL was taken and was added with 850 µL DPPH. The resulting solution was shaken and was kept in darkness for 20 minutes. After blank placement and instrument reset, first only DPPH was poured into coquette and it was read. Then, the sample was read by a spectrophotometer at 517 nm. The antioxidant capacity of the samples was calculated as DPPH inhibition percentage according to Eq 1 [16,17]:

$$\%\ DPPH_{sc} = \frac{A_{cont} - A_{samp}}{A_{cont}} \times 100 \qquad (1)$$

Where, % DPPHsc = inhibition percentage, Asamp = absorption rate (sample + DPPH), and Acont = DPPH absorption rate.

To measure chlorophyll content, 0.5 g of the sample was weighed and was pounded in a Chinese mortar with 50 mL 80% acetone. The extract was infiltrated and adjusted to 50 mL and was poured into a coquette. Chlorophyll content was read at 643 and 660 nm by a spectrophotometer. Then, they (A) were put in the following formula to derive the amount of chlorophyll a, chlorophyll b, and total chlorophyll according to Eqs 2, 3 and 4, respectively [18].

$$\text{Total chlorophyll (mg/ml)} = 7.12\,(A_{660}) + 16.8\,(A_{643}) \qquad (2)$$

$$\text{Chlorophyll a (mg/ml)} = 9.93\,(A_{660}) - 0.777\,(A_{643}) \qquad (3)$$

$$\text{Chlorophyll b (mg/ml)} = 71.6\,(A_{643}) - 2.81\,(A_{660}) \qquad (4)$$

To measure carotenoid content, 0.5 g of samples was weighed and was pounded in a Chinese mortar with 50 mL 80% acetone. The extract was infiltrated and poured into a coquette after being adjusted to 50 mL. They were read at 645, 663, and 660 nm. The results (A) were put in the Eq 5 to yield carotenoid contents [1].

$$\text{Carotenoid content} = 4.69\,(A_{660}) - 0.268\,(A_{645}) + 8.02\,(A_{663}) \quad (5)$$

Anthocyanin content was estimated in 0.5 g of each sample pounded in a Chinese mortar with 50 mL ethanol-hydrochloric acid (85% ethanol 95% + 15% hydrochloric acid). It was infiltrated, adjusted to 50 cc, and poured into coquettes. They were placed in a refrigerator at 4 °C for 24 hours followed by keeping in darkness for two hours. To determine anthocyanin content, the extracts were read at 535 nm by a spectrophotometer and the Eqs 6 and 7 were applied [18].

$$\text{Total sample absorption} = \frac{e \times b \times c}{d \times a} \times 100 \quad (6)$$

Where, a = sample size (0.5 g), b = the volume taken for measurement (5 mL), c = total volume (50 mL), d = fraction taken for 0.1 sample, and e = the figure read at 535 nm.

$$\text{Total anthocyanin content of sample (\%)} = \frac{\text{Sample total absorption}}{98.2} \quad (7)$$

One g of fresh leaves was rasped in 10 mL methanol for two minutes. The solution was infiltrated. Then, 0.5 mL of diluted extract (1:10 g/L) was added with 5 mL diluted folin-ciocalteu and then with 4 mL sodium carbonate solution (7.5% v/v). The samples were kept at laboratory temperature for 15 minutes and its absorption was read at 765 nm with a spectrophotometer.

The standard curve was prepared on the basis of gallic acid concentrations of 0, 10, 20, 30, 40 and 50 mg/L. Then, the line equation was obtained to bey bx a
. The read absorptions were put for y to yield the concentration x [19,20].
To measure peroxidase (POD) activity, the relevant extract was prepared and then, OD variation was read at 430 nm with a spectrophotometer once every 30 seconds for two minutes [21]. The procedure to measure catalase (CAT) activity was as below [22]:
0.01 mol phosphate buffer (pH = 7), 0.5 mL 0.2 mol H2O2, and 2 mL acid reagent (dichromate/citric acid mixture) were added to 1 g of plant tissue that had been ground in 4 mL ethanol. The absorption was read at 610 nm with a spectrophotometer.
Data were statistically analyzed in MSTATC Software Package; the means were compared by LSD test.

3. Results and discussion

According to the results of ANOVA (Table 1), the simple effects of BA and EPL and their interaction was not significant for chlorophyll b and total chlorophyll. Also, they implied the insignificant impact of BA on chlorophyll a. However, EPL and its interaction with BA changed chlorophyll a significantly ($p < 0.05$). We obtained the highest chlorophyll a from the plants treated with 5 mg/L EPL and the lowest one from those not treated with EPL (Table 2). A study on Eriobotrya japonica confirmed the desirable effect of EPL on chlorophyll content. The application of 24-EPL improved stem dry weight and leaf area versus control. The application of EPL improved plant growth by alleviating the harmful impacts of salinity [23].

The highest chlorophyll a content was observed in plants treated with 0 mg/L BA × 5 mg/L EPL and the lowest one was observed in those treated with 0 mg/L BA × 0 mg/L EPL (Table 3). The interaction between BA and EPL was not significant for chlorophyll b and total chlorophyll. A study on Zantedeschia aethiopica "Childsiana" revealed that the increase in BA rate improved chlorophyll

production [24].

ANOVA (Table 1) indicated that BA and EPL did not influence carotenoid content significantly but their interaction was significant ($p < 0.05$). The highest carotenoid content was obtained when the plants were treated with 1 mg/l BA × 5 mg/l EPL and the lowest one was related to those treated with 0 mg/l BA × 0 mg/l EPL and 10 mg/l BA × 5 mg/l EPL, showing an insignificant difference with 0 mg/l BA × 10 mg/l EPL (Table 3). Sardoei Kara et al. [25] reported that marigold seed priming and foliar application of 24-EPL improved growth parameters and photosynthesis pigments, which is consistent with our finding that 24-EPL influenced some growth parameters significantly.

ANOVA (Table 1) revealed that the simple effects of BA and EPL and their interaction were significant for anthocyanin. Plants treated with 1 mg/l BA showed the highest anthocyanin content and the lowest one was related to those treated with 0 mg/l BA or 5 mg/l BA (Table 4). This implies that the application of BA had a favorable impact on anthocyanin content of marigold plants, but this effect was not linear. Petridou et al. [26] reported that cytokinin positively impacted petal anthocyanin in chrysanthemum. In a study on cut Lisianthus, Karimi and Hassanpour Asil [27] found that short-term treatment with BA was effective on the maintenance of petal anthocyanin. They stated that BA postponed ethylene production and extended vase life by four days.

Table 1. Analysis of variance of the impact of experimental factors on the recorded traits.

S.O.V.	df	Means of squares									
		Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Phenol	Flavonoid (330 nm)	Flavonoid (300 nm)	Flavonoid (270 nm)	Antioxidant property	Catalase	Peroxidase
BA	3	0.33 ns	0.24 ns	0.81 ns	4.24**	2.83*	4.06 ns	9.19*	141.27*	0.01*	0.26**
EPL	3	1.38*	0.23 ns	1.81 ns	15.8**	10.67**	15.41**	10.25*	289.48**	0.01*	0.011 ns
BA × EPL	9	0.65*	0.12 ns	1.19 ns	4.05**	7.20**	15.33**	12.03**	412.43**	0.01*	0.24*
Error	30	0.3	0.12	0.68	0.43	0.87	2.22	2.76	46.58	0.005	0.05
C.V. (%)		11.58	15.34	11.73	15.7	13.56	20.46	16.03	8.97	15.31	27.43

**: Significant at $p < 0.01$; *: Significant at $p < 0.05$ level; ns: Non-significant.

Table 2. Means comparison for the simple effect of epibrassinolide (EPL) on the measured traits.

Treatments	Chlorophyll *a* (mg/L)	Anthocyanin (mg/100 g)	Phenol (mg/g)	Flavonoid at 270 nm (µmol/g FW)	Flavonoid at 300 nm (µmol/g FW)	Flavonoid at 330 nm (µmol/g FW)	Antioxidant property (% inhibition)	Catalase enzyme $(UNIT)^2$
0 mg/l	4.38 c	21.38 a	3.05 c	10.64 a	7.97 a	6.84 b	72.96 b	0.41 b
1 mg/l	4.63 bc	15.90 b	5.77 a	9.02 b	5.68 b	5.58 c	80.67 a	0.44 ab
5 mg/l	5.09 a	12.27 c	3.79 b	10.74 a	7.30 a	7.64 a	79.85 a	0.41 b
10 mg/l	4.87 ab	18.87 a	4.17 b	11.10 a	8.19 a	7.51 ab	70.85 b	0.48 a

Table 3. Means comparison for interactions of experimental hormones for the measured traits.

Treatments	Chlorophyll *a* (mg/L)	Carotenoid (mg/L)	Anthocyanin (mg/100g)	Phenol (mg GAE/ g DW)	Flavonoid (µmol/g FW)			Antioxidant property (% inhibition)	Catalase	Peroxidase
					270 nm	300 nm	330 nm			
0 mg/L BA ×0 mg/L EPL	4.03 c	1.05 d	8.33 f	3.12 eh	8.53 cde	5.85 de	5.64 eh	54.73 g	0.33 d	0.98 abc
0 mg/L BA ×1 mg/L EPL	4.64 bc	2.50 bcd	19.58 bc	5.64 b	6.81 e	4.35 e	4.66 h	71.17 def	0.37 cd	0.51 f
0 mg/L BA ×5 mg/L EPL	6.17 a	3.09 ad	12.50 ef	2.81 gh	1.48 ab	9.50 a	7.99 abc	88.03 a	0.50 abc	0.94 ae
0 mg/L BA ×10 mg/L EPL	5.10 b	1.37 d	21.97 b	3.97 def	10.90 abc	8.75 abc	7.12 cde	82.57 abc	0.44 ad	0.95 ad
1 mg/L BA ×0 mg/L EPL	4.67 bc	1.65 cd	34.14 a	2.31 h	10.55 bc	6.37 cde	6.30 def	80.95 ad	0.50 ab	1.10 abc
1 mg/L BA ×1 mg/L EPL	4.45 bc	2.77 bcd	10.32 ef	3.81 dg	8.54 cde	6.10 de	4.93 fgh	83.13 abc	0.56 a	1.22 ab
1 mg/L BA ×5 mg/L EPL	4.88 bc	5.25 a	13.17 ef	4.23 cde	8.60 cde	6.32 cde	8.84 ab	75.75 be	0.43 bcd	0.83 bf
1 mg/L BA ×10 mg/L EPL	4.36 bc	2.34 bcd	19.70 bc	3.73 dg	12.40 ab	9.09 ab	9.00 a	62.37 fg	0.47 abc	0.54 f
5 mg/L BA ×0 mg/L EPL	4.54 bc	1.67 cd	23.89	3.01 fgh	12.07 ab	10.22 a	8.83 ab	82.75 abc	0.35 cd	0.47 f
5 mg/L BA ×1 mg/L EPL	4.38 bc	3.84 ab	14.31 cde	8.54 a	9.78 bcd	6.93 bcd	6.50 cde	85.42 ab	0.39 bcd	1.05 abc
5 mg/L BA ×5 mg/L EPL	5.00 b	2.05 bcd	11.80 ef	3.54 dg	11.23 abc	6.48 cde	6.42 def	69.47 ef	0.39 bcd	0.58 def
5 mg/L BA ×10 mg/L EPL	5.02 b	2.19 bcd	13.24 def	3.81 dg	7.50 de	4.04 e	4.72 gh	56.67 g	0.56 a	0.55 ef
10 mg/L BA ×0 mg/L EPL	4.27 bc	2.38 bcd	19.13 bcd	3.78dg	11.41 ab	9.45 a	66.00 cde	73.38 cf	0.46 abc	0.96 ad
10 mg/L BA ×mg/L EPL	5.11 b	1.52 cd	19.41 bc	5.09 bc	10.95 abc	5.36 de	6.24 dg	82.95 abc	0.45 abc	0.80 cf
10 mg/L BA ×5 mg/L EPL	4.68 bc	1.05 d	11.60 ef	4.61 bcd	10.66 bc	6.92 bcd	7.33 bcd	76.17 ab	0.36 cd	1.00 abc
10 mg/L BA ×10 mg/L EPL	4.96 b	3.64 abc	20.58 b	5.19 bc	13.60 a	10.88 a	9.21 a	81.81 ad	0.45 abc	1.29 a

Similar letter(s) in each column shows insignificant differences. Phenol is expressed in terms of mg gallic acid per g DM, flavonoid in terms of µmol/g FW, and catalase and peroxidase in terms of µmol consumed H_2O_2 per min per mg protein.

Table 4. Means comparison for simple effects of benzyladenine (BA) on the measured traits.

Treatments	Anthocyanin (mg/100g)	Phenol (mg/g)	Flavonoid (330 nm)	Flavonoid (270 nm)	Antioxidant	Peroxidase	Catalase (Unit)[1]
0 mg/L	15.59 b	3.88 b	6.35 b	9.68 b	74.12 b	0.84 ab	0.40 b
1 mg/L	19.33 a	3.52 b	7.27 a	10.02 b	75.55 ab	0.92 a	0.49 a
5 mg/L	15.81 b	4.73 a	6.62 ab	10.15 b	73.58 b	0.66 b	0.42 b
10 mg/L	17.68 ab	4.66 a	7.34 a	11.66 a	81.08 a	1.01 a	0.43 b

Similar letter(s) in each column show insignificant difference. Catalase and peroxidase enzymes are expressed in terms of µmol consumed H_2O_2 per min per mg protein.

The highest anthocyanin contents of 21.38 and 18.87 mg/100 g were obtained from the plants treated with 0 and 10 mg/L EPL, respectively. The lowest one was extracted under 5 mg/L EPL (Table 2). A study on excised red cabbage showed that the application of 0.1 and 10 mmol EPL enhanced anthocyanin content at 21 and 52%, respectively [28]. The interaction "1 mg/L BA × 0 mg/L EPL" was associated with the highest anthocyanin content and the interaction "0 mg/L BA × 0 mg/L EPL" with the lowest one. According to Petridou et al. [26], the treatment of chrysanthemum with cytokinin influenced petal anthocyanin positively.

Analysis of variance (Table 1) indicated that the simple effects of BA and EPL and their interactions were significant for phenol index ($p < 0.01$). Plants treated with 5 and 10 mg/L BA exhibited the highest phenol contents and those treated with 1 mg/L BA had the lowest one, showing no significant difference with those not treated with BA (Table 4). Karimi Qale'ehTaki [29] observed the highest phenol content at BA rate of 1 mg/L. The highest phenol content was obtained from the plants treated with 1 mg/L EPL and the lowest one from those that did not receive EPL (Table 2). The higher EPL rate that was used to treat maize plants resulted in the decrease in phenolic compounds [30]. Similar results have been reported for cucumber [31]. In our experiment, the application of 1 mg/L EPL increased phenol content as compared to control, but as EPL rate was increased, phenol content showed a descending trend.The highest phenol content was obtained from the interaction "5 mg/L BA × 1 mg/L EPL" and the lowest one was obtained under "1 mg/LBA × 0 mg/L EPL" (Table 3). It has been reported that 3 and 6 µmol brassinosteroid increased total phenol content of the tomato fruits significantly, but they did not differ significantly with one another ($p < 0.05$) [32].

The simple effect of BA, EPL and their interactions were significant for flavonoid at 270 nm. According to Table 1, the simple effect of BA was insignificant on flavonoid at 300 nm, whereas the simple effect of EPL and its interaction with BA was significant for it ($p < 0.01$). Table 1 depicts that flavonoid at 330 nm was significantly influenced by BA ($p < 0.05$) and by EPL and BA × EPL ($p < 0.01$).The highest flavonoid at 270 nm was obtained from plants treated with 10 mg/L BA. No BA application exhibited the lowest flavonoid (Table 4). BA did not change flavonoid at 300 nm significantly. The highest flavonoid at 330 nm was observed in plants treated with 10 mg/L BA and the lowest one was related to no BA treatment (Table 4). The results revealed that the highest flavonoid at 270 and 300 nm was related to plants treated with 10 mg/L EPL. But, they did not differ significantly with that obtained from plants treated with 5 or 0 mg/L EPL. The lowest one was observed in plants treated with 1 mg/L EPL. Flavonoid at 330 nm was the maximum in plants treated with 5 mg/L EPL and was the minimum in those treated with 1 mg/L EPL (Table 2). This implies that EPL changes flavonoid favorably at all three wavelengths. Accordingly, EPL at higher rates was found to be effective on flavonoid content of marigolds.

The highest flavonoid at 270 nm was obtained from "10 mg/L BA × 10 mg/L EPL" and the lowest one from "0 mg/L BA × 1 mg/L EPL". The highest flavonoid at 300 nm was observed in plants treated with "10 mg/L BA × 10 mg/L EPL". It did not differ significantly from "5 mg/LBA × 0 mg/L EPL", "0 mg/L BA × 5 mg/L EPL" and "10 mg/L BA × 0 mg/L EPL". The lowest one was related to "0 mg/L BA × 1 mg/L EPL", showing insignificant difference with "5 mg/LBA × 10 mg/L EPL". The highest flavonoid contents at 330 nm (9.21 and 9.00 μmol/g FW, respectively) were found in plants treated with "10 mg/L BA × 10 mg/L EPL" and "1 mg/L BA × 10 mg/L EPL". The lowest one was related to the treatment of "0 mg/L BA × 1 mg/L EPL" (Table 3). The highest flavonoid concentrations at 270, 300 and 330 nm were produced at BA rate of 1 mg/L in Lilium ledebourii "Bioss" [29].

Results (Table 1) indicated that the simple effects of BA and EPL and their interactions were significant for antioxidant property ($p < 0.05$). The highest antioxidant property with 81.08% inhibitionwas observed in plants treated with 10 mg/L BA and the lowest one was obtained when no BA was applied (Table 4). This shows that as BA rate is increased, antioxidant property is linearly strengthened.The highest antioxidant property was associated with plants treated with 1 mg/L and 5 mg/L EPL. The lowest one was related to plants treated with 10 mg/L EPL, showing insignificant differences with no EPL application (Table 2).

Among interactions, "0 mg/L BA × 5 mg/L EPL" yielded the highest antioxidant property and "0 mg/L BA × 0 mg/L EPL" resulted in the lowest one with no significant difference with that of "5 mg/L BA × 10 mg/L EPL" (Table 3). It implies that the use of EPL had favorable impact on antioxidant property, but its excessive use lessened its favorable impact.

As analysis of variance showed (Table 1), the simple effect of BA and EPL and their interactions were significant for catalase ($p < 0.05$). Also, BA effects and BA × EPL interactions were significant for peroxidase ($p < 0.01$). But, EPL did not account for peroxidase significantly. Plants treated with 1 mg/L BA showed the highest catalase content of 0.49 μmol consumed H2O2min/mg/protein and those that were not treated with BA showed the lowest one (Table 4). It seems that the use of BA had a positive impact on catalase of marigold, but this impact was not linear so that as BA rate exceeded the optimum level, catalase activity was suppressed. Catalase is an effective antioxidant in the defense system of most plants against abiotic stresses. It can directly transform hydrogen peroxide into water and oxygen and remove the toxicity of this free oxygen radical [33].

The treatment of 10 mg/L BA resulted in the highest peroxidase (1.01 µmol consumed H2O2 /min/mg protein) with no significant difference with BA rates of 1 and 5 mg/L. The lowest one was obtained when no BA was applied (Table 2). The results of a research on Alstroemeria showed that as plants were treated with higher concentrations of BA, peroxidase was linearly decreased [34]. Our results were inconsistent with this study. In a study on tomato, significant reductions were observed in relative water content, stomatal conductance, intercellular CO2 concentration, and photosynthesis activity under water stress. But, EPL treatment alleviated the adverse effects of water stress to a great extent and improved relative water content and photosynthesis activity. Also, it was revealed that the application of 24-epibrassinolide enhanced the activities of antioxidant enzymes significantly [35].

The highest peroxidase content (1.29 µmol H2O2 min/mg/protein) was obtained from the treatment of "10 mg/L BA × 10 mg/L EPL" and the lowest one from "5 mg/L BA × 0 mg/L EPL" (Table 3). The highest amount of catalase was observed in plants treated with "1 mg/L BA × 1 mg/L EPL" and "5 mg/L BA × 10 mg/L EPL" and the lowest in plants that were not treated with BA and EPL (Table 3). This shows that the simultaneous use of BA and EPL favorably impacted catalase activity so that its content was much lower when none of them was applied. Behnamnia et al. [36] reported that brassinolide application improved root volume, antioxidant content, free proline content, and APX, POD, CAT and SOD activity in tomato seedlings. On the contrary, MDA and H2O2 were significantlydecreased in plants treated with brassinosteroid.

In conclusion, we found that BA and EPL positively influenced the recorded traits of marigold. They enhanced catalase activity, anthocyanin, antioxidant property, phenol, and flavonoid. Both BA and EPL increased marigold petal flavonoid content and similarly, improved antioxidant capacity. According to the results, BA and EPL were influential on the quality of marigold flowers and enhanced their pigments, essential oils, and yield.

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Conflict of interest

All authors declare that they have no conflict of interests.

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Soil management practices affect arbuscular mycorrhizal fungi propagules, root colonization and growth of rainfed maize

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ABSTRACT

Agronomic management practices influence beneficial soil biota, especially arbuscular mycorrhizal fungi (AMF). AMF colonizes about eighty percent of land plants, promoting absorption of essential nutrients and crop growth. Here, a 5-year field experiment was carried out in Central Kenyan Highlands to determine the effect of tillage, mulching and inorganic fertilizers on the number of infective AMF propagules in the soil, mycorrhizal root colonization of maize and uptake of P and N from the soil. The study involved conventional and minimum tillage systems, mulching using dried maize stovers and inorganic fertilizers (120 kg N/ha). The experiment was set up in randomized complete block design and replicated thrice. The number of infective AMF propagules decreased in the following order; V4 stage ($p < 0.0001$), V6 stage ($p < 0.0001$), maize harvest ($p = 0.0076$) and before maize planting ($p = 0.0061$). Minimum tillage + mulch + no NP fertilizer (ZRO) treatment recorded the highest number of infective AMF propagules with an average of 90 propagules g-1 of soil whereas conventional tillage + mulch + NP fertilizer (CRF) and conventional tillage + no mulch + NP fertilizer (CWF) treatments recorded the lowest number of AMF propagules with an average of 1.33 propagules g-1 of soil. Besides, AMF colonization of maize roots at V4, V6 and harvest stages was significantly affected by tillage ($p < 0.0001$), mulch ($p = 0.0001$) and fertilizer ($p < 0.0001$). Results at juvenile stage showed a strong positive correlation between AMF colonization and shoot P ($r = 0.933$, $p < 0.0001$) and N ($r = 0.928$, $p < 0.0001$). These findings demonstrate a strong effect of agronomic management practices on soil AMF propagules which subsequently affected root colonization and uptake of essential nutrients such as P and N.

Keywords: *arbuscular mycorrhizal fungi; root colonization; soil management practices; P uptake; maize*

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are beneficial soil biota that form associations with roots of about eighty percent of land plants, including agricultural crops [1]. AMF increase the plant-root absorptive surface area, enabling plants to get access to a variety of essential nutrients from the soil [2]. Phosphorous (P) and nitrogen (N) are the essential nutrients which plants acquire as a result of AMF colonization [3], although these beneficial fungi can also increase uptake of K, Zn, Cu and nitrates from the soil [4]. In return, the AMF acquire plant-derived C which is necessary for their growth [5].

In addition, AMF provide other agroecosystem services such as carbon sequestration and formation of good soil aggregation [3,6]. AMF are associated with glomalin related soil protein which binds together soil particles, increasing the stability of the soil against agents of erosion [7]. Also, plants colonized by AMF have enhanced resistance to biotic and abiotic stresses [8] as well as increased tolerance to water stress, especially in areas with limited water supply [1]. As a result, AMF communities are essential in enhancing sustainable agriculture because of their contribution to increased crop production and soil fertility [3].

Soil management practices like tillage, crop rotation, fertilizer application and crop protection affect AM fungi symbiosis, particularly in arable lands [9,10]. Tillage is a common soil management practice used in modern agriculture mainly to control weeds, prepare seedbeds prior to planting and incorporate cover crops, pesticides as well as fertilizers [11]. Additionally, tillage can also be used to minimise the occurrence of plant diseases and enhance decomposition of crop residues [12]. However, deep tillage can lead to disruption of AMF extraradical hyphal network, thereby contributing to low levels of AMF active propagules in the soil [9]. Consequently, conventional tillage practices reduce the abundance of AMF and other beneficial soil microorganisms when compared with conservation tillage practices [1]. However, most studies on impacts of conventional tillage on AMF involve the use of high-level mechanization, and there have been no studies which have been done to elucidate the impact of low-cost manual tillage practiced by smallholder farmers on AMF communities [13]. This study involved the use of low-cost manual tillage practices such as hand hoes in preparation of land.

Conservation tillage involves use and management of crop residues which cover about 30% of the soil surface, thereby reducing soil erosion and degradation [12]. Moreover, conservation tillage restores the fertility status of the soil, lowers soil temperature, minimizes application of chemical fertilizers, and increases water retaining capacity of the soil [14]. Therefore, conservation tillage practices enhance establishment of more AMF in the soil [9]. Forms of conservation tillage include minimum tillage, no-till and reduced tillage [11]. No-till involves cultivation of crops without subjecting the soil to any form of tillage while minimum tillage is cultivation of crops with little soil disturbance without turning the soil over [15].

Use of organic materials enhance survival of AMF propagules and colonized roots in the soil [14]. Organic materials release water-soluble C which stimulates AMF growth in the soil [16]. High-input agricultural systems like the application of inorganic fertilizers have negatively affected AMF symbioses [9] especially use of soluble mineral fertilizers have contributed to reduction in diversity of AMF and other beneficial microorganisms in the soil [10]. Inorganic fertilization increases available soil P which suppresses AMF in the soil [16]. Additionally, use of inorganic fertilizers results in poor spore production by AMF communities in the soil [3,10].

In this study, we tested the hypotheses that tillage, mulching and inorganic fertilization influence soil mycorrhizal infection potential, root colonization and growth of maize. The specific objectives were to monitor the effect of tillage, mulching and inorganic fertilization on (1) soil AMF propagules (2) maize AMF root colonization at different growth stages, and (3) its relationship with growth and shoot P and N concentration.

2. Materials and methods

2.1. Experimental site

Experimental fields werelocated at Kirege Primary School (S 00°20’07.0”; E 037°36’46.0”), Chuka Division in Tharaka-Nithi County, Kenya. The study site lies at an altitude of 1526 metersabove sea level on the Eastern slopes of Mt. Kenya. The site is characterized by annual precipitation of between 900–1400 mm and annual mean temperature of 20 °C. The rainfall is bimodal with long rains from March to June and short rains from October to December [17]. The experimental fields were part of a field experiment which was established in April 2013. The soil is Humic Nitisol with the following

physicochemical properties; clay 72%, silt 20%, sand 8%, pH (water) 4.75, total N (Kjeldahl method) 0.02%, organic carbon 0.15% and available P 0.01%. The study site is mainly a maize growing area with each household having an average farm size of 1 acre [18]. Other food and horticultural crops grown include beans (Phaseolus vulgaris L.), sweet potatoes (Ipomoea batatas L.), irishpotatoes (Solanum tuberosum L.), kales (Brassica oleracea L.), onions (Allium cepa L.) and tomatoes (Solanum lycopersicum L.) [19].

2.2. Experimental design

The study was set up as a randomized complete block design with eight treatments which were replicated three times. The soil management practices tested included;
(1) Conventional tillage + mulch + NP fertilizer (CRF),
(2) Conventional tillage + mulch + no NP fertilizer (CRO),
(3) Conventional tillage + no mulch + NP fertilizer (CWF),
(4) Conventional tillage + no mulch + no NP fertilizer (CWO),
(5) Minimum tillage + mulch + NP fertilizer (ZRF),
(6) Minimum tillage + mulch + no NP fertilizer (ZRO),
(7) Minimum tillage + no mulch + NP fertilizer (ZWF), and
(8) Minimum tillage + no mulch + no NP fertilizer (ZWO).

In conventional tillage, plots were hand hoed to a depth of about 0.15 m at the beginning of the season and weeded if required using a hand hoe. In minimum tillage, manual uprooting of weeds was also done in the course of the season to reduce soil disturbance. For residue retention treatments, maize stovers from the previous cropping season were broadcasted at the rate of 3 Mg ha-1, a week after emergence. Inorganic fertilizers used in this study were NP (23:23) and triple superphosphate (TSP). Nitrogen was applied at a rate of 120 kg N ha-1 in split whereby 60 kg N ha-1 was applied as the starter fertilizer whereas the remaining 60 kg N ha-1 was applied by top dressing with urea 30 days after planting. TSP fertilizer was used to supply phosphorous at a rate of 90 kg P ha-1 in plots which did not receive NP fertilizer. The whole experimental trial included the 24 plots measuring 7.0 m by 7.0 m with 1 m and 2 m wide alleys separating plots within a block and between the blocks respectively.

2.5. Enumeration of infective AMF propagules

Estimates of infective AMF propagules (spores, hyphae and colonized roots) were determined by Most Probable Number (MPN) method [20]. Thirty g of the site soil was diluted serially with 270 g of sterilized sand in a 10-fold dilution series. Five levels were performed with 5 replicate tubes in each dilution to yield a five by five MPN matrix for every soil sample. Sand was sterilized by autoclaving at 121 °C for 60 minutes. Seeds of bermuda grass (Cynodon dactylon) were sown into all tubes and watered as required. Seven days after germination, the grass was thinned to four plants per tube. In the greenhouse, completely randomized design was used. After 4 weeks, the grass was harvested, roots washed and stained with 0.05% trypan blue stain in lactic acid. Tubes containing mycorrhizal colonization (positive tubes) in different dilutions were recorded, and the number of infective AMF propagules in the soil calculated [21].

2.6. Plant sampling

Maize plants were sampled from every plot for determination of AMF root colonization at V4 stage (22nd November 2016), V6 stage (3rd January 2017) and at maize harvest (28th March 2017). Four maize plants were uprooted from every plot to obtain the whole root system. Sampled plants were placed in labelled polyethene bags and transported to the laboratory for analyses. Roots were processed for AMF colonization while the shoots were oven-dried to a constant weight and preserved for N and P analyses.

2.7. Determination of maize shoot P and N

Dried maize plants from each subplot were analysed for shoot N and P using wet digestion method [22]. Shoot P was determined using spectrophotometry method at a wavelength of 880 nm after the development of blue colouration [23] while shoot N was determined using Kjeldahl method [24].

2.8. Assessment of mycorrhizal root colonization

Finer and fibrous roots from the sampled maize crops were cleared and washed free of the soil particles and placed in falcon tubes [25]. Ten percent potassium hydroxide was added to the roots in falcon tubes and placed in a waterbath at 80 °C for 15 minutes. After the heat treatment, roots were rinsed with tap water and five percent hydrochloric acid added and allowed to acidify the roots for 10 minutes. Staining of the roots was done using 0.05% trypan blue stain in lactic acid at 80 °C for 15 minutes. Ten percent lactic acid was added to destain the roots. The percentage of mycorrhizal root colonization was assessed under dissecting microscope at x40 magnification using gridline intersect method [26].

2.9. Data analyses

Data was tested for homogeneity of variance by Bartlett test. The percentage data was arcsine ($\sqrt{x}$) transformed to fulfill assumptions of ANOVA. Data reported in figures and tables were back transformed. Data on percentage of mycorrhizal root colonization and numbers of infective AMF propagules was analyzed by two-way ANOVA and wherever feasible means separated by Tukey's Honest Significance Difference (HSD) at $p < 0.05$. The relationship between mycorrhizal root colonization and shoot N and P was tested by Pearson's Product Moment correlation. All statistical analyses were performed using SAS (version 9.0) software.

3. Results

3.1. Effects of tillage, mulch and inorganic fertilizers on number of infective AMF propagules

Minimum tillage treatments produced the highest number of infective AMF propagules at V4, V6 and harvest stages compared to conventional tillage treatments. The number of infective AMF propagules was highest at juvenile stage ($p < 0.0001$), followed by V6 stage ($p < 0.0001$), harvest stage ($p = 0.0076$) and before maize planting ($p = 0.0061$). The highest number of AMF propagules in the soil was recorded in ZRO treatment with an average of 90 propagules g-1 of soil whereas CRF and CWF treatments recorded the lowest number of AMF propagules with an average of 1.33 propagules g-1 of soil (Figure 1).

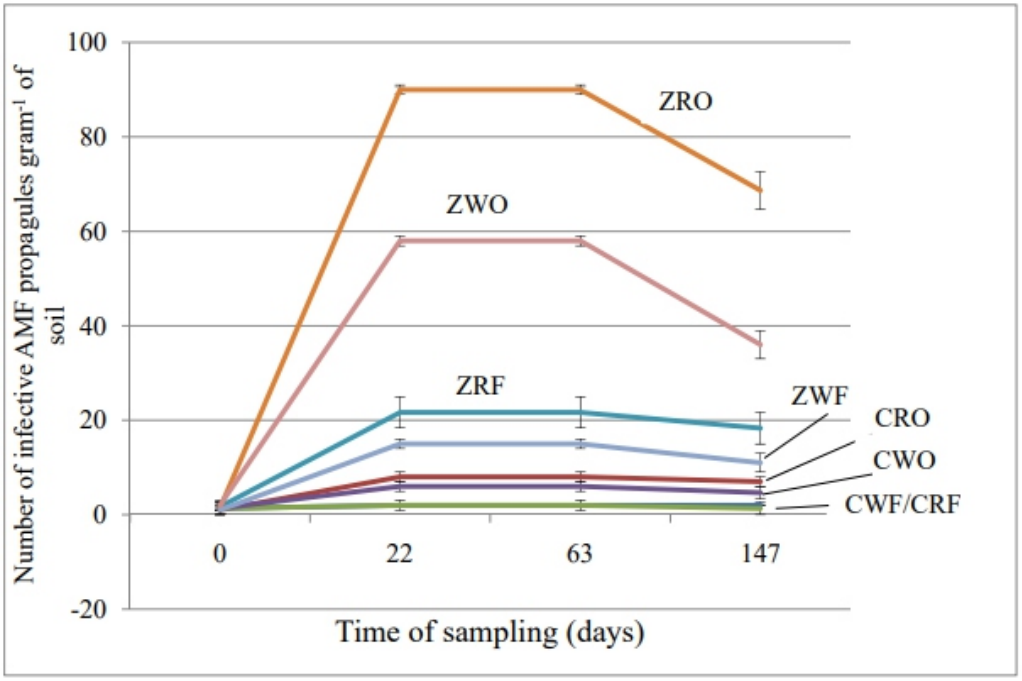

Figure 1. Number of infective AMF propagules as influenced by tillage, mulch and inorganic fertilizers. Time of sampling (days) were; 0 days: before maize planting, 22 days: maize at V4 stage, 63 days: maize at V6 stage: 147 days: maize at harvest. Vertical bars represent ± standard error.CRF, Conventional tillage + mulch + NP fertilizer treatment; CWO, Conventional tillage + no mulch + no NP fertilizer treatment; CWF, Conventional tillage + no mulch + NP fertilizer treatment; CRO, Conventional tillage + mulch + no NP fertilizer treatment; ZRO, Minimum tillage + mulch + no NP fertilizer treatment; ZWO, Minimum tillage + no mulch + no NP fertilizer treatment; ZWF, Minimum tillage + no mulch + NP fertilizer treatment, ZRF, Minimum tillage + mulch + NP fertilizer treatment.

3.2. Maize mycorrhizal colonization at different growth stages

Fertilizer ($p < 0.0001$), mulch ($p = 0.0001$) and tillage ($p < 0.0001$) significantly affected mycorrhizal colonization at juvenile, V6 and maize harvest stages. At juvenile stage, the percentage of mycorrhizal colonization ranged from 41.33% for CWF treatment to 67.33% for ZRO treatment (Table 1). Similarly, at V6 stage ZRO treatment recorded the highest percentage of mycorrhizal colonization of 59% while CWF treatment recorded the lowest percentage of mycorrhizal colonization of 31.33%. Moreover, the same trend was observed at maize harvest stage (Table 1). At all stages, there was no significant interaction between the treatments.

Table 1. Percentage of mycorrhizal colonization of maize roots as influenced by mulch, tillage and inorganic fertilization, and their interactions.

Treatments	Percentage of mycorrhizal colonization		
	V4 stage	V6 stage	Harvest
ZRO	67.33 (3.71) a	59.00 (3.51) a	51.00 (3.51) a
ZRF	59.33 (0.67) b	50.67 (0.88) b	42.33 (1.20) b
ZWO	51.00 (0.58) cd	41.33 (0.33) cd	30.67 (0.68) cd
ZWF	44.00 (0.58) e	33.67 (1.20) ef	23.33 (1.22) e
CRO	53.33 (0.33) bc	43.33 (0.88) c	34.33 (0.88) c
CRF	47.67 (0.33) cde	39.33 (0.33) cde	30.00 (0.58) cde
CWO	45.67 (0.33) de	34.33 (0.34) def	25.33 (0.67) de
CWF	41.33 (0.67) e	31.33 (0.88) f	23.00 (0.58) e
Tillage			
Conventional tillage	47.00 (1.31) b	35.08 (1.42) b	25.17 (1.35) b
Minimum tillage	55.42 (2.77) a	46.17 (2.99) a	36.83 (3.31) a
Mulch			
Mulch	56.92 (2.34) a	48.08 (2.40) a	39.42 (2.55) a
No mulch	45.50 (1.09) b	37.17 (1.17) b	27.58 (0.99) b
Fertilizer			
NP fertilizer	48.08 (2.09) b	38.75 (2.28) b	29.67 (2.39) b
No NP fertilizer	54.33 (2.54) a	44.50 (2.83) a	35.33 (3.00) a
P values for main factors and their interactions			
Tillage	<0.0001	0.0021	0.0201
Mulch	0.0001	0.0001	<0.0001
Fertilizer	<0.0001	0.0301	0.0011
Tillage × mulch	0.1004	0.5523	0.0601
Tillage × fertilizer	0.2246	0.0613	0.0601
Mulch × fertilizer	0.5638	0.6865	0.4379
Tillage × mulch × fertilizer	0.9339	0.9355	0.8756

*Note: The mean standard errors are presented in parentheses. Values followed by the same letter are not significantly different at $p < 0.05$ (Tukey's HSD test). CRF, Conventional tillage + mulch + NP fertilizer treatment; CWO, Conventional tillage + no mulch + no NP fertilizer treatment; CWF, Conventional tillage + no mulch + NP fertilizer treatment; CRO, Conventional tillage + mulch + no NP fertilizer treatment; ZRO, Minimum tillage + mulch + no NP fertilizer treatment; ZWO, Minimum tillage + no mulch + no NP fertilizer treatment; ZWF, Minimum tillage + no mulch + NP fertilizer treatment, ZRF, Minimum tillage + mulch + NP fertilizer treatment

3.3. Growth of maize, P and N uptake at juvenile stage

Maize shoot dry matter was significantly influenced by tillage ($p < 0.0301$), mulch ($p = 0.0071$) and fertilizer ($p = 0.0301$). CRF treatment recorded the highest shoot dry matter with an average of 5.78 g plant-1 whereas ZWO treatment recorded the lowest shoot dry matter with an average of 2.21 g plant-1.

Phosphorous concentration in maize shoots at juvenile stage was significantly influenced by tillage ($p = 0.0401$), mulch ($p = 0.0049$) and fertilizer ($p = 0.0361$). Interestingly, ZRO treatment recorded the highest P concentration of 9.96 mg kg-1 on average whereas CWF treatment recorded the lowest P concentration of 4.55 mg kg-1 in average. Moreover, N concentration in maize shoots at juvenile stage was also significantly influenced by tillage ($p = 0.0231$), mulch ($p = 0.0001$) and fertilizer ($p = 0.0081$). The highest N concentration was recorded in ZRO treatment (3.37 g 100g-1) whereas the lowest N concentration was recorded in CWF treatment (1.32 g 100g-1) (Table 2).

Remarkably, AMF root colonization significantly affected P concentration ($r = 0.933$, $p < 0.0001$) and N concentration ($r = 0.928$, $p < 0.0001$) at juvenile stage of maize. Besides, there was a strong positive correlation between AMF colonization and shoot P and N concentration ($R2 = 0.87$, $p < 0.0001$; $R2 = 0.8619$, $p < 0.0001$ respectively) at juvenile stage (Figures 2 and 3).

Table 2. Shoot dry matter (DM), P and N concentration of maize plant at V4 stage as influenced by mulch, tillage and inorganic fertilization, and their interactions.

Treatments	Shoot DM (g plant^{-1})	P (mg kg^{-1})	N (g 100g^{-1})
ZRO	2.43(0.03) e	9.96 (0.12) a	3.37 (0.13) a
ZRF	4.32 (0.65) b	8.30 (0.22) c	2.79 (0.18) bc
ZWO	2.21 (0.25) f	8.83 (0.39) b	3.11 (0.28) ab
ZWF	4.11 (0.38) bc	8.03 (0.07) c	2.43 (0.22) cd
CRO	3.56 (0.39) d	6.71 (0.48) d	2.35 (0.86) d
CRF	5.78 (0.63) a	5.48 (0.44) e	1.79 (0.08) e
CWO	3.86 (0.33) c	6.39 (0.74) d	2.11 (0.37) de
CWF	5.67 (0.67) a	4.55 (0.18) f	1.32 (0.27) f
Tillage			
Conventional tillage	4.21 (0.31) a	4.37 (0.42) b	2.33 (0.85) b
Minimum tillage	2.97 (0.23) b	7.39 (1.02) a	3.02 (0.32) a
Mulch			
Mulch	4.04 (0.34) a	9.38 (0.50) a	3.22 (0.22) a
No mulch	3.02 (0.09) b	4.96 (0.19) b	2.03 (0.09) b
Fertilizer			
NP fertilizer	5.34 (1.09) a	4.45 (0.33) b	1.21 (2.39) b
No NP fertilizer	2.51 (0.54) b	8.56 (0.83) a	3.01 (0.07) a
P values for main factors and their interactions			
Tillage	<0.0001	0.0401	0.0231
Mulch	0.0071	0.0049	0.0001
Fertilizer	0.0301	0.0361	0.0081
Tillage × mulch	0.1704	0.5556	0.0691
Tillage × fertilizer	0.2996	0.1612	0.0645
Mulch × fertilizer	0.5638	0.6945	0.9379
Tillage × mulch × fertilizer	0.9342	0.9305	0.2359

*Note:The mean standard errors are presented in parentheses. Values followed by the same letter are not significantly different at $p < 0.05$ (Tukey's HSD test). CRF, Conventional tillage + mulch + NP fertilizer treatment; CWO, Conventional tillage + no mulch + no NP fertilizer treatment; CWF, Conventional tillage + no mulch + NP fertilizer treatment; CRO, Conventional tillage + mulch + no NP fertilizer treatment; ZRO, Minimum tillage + mulch + no NP fertilizer treatment; ZWO, Minimum tillage + no mulch + no NP fertilizer treatment; ZWF, Minimum tillage + no mulch + NP fertilizer treatment, ZRF, Minimum tillage + mulch + NP fertilizer treatment.

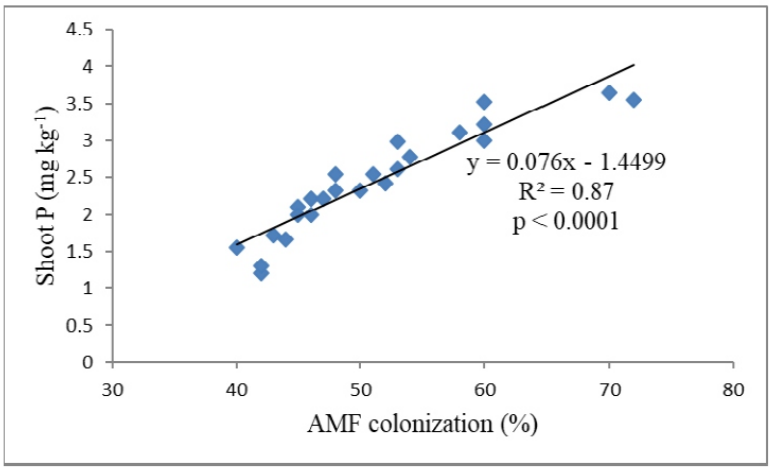

Figure 2. The relationship between AMF root colonization and shoot P on maize at juvenile stage.

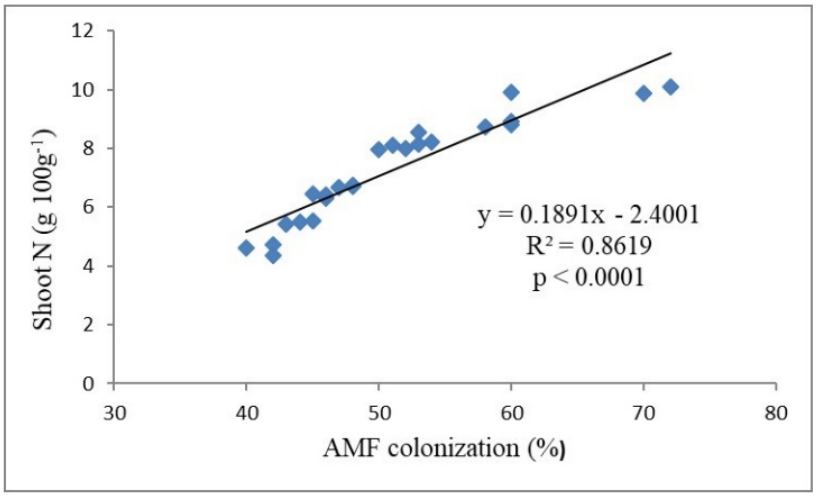

Figure 3. The relationship between AMF root colonization and shoot N on maize at juvenile stage.

4. Discussion

4.1. Number of infective AMF propagules

The number of infective AMF propagules in the soil decreased in the following order; V4 stage, V6 stage, maize harvest and before maize planting. This could be ascribed to the fact that AMF species are biotrophic, thus senesce in the absence of host crops [27]. At V4 stage, the secretion of compounds by maize is higher than the other stages because the tissues are actively growing. These compounds may include nutrients and energy sources required by AMF to grow and sporulate [28]. Consequently, the high number of AMF propagules in the soil at V4 stage colonizes a large number of roots promoting

the acquisition of essential nutrients from the soil which enhance plant growth. As the plant develops towards maturity, the quantity of these compounds secreted to the plant rhizosphere decreases [27] thus decreasing the number of infective AMF propagules obtained at V6 and harvest stages.

Tillage is a common practice used by farmers to control weeds, enhance crop residues decomposition, preparation of seedbeds prior to planting and also the incorporation of fertilizers into the soil [11,29]. However, previous studies on the impact of tillage on AMF mainly involved high level mechanization in conventional tillage [11,30]. By contrast, this study involved low cost manual tillage using hand hoe in conventional tillage showing that the number of infective AMF propagules were higher in minimum tillage than in conventional tillage system. Most AMF communities are found in the topsoil [12], therefore soil manipulation using low cost manual tillage like hand hoe can disrupt the hyphal networks of these fungi resulting in less number of AMF infective propagules in conventional tillage system. Minimum tillage is a form of conservation tillage which involves little soil manipulation, thus enhancing establishment of more AMF communities in the soil [3,31].

4.2. Mycorrhizal colonization of maize roots

AMF form symbiotic associations with about eighty percent of land plants, enhancing absorption of essential nutrients from the soil and protection against biotic and abiotic stresses [6]. However, the effectiveness of AMF symbiosis is influenced by the type of agronomic practices used by farmers [9]. In this work application of NP fertilizer treatment recorded lower percentage of mycorrhizal colonization compared to no application. Application of fertilization, especially soluble inorganic fertilizers negatively effects establishment of AMF communities in the soil [10].Mineral fertilizers increase soil acidity which reduces the numbers of viable AMF spores in the soil [1,32] and also increase amount of P in the soil which inhibit production of spores by AMF [33]. The soil pH was acidic, partly because of addition of NP fertilizers and these negatively affected AMF communities, leading to low colonization of maize roots in NP fertilizer treatment. Organic materials such as mulch increase the amount of water-soluble C in the soil, water retention capacity and fertility status of the soil [34,35]. Therefore, there are more AM fungi communities in soils containing organic inputs which can lead to higher percentage of mycorrhizal root colonization in mulch treatment than in no mulch treatment.

The hyphal fragments of AMF communities tend to lose their viability when they are exposed to well aerated soils [36]. Conventional tillage loosens soil particles and breaks extraradical hyphal network of AMF communities, thereby reducing AMF root colonization [33]. In this study, conventional tillage recorded lower percentage of mycorrhizal colonization compared to minimum tillage. This could be ascribed to better AMF hyphal network development in minimum tillage treatment which favoured more root colonization [9]. In addition, conventional tillage using a hand hoe disrupted extraradical hyphae of AMF communities in the soil resulting in less AMF root colonization in conventional tillage soils. These findings showed that juvenile stage recorded highest percentage of mycorrhizal colonization compared to V6 and maize harvest stages. At juvenile stage, the plant produces greater quantity of beneficial exudates rich in carbon which are essential for sporulation of AMF in the soil [28]. Increase in AMF communities lead to more colonization of maize roots at V4 stage. The quantity of these exudates decreases as the plant develops from to silking stage [27]. As a result, percentage of mycorrhizal colonization was lower at V6 than at harvest stages.

4.3. Growth of maize, uptake of P and N at juvenile stage

Nutrients in the soil must be available in sufficient amounts for the plants to grow optimally [37]. Natural reserves of most essential plant nutrients occur in forms which are unavailable to plants [38]. As a result, fertilizers are applied to supplement essential plant nutrients in the soil [39]. In this study, maize shoot dry matter was higher in NP fertilizer compared to no application. Application of inorganic fertilizers releases nutrients which are required for rapid growth of the shoot [8]. NP fertilizers contain nutrients which are soluble in the soil, thus readily available to plants for use even in small quantities [10,39]. Organic materials also contain nutrients required for plant growth. However, nutrients in organic inputs occur in balanced quantities and this is essential for making the plant to be healthy [15]. NP fertilizer treatment recorded higher shoot dry matter than mulch treatment because the concentration of nutrients in organic materials such as mulch is lower compared to concentration of nutrients in inorganic fertilizers [39].

Mulch treatment recorded highest concentration of maize shoot P and N whereas conventional tillage and NP fertilizer recorded lowest concentration of maize shoot P and N. Organic inputs enhance AMF colonization which subsequently improves uptake of P and N from the soil [16]. Organic materials also ameliorate soil structure which increases the growth of plant roots, enabling plants to absorb substantial amounts of beneficial nutrients like P and N from the soil [40]. On the other hand, conventional tillage breaks extraradical hyphae of AMF [36], reducing the extent of maize root colonization by these beneficial soil microorganisms. Consequently, there were low P and N concentrations in maize crops grown in conventional tillage soils. Inorganic fertilization inhibits plant root colonization by AM fungi because of the acidification of the soil [9]. Inorganic fertilizers also increase available P in the soil which suppresses establishment of AMF [10]. Therefore, low AMF colonization in conventional tillage and inorganic fertilizer treatments led to little uptake of P and N from the soil.

There was a positive linear correlation between AMF root colonization and shoot P concentration. The hyphae networks of AMF communities increase the surface area for absorption of nutrients from the soil which contribute to increased levels of shoot P in plants colonized by these microorganisms [41]. Hyphal networks of AMF species also serve as an extension of maize roots and bypass the P-depletion zone to absorb more P [38]. The findings obtained in this study are congruent to those obtained by [41,42] who reported high levels of shoot P in plants colonized by AMF.

Nitrate or ammonium are the chief inorganic forms in which N is acquired by most plants [43]. Inadequate concentrations of N in the soil can limit growth of both soil microorganisms and plants [44]. Correlation analysis also showed that there was a positive linear correlation between AM fungi root colonization and N concentration in maize shoots at juvenile stage. AMF communities in the soil play a pivotal role in mineralization of N in the soil, enabling plants to obtain substantial amounts of this nutrient [44]. Arbuscular mycorrhizal fungi produce extensive hyphae networks which explore the soil and mineralize more N for the host plants [45]. Some AMF in the soil also produces extracellular enzymes which help in breakdown of organic materials which contain N, making it available for plant uptake [44]. There is always competition for mobile N by plants and microorganisms but the latter tend to outcompete the plants because of their ability to get access to micropores in the soil [45]. However, plants colonized by AMF can acquire nutrients from the soil especially N because of the extensive hyphae of these soil microorganisms [36]. The findings of this study are in agreement with those obtained by [6], who reported increased uptake of N following .

5. Conclusions

Our experimental findings show that minimum tillage and mulching positively affected the numbers of AMF propagules in the soil. Inorganic fertilization and conventional tillage using low cost manual tillage such as hand hoe negatively affected numbers of infective AMF propagules, reducing the benefits provided to plants by AMF communities. In this study, application of tillage, mulching and inorganic fertilizers also affected colonization of maize roots at V4, V6 and harvest stages. Mulching and minimum tillage enhanced colonization of maize roots which subsequently increased uptake of more P and N from the soil, enhancing better growth of maize. However, inorganic fertilization negatively affected maize root colonization by AMF. This study points out that conservation agricultural practices such as minimum tillage and mulch should be used as agronomic practices because they favour establishment of AMF communities and associated agroecosystem services. Further investigations will provide the correct balance for incorporation of conventional and conservation agricultural practices to increase crop production as well as enhance protection of beneficial soil microorganisms such as AMF.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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Increasing efficiency of crop production with LEDs

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A B S T R A C T

Light-emitting diode (LED) technology is paving the way to increase crop production efficiency with electric lamps. Users can select specific wavelengths to elicit targeted photomorphogenic, biochemical, or physiological plants responses. In addition, LEDs can help control the seasonality of flowering plants to accurately schedule uniform flowering based on predetermined market dates. Research has shown that the monochromatic nature of LEDs can help prevent physiological disorders that are common in indoor environments, and help reduce incidence of pest and disease pressure in agriculture, which could ultimately increase crop production efficiency by preventing crop losses. Furthermore, a significant attribute of LED technology is the opportunity to reduce energy costs associated with electric lighting. Studies have shown that by increasing canopy photon capture efficiency and/or precisely controlling light output in response to the environment or to certain physiological parameters, energy efficiency and plant productivity can be optimized with LEDs. Future opportunities with LED lighting include the expansion of the vertical farming industry, applications for space-based plant growth systems, and potential solutions to support off-grid agriculture.

Keywords: *controlled environment agriculture; dynamic lighting; intracanopy lighting; light-emitting diode; light quality; targeted lighting; vertical farming .*

Abbreviations: CO2: carbon dioxide; DE: day extension; DLI: daily light integral; EOD: end-of-day; HPS: high-pressure sodium; ICL: intracanopy lighting; ISS: international space station; LED: light emitting diode; LD: long-day; NASA: national aeronautics and space administration; NI: night interruption; PAR: photosynthetically active radiation; PD: pre-dawn; PPF: photosynthetic photon flux; RQE: relative quantum efficiency; SD: short-day; UV: ultraviolet; UV-B: ultraviolet-B; VF: vertical farms

1. Introduction

The use of light-emitting diodes (LEDs) for plant lighting has revolutionized the greenhouse and controlled-environment industry. Initial interest in LEDs as a radiation source for plants centered on the opportunity to improve light sources for space-based plant growth systems [1–4]. Within the last two decades, horticultural researchers have proven that LEDs can serve as an energy-efficient replacement for incandescent lamps to control photoperiodic responses in flowering plants. Studies have also demonstrated that LEDs are viable alternatives to fluorescent lamps for sole-source lighting in growth rooms, and are currently major competitors of high-pressure sodium (HPS) lamps for supplemental lighting in greenhouses. A myriad of recent studies describe the many advantages of using LEDs for plant production, which range from the application of narrowband radiation to serve as cues that drive specific photomorphogenic, biochemical, or physiological plants responses, to applications for pest and disease management, and reductions in energy consumption from plant lighting. A review of key studies focused on increasing the efficiency of crop production with LEDs, and discussion of current and potential applications follows.

2. Monochromatic LEDs for plant production

2.1. Light-quality control of plant growth and development

A valuable attribute of using LEDs for plant lighting is the option to select specific wavelengths for a targeted plant response [5]. Broadband red light (600 to 700 nm), which typically promotes dry mass gain, stem elongation, and leaf area expansion of many plants species, has the highest relative quantum efficiency (RQE) for driving single-leaf photosynthesis [6,7]. Initial LED plant-lighting research in the 1990s proved that plants could grow and complete their life cycle with red LEDs alone, but growth and development was significantly improved when red LEDs were supplemented with small proportions of blue light [8–12]. Because blue LEDs were not widely available at the time, initial studies were conducted using red LEDs (660 nm) supplemented with blue fluorescent lamps.

Relative quantum efficiency curves indicate that broadband blue light (400 to 500 nm) is 25 to 35% less efficient that red light in driving single-leaf photosynthesis [6,7]. Cope et al. [13] described the potential factors that limit the RQE of blue light for photosynthesis: (1) approximately 20% of blue photons are absorbed by non-photosynthetic pigments (e.g. anthocyanins), which result in energy lost as heat and/or fluorescence; and (2) some blue photons are absorbed by accessory pigments (e.g., carotenoids), which can be 10 to 65% less efficient than chlorophyll molecules at transferring light-energy to the photosynthetic reaction center [13]. However, studies have shown that up to a species- or cultivar-specific threshold, increasing the proportion of blue light can increase single-leaf photosynthetic capacity and efficiency [14,15]. Increasing blue light often inhibits cell division expansion, reducing leaf area (i.e., radiation capture) and stem elongation and increasing leaf thickness in most plant species. Bugbee [16] suggested that the reduction in radiation capture is the primary reason for reduced growth (dry mass gain) in response to higher blue light. Blue light is also known to affect leaf stomatal aperture, regulate chloroplast development, and control photomorphogenic and phototrophic plant responses primarily through the action of cryptochrome and phototropin photoreceptors [17–19]. Several studies indicate that 5 to 20% of blue light within the total photosynthetic photon flux (PPF) is needed to improve growth and development and minimize shade-avoidance responses (e.g., elongated internodes, petioles, and hypocotyls, larger, thinner leaves, decreased chlorophyll production, and early flowering) in controlled environments [10,20–24].

A general conclusion from sole-source light-quality research suggests that plant responses to LEDs are species- and sometimes cultivar-specific, and greatly depend on the stage of plant development, light intensity, duration of treatment, or other environmental interactions [23]. Dissolved chlorophyll pigments absorb light most effectively in the red and blue regions of the photosynthetically active radiation (PAR) spectrum (400 to 700 nm). Therefore, early LED systems were equipped with red and blue LEDs alone. However, because other accessory pigments (e.g., carotenoids) efficiently absorb much of the light that is poorly absorbed by chlorophyll, plants can use most of the light within PAR for photosynthesis [25]. Thus, commercial fixtures for plant production now include LEDs with peak wavelengths beyond red and blue. In fact, white LED fixtures are increasingly being used in growing environments because they help overcome some of the complications involving LED color selection and, depending on the desired growth characteristic, may minimize unwanted responses from the range of possible plant responses to narrowband red and blue light [26]. White LED fixtures can be produced either by combining LEDs with different peak wavelengths or, more commonly, by using blue LEDs with a phosphor coating. At the expense of efficiency, the phosphor absorbs some fraction of the photons

emitted by the blue LEDs and re-emits light with longer wavelengths through luminescence, generating white light [27]. The components of the phosphor coating will typically dictate the percentages of red, green, and blue light available for plant growth with broadband white LEDs.

Although green (500 to 600 nm) and far-red light (700 to 800 nm) are often disregarded as useful wavebands for photosynthesis because of their minimal absorption by chlorophyll pigments, studies suggest that they can have positive direct and indirect effects in plant growth and photosynthesis. Because red and blue photons are efficiently absorbed by chlorophyll, most red and blue light is absorbed within a few cell layers from the leaf surface, while green photons can penetrate deeper into the leaf [28]. Accordingly, Sun et al. [29] found that red and blue light drive CO2 fixation primarily in the upper palisade mesophyll of the chloroplast, while green light drives CO2 fixation in the lower palisade. Similarly, Terashima et al. [30] reported that with high PPF, once the upper chloroplasts of individual leaves are saturated by white light, additional green light can increase photosynthesis by penetrating deeper into the leaf and driving CO2 fixation of inner chloroplasts that are not light-saturated by white light. Green light has also been shown to penetrate deeper into the foliar canopy than red and blue light, and can therefore increase whole-plant photosynthesis by stimulating CO2 fixation of inner- and lower-canopy leaves [31–33]. What's more, depending on species, the RQE of absorbed broadband green light can be comparable with that of red, and higher than that of blue [6,7]. Another useful feature of green LEDs, particularly when used to create white light with narrowband red and blue LEDs, is that it can allow for a better visual assessment of plant-status and true-leaf color, something that is typically hard to do when plants are irradiated with purple light from red and blue LEDs only.

Far-red wavelengths can regulate phytochrome-mediated morphological and developmental plant responses. In an effort to promote radiation capture and survival under a low red-to-far red spectra (i.e., similar to shade), plants develop a shade-avoidance response resulting in stem elongation and larger, thinner leaves. Park and Runkle [34] found that supplementing red and blue LEDs with far-red increased plant growth indirectly through leaf expansion, and directly through an increase in whole-plant net assimilation, defined as the rate of increased dry mass per unit leaf area. Zhen and van Iersel [35] evaluated the potential of enhancing photosynthesis in plants grown under red + blue or broadband white LEDs supplemented with far-red LEDs. The authors found that far-red light, which preferentially excites photosystem I, can increase the photosynthetic efficiency of shorter-wavelengths that over-excite photosystem II; their findings prove that different wavelengths of light can have synergistic effects that improve the overall rate of photochemistry and CO2 assimilation [35]. Both of these studies suggest that adding far-red to fixtures with monochromatic LEDs could improve photosynthetic light-use efficiency and increase crop growth in controlled environments.

Except for studies evaluating photoperiodic control of flowering plants, most horticultural research focused on plant growth-responses to LEDs have used a constant spectral environment throughout the day, and typically, during an entire crop cycle. However, dynamic control of LED-light quality can provide the opportunity to change the spectral environment overtime, which may be required to optimize growth and development throughout a plant's life cycle. Several studies have demonstrated that end-of-day (EOD) (i.e., light applied at the end of the photoperiod) far-red can be used as an effective non-chemical means to control plant morphology in a number of crops [36–42]. Moreover, short-term exposure to pre-dawn (i.e., light applied before the start of the photoperiod) or EOD light-quality treatments can have significant effects on plant growth and morphology [43–46]. Although the mechanisms that drive biomass increase under short exposure to PD or EOD-light are unknown, they

may be related to hormonal changes that affect the circadian rhythm of plants and induce instantaneous changes in stomatal conductance and transpiration, which have been shown to strongly respond to light quality [15,47,48].

As stated by Mitchell and Stutte [26], there is no single light-quality recipe that serves all species and every stage of plant growth. However, a compromise between red and blue LEDs can typically drive photosynthesis and regulate vegetative growth of most plants. As suggested by Cope and Bugbee [20], it is likely that the optimal light spectrum for plant growth and development changes with plant age, as plants need to balance leaf area expansion (to maximize radiation capture) with stem elongation and reproductive growth. A thorough understanding of the energy balance needed by plants to regulate growth throughout their life cycle is essential to the development of LED light sources for plant applications. Furthermore, it is important to consider that plants grown indoors are typically exposed to a light spectrum that depends on the electric-lamp type used. In contrast, greenhouse-grown plants develop under broad-spectrum sunlight and sometimes receive supplemental lighting from a specific spectra provided by electric lamps. Thus, if LEDs are used to supplement sunlight, additional blue light may not be as critical as it seems to be for indoor production; that is because sunlight's broad spectrum contains significant amounts of blue light at midday, which may be sufficient for plant growth and development [49]. In addition, because supplemental lighting typically constitutes only a fraction of the total irradiance received by plants, mostly during light-limited periods, photomorphogenic and physiological disorders that have been reported for plants grown under narrowband lighting in growth chambers (see Controlling physiological disorders) are potentially less likely to occur in greenhouse production using narrowband supplemental lighting.

2.2. Controlling seasonality of flowering plants

Similar to growth and development, flowering responses to light quality are species- or cultivar-specific and are primarily determined by the duration of the continuous dark period within a day, also known as the critical night length [50]. Plants are typically classified into response groups based on how that critical night length affects flower regulation. Day-neutral plants flower regardless of photoperiod, assuming other environmental and cultural factors are not limiting. Short-day (SD) plants flower most rapidly when uninterrupted dark periods are longer than some species-specific critical night length. In contrast, flower induction of long-day (LD) plants is most rapid when dark periods are shorter than a critical duration. Therefore, when the photoperiod is short, longer days (i.e., shorter nights) from day-extension lighting with electric lamps can induce flowering of LD plants or inhibit flowering of SD plants to enable vegetative growth. Similarly, night interruption (NI) or PD lighting can be effective at interrupting the dark period and thus, promoting LD photoperiodic responses [51,52]. However, NI has been shown to induce flowering in LD plant more effectively than day extension or PD lighting [50].

In addition to a critical night length, light quality is essential to control the seasonality of flowering plants. Red and far-red light-absorbing phytochromes are the primary photoreceptors that regulate flowering of photoperiodic species, although blue light, which is also weakly absorbed by phytochromes, has been shown to regulate flowering at higher intensities (e.g., 30 μmol·m−2·s−1) than the typical <2 μmol·m−2·s−1 required from red and/or far-red light [53–56].

In the floriculture industry, commercial growers typically extend or truncate the photoperiod to accurately schedule uniform flowering of most photoperiodic-sensitive species based on a predetermined market date (e.g., mother's day, Easter, Christmas). Because of their high far-red photon emission, incandescent light bulbs used to be the lamp of choice for low-intensity photoperiodic lighting. However, with the advancement of LEDs, incandescent bulbs were quickly replaced as photoperiodic lamps because of their short-life span, and more importantly, because they were being phased out of production in many countries due to their electrical inefficiency [57,58]. Compact fluorescent lamps are more energy efficient and longer lasting than incandescent bulbs. However, their spectra has little or no effect on regulating flowering [58]. The narrow bandwidth of LEDs makes precise control of light quality possible, which has significantly broadened our understanding of how different wavebands regulate flowering. Compared to incandescent bulbs, LEDs provide significant advantages such as reducing energy and maintenance costs, accelerating flowering, or preventing excessive stem elongation in some plant species.

In a coordinated greenhouse grower trial, Meng and Runkle [59] compared LED lamps emitting primarily red and far-red radiation with incandescent bulbs to create LDs with NI; the authors confirmed that LEDs were as effective as incandescent bulbs at regulating flowering of several herbaceous ornamental crops. However, research has shown that not all LED lamps are effective at regulating flowering; their effectiveness depends on their spectral composition. Craig and Runkle [60] found that a balanced combination of red and far-red radiation from LEDs promotes flowering of several LD plants. In contrast, red-enriched radiation with LEDs works best at delaying flowering in SD plants [61]. Furthermore, Meng and Runkle [62,63] reported that NI with low far-red radiation from LEDs may not be perceived as a LD; the authors suggested that LD plants can be classified into far-red-dependent and far-red-neutral varieties based on their flowering responses to far-red radiation. Meng and Runkle [62] also found that cool-white LEDs and warm-white LEDs have a similar effectiveness at regulating flowering than red or blue + red LEDs. Relatively few studies have explored the efficacy of green radiation at regulating photoperiodic flowering. Under SDs, NI or DE (day extension) with low or high intensity green LEDs were shown to inhibit flowering of SD plants [64–66]. However, similar to blue-light flowering responses, the degree of flowering regulation with green LEDs seems to depend on intensity and/or treatment duration, and are most likely species-specific. Meng and Runkle [55] suggested that because green radiation can exert an inhibitory flowering effect in some species similar to that of low-intensity red light, a combination of green and red LEDs could be more effective at inhibiting flowering of SD plants than either waveband alone. Lastly, flowering responses to light quality also seem to be dependent on daily light integral (DLI), which refers to the cumulative number of photons within PAR received during a 24h period. Kohyama et al. [67] found that adding far-red to red + white radiation in NI promotes flowering of some ornamental species under a low DLI ($\leq$6 mol·m−2·d−1) but not under a DLI <12 mol·m−2·d−1.

2.3. Controlling physiological disorders

The monochromatic nature of LEDs can lead to physiological disorders in some plant species or cultivars that are typically not present when plants are grown under broadband light. For example, intumescence, a cultivar-specific physiological disorder that is characterized by abnormal outgrowth of cells on plant surfaces (typically induced by abiotic stress), was first associated with a lack of ultraviolet (UV; 300 to 400 nm) and far-red radiation in the spectral environment [68,69]. Others have found that UV radiation can prevent intumescence development on susceptible cultivars of tomato (Solanum

lycopersicum) and ornamental sweet potato (Ipomoea batatas) [70–72]. Similarly, far-red and/or blue light have been shown to mitigate intumescence injury in tomato and cowpea (Vigna unguiculata) in UV-deficient light environments [5,69,73–75]. Due to the damage in the photosynthetic tissue, intumescence not only impairs the physiological processes of plants, but also negatively affects the overall aesthetic quality of plant products, which is a significant concern with ornamental crops grown under sole-source lighting with narrowband LEDs.

As previously mentioned, red light alone can result in suboptimal growth and development of many plant species [8,10–12,14]. Research has shown that 100% red light can lead to physiological disorders that can result in undesirable stem elongation and/or deficient chlorophyll biosynthesis, leading to low photosynthetic rates due to a dysfunctional photosynthetic apparatus [15,76,77]. The mechanisms behind the suboptimal growth under monochromatic red light are not yet fully understood and seem to be species-specific, as some plants have been shown to produce higher biomass under 100% red light compared to a combination of wavelengths [75,78,79].

2.4. Reducing incidence of pest and disease pressure

Irradiating plants with specific wavebands of LEDs has potential for reducing pest pressure and suppressing plant disease in production environments, which could ultimately increase crop production efficiency by preventing crop losses. The role of light quality in plant disease resistance has been correlated with light-induced signaling pathways that interact with plant-defense regulatory mechanisms. Effects of light quality on secondary metabolite accumulation (e.g., flavonoids) have been associated with plant immunity, disease development, and insect interactions [80–82]. A large number of studies have reported increases in light-quality-induced secondary metabolite as means to increase nutritional attributes of plant products [83]. However, fewer studies have focused on evaluating the level of plant protection in response to light-induced, metabolite-based resistance.

Ultraviolet LEDs, namely those with peak wavelengths in the ultraviolet-B (UV-B; 320 to 290 nm) region of the spectrum, can effectively control powdery mildew caused by Sphaerotheca aphanis in strawberry (Fragaria × ananassa) [84] and Podosphaera pannosa in rose [85]. Similarly, blue LEDs have been shown to inhibit the development of Botrytis cinerea in detached tomato leaves and, when used as PD and EOD treatments, can reduce incidence of black leaf mold (Pseudocercospora fuligena) in greenhouse-grown tomato [86,87]. Green LEDs have been proven to be effective for controlling strawberry anthracnose (Glomerella cinglata) [88], leaf spot disease (Corynespora cassiicola) in perilla (Perilla frutescens) [89], and cucumber anthracnose (Colletotrichum orbiculare) and gray mold (Botrytis cinerea) [90]. It has also been reported that red LEDs can induce resistance to powdery mildew caused by Podosphaera pannosa in roses [91], Sphaerotheca fuliginea in cucumber (Cucumis sativus) [92], and downy mildew in basil caused by Peronospora belbahrii [93].

The use of narrowband LEDs is also a promising approach for increasing the attractiveness, specificity, and adaptability of conventional insect traps. Adding UV, green, and/or yellow LEDs to insect traps has been shown to increase the capture efficacy of fungus gnats (Bradysia difformis), greenhouse whitefly (Trialeurodes vaporariorum), oriental fruit fly (Bactrocera dorsalis), biting midges (Culicoides brevitarsis), red flour beetle (Tribolium castaneum), sweet photo weevil (Euscepes postfasciatus), and cotton bollworm (Helicoverpa armigera), among others, compared to non-LED-supplemented traps [94–103].

A variety of factors can influence the effectiveness of light quality to reduce incidence of pest and disease pressure, including the specific peak wavelength of LEDs, light intensity, and time of exposure. However, with the advancement of LED technologies, there is an increasing interest in using light quality as an integral component of pest management programs that can reduce the dependence on environmentally hazardous pesticides. Although research has shown that LEDs can suppress some diseases and reduce pest pressure that are of economic importance in major crops, consideration should be placed to the indirect effects that applying this light-quality treatments will have on plant growth and development.

3. Reductions in energy consumption

The cost of electricity to provide electric light in controlled and semi-controlled (greenhouse) environments is high. In 2014, Nelson and Bugbee [104] published an economic analysis comparing electric costs of using multiple lighting technologies, including ten types of LED fixtures. The authors concluded that the cost per photon delivered from LEDs was higher than that of all traditional horticultural lamps (e.g., HPS, cool-white fluorescent, metal halide), and that at the time, the best HPS and LED fixtures had nearly identical efficiencies (μmol·J−1) [104]. More recently, Wallace and Both [105] compared the energy efficiency of various LED and HPS lamps and also concluded that the best HPS and LED fixtures had similar efficiency. However, electrical efficiency of LEDs continues to increase, and as the technology improves and the capital cost for purchasing LED equipment decreases, the cost per photon will likely continue to decrease. Moreover, if canopy photon capture efficiency is maximized, lighting system efficiency of LEDs can be significantly increased by capitalizing from "precision lighting".

3.1. Intracanopy lighting (ICL)

The relative coolness (i.e., low radiant heat output) of LED surfaces allows for high flexibility in lamp placement and resulting light distribution within plant canopies. The ability to focus radiation close to plant canopies means that less energy is needed to achieve target PPFs than if a hot light-source is located further away from the crop surface. In an effort to increase the efficiency of irradiation by allowing direct light into the inner canopy of crop stands, Frantz et al. [31,106] performed initial proof-of-concept studies with ICL using 15-watt fluorescent lamps; the authors demonstrated that by maintaining irradiance of the inner foliar canopy above the light-compensation point, ICL-grown cowpea could yield 50% of the edible biomass using only 10% of the total input energy compared to traditional top lighting. Subsequently, when LEDs became readily available for research, Massa et al. [107] validated previous ICL studies using LED "lightsicles" that were individually energized at different vertical planes to keep pace with plant growth. Others have confirmed that ICL with LEDs can prevent a decrease in photosynthesis and premature senescence of lower-canopy leaves grown with sole-source lighting [107] or supplemental lighting [108–111]. Moreover, Gómez and Mitchell [112] reported significant energy savings from supplemental lighting when using ICL with LEDs compared to HPS lamps. In contrast, Dueck et al. [108] reported an increase in energy consumption when growing tomatoes with intracanopy supplemental lighting with LEDs. However, the higher energy consumption was attributed to the higher heating requirements with LEDs compared to HPS, as 'waste' thermal energy from HPS lamps typically helps offset winter heating costs in greenhouses [113].

3.2. Targeted lighting

Because 'waste' heat is removed remotely from the photon-emitting surface of LEDs, lamps can be placed close to crop surfaces without overheating or scorching plants. Moreover, because LEDs and their fixtures can be designed to cast narrow beams of light, targeted lighting can be applied by selectively switching on LEDs positioned directly above individual plants as they grow. Poulet et al. [21] reported that targeted, close-canopy lighting of lettuce using red and blue LEDs reduced energy consumption per unit dry mass by 32 or 50% compared to total coverage sole-source lighting using either broadband LEDs or red + blue LEDs, respectively.

3.3. Dynamic control of LEDs

An underutilized property of LED fixtures is their ability to precisely control PPF with dimming in response to the environment or to certain physiological parameters. As described by van Iersel [114], controlling the intensity of the light output of LEDs can be accomplished using one of two methods: (1) current control or pulse width modulation (i.e., control of the frequency at which LEDs are turned on and off; typically thousands of times per second); or (2) duty cycle control (i.e., fraction of time the LEDs are energized during each on/off cycle).

Pinho et al. [115] evaluated dynamic lighting as a way to control LED supplemental lighting; their system automatically compensated for variation of sunlight PPF at plant canopy level. The authors used an on-off switching algorithm in order to maintain a constant PPF with LEDs and reported a 20% reduction in energy consumption compared to HPS lamps [115]. Similarly, Clausen et al. [116] and Schwend et al. [117] reported 25% and 21% reduction in energy consumption, respectively, when sensor-based dynamic LED lighting was adjusted based on the environment. More recently, van Iersel and Gianino [118] demonstrated that by adjusting the duty cycle of LEDs based on the ability of plants to use light efficiently, an adaptive LED light controller can reduce the energy costs of supplemental LED lighting by preventing the PPF at canopy level from dropping below a user-defined threshold. In practice, their system allows for supplemental lighting with LED fixtures to automatically provide more light when there is little sunlight and dim as the amount of sunlight increases [118]. Early trials with this adaptive system showed that energy consumption can be reduced by 60% with only a 10% decrease in crop biomass, as compared to timer-controlled LED fixtures [119]. In a separate set of studies, van Iersel et al. [120,121] focused on adjusting PPF based on the physiological properties of crops, rather than on changing light intensities. They showed that a biofeedback system that relies on a chlorophyll fluorometer and a quantum sensor to measure the quantum yield of photosystem II and PPF, respectively, can determine the electron transport rate, compare that value to a user-defined threshold, and then change the light output of the LED light (either by changing the duty cycle or current) to maintain a range of different electron transport rates in a variety of species [120,121]. Similarly, Carstensen et al. [122] used a remote-sensing approach with a spectrophotometer to sense the dynamics of chlorophyll fluorescence emission from a plant canopy; the authors developed a model that appears to be indicative of the light-use efficiency and light-induced stress of plants [122]. As shown in these studies, dynamic control of LED lighting can help optimize energy efficiency and plant productivity with LEDs.

4. Future of LEDs

4.1. Vertical farms (VFs)

Commercial VFs produce high-value plant products in multi-tiered, high-density growing systems. As suggested by others [123], LEDs are adequate candidates for sole-source photosynthetic lighting in VFs because fixtures typically have low power density per unit growth area (kW·m–2) and can deliver high light intensities with low radiant heat delivered to crops. Initial efforts to produce high-value crops in warehouse-based plant factories used water-cooled HPS lamps; however, the high-energy consumption needed to produce with HPS lamps negated economic viability [26]; follow-up research used fluorescent lamps, which became standard in controlled environments [124]. However, LEDs are now widely used in VFs in Asia and are gaining popularity in other countries, where commercial VFs produce a variety of leafy greens, young plants, and low-profile fruit crops. Kozai et al. [125] includes a comprehensive review of the of the many potential applications of LEDs in urban agriculture. Akiyama and Kozai [126] described the impact of LED fixture design (lamp and plant spacing) on the spatial distributions of PPF in a simulated VF. Ibaraki [127] showed that depending on canopy structure, LEDs can be used to control the direction of light and reduce the distance between lamps and plants, thus maximizing light-use efficiency in terms of irradiance (W·m−2) and PPF (μmol·m−2·s−1). According to Hayashi [128], improvements in cost reduction, energy efficiency, quality, intensity, and flexibility of LED fixtures have driven VF research in Japan, the Netherlands, England, Taiwan, South Korea, and the U.S. In addition, future projects are targeting applied research to support relevant VF concepts in the Middle East, Central and America, and Africa. Currently, the VF industry in North America is considered to be in its initial stage, with many entrepreneurs and growers investing in different lighting technologies given the lack of an acceptable business model or standard method of implementing LED technologies [129]. However, some expect that the increasing number of horticultural-grade LED manufacturers establish models to help improve production efficiency with standardized light-quality formulas and suggested methods for minimizing production costs [130]. One critical aspect to consider is the cost-effectiveness of VF, particularly when used to produce food crops. Most Asian and European countries that have successfully adopted VF with LEDs have land and/or environmental limitations for producing high-quality fresh food, which, coupled with food-safety concerns, justify the high sale-value in those regions of the world. However, research has shown that although some U.S. consumers are willing to pay premium prices for locally-grown produce, the average consumer is hesitant to purchase costly food grown in VFs [131]. Moreover, Yano et al. [132] showed that consumers believe that LEDs could negatively affect the nutritional attributes and overall taste desirability of plant products. There is a need to improve public perception about VF in an effort to establish a reliable consumer base that will drive the industry forward.

4.2. Space farming

The development of LED-based plant growth systems has been supported by NASA since the late 1980s for research in the International Space Station (ISS), to evaluate bioregenerative life-support systems, and to support future colonies on the Moon and Mars [133,134]. The first use of LEDs to grow plants in space in 1995 [135] paved the way for the development of the Vegetable Production Chamber (VEGGIE), which has demonstrated the feasibility of supporting a space garden in the ISS [136,137]. Mitchell et al. [138] estimated that up to 50 m2 of cropping area are needed to sustain one crewmember on a mission, which highlighted a challenging energetic cost for the NASA Biomass Production Chamber [139]. In this context, LEDs play a key role at enabling energy-affordable food production in controlled environments intended for life support in space [21,107,112,140]. Furthermore, the small size

of LEDs contribute to reducing the equivalent system mass of a lighting system, which can attenuate the overall cost of a space mission [141]. Another attractive feature of LEDs for space applications is that their long lifetime and reliability can significantly reduce maintenance costs and astronaut labor requirements for plant growth systems [142]. In addition, the solid-state electronics of LEDs ensure safety and affordable risk management strategies that are highly important in manned space missions [2]. The influence of the space-flight environment on plant growth has been highlighted in several studies [143–148], which indicate that there is a critical need to conduct research that will support the goal of providing efficient plant-based bioregenerative life support systems in extreme environments (e.g., altered gravity, ionizing radiations, ultradian rhythms). The implementation of LEDs in the ISS laboratories allows evaluations of important questions in fundamental biology aiming at improving our knowledge about plant production in space [149,150].

4.3. Off-grid plant production

Improvements in robustness and cost reduction of LEDs have made access to electric lighting a reality for rural communities that used to solely depend on fuel-based lighting [151]. The low-energy requirement of LEDs in combination with photovoltaics has led to the development of solar-powered LED systems, which may offer significant opportunities for off-grid agricultural applications [152].

Conflict of interest

All authors declare no conflicts of interest in this paper.

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