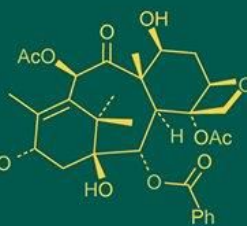
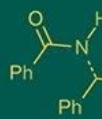


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Interactive effects of pre-harvest foliar sprays and packaging materials on xanthophyll retention in marigold (*Tagetes erecta* L.) cv. Pusa Narangi Gaiinda

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Abstract

This study evaluated the effects of pre-harvest foliar sprays and different packaging materials on the xanthophyll content of marigold (*Tagetes erecta* L.) flowers during postharvest storage at 10 °C. Flowers from the selected pre-harvest foliar spray treatments (based on superior growth and yield performance) viz., salicylic acid (200 ppm), calcium nitrate (2%), calcium chloride (2%), along with an untreated control were packaged in Leno bags (onion mesh bags) and polyethylene bags with varying levels of ventilation (0%, 1%, 2%, and 3%) and assessed for changes in xanthophyll content throughout the storage period at 10 °C. The results revealed that the pre-harvest application of calcium nitrate at 2%, in combination with polyethylene packaging without ventilation (0%), was most effective in preserving xanthophyll content during storage. These findings indicate that the integration of appropriate pre-harvest foliar nutrition with optimal postharvest packaging significantly enhances pigment retention of marigold flowers during storage.

Keywords: Marigold, xanthophyll content, pre-harvest sprays, packaging materials

1. Introduction

Marigold (*Tagetes* spp.) is a commercially important ornamental crop widely cultivated for its bright yellow to orange flowers, the coloration of which is primarily due to the presence of xanthophyll pigments, particularly lutein. Lutein extracted from marigold flowers is extensively utilized in the food, pharmaceutical and poultry industries as a natural colorant and nutritional supplement (Goodwin, 1980; Britton *et al.*, 1995) [3, 1]. The concentration and stability of these pigments play a pivotal role in determining the visual quality, consumer preference, and market value of marigold flowers. Consequently, the maintenance of xanthophyll content during postharvest handling is essential for preserving flower quality and enhancing economic returns.

Xanthophylls are highly sensitive to oxidative degradation and are adversely affected by environmental factors such as oxygen exposure, temperature fluctuations, and moisture loss during storage, leading to rapid pigment deterioration and reduced flower quality (Rodriguez-Amaya, 2001; Kader, 2002) [12, 6]. Pre-harvest foliar application of plant growth regulators and mineral nutrients has been reported to improve flower quality by enhancing pigment biosynthesis, maintaining membrane integrity, and delaying senescence processes in ornamental crops (Davies, 2010; Sajid *et al.*, 2016) [2, 13]. Additionally, postharvest packaging materials play a crucial role in modifying the storage microenvironment by regulating gaseous exchange and moisture retention, thereby influencing pigment stability and overall shelf life of flowers (Paull & Duarte, 2011; Joyce *et al.*, 2001) [9, 5].

Despite the recognized individual roles of pre-harvest treatments and postharvest packaging in maintaining flower quality, limited information is available on their combined effects on xanthophyll retention in marigold flowers during storage. Therefore, the present study was undertaken to evaluate the influence of different pre-harvest foliar sprays and packaging materials on xanthophyll content of marigold flowers during the postharvest storage period.

2. Material and Methods

2.1 Plant material and packaging material

The African marigold (*Tagetes erecta* L.) cultivar 'Pusa Narangi Gaiinda' was selected for the present investigation.

Flowers were harvested from plants subjected to selected pre-harvest foliar spray treatments, namely salicylic acid (200 ppm), calcium nitrate (2%), and calcium chloride (2%), which were chosen based on their superior performance in enhancing plant growth and yield, along with an untreated control. For each treatment, a uniform sample of 100 g of freshly harvested flowers was used.

The flowers were packed in different packaging materials, namely Leno bags (onion mesh bags) and polyethylene bags of 200-gauge thickness. The surface area of each bag was calculated and ventilation was provided by uniformly perforating the bags with holes of 0.5 cm² each to achieve ventilation levels of 1%, 2%, and 3% of the total bag area, as per the respective treatments. Bags without perforations served as the 0% ventilation treatment.

2.2 Experimental Design

The experiment was laid out in a completely randomized design (CRD) with a factorial arrangement and three replications. The treatments consisted of two factors, namely pre-harvest foliar sprays and packaging materials.

Pre-harvest foliar sprays comprised four treatments: salicylic acid at 200 ppm (T₁), calcium nitrate at 2% (T₂), calcium chloride at 2% (T₃), and an untreated control (T₄). Postharvest packaging treatments included five packaging materials: leno bags (P₁), polyethylene bags without ventilation (P₂), and polyethylene bags with 1% (P₃), 2% (P₄), and 3% (P₅) ventilation. All possible combinations of foliar spray and packaging treatments (4 × 5 = 20) were included in the study.

2.3 Estimation of Xanthophyll Content (g/kg petal meal)

Xanthophyll content of marigold flower petals was estimated at 3 days interval following the AOAC method as described by Lawrence (1990) [7], using a UV-Visible spectrophotometer.

a. Preparation of Extractant

The extractant solution was prepared by mixing hexane, acetone, absolute alcohol, and toluene in the ratio of 10:7:6:7 (v/v), respectively.

a. Sample Preparation: Fresh marigold petals were dried in a hot air oven and ground into a fine powder. A known quantity (0.05 g) of the dried petal powder was transferred into a 100 mL volumetric flask, to which 30 mL of the extractant was added. The mixture was shaken thoroughly for 10-15 minutes to ensure complete extraction.

b. Hot Saponification and Extraction

To the extract, 2 mL of 40% methanolic potassium hydroxide (KOH) solution (prepared by dissolving 40 g KOH pellets in 100 mL methanol) was added. The contents were shaken for one minute and refluxed in a water bath at 56 °C using an air condenser to prevent solvent loss. The mixture was then cooled and kept in the dark for one hour. Subsequently, 30 mL of hexane was added, and the flask was shaken for one minute. The volume was then made up to 100 mL with 10% sodium sulphate (Na₂SO₄) solution (10 g Na₂SO₄ dissolved in 100 mL distilled water) and kept in the dark for an additional one hour to allow phase separation. The upper hexane layer was collected in a 50 mL volumetric flask, and a 3 mL aliquot was further diluted to 100 mL with hexane.

c. Spectrophotometric Measurement

The absorbance of the diluted extract was measured at 474 nm using a UV-Visible spectrophotometer. Total xanthophyll content was calculated using the standard AOAC formula

$$\text{Total xanthophyll content (g/kg petal meal)} = \frac{A_{474} \times D}{W \times 236}$$

Where,

A₄₇₄ = Absorbance at 474nm

W = weight of the sample (petal meal) in g

D = Final dilution = $\frac{50 \times 100}{3}$

236 = Specific absorption coefficient for xanthophylls (1 g L⁻¹)

2.4 Statistical Analysis

Data were analysed using two-way ANOVA to determine the effects of foliar sprays, packaging materials, and their interaction on xanthophyll content over time. Mean comparisons were performed using Tukey's HSD test at a 5% significance level.

3. Results and Discussion

The pre harvest foliar sprays, packaging material and their interaction showed significant effect on xanthophyll retention during storage in the present study and results were presented in Table 1.

3.1 Influence of Pre-Harvest Foliar Sprays

Maximum content of xanthophyll in petals was recorded in flowers from Ca (NO₃)₂ treatment (T₂) on all days of observation *i.e.*, on 3rd, 6th and 9th day of storage as 11.58, 9.03 and 3.67 g/kg petal meal respectively. The xanthophyll continued to decline during storage in all the treatments. Calcium nitrate (2%) consistently maintained significantly higher xanthophyll content compared to the other pre-harvest foliar treatments (Table 1). The enhanced pigment retention was observed in calcium nitrate-treated flowers may be attributed to the role of calcium in maintaining cell wall integrity and membrane stability, thereby reducing the rate of enzymatic degradation of carotenoids. Calcium ions interact with cell wall pectins to form calcium pectate cross-links, which strengthen cell wall structure and reduce membrane permeability (Poovaiah, 1986; Marschner, 2012) [10, 8]. This structural reinforcement limits the leakage of degradative enzymes into plastids, the primary sites of xanthophyll accumulation thereby contributing to improved pigment stability during postharvest storage (Valero and Serrano, 2010) [14].

3.2 Effect of Packaging Material

Polyethylene bags with 0% ventilation (P₂) were more effective in preserving xanthophyll content than leno bags and polyethylene bags with higher ventilation levels on all days of observation *i.e.*, on 3rd, 6th and 9th day of storage as 10.11, 8.94 and 7.51 g/kg petal meal respectively. Packaging plays a critical role in modifying the postharvest microenvironment surrounding the flowers. Low-ventilation packaging creates conditions of reduced oxygen availability, slightly elevated carbon dioxide concentration and improved

moisture retention, which collectively contribute to delayed senescence and enhanced pigment stability (Kader, 2002; Valero and Serrano, 2010) [6, 14].

The superior pigment retention observed in non-ventilated polyethylene bags may be attributed to the inhibition of oxidative enzymes, particularly lipoxygenase, which is a key enzyme involved in the oxidative bleaching of carotenoids, including xanthophylls (Gross, 1991; Yahia, 2009) [4, 15]. By minimizing oxygen diffusion, polyethylene bags with 0% ventilation effectively reduce oxidative degradation of pigments and limit moisture loss both of which are major factors accelerating postharvest deterioration. Since xanthophyll degradation is primarily an oxidation-driven process, the reduced oxygen environment significantly slows the breakdown of the polyene (hydrocarbon) chain of the lutein molecule, thereby maintaining higher pigment levels during storage (Rodriguez-Amaya, 2016) [12].

3.3 Interaction Between Foliar Spray and Packaging

The highest retention of xanthophyll content was recorded in flowers subjected to pre-harvest foliar application of calcium nitrate (2%) and subsequently packaged in polyethylene bags with 0% ventilation (T₂P₂), indicating a strong synergistic interaction between pre-harvest nutrition and postharvest packaging conditions. This synergistic response is attributed to the combined inhibition of oxidative enzymes, particularly lipoxygenase (LOX) and peroxidase (POD), which are primary contributors to carotenoid degradation during storage (Gross, 1991; Yahia, 2009) [4, 15]. Pre-harvest calcium nitrate application enhanced cell wall and membrane stability through calcium-pectate complex formation, thereby reducing enzymatic leakage (Poovaiah, 1986; Marschner, 2012) [10, 8], while non-ventilated polyethylene packaging maintained a low-oxygen, moisture-retentive microenvironment that suppressed oxidative activity and delayed senescence (Kader, 2002; Valero and Serrano, 2010) [6, 14].

Table 1: Effect of pre-harvest foliar sprays and packaging material on xanthophyll content of marigold flowers stored at 10 °C (g/kg of petal meal)

Packaging material (P)					Days										
	Day 3					Day 6					Day 9				
	Treatment				Package (P _j) mean	Treatment				Package (P _j) mean	Treatment				Package (P _j) mean
	T ₁	T ₂	T ₃	T ₄		T ₁	T ₂	T ₃	T ₄		T ₁	T ₂	T ₃	T ₄	
P ₁	6.70	10.93	7.76	9.78	8.79	4.63	7.47	5.73	6.81	6.16	*0.00	*0.00	*0.00	*0.00	*0.00
P ₂	8.11	12.11	9.00	11.23	10.11	7.18	10.52	7.89	10.16	8.94	5.97	9.14	6.08	8.84	7.51
P ₃	7.47	12.38	8.58	11.35	9.94	6.52	10.24	7.55	10.34	8.66	5.42	9.16	6.49	8.48	7.39
P ₄	7.39	11.51	8.67	10.37	9.48	5.75	9.11	6.62	7.48	7.24	*0.00	*0.00	*0.00	*0.00	*0.00
P ₅	6.72	10.97	8.06	9.80	8.88	5.15	7.80	6.21	6.52	6.42	*0.00	*0.00	*0.00	*0.00	*0.00
Treatment (T _i) mean	7.28	11.58	8.41	10.50		5.85	9.03	6.80	8.26		2.28	3.67	2.51	3.46	

	SEm ±	CD at 5%	SEm ±	CD at 5%	SEm ±	CD at 5%
Packaging	0.15	0.43	0.14	0.40	0.11	0.32
Treatment	0.13	0.38	0.12	0.35	0.10	0.29
Packaging X treatment	0.30	0.85	0.28	0.79	0.22	0.63

* Complete wilting observed under this treatment by the indicated day

Packaging material: P₁-Leno bags P₂-PE bags with 0% ventilation P₃-PE bags with 1% ventilation P₄-PE bags with 2% ventilation P₅-PE bags with 3% ventilation

Pre-harvest foliar sprays: T₁-SA @ 200 ppm T₂-Ca (NO₃)₂ @ 2% T₃-CaCl₂ @ 2% T₄-control (No spray)

4. Conclusion

These findings provide practical strategies for improving flower quality under commercial conditions during storage. To maximize xanthophyll retention in marigold production, a combined pre-and postharvest management approach is recommended. Pre-harvest application of calcium nitrate (2%) at 35-40 days after transplanting enhances flower quality and pigment stability. Postharvest use of low-density polyethylene (LDPE) bags during storage and transportation minimizes pigment degradation. Adoption of this integrated protocol can extend the shelf life of marigold flowers by approximately 8-10 days while retaining more than 80% of the original xanthophyll content.

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