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Phytochemical characterization and antimicrobial potential of colored chrysanthemum extracts for postharvest preservation of table grapes

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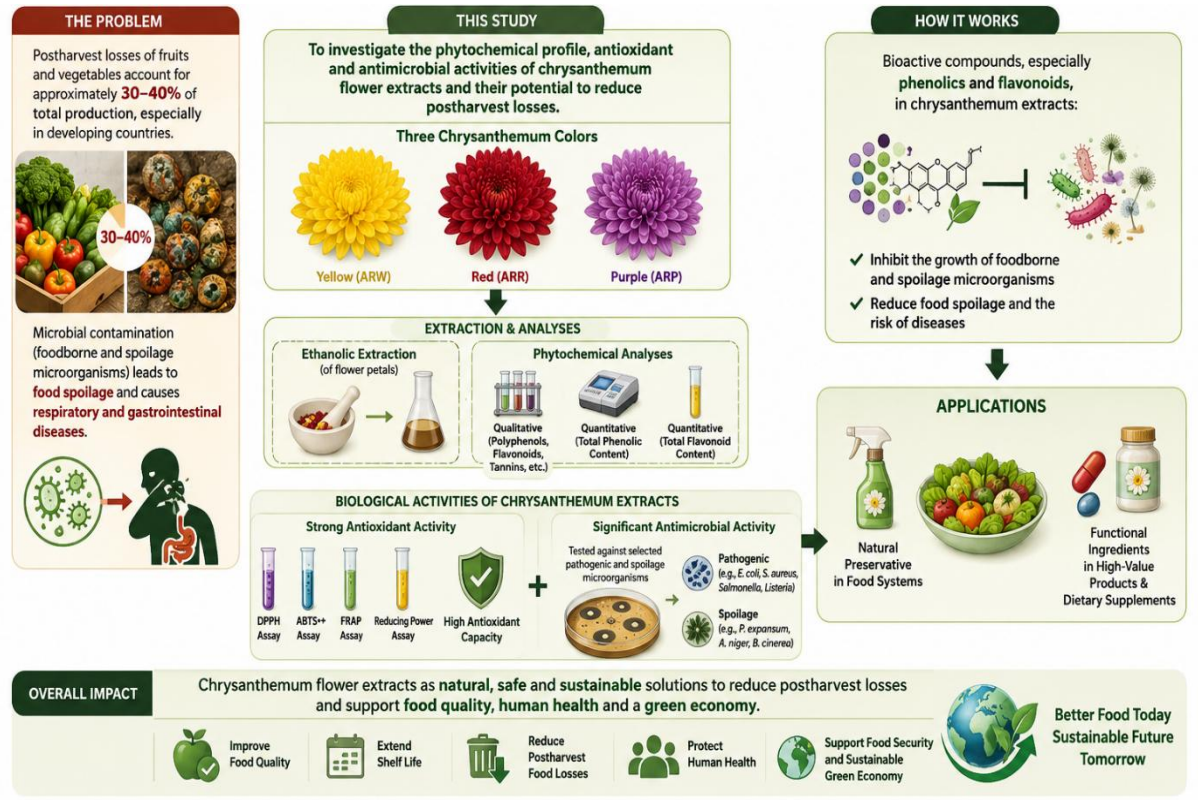
Abstract

Postharvest deterioration of fresh fruits remains a major challenge in the food industry due to microbial spoilage, moisture loss, and physicochemical degradation. Growing demand for safe and environmentally friendly alternatives to synthetic preservatives has increased interest in natural bioactive compounds and edible coatings. This study investigated the phytochemical characteristics and postharvest preservation efficiency of yellow (ARW), red (ARR), and purple (ARP) chrysanthemum flower extracts incorporated into carboxymethyl cellulose (CMC)-based edible coatings for maintaining grape quality during room-temperature storage. Phytochemical screening revealed abundant bioactive compounds, including phenolics, flavonoids, tannins, alkaloids, terpenoids, anthocyanins, and glycosides. Purple chrysanthemum extract (ARP) exhibited the highest total phenolic and flavonoid contents (40.68 ± 0.37 mg GAE/g DW and 35.92 ± 0.23 mg QE/g DW, respectively) and superior antioxidant activity in DPPH, ABTS, and FRAP assays. Ethanolic extracts also demonstrated pronounced antimicrobial activity against bacterial and fungal pathogens

associated with food spoilage. CMC coatings enriched with chrysanthemum extract significantly improved the postharvest quality and storage stability of grapes stored at 20 ± 1 °C. Among the treatments, CMC + ARP (3%) showed the greatest preservation efficiency by reducing microbial growth, delaying physicochemical deterioration, minimizing weight loss, and maintaining total soluble solids compared with untreated grapes. These findings demonstrate a strong relationship between flower pigmentation, phytochemical accumulation, antioxidant potential, and preservation efficiency. Overall, chrysanthemum flower extract represents a promising multifunctional natural preservative for edible coatings, sustainable food packaging, and postharvest management, with potential to extend fruit shelf life while reducing reliance on synthetic chemical preservatives.

Keywords: bioactive phytochemicals, edible composite coatings, plant-derived preservatives, oxidative stability, shelf-life extension, and sustainable food packaging.



Graphical abstract

Introduction

Postharvest losses of fresh fruits and vegetables remain a major challenge that affects food quality, shelf life, and economic sustainability worldwide. Microbial spoilage caused by fungal and bacterial contamination is considered one of the primary factors responsible for postharvest deterioration, particularly in highly perishable fruits **(Nain *et al.,* 2020**). During storage, oxidative stress and microbial proliferation accelerate tissue degradation, resulting in significant quality loss and reduced marketability. Grapes (*Vitis vinifera L.*) are particularly susceptible to postharvest spoilage due to their high moisture content, delicate epidermal tissues, and sensitivity to fungal pathogens and foodborne microorganisms (**Freire *et al.,* 2017**). Therefore, developing safe and effective preservation approaches for fresh grapes is of considerable importance for improving their postharvest quality and extending their shelf life.

Synthetic preservatives, such as sulfur dioxide, sodium benzoate, and potassium sorbate, are widely used to inhibit microbial growth and delay spoilage in food systems. However, increasing concerns regarding the potential health risks and environmental impacts associated with synthetic additives have encouraged the development of natural and sustainable alternatives **(Bensid *et al.,* 2022**). Consequently, plant-derived bioactive compounds have attracted increasing attention because of their antioxidant and antimicrobial properties and potential applications in food preservation systems.

Medicinal and aromatic plants are rich sources of phenolic compounds, flavonoids, terpenoids, and other secondary metabolites with strong biological activity (**Lin *et al.,* 2010**). Among these plants, Chrysanthemum species belonging to the family Asteraceae have been extensively utilized in traditional medicine and functional foods because of their diverse pharmacological properties (**Shunying *et al.,* 2005**). Chrysanthemum flowers contain abundant bioactive constituents, including flavonoids, phenolic acids, glycosides, terpenoids, and anthocyanins, which contribute to their antioxidant, antimicrobial, anti-inflammatory, and protective activities (**Kuang *et al.,* 2018; Samiei and Shakeri, 2022**). Recent studies have demonstrated that chrysanthemum extracts possess strong antioxidant capacity owing to their high phenolic and flavonoid content (**Mohamad and Zahari, 2024; Neatpatiparn *et al.,* 2025).** In addition, flower color variation has been associated with differences in phytochemical composition and antioxidant potential, particularly in pigmented chrysanthemum flowers that are rich in anthocyanins and flavonoids (**Li *et al.,* 2024**).

Edible coatings based on biodegradable biopolymers have recently emerged as promising strategies for maintaining postharvest quality and reducing microbial spoilage in fresh produce. Carboxymethyl cellulose (CMC) is widely used as an edible coating material because of its film-forming ability, biodegradability, and capacity to reduce moisture loss and

microbial contamination during storage (**Dong *et al.,* 2017**). The incorporation of natural plant extracts into CMC-based coatings may further enhance their antioxidant and antimicrobial properties, thereby improving food safety and extending shelf life.

Although several studies have investigated the biological activities of chrysanthemum extracts, limited information is available regarding the comparative phytochemical composition and antimicrobial potential of chrysanthemum flowers of different colors and their application in edible coating systems for postharvest fruit preservation. Therefore, this study aimed to evaluate the phytochemical profiles, antioxidant activities, and antimicrobial properties of yellow, red, and purple chrysanthemum flower extracts. Furthermore, this study investigated the effectiveness of incorporating chrysanthemum extracts into CMC-based edible coatings to reduce microbial growth and improve the postharvest quality of table grapes stored at room temperature.

Materials and Methods

Plant Material and Preparation of Chrysanthemum Extracts

Fresh yellow (ARW), red (ARR), and purple (ARP) chrysanthemum flowers were obtained from the experimental farm of the Faculty of Agriculture at Minia University, Egypt. The flowers were thoroughly washed with distilled water and air-dried under shaded conditions at room temperature for five days. The dried flower petals were ground into a fine powder using an electric grinder and stored at 4 °C until extraction was performed. For extract preparation, 100 g of powdered flower material was mixed with 70% ethanol (700 mL ethanol and 300 mL distilled water) and continuously stirred for 24 h at room temperature using a magnetic stirrer. The extracts were filtered through a double-layer cheesecloth and centrifuged at 3000 rpm for 10 min. The residues were re-extracted under identical conditions, and the filtrates were then combined. The solvent was removed under reduced pressure using a rotary evaporator at 40 °C until dry. The obtained extracts were stored at 2–8 °C in the dark until further analysis **(Neatpatiparn *et al.,* 2025).**

Determination of Bioactive Compounds in Chrysanthemum Extract

Secondary Metabolite Screening

Preliminary phytochemical screening of chrysanthemum extracts was carried out following previously validated qualitative procedures to identify major secondary metabolites, including phenolics, flavonoids, tannins, alkaloids, terpenoids, glycosides, saponins, anthocyanins, steroids, coumarins, and emodins **(Ayoola *et al.,* 2008; Kumar *et al.,* 2009).**

Quantitative phytochemical analysis

Total Phenolic Content (TPC)

The total phenolic content (TPC) of chrysanthemum extracts was determined using the Folin–Ciocalteu colorimetric method, as described previously (**Kim *et al.,* 2009)**. Briefly, 200 µL of the extract or gallic acid standard solution was mixed with 2.6 mL distilled water and 200 µL Folin–Ciocalteu reagent. After 6 min, 2 mL of 7% (w/v) sodium carbonate solution was added, and the reaction mixture was incubated at room temperature for 90 min. Absorbance was measured at 750 nm using a spectrophotometer. Gallic acid was used as the calibration standard at concentrations ranging from 0.1 to 0.8 µg/mL. The results were expressed as mg gallic acid equivalents per gram dry weight extract (mg GAE/g DW). All measurements were conducted in triplicate.

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of chrysanthemum extracts was determined using the aluminum chloride colorimetric method described by **Jia *et al.,* (1999).** Briefly, 500 µL of the extract solution (600 µg/mL in methanol) was mixed with 0.1 mL of 10% (w/v) aluminum chloride, 0.1 mL of 1 M potassium acetate, 1.5 mL methanol, and 2.8 mL distilled water. The reaction mixture was incubated at room temperature for 30 min, and the absorbance was measured at 415 nm. Quercetin was used as the calibration standard at concentrations ranging from 1 to 25 µg/mL. The results were expressed as mg quercetin equivalents per gram dry weight extract (mg QE/g DW). All analyses were performed in triplicate.

Determination of Antioxidant Activity

Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The DPPH radical-scavenging activity of the chrysanthemum extracts was determined according to **the method described by Saija *et al.,* (1998).** Briefly, 100 µL of freshly prepared DPPH solution (0.1% in methanol) was mixed with 100 µL of the extract in a 96-well microplate and incubated in the dark at room temperature for 30 min. Absorbance was measured at 540 nm using a microplate reader. The antioxidant activity was expressed as µmol Trolox equivalents per gram dry weight extract (µmol TE/g DW). The IC_{50} value was calculated from the dose–response curve. All measurements were performed in triplicate.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) assay was performed according to **Jones *et al.,* (2020).** The FRAP reagent containing acetate buffer, ferric chloride, and TPTZ solution was mixed with the extract samples and incubated at 37 °C for 40 min. Absorbance was

measured at 593 nm. Antioxidant activity was expressed as µmol Trolox equivalents per gram dry weight extract (µmol TE/g DW). All measurements were conducted in triplicate.

ABTS Radical Scavenging Assay

ABTS radical-scavenging activity was evaluated according to **the method described by Zielinski *et al.*, (2014).** The ABTS radical solution was prepared using ABTS and potassium persulfate and incubated in the dark for 24 h before use. Briefly, 190 µL of ABTS solution was mixed with 10 µL of the extract in a 96-well microplate and incubated at room temperature in the dark for 120 min. Absorbance was measured at 734 nm. Antioxidant activity was expressed as µmol Trolox equivalents per gram dry weight extract (µmol TE/g DW). All analyses were performed in triplicate.

Determining antimicrobial activity

Media used

- **Topping's medium** consisted of beef extract (3 g), yeast extract (2 g), peptone (5 g), agar (15 g), and distilled water to 1000 mL, and pH (7.0). (**Topping medium 1937**).
- **The potato dextrose agar** (PDA) medium consisted of potatoes (400 g), dextrose (10 g), agar (15 g), distilled water (1000 mL), and pH (6.4). (**Beever and Bollard, 1970**).
- **Martin's Medium** for Fungi consisted of Glucose (10 g), Peptone (5 g), KH_2PO_4 (1 g), $MgSO_4.7H_2O$ (0.5 g), Agar (20 g), Distilled water to 1000 mL and Rose Bengal 1 part in 30.000 parts of medium. **(Martin 1950**).

Preparation of Inoculum

The antibacterial activity of chrysanthemum extracts was evaluated against *Escherichia coli* GIM1.702 and *Erwinia carotovora*. Bacterial strains were cultured overnight in Mueller–Hinton broth (MHB) at 37 °C under continuous shaking. Bacterial suspensions were adjusted to approximately 1×10^8 CFU/mL, corresponding to the 0.5 McFarland standard (**Bhalodia and Shukla, 2011).** Antifungal activity was evaluated against *Fusarium solan*i and *Penicillium digitatum*. Fungal inoculate were prepared from 48-h-old cultures grown in potato dextrose broth (PDB), and spore suspensions were adjusted to approximately 1×10^6 spores/mL according to **Nisha *et al.*, (2010).**

Antimicrobial Screening of Plant Extracts

The total number of bacteria and fungi present in the chrysanthemum extracts was determined prior to conducting antimicrobial activity tests. Briefly, 10 g of each extract was suspended in 90 mL of sterile distilled water, and the mixture was shaken for 5 min to obtain a homogeneous mixture. Sequential decimal dilutions (10^{-1} to 10^{-6}) were then prepared.

Samples of each dilution were spread on Topping medium (**Topping, 1937**) for bacterial counting and on Martin medium **(Martin, 1950**) for fungal counting. All plates were incubated at 30 ± 5 °C for 72 h. After incubation, colony-forming units (CFU) were counted, confirming the absence of both bacterial and fungal contaminants (**Table 1**). Therefore, the chrysanthemum extracts were not sterilized prior to antimicrobial testing, in accordance with the procedure described by **Saxena *et al.,* (1995).**

Table 1. Total count of bacteria and fungi ($\times 10^2$ CFU/mL) in the 70% ethanol extract of chrysanthemum extract.

Extraction	**Ethanol 70%**		
Plants	**P1**	**P2**	**P3**
Bacteria	0	0	0
Fungi	0	0	0

Agar Well Diffusion Assay

The antimicrobial activity of chrysanthemum extracts was evaluated using the agar well diffusion method, as described by **Daoud *et al.,* (2015**). Briefly, sterile Mueller–Hinton agar plates for bacterial strains and potato dextrose agar (PDA) plates for fungal strains were inoculated with 100 µL of the prepared microbial suspensions using the spread plate technique. Wells with a diameter of 6 mm were aseptically prepared using a sterile corkborer. Each well was filled with 100 µL of chrysanthemum extract solution (20%, w/v). The plates were kept at 4 °C for 30 min to allow diffusion of the extracts into the agar medium, followed by incubation at 37 °C for 24 h for bacterial strains and 30 °C for 48–72 h for fungal strains. The antimicrobial activity was determined by measuring the diameter of the inhibition zones (mm), including the well diameter. Sulfur dioxide (SO_2; 150 ppm) was used as a positive control preservative and evaluated using the disc diffusion method, as described by **Selim *et al.,* (2012)**. Sterile Whatman No. 3 filter paper discs (6 mm diameter) were impregnated with the preservative solution and placed on inoculated agar plates. The inhibition zones were measured after incubation under the same conditions.

Preparation of CMC-Based Edible Coatings

CMC-based edible coatings were prepared according to **Dong *et al.,* (2017)**, with slight modifications. Briefly, 1 g of carboxymethyl cellulose (CMC) was dissolved in 100 mL distilled water under continuous stirring at 70 °C for 45 min. Glycerol (0.5 mL) was added as a plasticizer, and the solution was further stirred for 10 min. Chrysanthemum extracts were

incorporated into the coating solutions at concentrations of 1%, 2%, and 3% (w/v) to obtain bioactive edible coatings with antioxidant and antimicrobial properties.

Coating Application and Storage Conditions

Fresh table grapes (*Vitis vinifera L.*) were obtained from a local market in Minia, Egypt. Damaged, bruised, or diseased fruits were discarded, and healthy grapes were washed with a 50 ppm hydrogen peroxide solution for 20 s, followed by air drying at room temperature. The grapes were randomly divided into different treatment groups for analysis. The first group was treated with 1 %, 2 %, and 3 % (w/v) chrysanthemum extract solutions. The second group was coated with CMC-based edible coatings containing chrysanthemum extract at the same concentrations (1% CMC + 1%, 2%, or 3% chrysanthemum extract). Untreated grapes were used as the control. After treatment, the grapes were air-dried at room temperature for 2 h and packed in perforated polypropylene plastic containers. Each treatment was performed in triplicate, with each replicate containing 15–20 grapes (approximately 100 g). The samples were stored at 20 ± 1 °C for up to 10 days. Microbiological analyses were performed on days 3, 5, 7, and 10 of storage **(Abdullah *et al.*, 2023).**

Weight Loss (%)

Fruit weight was measured periodically during storage using a digital balance to evaluate moisture loss throughout the storage period. Weight loss (%) was calculated based on the reduction in fruit weight relative to the initial recorded weight according to the standard gravimetric method. All measurements were performed in triplicate to ensure analytical accuracy and reproducibility **(Costa *et al.*, 2023).**

$$\text{Weight loss (\%)} = \frac{W_0 - W_t}{W_0} \times 100 \qquad (1)$$

W_0; Initial weight (g) **W_t.;** Weight at storage day *t* (g**)** **t:** Storage time (days)

Decay Percentage

The percentage of fruit decay was evaluated periodically during storage by visually examining the grapes for signs of fungal mycelial growth, tissue softening, or bacterial lesions. Fruits exhibiting visible spoilage symptoms were considered decayed, and the decay percentage was calculated relative to the total number of fruits in each treatment group, according to the method described **by Khodaei *et al.* (2021).**

$$\text{Decay (\%)} = \frac{N_d}{N_t} \times 100 \qquad (2)$$

Nd; Number of decayed fruits **N_t:** Total number of fruits examined

Total Soluble Solids (TSS)

Total soluble solids (TSS) were determined using a handheld refractometer (ORA 80BB). Fruit samples were homogenized thoroughly using a laboratory blender, and the resulting juice was filtered prior to analysis. A few drops of the filtrate were placed on the prism surface of the refractometer, and TSS values were recorded directly as °Brix according to the method described **by Bhati and Raliya (2022).**

Microbiological tests

Total aerobic bacterial counts were determined using the Topping medium after incubation for 3, 5, 7, and ٩ days. Briefly, 10 g of grape samples from each treatment were homogenized in 90 mL of sterile distilled water under aseptic conditions. Serial decimal dilutions were prepared, and aliquots were plated on the appropriate culture media for microbial enumeration. Bacterial counts were determined using Topping medium after incubation at 37 °C for 24 h, whereas fungal counts were determined using Martin's medium after incubation at 30 °C for 72 h (**Johnny *et al.,* 2021**). All analyses were performed in triplicate, and the results were expressed as $\log_{10}$ CFU/g of sample.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as the mean ± standard error (SE). Statistical analyses were performed using the SPSS software (IBM SPSS Statistics, version ٢٥; IBM Corp., Armonk, NY, USA). Data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significant differences among treatments at $p < 0.05$.

Results and dissection

Phytochemical screening

Phytochemical screening revealed that the ethanolic chrysanthemum extracts contained a wide range of biologically active secondary metabolites, including steroids, alkaloids, glycosides, terpenoids, saponins, anthocyanins, tannins, emodins, coumarins, flavonoids, and phenolic compounds **(Table 2).** Considerable variations in phytochemical abundance were observed among the investigated flower colors. Red (ARR) and purple (ARP) chrysanthemum extracts exhibited higher concentrations of most detected phytochemicals, whereas yellow chrysanthemum extract (ARW) showed comparatively lower levels. The elevated accumulation of phytochemicals observed in ARR and particularly

ARP may be attributed to enhanced pigment-associated secondary metabolism in colored chrysanthemum flowers. Anthocyanin-rich flowers are generally characterized by increased biosynthesis of flavonoids and phenolic compounds, which function as protective metabolites against oxidative stress and environmental damage. These compounds are known to contribute significantly to antioxidant and antimicrobial activities through hydrogen donation, free radical scavenging, metal chelation, and microbial membrane disruption mechanisms. The obtained results are consistent with **those of Dahiya *et al.,* (2025)**, who reported that *Chrysanthemum indicum* extracts contain glycosides, tannins, flavonoids, and phenolic acids. Similarly, **Kumar *et al.,* (2021)** demonstrated the presence of saponins, flavonoids, terpenoids, and steroids in chrysanthemum flower extracts. **Apriliyani *et al.,* (2021)** additionally reported that chrysanthemum flowers contain antioxidant phytochemicals, including carotenoids and tannins, whereas **Rao *et al.,* (2020)** attributed the antioxidant activity of chrysanthemum extracts to the presence of flavonoids, triterpenoids, alkaloids, glycosides, and steroids. Comparable phytochemical profiles rich in phenolics, alkaloids, flavonoids, tannins, saponins, and terpenoids were also reported by **Belhachat *et al.,* (2023).** Although previous studies mainly focused on the medicinal and antioxidant properties of chrysanthemum extracts, the present work further demonstrates the relationship between flower pigmentation, phytochemical abundance, and postharvest preservation efficiency in grapes. The superior phytochemical composition observed in purple chrysanthemum extract (ARP) was directly associated with enhanced antioxidant and antimicrobial performance, as well as improved preservation efficacy when incorporated into CMC-based edible coatings. This integrated relationship between flower color, phytochemical functionality, and edible coating application represents an important novelty aspect of the current study.

Table 2. Phytochemical screening of colored chrysanthemum flower ethanolic extracts

Chemical Constituents	ARW	ARR	ARP
Anthocyanins	+	++	++
Tannins	++	++	++
Flavonoids	+	++	+++
Coumarins	+	+	++
Saponins	+	+	+
Steroids	++	++	++
Glycosides	+	++	++
Phenols	+	++	+++
Terpenoids	++	++	++
Emodins	++	++	++
Alkaloids	+	++	+++

(+++), (++), (+), and (-) refer to high, moderate, low, and absent amounts, respectively ; ARW: Yellow chrysanthemum flower extract; ARR: Red chrysanthemum flower extract; ARP: Purple chrysanthemum flower extract.

Total phenolic and flavonoid content

Quantitative determination of total phenolic content (TPC) and total flavonoid content (TFC) demonstrated considerable variations among the investigated chrysanthemum extracts (**Table 3**). Purple chrysanthemum extract (ARP) exhibited the highest TPC value (40.68 ± 0.37 mg GAE/g DW), followed by red chrysanthemum extract (ARR; 38.34 ± 0.46 mg GAE/g DW), whereas yellow chrysanthemum extract (ARW) recorded the lowest phenolic content (31.16 ± 0.24 mg GAE/g DW). A similar trend was observed for TFC values, where ARP showed the highest flavonoid concentration (35.92 ± 0.23 mg QE/g DW), followed by ARR (32.54 ± 0.27 mg QE/g DW) and ARW (27.19 ± 0.36 mg QE/g DW).

The elevated phenolic and flavonoid content observed in ARP may be associated with enhanced pigment-related secondary metabolism in purple chrysanthemum flowers. Anthocyanin-rich flowers are characterized by increased biosynthesis of flavonoid-derived compounds, which play essential protective roles against oxidative stress and environmental damage. This observation suggests a strong relationship between flower pigmentation intensity and phytochemical accumulation, which may directly influence the biological functionality of the chrysanthemum extracts.

Phenolic compounds are major contributors to antioxidant activity because of their ability to donate hydrogen atoms, transfer electrons, scavenge reactive oxygen species, and inhibit oxidative chain reactions **(Karthivashan *et al.,* 2013).** In addition, flavonoids act as reducing agents and metal chelators, contributing significantly to the reduction of oxidative stress and cellular protection. Therefore, the significantly higher TPC and TFC values observed in ARP may explain its superior antioxidant and antimicrobial activities reported in this study.

The obtained findings are consistent. **Apriliyani *et al.,* (2021)** reported that red and blue chrysanthemum flowers contain elevated concentrations of flavonoids, anthocyanins, and cyanidin pigments compared with yellow cultivars. Similarly, **Malpani *et al.,* (2019)** demonstrated a positive correlation between antioxidant activity and total phenolic content in chrysanthemum flowers**. Yuan *et al.,* (2025)** additionally reported that chrysanthemum extracts exhibiting high phenolic and flavonoid contents showed strong reducing power and DPPH radical-scavenging activity**. Mohamad and Zahari (2024)** also attributed the

antioxidant and antimicrobial activities of chrysanthemum extracts to their richness in phenolic bioactive compounds. Comparable observations were reported by **Alexieva *et al.*, (2013),** who detected considerable phenolic accumulation associated with enhanced antioxidant potential in chrysanthemum flowers.

Unlike previous studies that mainly focused on the medicinal importance of chrysanthemum phytochemicals, the present study further establishes a direct relationship between flower color, phytochemical accumulation, antioxidant activity, antimicrobial performance, and postharvest preservation efficiency in grape storage systems. The superior phenolic and flavonoid contents observed in purple chrysanthemum extract (ARP) highlight its strong potential as a multifunctional natural preservative for edible coating applications and postharvest fruit protection.

Table 3. Total phenolic content (TPC) and total flavonoid content (TFC) of colored chrysanthemum ethanolic extracts

Used Free radical	ARW	ARR	ARP
Total phenolic content (TPC) (mg GAE/mL)	31.16 ± 0.24^{c}	38.34 ± 0.46^{b}	40.68 ± 0.37^{a}
Total flavonoids content (TFC) (mg QE /mL)	27.19 ± 0.36^{c}	32.54 ± 0.27^{b}	35.92 ± 0.23^{a}

ARW: Yellow chrysanthemum extract; ARR: Red chrysanthemum extract; ARP: Purple chrysanthemum extract. Different superscript letters indicate significant differences ($p < 0.05$).

Antioxidant activity

The antioxidant activities of yellow (ARW), red (ARR), and purple (ARP) chrysanthemum extracts were evaluated using DPPH, FRAP, and ABTS assays (**Table 4)**. Significant variations in antioxidant capacity were observed among the investigated flower colors. The DPPH assay demonstrated that ARP exhibited the strongest radical-scavenging activity (53.91 ± 2.19 µmol TE/g DW), followed by ARR (45.96 ± 2.37 µmol TE/g DW), whereas ARW showed the lowest activity (40.24 ± 2.25 µmol TE/g DW). DPPH is a stable free radical commonly used for evaluating antioxidant activity because antioxidants can neutralize DPPH radicals through hydrogen donation and electron transfer mechanisms, resulting in radical reduction and discoloration (**Kumar *et al.*, 2012**).Similarly, the ABTS assay showed high antioxidant activity in all extracts, with ARP recording the highest scavenging activity (19.96 ± 1.28 µmol TE/g DW), followed by ARR (17.89 ± 1.40 µmol TE/g DW) and ARW (14.36 ± 1.37 µmol TE/g DW). Comparable trends were observed in the FRAP assay, where ARR and ARP exhibited higher ferric-reducing power (13.63 ± 1.21

and 13.24 ± 1.24 µmol TE/g DW, respectively) than ARW (11.24 ± 1.36 µmol TE/g DW). The FRAP assay measures the ability of antioxidant compounds to reduce Fe^{3+} to Fe^{2+} under acidic conditions (**Carlsen *et al.,* 2010**).

The superior antioxidant activity observed in ARP may be directly associated with its elevated phenolic and flavonoid contents. Phenolic compounds are recognized as effective antioxidants because of their ability to scavenge reactive oxygen species, donate hydrogen atoms, stabilize free radicals, and inhibit oxidative chain reactions. In addition, anthocyanin-rich pigmented flowers generally possess enhanced antioxidant metabolism owing to the accumulation of flavonoid-derived secondary metabolites.

The obtained findings are consistent with **those of Ley *et al.,* (2025)**, who reported strong antioxidant activity in chrysanthemum extracts based on DPPH, ABTS, and FRAP assays. Similar observations were reported by **Ozsoy *et al.,* (2018)**, who demonstrated that chrysanthemum ethanolic extracts possess considerable antioxidant potential because of their phytochemical composition. **Popova *et al.,* (2015)** additionally reported that purple and yellow chrysanthemum flowers contain high concentrations of total phenolics and flavonoids associated with enhanced antioxidant activity. Comparable antioxidant activities based on ABTS and FRAP assays were also observed by **Alexieva *et al.,* (2013**).

The consistently high antioxidant activities observed across the DPPH, FRAP, and ABTS assays indicate that chrysanthemum extracts possess multifunctional antioxidant mechanisms involving radical scavenging and reducing activities. Among the investigated extracts, purple chrysanthemum extract (ARP) demonstrated the strongest antioxidant potential, highlighting its promising applicability as a natural antioxidant source for food preservation and bioactive edible coating systems.

able 4. Antioxidant activities of colored chrysanthemum ethanolic extracts determined by DPPH, ABTS, and FRAP assays

Used Free radical	ARW	ARR	ARP
DPPH (µmol TE/mg filtrate**)**	40.24±2.25^{c}	45.96±2.37^{b}	53.91±2.19^{a}
FRAP (µM TE/mg filtrate**)**	11.24 ± 1.36^{c}	13.63 ± 1.21^{a}	13.24 ± 1.24^{b}
ABTS (µmol TE/ mg filtrate**)**	14.36 ± 1.37^{c}	17.89 ± 1.40^{b}	19.96 ± 1.28^{a}

TE: Trolox equivalent; each value is expressed as the mean ± SE (n = 3). ARW: Yellow chrysanthemum flower extract; ARR: Red chrysanthemum flower extract; ARP: Purple chrysanthemum flower extract. Different superscript letters indicate significant differences ($p < 0.05$).

Mechanistic Interpretation of the Antimicrobial Activity of Chrysanthemum Extracts

The antimicrobial activities of chrysanthemum extracts against bacterial and fungal pathogens are presented in **Tables 5 and 6 and illustrated in Figure 1**. The investigated extracts exhibited inhibitory activity against both Gram-negative bacteria *(Escherichia coli and Erwinia carotovora*) and fungal pathogens (*Penicillium digitatum and Fusarium solani).* However, considerable differences in antimicrobial efficacy were observed according to flower color and extraction solvent. The 70% ethanolic extracts exhibited significantly higher antimicrobial activity than aqueous extracts. This enhanced activity may be attributed to the superior extraction efficiency of ethanol for moderately polar bioactive compounds, including phenolics, flavonoids, tannins, terpenoids, and anthocyanins. These phytochemicals are widely recognized for their antimicrobial properties and are generally extracted more efficiently using hydroalcoholic solvents than water alone (**Liu *et al.,* 2020**). Consequently, the stronger inhibitory effects observed for ethanolic extracts likely reflect their richer phytochemical composition and higher concentrations of biologically active metabolites. Among the investigated extracts, purple chrysanthemum extract (ARP) exhibited the strongest antimicrobial activity against all tested microorganisms. The highest antibacterial inhibition zone was recorded against *Escherichia coli* (32.5 mm), whereas inhibition zones of 28.5 mm were observed against *Erwinia carotovora* using the 70% ethanolic extract. Similarly, ARP exhibited remarkable antifungal activity, producing inhibition zones of 28.7 mm against *Penicillium digitatum* and 25.5 mm against *Fusarium solani* compared with the chemical preservative control (SO_2). The pronounced antibacterial activity observed against Gram-negative bacteria is particularly noteworthy because these bacteria possess an outer membrane rich in lipopolysaccharides that commonly limits antimicrobial penetration and increases resistance to antimicrobial agents (**Jalali *et al.,* 2016**). The obtained results therefore suggest that the bioactive compounds present in chrysanthemum extracts may interfere with bacterial membrane integrity, increase membrane permeability, and disrupt essential cellular processes such as enzyme activity and protein synthesis.

Similarly, the strong antifungal activity of ARP may be attributed to the interaction between phenolic and flavonoid compounds and fungal cell wall components and membrane-bound ergosterol. These interactions may result in membrane destabilization, leakage of intracellular components, inhibition of spore germination, and suppression of mycelial growth (**Xing *et al.,* 2022**). The comparatively lower activity observed in aqueous extracts

426 further emphasizes the importance of solvent polarity in the extraction of fungitoxic
427 phytochemicals.
428 The superior antimicrobial performance of ARP strongly correlates with its elevated phenolic
429 and flavonoid contents as well as its higher antioxidant activity, as observed in previous
430 sections. This relationship highlights the important role of pigment-associated secondary
431 metabolites, particularly anthocyanins and flavonoids, in determining the biological
432 functionality of chrysanthemum extracts.
433 These findings are consistent with those of previous studies reporting strong antimicrobial
434 activities of phenolic-rich chrysanthemum extracts against bacterial and fungal pathogens.
435 However, the present study further demonstrates the direct relationship between flower color,
436 phytochemical accumulation, antioxidant potential, and antimicrobial efficiency concerning
437 postharvest preservation applications. The strong antimicrobial activity exhibited by purple
438 chrysanthemum extract (ARP) highlights its promising potential as a natural alternative to
439 synthetic preservatives for food preservation and postharvest disease control. **Table 5.**
440 **Inhibition zones (mm) of 70% ethanolic chrysanthemum extracts against *Escherichia***
441 ***coli* and *Erwinia carotovora***

Bacteria	*E. coli*	*Erwinia carotovora*
Control	8.5 ± 1.00^{f}	7.8 ± 1.15^{g}
ARW	14.3 ± 1.53^{d}	12.8 ± 1.00^{e}
ARR	16.8 ± 1.00^{c}	14.2 ± 1.53^{d}
ARP	32.5 ± 1.15^{a}	28.5 ± 1.00^{b}

Within the same column, different superscript letters indicate significance. ($p<0.05$).

442 ARW, Yellow chrysanthemum flower extract; ARR, Red chrysanthemum flower extract; ARP, Purple
443 chrysanthemum flower extract.

444 **Table 6. Inhibition zone (mm) of natural plant 70% ethanol extract compared with**
445 ***Fusarium solani. and Penicillium digitatum.***

Fungi	*Fusarium solani*	*Penicillium digitatum*
Control	11.4 ± 2.08^{g}	9.3 ± 0.58^{h}
ARW	20.5 ± 1.15^{c}	14.8 ± 1.15^{e}
ARR	18.0 ± 1.00^{d}	11.8 ± 0.58^{f}
ARP	28.7 ± 1.73^{a}	25.5 ± 1.00^{b}

Within the same column, different superscript letters indicate significance. ($p<0.05$).

446 ARW, Yellow chrysanthemum flower extract; ARR, Red chrysanthemum flower extract; ARP, Purple
447 chrysanthemum flower extract.

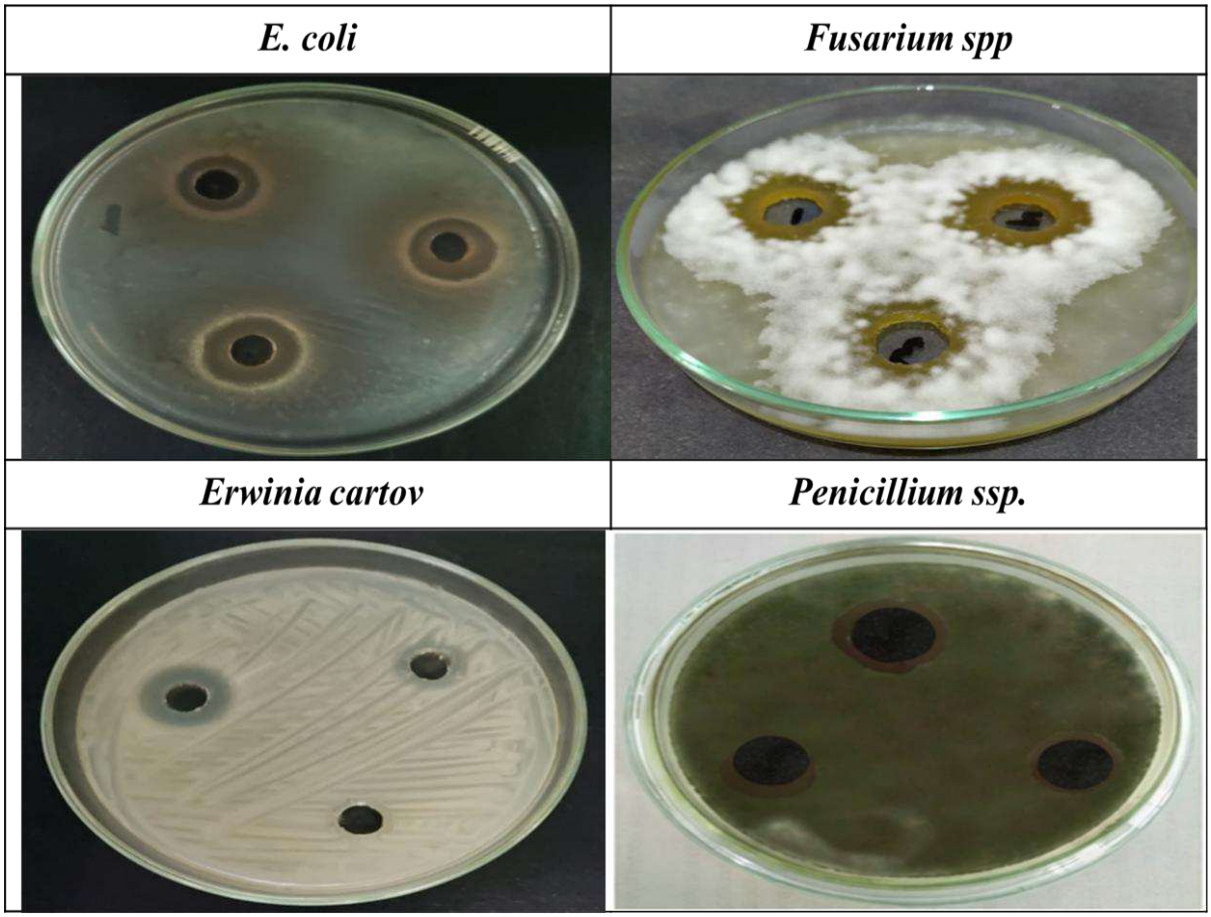


Figure (1) Inhibition zone (mm) of natural plant 70% ethanol extraction compared with *E. coli, Erwinia carotovora, Fusarium spp., and Penicillium spp*.

Weight Loss (%) of Grapes Stored at 20 ± 1 °C

The changes in weight loss (%) of the coated and uncoated grapes during storage at 20 ± 1 °C are presented in **Figure 2**. Weight loss progressively increased throughout the storage period for all treatments, reflecting continuous moisture loss and transpiration during ambient storage. However, the rate of weight loss differed significantly according to the treatment type and extract concentration. The control treatment exhibited a rapid increase in weight loss, reaching 9.30 ± 0.17% by day 7, indicating severe moisture loss and accelerated deterioration in the untreated grapes. In contrast, grapes treated with chrysanthemum flower extract (CFE) showed significantly lower weight loss than the control. Moreover, increasing the CFE concentration gradually reduced the weight loss during storage. The CFE (3%) treatment maintained lower weight loss values, reaching 7.39 ± 2.00% on day 10 compared

with 11.01 ± 1.17% for CFE (1.5%). CMC coatings alone also reduced moisture loss compared to untreated grapes because of their semipermeable barrier properties. However, the greatest reduction in weight loss was observed when CMC was combined with CFE. Among all treatments, CMC + CFE (3%) exhibited the lowest weight loss values throughout the storage period, recording only 0.89 ± 0.43%, 1.38 ± 0.54%, 2.69 ± 1.39%, and 4.40 ± 1.29% at 3, 5, 7, and 10 days, respectively. The improved retention of fruit weight in the combined treatments may be attributed to the synergistic interaction between the barrier properties of CMC coatings and the bioactive phytochemicals present in chrysanthemum extracts. The CMC coating likely reduced transpiration and respiration rates by limiting moisture evaporation and gas exchange, while the incorporated phytochemicals may have contributed to maintaining cellular integrity and delaying tissue deterioration. **(Shiekh *et al.*, 2022).**

The results indicate that chrysanthemum extract-enriched edible coatings effectively minimized water loss and improved the storage stability of grapes during room-temperature storage. The superior performance of CMC + CFE (3%) highlights its strong potential as a natural preservation system for maintaining grape quality and extending the shelf life.

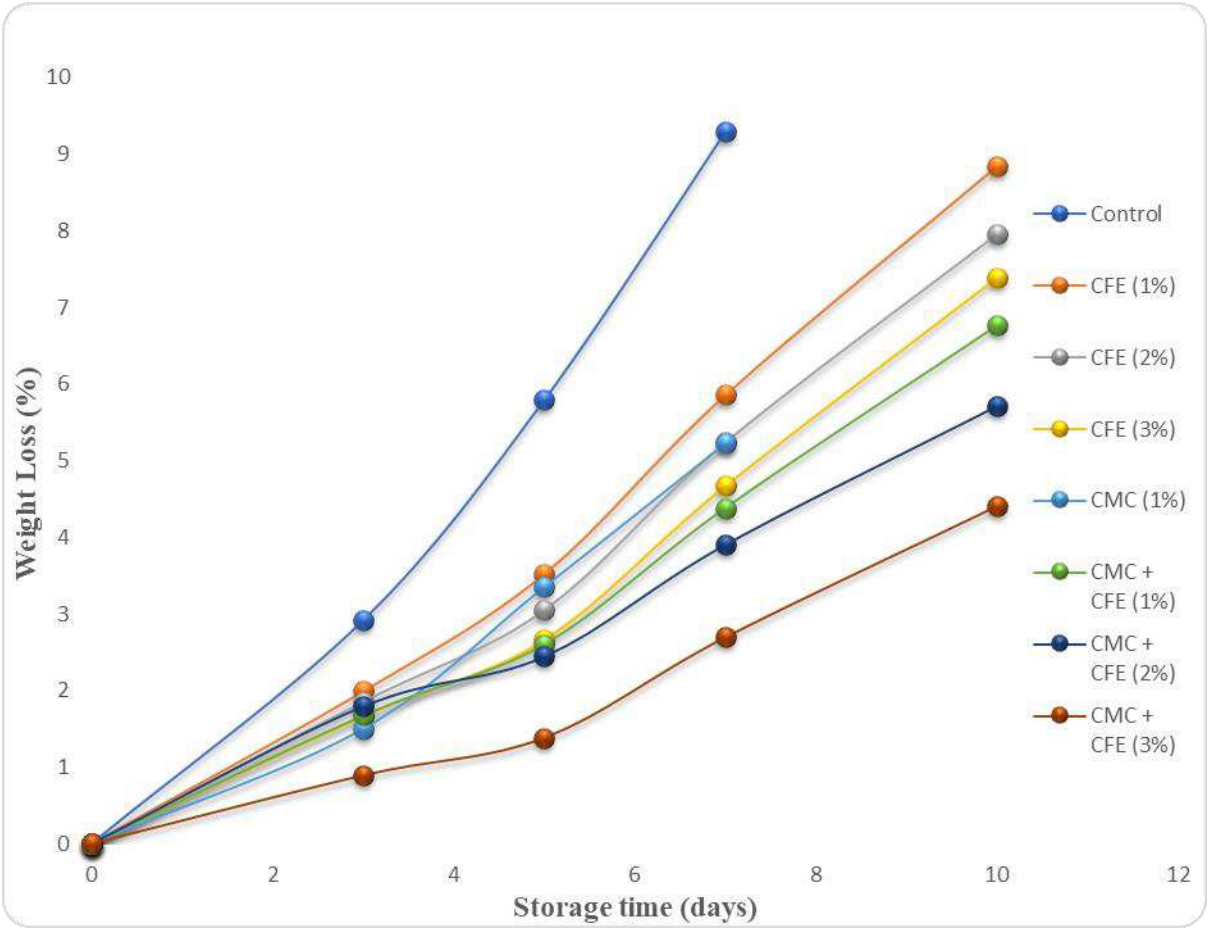


Figure 2. Effect of chrysanthemum flower extract and CMC edible coatings on the weight loss (%) of grapes during storage at 20 ± 1 °C.

Decay Percentage (%)

The decay percentage progressively increased during storage at 20 ± 1 °C in all treatments, reflecting natural postharvest senescence and microbial deteriorationFigures ٣) (and ٤**.** The untreated control showed the highest decay incidence, reaching 75.0 ± 0.58% by day 7, whereas CMC alone also exhibited relatively high spoilage levels (65.0 ± 1.15%). By day 10, both treatments became unsuitable for further evaluation because of severe structural collapse and advanced microbial spoilage. Similar findings were reported by **Priyadarshi *et al.,* (2024)**, who demonstrated that polysaccharide coatings without active antimicrobial compounds provide limited protection during prolonged storage.

In contrast, incorporation of Chrysanthemum Flower Extract (CFE) significantly reduced decay development in a concentration-dependent manner. Higher CFE concentrations showed greater preservation efficiency, with CFE (3%) exhibiting lower spoilage than lower concentrations throughout storage.

The greatest preservation effect was observed in the composite CMC + CFE coatings, particularly CMC + CFE (3%), which maintained the lowest decay values, reaching only 25.7 ± 0.58% on day 7 and 38.7 ± 0.58% by day 10. This superior performance may be attributed to the synergistic interaction between the semipermeable barrier properties of CMC and the antimicrobial phytochemicals present in the chrysanthemum extract. Similar preservation mechanisms have been described by **Van *et al.,* (2023**), who reported that edible coatings reduce gas exchange and delay senescence during storage.

Furthermore, polymeric encapsulation may protect phenolics and flavonoids from premature degradation, thereby allowing sustained antimicrobial activity during storage. In agreement with **Priyadarshi *et al.* (2024**), the continuous release of bioactive compounds likely contributed to reduced microbial proliferation, delayed tissue deterioration, and lower decay progression. These findings highlight the strong potential of chrysanthemum-enriched edible coatings as sustainable natural alternatives for improving grape shelf life and postharvest quality under room-temperature storage conditions.

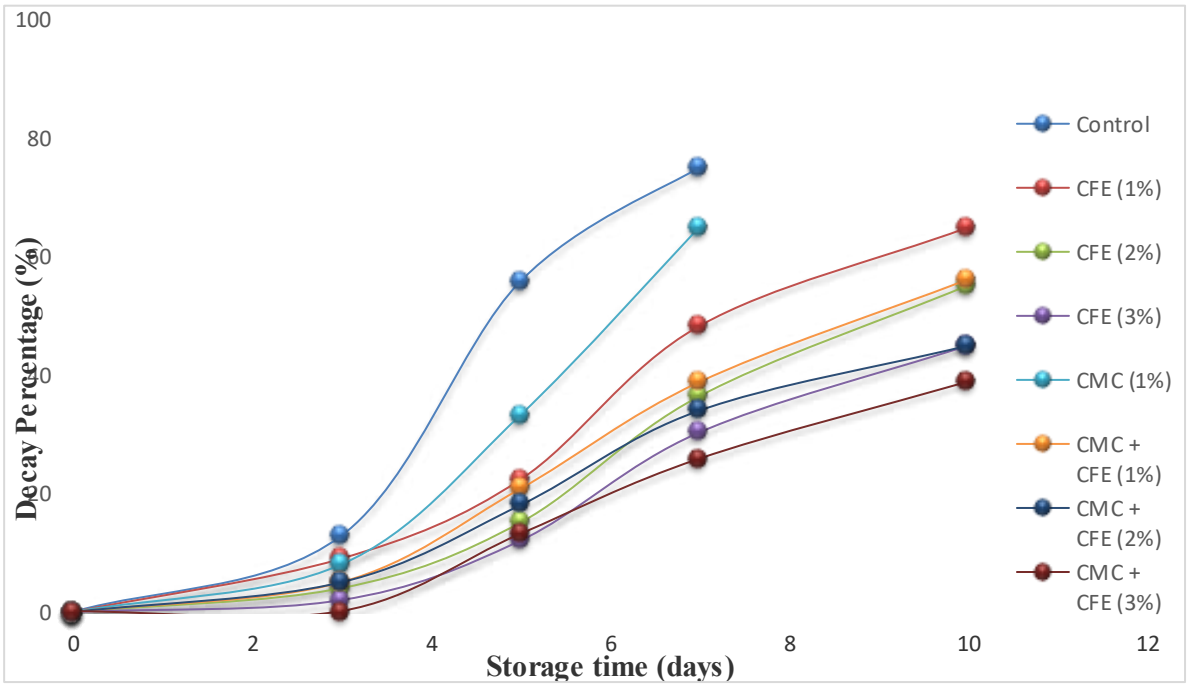


Figure 3. Effect of chrysanthemum flower extract and CMC edible coatings on the decay percentage (%) of grapes during storage at 20 ± 1 °C.



Figure 4. Effect of chrysanthemum flower extract and CMC edible coatings on the shelf life of grapes during storage at 20 ± 1 °C.

Total Soluble Solids (TSS)

The changes in total soluble solids (TSS) of coated and uncoated grapes during storage at 20 ± 1 °C are presented in **Figure 5.** TSS values progressively increased throughout the storage period in all treatments, reflecting continuous physicochemical changes associated with fruit ripening and senescence. The increase in TSS during storage is mainly attributed to moisture loss, polysaccharide degradation, and the subsequent concentration of soluble sugars and organic compounds within grape tissues.

Untreated grapes exhibited the highest increase in TSS values during storage, increasing from 15.13 °Brix on day 0 to 20.04 °Brix by day 7, indicating accelerated ripening and rapid deterioration under ambient storage conditions. Similarly, grapes coated with CMC alone showed relatively elevated TSS values, although lower than those of the control, suggesting that the coating partially reduced metabolic changes during storage.

In contrast, treatments containing CFE significantly moderated TSS accumulation during storage, with higher extract concentrations exhibiting greater preservation efficiency. Grapes treated with CFE (3%) maintained lower TSS values throughout storage compared with lower extract concentrations, reaching 18.43 °Brix at day 10 compared with 19.81 °Brix for CFE (1%). This concentration-dependent response suggests that increasing the concentration of chrysanthemum extract enhanced the preservation efficiency of the treatment system.

The most pronounced effect was observed in the combined treatments containing both CMC and CFE. Among all treatments, CMC + CFE (3%) exhibited the lowest TSS values during storage, increasing only from 14.44 °Brix at day 0 to 18.02 °Brix at day 10. The superior performance of this treatment suggests a synergistic interaction between the semipermeable barrier properties of CMC and the bioactive phytochemicals present in chrysanthemum extract. The reduced TSS accumulation observed in coated grapes may be mechanistically associated with lower respiration and transpiration rates, delayed senescence, and reduced moisture loss during storage. The semipermeable coating likely limited gas exchange and water vapor transfer, thereby slowing carbohydrate metabolism and delaying the concentration of soluble solids within fruit tissues. In addition, the antioxidant

phytochemicals present in chrysanthemum extract may have contributed to preserving membrane integrity and reducing oxidative deterioration during storage.

Interestingly, the lower TSS accumulation observed in the CMC + CFE treatments corresponded with the lower weight loss and microbial counts recorded in the same treatments, suggesting that improved moisture retention and reduced microbial proliferation collectively contributed to maintaining grape quality during storage. This integrated preservation effect highlights the multifunctional role of chrysanthemum-enriched edible coatings in improving postharvest stability. **(Nguyen *et al.*, 2020)**

Unlike conventional edible coating studies that mainly focus on barrier properties alone, the present findings demonstrate that the incorporation of chrysanthemum flower extract into CMC coatings not only reduced moisture loss but also effectively delayed physicochemical deterioration and metabolic changes during storage. These findings highlight the potential of chrysanthemum phytochemicals as multifunctional natural additives for developing advanced edible coating systems capable of improving both microbiological and physicochemical stability of fresh grapes under room-temperature storage conditions.

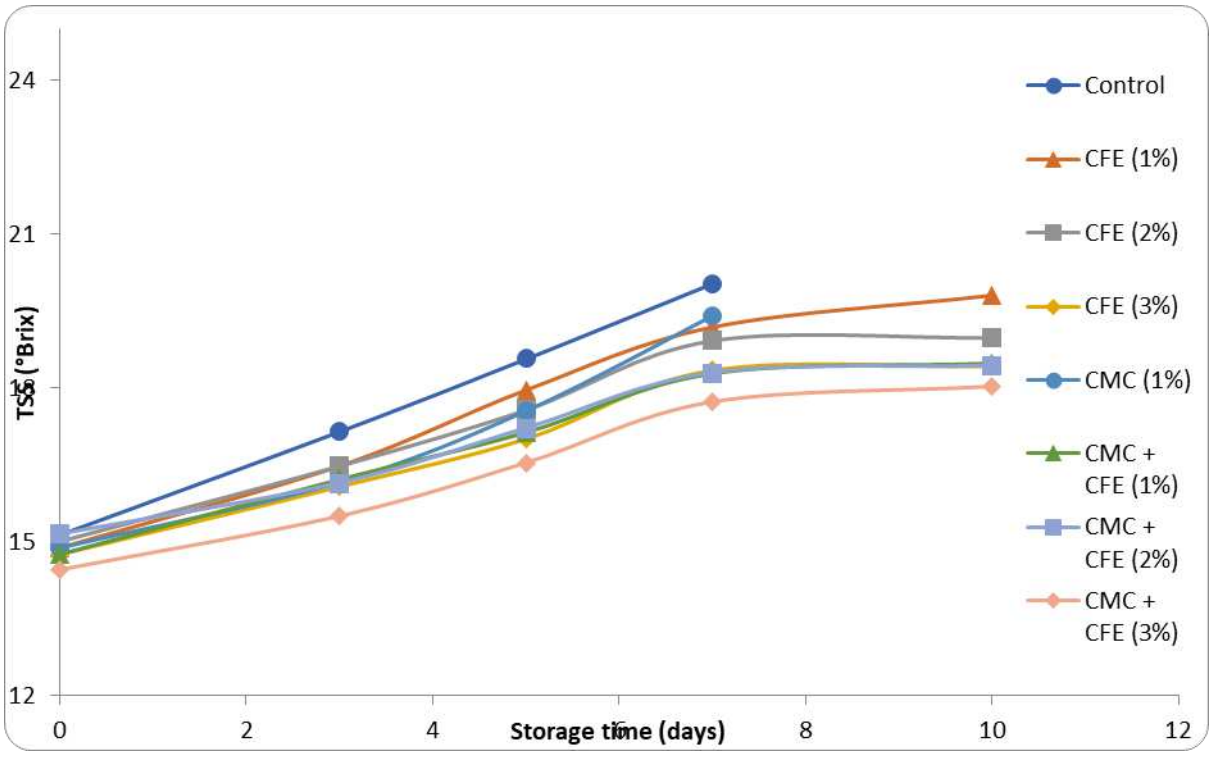


Figure 5. Effect of chrysanthemum flower extract and CMC edible coatings on total soluble solids (TSS, °Brix) of grapes during storage at 20 ± 1 °C.

Total bacterial count of grapes stored at a temperature of (20±1°C)

The total bacterial counts of coated and uncoated grapes during storage at 20 ± 1 °C are shown in **Figures 6 and 7**. A gradual increase in bacterial load was observed throughout the storage period for all treatments, confirming that room-temperature storage favors microbial proliferation in grapes. However, the treated samples exhibited significantly lower bacterial counts than untreated grapes.

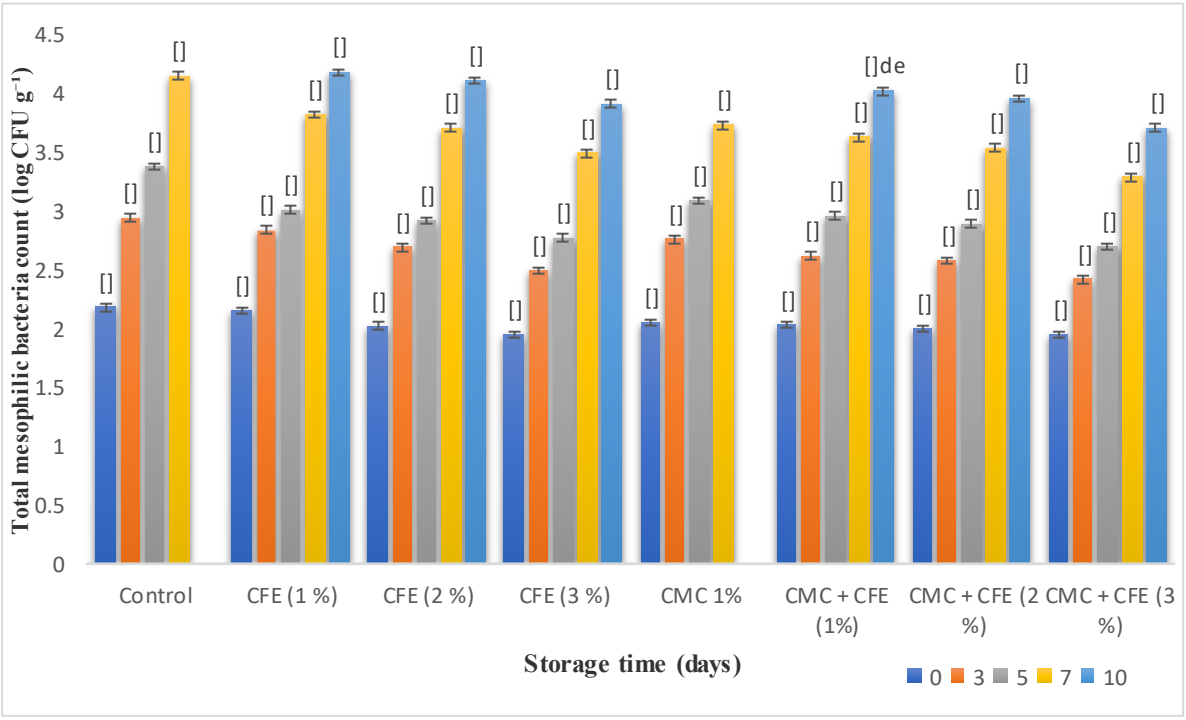
The control treatment showed the highest bacterial growth, with counts increasing from 2.18 $\log_{10}$ CFU/g at day 0 to 4.15 $\log_{10}$ CFU/g by day 7, indicating rapid microbial deterioration under unprotected storage conditions. In contrast, grapes treated with chrysanthemum flower extract (CFE) demonstrated reduced bacterial growth, particularly at higher extract concentrations. The 3% CFE treatment maintained lower bacterial counts throughout storage, reaching 3.91 $\log_{10}$ CFU/g at day 10 compared with 4.18 $\log_{10}$ CFU/g for the 1% treatment.

CMC coatings alone also reduced bacterial proliferation compared to the control treatment. Nevertheless, the strongest antibacterial activity was observed when CMC was combined with CFE. Among all treatments, CMC + CFE (3%) exhibited the lowest bacterial counts during storage, recording values of 1.95, 2.42, 2.70, 3.29, and 3.71 $\log_{10}$ CFU/g at 0, 3, 5, 7, and 10 days, respectively.

The enhanced antibacterial activity observed in treatments containing chrysanthemum extract may be attributed to the presence of phenolics, flavonoids, tannins, and other bioactive phytochemicals capable of disrupting bacterial membrane permeability and interfering with essential metabolic pathways. In addition, the semipermeable structure of CMC coatings likely reduced oxygen transfer and environmental contamination, thereby limiting bacterial proliferation during storage. The superior performance of the combined treatments suggests a synergistic interaction between the physical barrier properties of CMC and the antimicrobial activity of chrysanthemum phytochemicals. This combined effect effectively delayed bacterial spoilage and improved the microbiological stability of grapes during storage.

The obtained findings are consistent with **those of Cefola *et al.,* (2020) and Zhang *et al.,* (2025),** who reported that edible coatings enriched with natural antimicrobial compounds effectively reduced microbial growth and improved the postharvest stability of fresh fruits.

Overall, the present results demonstrate that CMC-based edible coatings enriched with chrysanthemum flower extract represent a promising natural preservation strategy for controlling bacterial growth and extending the shelf life of grapes during storage at room temperature.



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613 **Figure 6.** Effect of coating and uncoating with chrysanthemum flower extract on total mesophilic
614 bacterial count of grapes during storage at 20 ± 1 °C. Different superscript letters indicate significant
615 differences ($p < 0.05$).

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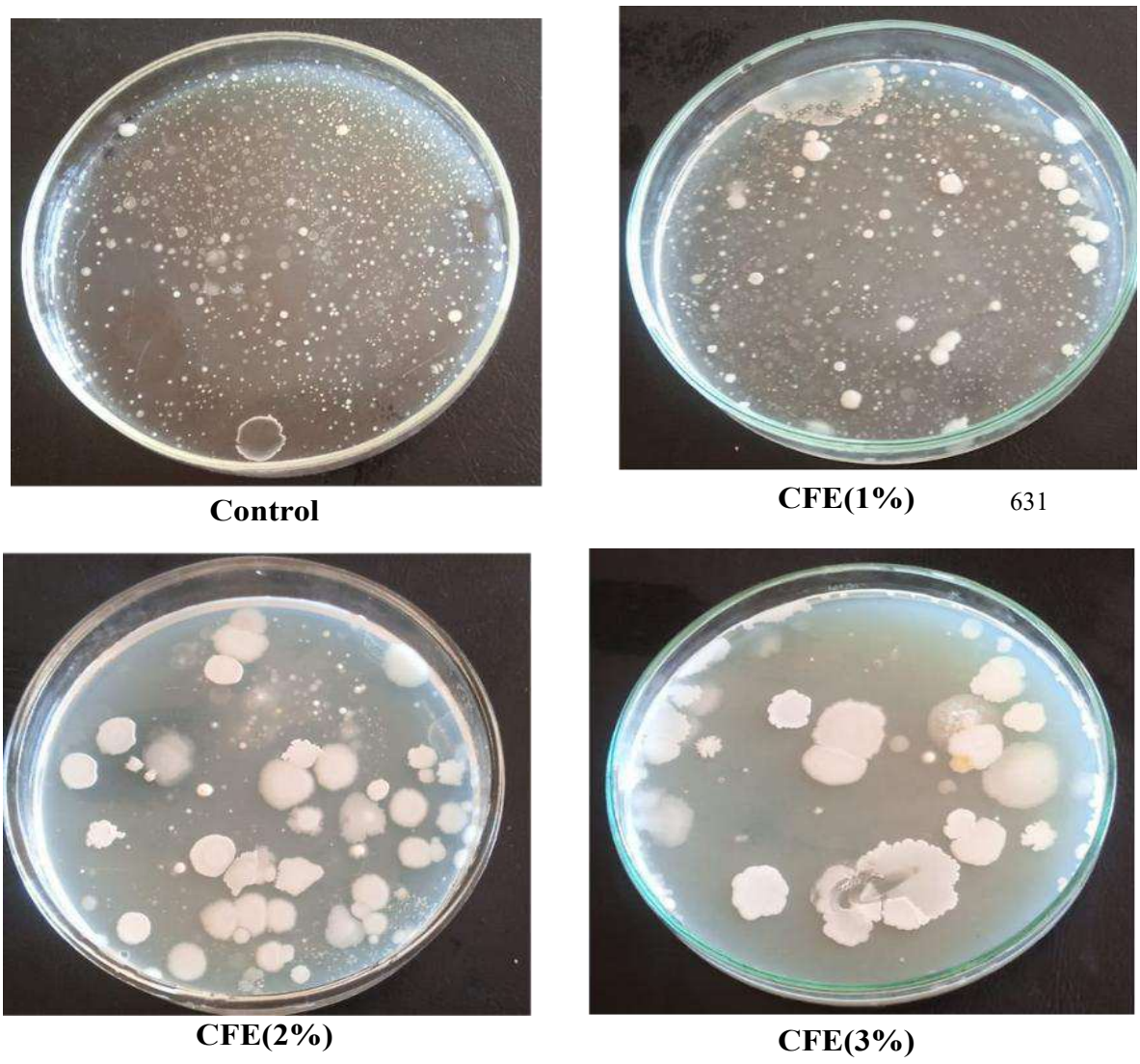

Figure 7. Total mesophilic bacterial growth in grapes treated with chrysanthemum flower extract and CMC edible coatings during storage at 20 ± 1 °C.

Total molds count and yeast of grapes stored at a temperature of (20±1°C)

The occurrence of filamentous molds and associated mycotoxins in grapes is strongly influenced by storage conditions, grape composition, and postharvest handling practices. Grapes are highly susceptible to fungal colonization because of their elevated moisture content, high sugar levels, and soft epidermal tissues, which provide favorable conditions for fungal proliferation during their storage **(Freire *et al.,* 2017).**

As shown in **Figures 8 and 9,** fungal counts progressively increased throughout storage in all treatments, confirming the stimulatory effect of room-temperature storage on fungal growth. However, untreated grapes exhibited the highest fungal proliferation, indicating rapid deterioration in the absence of preservative treatments. Treatments

containing chrysanthemum flower extract (CFE) significantly suppressed fungal growth compared with the control, with the antifungal effect becoming more pronounced at higher extract concentrations. This inhibitory activity may be attributed to the elevated concentrations of phenolics, flavonoids, tannins, and anthocyanins present in chrysanthemum extracts, which are known to disrupt fungal membrane integrity, inhibit spore germination, and interfere with essential fungal metabolic pathways. (**Prabawa *et al.*,2023)** CMC coatings alone exhibited moderate antifungal activity, likely because of their semipermeable barrier properties that reduce oxygen transfer and moisture loss. Nevertheless, the strongest antifungal effect was observed when CMC was combined with CFE, particularly at a 3% concentration, suggesting a synergistic interaction between the coating matrix and chrysanthemum phytochemicals. The improved preservation efficiency of the combined treatments may result from the simultaneous action of the physical barrier provided by CMC and the sustained release of antifungal bioactive compounds from the incorporated extract. Although fungal growth gradually increased during prolonged storage, the treated samples maintained substantially lower fungal counts than untreated grapes, indicating delayed fungal proliferation and improved microbiological stability. These findings are consistent with **Ali *et al.*, (2026)**, who reported that edible coatings enriched with plant-derived bioactive compounds effectively reduced fungal spoilage during fruit storage.

Overall, the results demonstrate that CMC-based edible coatings enriched with chrysanthemum extract represent a promising natural strategy for suppressing fungal growth and improving the postharvest quality of grapes during room-temperature storage.

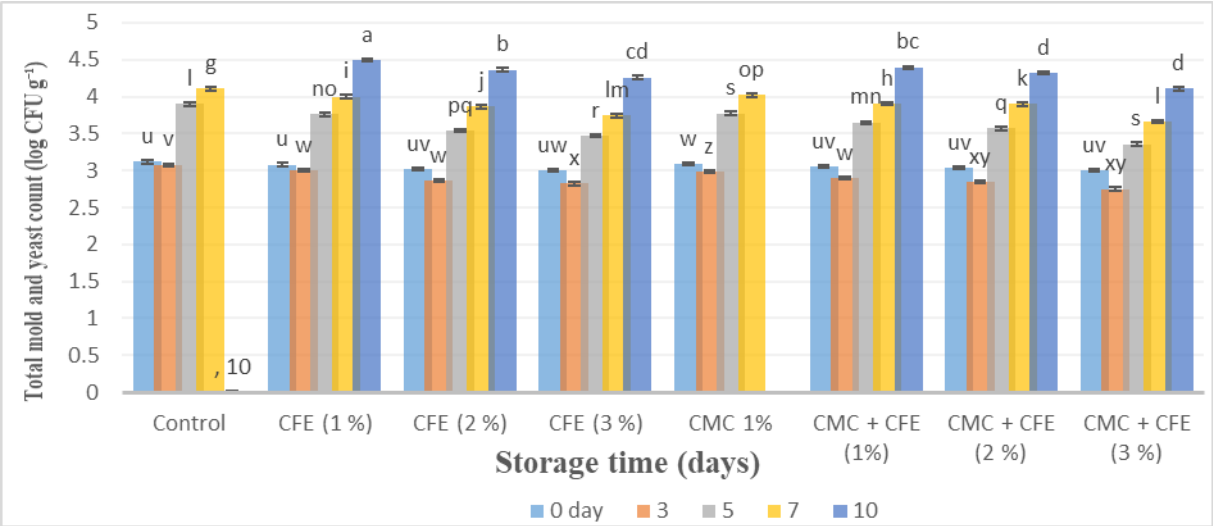

Figure 8. Effect of coating and uncoating with chrysanthemum flower extract on total mold count and yeast count of grapes during storage at 20 ± 1 °C. Different superscript letters indicate significant differences ($p < 0.05$).

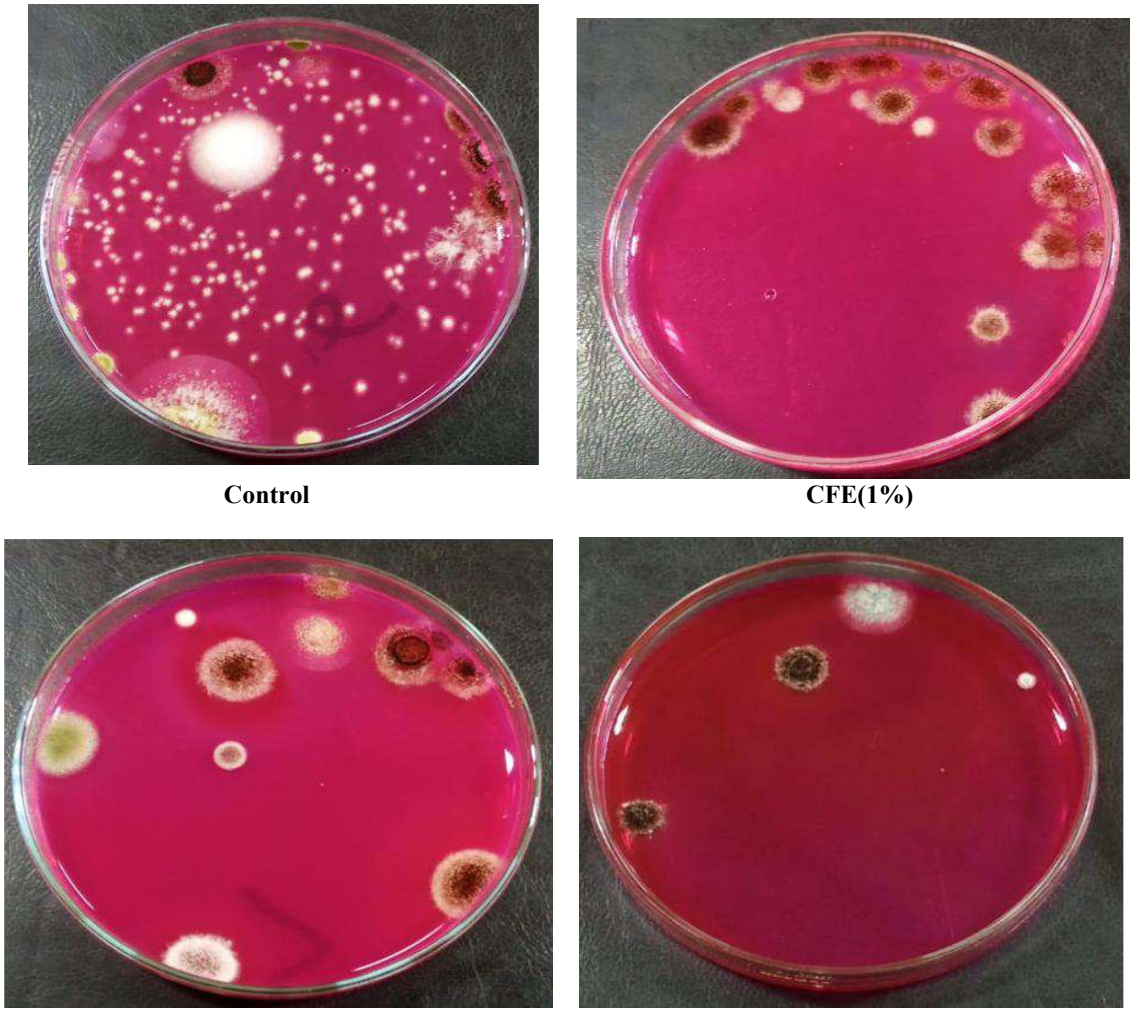

Figure 9. Total mold count and yeast growth in grapes treated with chrysanthemum flower extract and CMC edible coatings during storage at 20 ± 1 °C.

Conclusion

This study underscores the significant practical value of deploying natural, eco-friendly bioactive alternatives to mitigate postharvest fungal decay in table grapes. The incorporation of colored Chrysanthemum morifolium flower extracts ,particularly the purple variant (ARP) ,into carboxymethyl cellulose (CMC)-based edible coatings demonstrated an exceptional capacity to suppress fungal proliferation and potentially reduce the risk of mycotoxin contamination during ambient storage. These findings firmly validate the feasibility of integrating pigment-rich botanical extracts into sustainable postharvest

management strategies. Ultimately, this approach offers a multifunctional solution to enhance fruit quality, extend shelf life, and fortify food safety while substantially decreasing commercial reliance on synthetic chemical fungicides.

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Author contribution

AS: Conceptualization, Formal analysis, Investigation, Visualization, Data curation, Methodology, Project administration, Resources, Software Writing – original draft, Writing – review & editing. **SAH:** Formal analysis, Investigation, Visualization, Data curation, Methodology, Project administration, Resources, Software Writing – original draft, Writing – review & editing. **SAM**: Formal analysis, Investigation, Visualization, Data curation, Methodology, Project administration, Resources, Software Writing – original draft, Writing – review & editing. **MOS**: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **AMA**: Formal analysis, Investigation, Visualization, Data curation, Methodology, Project administration, Resources, Software Writing – original draft, Writing – review & editing. **EMM:** Formal analysis, Investigation, Visualization, Data curation, Methodology, Project administration, Resources, Software Writing – original draft, Writing – review & editing. **IFR**: Formal analysis, Investigation, Visualization, Data curation, Methodology, Project administration, Resources, Software Writing – original draft, Writing – review & editing. **SKT**: Conceptualization, Formal analysis, Investigation, Visualization, Data curation, Methodology, Project administration, Resources, Software Writing – original draft, Writing – review & editing.

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Data availability

Datasets are available at the corresponding author up on the request.

Declarations

Ethics approval:

Not applicable. This study did not involve human participants or animals.

Consent for publication:

Not applicable.

Competing interests:

The authors have no conflicts of interest to declare.

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