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Efficacy of peppermint oil-incorporated tragacanth gum coating on postharvest quality and antioxidant enzyme activity in banana fruits

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ABSTRACT

Purpose: This study investigated the effectiveness of tragacanth gum (TG) coatings integrated with peppermint oil (PO) in controlling pathogenic fungi in banana fruits. **Research Method**: The research specifically evaluated the *in-vitro* and *in-vivo* responses of these coating agents on fungal pathogens and assessed their impact on peroxidase (POD) and catalase (CAT) activities in banana fruits. **Findings**: The study revealed that tragacanth gum integrated with peppermint oil (TGPO) effectively inhibited the growth of *Colletotrichum musae* and *Aspergillus fumigatus* in bananas. TGPO-treated fruits exhibited significantly lower disease incidence (32.67±1.00 % for *C. musae* and 28±1.00 % for *A. fumigatus*) and severity compared to the controls after 14 days. The treatment also maintained higher catalase and peroxidase enzymatic activities, indicating enhanced disease resistance. Furthermore, TGPO-treated bananas retained better quality parameters during the 15-day storage period, including optimal pH levels, lower total soluble solids, reduced water activity, greater firmness, and decreased electrical conductivity. **Research limitations**: No limitations were identified during the course of carrying out this study. **Originality/Value**: The originality and value of this work lie in being the first study to investigate TGPO as a natural preservative coating for bananas. While previous research has examined various edible coatings and essential oils separately, this study uniquely demonstrates how integrating tragacanth gum and peppermint oil creates an effective, eco-friendly solution for extending banana shelf life by controlling fungal pathogens (particularly *C. musae* and *A. fumigatus*) while maintaining fruit quality. This novel approach addresses growing consumer demand for natural food preservation methods while potentially reducing post-harvest losses in the banana industry.

INTRODUCTION

Fruits and vegetables are among the most widely consumed plant-based foods, recognized for their high concentrations of essential vitamins, minerals, fibres, and phytochemicals, which contribute to their numerous health benefits (Samtiya et al., 2021; Slavin & Lloyd, 2012). Consumer preferences for fresh produce are greatly influenced by visual qualities such as size, freshness, appearance, and shelf life, which play a significant role in purchasing decisions (Slavin & Lloyd, 2012; Török et al., 2023). Increasing consumer demand for fresh, natural, minimally processed, and additive-free foods has heightened the importance of maintaining post-harvest quality (Mesías et al., 2021).

However, substantial post-harvest losses of fruits can occur due to a range of factors, including premature harvesting, improper handling, and substandard materials during processing, inappropriate transportation, and poor retail practices (Rajapaksha et al., 2021). In particular, pest infestations, fungal infections, and inadequate storage conditions accelerate spoilage, leading to significant reductions in both fruit quality and marketability (Jiao et al., 2022; Wang et al., 2022).

To address these challenges and extend the shelf life of fresh produce, edible coatings derived from natural sources, including plants and animals, have emerged as an eco-friendly solution. Edible coatings composed primarily of polysaccharides, proteins, and lipids, form a protective barrier around food products, helping to control moisture loss, gas exchange, and microbial contamination (Mendy et al., 2019; Thakur et al., 2018). Such coatings, applied via dipping, brushing, or spraying, create a semi-permeable membrane that suppresses respiration and enhances food preservation (Raghav et al., 2016). Protein-based coatings typically exhibit superior mechanical properties, while polysaccharide-based coatings are more effective in reducing gas permeability and water transfer (Purewal et al., 2024). Understanding the water activity ($^{a}_{w}$) of coated fruits and vegetables is essential for determining the effectiveness of these coatings, as $^{a}_{w}$ directly influences microbial growth, enzymatic reactions, and overall food stability (Perez-Vazquez et al., 2023; Tapia et al., 2008). Lowering $^{a}_{w}$ can significantly reduce spoilage rates by restricting the availability of free water, which is necessary for microbial proliferation (Tapia et al., 2008).

Several studies have explored the effectiveness of edible coatings in extending the shelf life of bananas (Iacovino et al., 2024). Chitosan-based coatings, for instance, have been widely studied for their antifungal properties and ability to reduce weight loss and delay ripening in bananas (Hossain & Iqbal, 2016). Similarly, pectin-based coatings have demonstrated potential in maintaining fruit firmness and minimising microbial decay (Moalemiyan et al., 2012; Sanchís et al., 2017). In addition to polysaccharide-based coatings, research has also investigated the incorporation of natural compounds such as essential oils to enhance the antimicrobial and antioxidant properties of edible coatings (Anis et al., 2021; de Souza et al., 2019; López et al., 2015). Essential oils, including thyme, peppermint and cinnamon oil, have been shown to improve post-harvest quality and reduce fungal infections in various fruits, including bananas (Mohammadi et al., 2020; Pawar et al., 2024). The established literature highlights the potential of integrating natural bioactive compounds into edible coatings to improve shelf life and postharvest management, particularly for fruits like bananas.

Bananas (*Musa sapientium*), a major staple in tropical and subtropical regions, are particularly susceptible to post-harvest spoilage (Alhassan & Ndomakaah, 2024). Rich in essential nutrients such as vitamins A and C, potassium, and fibres, bananas play a crucial role in global food security (Rajapaksha et al., 2021). Tragacanth gum (TG), a natural polysaccharide derived from various *Astragalus* species, has gained attention for its potential in edible coatings due to its emulsifying properties and water-soluble components (Azarikia &

Abbasi, 2016; Gavlighi et al., 2013). When combined with essential oils, such as peppermint oil (*Mentha piperita* L.), tragacanth gum-based coatings may offer both preservative and antioxidant benefits, potentially extending the shelf life of perishable fruits like bananas (Singh et al., 2015).

Given the growing interest in natural alternatives instead of chemical preservatives, this study aims to evaluate the efficacy of tragacanth gum coatings impregnated with peppermint oil in assessing the *in-vitro* and *in-vivo* responses of these coating agents on pathogenic fungi associated with banana fruits. In addition, the study also investigates the impact of tragacanth gum coatings impregnated with peppermint oil on peroxidase (POD) and catalase (CAT) activities in banana fruit.

MATERIALS AND METHODS

Sources of test samples

Healthy, green *Musa sapientum* fruits, which had not yet fully ripened, were collected from the middle part of the bunch to ensure uniform exposure to sunlight and consistent ripening conditions. These fruits were free from peel damage, insect infestation, and fungal infection, with a maturity index of 22% by dry matter prior to treatment. They were obtained through a fruit vendor at a commercial market in Yaba, Lagos State, Nigeria. Tragacanth gum (TG) and peppermint oil (PO) were sourced from the Mycology Laboratory, Department of Botany, University of Lagos.

Media preparation and isolation of fungal pathogens

Potato Dextrose Agar (PDA) was prepared by dissolving 40 g of PDA powder in 1,000 mL of distilled water. The mixture was sterilized at 121°C for 15 minutes under 15 lbs of pressure in an autoclave. Once cooled to 45–50°C, 0.5 g/L of chloramphenicol was added to inhibit bacterial growth. The medium was poured into Petri dishes (15–20 mL per plate) and allowed to solidify. Small sections (5 mm) of banana tissue showing infection symptoms were surface-sterilized with 40% sodium hypochlorite for 3 minutes, rinsed three times with sterile distilled water, and air-dried on sterile filter paper. The tissue sections were then aseptically placed on PDA plates and incubated at room temperature (25 ± 2°C). Pure fungal isolates were obtained by repeated subculturing on fresh PDA plates (Matche & Adeogun, 2022). Thereafter, a pathogenicity assay was conducted to ascertain the pathogenicity of the isolated fungi on the fruits. The symptomless and surface-sterilized banana fruits were wounded and inoculated with fungal cultures. The fruits were incubated under high humidity for six days then examined for disease symptoms. Morphological and microscopic analyses confirmed the pathogenicity of the reisolated fungi (Matche & Adeogun, 2022).

Preparation of tragacanth gum (TG) and peppermint oil (PO) coating

The coating formulation was prepared by dissolving 10 g (10% $^{w}/_{v}$) of Tragacanth gum (TG) into 90 mL of deionized water, followed by equilibration at 70°C for 25 minutes (Adekunle et al., 2021; Adeogun et al., 2023). The mixture was then stirred using a magnetic stirrer, and 2 mL of glycerol was introduced as a plasticizer to improve the mechanical properties of the coating. The pH was adjusted to 5.6 using 1N sodium hydroxide (NaOH) while homogenizing the solution for 5 minutes. Thereafter, the solution was filtered using Mira-cloth and 0.5 mL (0.5%) of peppermint oil (PO), pre-dissolved in 99.5 mL of ethanol, and incorporated into the filtered Tragacanth gum solution (Adeogun et al., 2023; Nasiri et al., 2018). The same preparation was made for the control sample without the addition of the oil.

Preparation of spore suspension

Fungal cultures aged 8–10 days, used for spore preparation, were used based on a modified method of Hojnik et al. (2019). Individual cultures were flooded with 10 mL of an aqueous Tween 80 solution (1 drop in 1000 mL sterile water) and gently scraped with a sterile loop to dislodge the spores. The resulting crude suspension was filtered through a layer of Miracloth to remove mycelial fragments. One gram of fungal mycelium was suspended in 9 mL of sterile distilled water. The spore suspension was serially diluted seven times with sterile distilled water and then stored for subsequent use.

***In vitro* antifungal assay**

The antifungal efficacy of TG and TG-PO was tested against isolated pathogenic fungi from banana fruit. PDA was amended with: TG alone (10%, w/v), and TG (10%, w/v) incorporated with PO (0.5%, v/v). Fungal discs (5 mm) from 14-day-old cultures of the pathogenic fungi were placed at the centre of Petri dishes containing the treated PDA. Control plates containing only PDA were also prepared. The plates were incubated at 25 ± 2°C, and radial mycelial growth was measured daily for 6 days. Antifungal activity was assessed by measuring the colony diameter (cm) using a ruler (Matche & Adeogun, 2022).

In vivo antifungal assay on banana fruits using poisoned food technique

The *in-vivo* antifungal effects of tragacanth gum (TG) and TG incorporated with peppermint oil (TGPO) were evaluated on the fruit of *Musa sapientum* (banana) using the poisoned food technique. Bananas that were not yet fully ripe, with a 22% maturity index based on dry matter and exhibiting yellow colouration, were washed with 20% sodium hypochlorite, rinsed three times with distilled water, and air-dried. Some of the fruit were dipped in TG alone, while others were dipped in the TGPO solutions for 2–3 minutes. The last set, acting as a control, was dipped in sterile distilled water for 2–3 minutes, and all treatments were then air-dried. After treatment, the fruit was inoculated with spore suspensions of the test pathogens using a sterile needle. All treated fruit was wrapped in aluminum foil, packed in commercial cartons, and stored at room temperature. Disease incidence and severity were monitored at 2-day intervals for up to 14 days.

Disease severity was assessed on a scale of 1 to 5, with 1 indicating that 0% of the fruit surface was rotted, and 5 indicating that 76–100% of the fruit surfaces were rotted. Disease incidence was recorded as the percentage of fruits showing symptoms of rot (Kumar et al., 2021; Mohammed Idris et al., 2015).

Measurement of antioxidant enzymatic activities

The extraction of tissue samples for the determination of antioxidant enzyme activities follows the methods described by Chen et al. (2015). Tissue samples (1 g) were collected from an area 2 mm away from the inoculation site on 20 bananas. The samples were homogenized in appropriate buffer solutions and then centrifuged at 15,000 g for 30 minutes at 4°C. Supernatants were used to assess peroxidase (POD) and catalase (CAT) activities, using a sodium phosphate buffer (100 mM, pH 7).

Peroxidase (POD) activity assay

POD activity was determined following the method of Sellamuthu et al. (2013), with modifications. The reaction mixture contained 144 µL of buffered substrate (100 mM sodium phosphate, pH 7.0, and 20 mM guaiacol) and 36 µL of tissue extract. After adding 72 µL of H_2O_2 (100 mM), the increase in absorbance at 460 nm was measured over 120 seconds. Enzyme activity was expressed as ΔA_{460} (min^{-1} mg $protein^{-1}$).

Catalase (CAT) activity assay

CAT activity was measured according to Beers and Sizer (1952), with slight modifications. The reaction mixture included 150 µL of sodium phosphate buffer (pH 7.0, 100 mM), 50 µL of H_2O_2 (100 mM), and 50 µL of enzyme extract. The breakdown of H_2O_2 was monitored at 240 nm, and enzyme activity was expressed as units per mg of protein, where one unit is defined as the conversion of 1 µmol of H_2O_2 per minute.

Quality assessment of treated banana fruits

The banana fruits were coated with Tragacanth gum, either incorporated with peppermint oil (TGPO), alone (TG), or treated separately with double-distilled water (CTRL). They were then stored and analyzed at intervals of Days 0, 5, 10, and 15, at a temperature of 29.63±0.072°C and a humidity of 67.33±0.272%.

pH measurement

The pH of the samples was determined using a pH probe (Hanna 37030, Germany) at ambient temperature with consistent stirring. The hydrogen ion concentration in the solution was represented as the negative logarithm of its activity (Adeogun et al., 2020).

Total soluble solids (TSS)

Total soluble solids were assessed following the procedure outlined by Adeogun et al. (2023). Measurements were taken using a handheld refractometer (Erma, Japan) at 20°C. The refractive index was recorded and expressed in degrees Brix (°Brix).

Water activity

Water activity of the samples was evaluated with an Amtast Water Activity Analyzer (WA-60A, USA) set at 20°C. Calibration of the device was performed using two reference standards: 6.0 Molal NaCl in water (aw = 0.760) and 0.5 Molal KCl in water (a_w = 0.984). Samples were cut into pieces, placed in sealed plastic containers, and introduced into the calibrated instrument's chamber (Parreidt, 2018).

Firmness

The firmness of banana fruits was measured using a manual firmness tester (Graigar, China). For each fruit, two firmness readings were taken at its equatorial section, rotating the fruit by 180° between measurements (Tesfay et al., 2017).

Electrical conductivity analysis

Five discs, each with a 1 cm diameter, were cut from the banana fruits and submerged in separate test tubes containing 25 mL of deionized water under continuous agitation. After 5 hours of incubation, the solution's electrical conductivity was recorded using a Hanna HI 98192 conductivity meter. Subsequently, the discs were boiled in the same water for 20 minutes, and the conductivity was re-measured. The electrical conductivity (EC) was determined by calculating the electrical conductivity values through subtracting the initial electrical conductivity reading from the final electrical conductivity reading and thereafter divided by the number of samples evaluated (Tesfay et al., 2017; Venkatarayappa et al., 1984).

Statistical analysis

All results are presented as means ± standard deviations using Statistical Package for the Social Sciences (SPSS) 26.0. A completely randomized design (CRD) was used for all experiments. Pathogenicity tests were conducted on four bananas per treatment. *In vitro* tests were performed

in triplicate with five Petri dishes per treatment, while *in vivo* tests involved five bananas per treatment with three replications. Quality assessments of the banana fruits coated with Tragacanth gum containing peppermint oil were conducted. Data were analyzed for significance using ANOVA. Assessment times for the *in vitro* study were days 0, 1, 2, 3, 4, 5, and 6; for the *in vivo* study, they were days 0, 2, 4, 6, 8, 10, 12, and 14; and for antioxidant enzymatic activities, they were days 0, 2, 4, 6, and 8. Quality assessments of the fruits were performed on days 0, 5, 10, and 15. These time points were considered as factors influencing the inhibitory and responsive activities of TGPO.

RESULTS

Fungal isolation and pathogenicity

Colletotrichum musae, Aspergillus niger, and Aspergillus fumigatus were isolated from banana fruits. Figures 1a-c show the micrographs of the isolated fungi from banana fruits. The results of pathogenicity assessment indicated that a significant proportion of the fruits exhibited symptoms and cultural traits similar to those caused by *Colletotrichum musae* and *Aspergillus fumigatus.* This finding was further confirmed through re-inoculation of the two organisms on potato dextrose agar (PDA), which revealed successful re-isolation of *C. musae* and *A. fumigatus.* These findings indicate that *C. musae* and *A. fumigatus* are the causative agents responsible for the rot observed in the tested banana fruits.

The *in-vitro* assessment of peppermint oil integrated with tragacanth gum on the pathogenic fungi

Figure 1 shows the micrographs of the isolated fungi from banana fruits. Figure 2 illustrates significant differences in the efficacy of peppermint oil incorporated with Tragacanth gum, Tragacanth gum solution alone, and the control solution (distilled water) on fungal growth. The inhibitory effects of the Tragacanth gum solution on *C. musae* (TGCM: Day 0, 0.5 ± 0.05 cm; Day 6, 6.3 ± 0.12 cm) and *A. fumigatus* (TGAF: Day 0, 0.58 ± 0.02 cm; Day 6, 3.85 ± 0.03 cm) were greater than those of the control solutions (CTRLCM: Day 0, 1.13 ± 0.06 cm; Day 6, 6.3 ± 0.12 cm and CTRLAF: Day 0, 1.3 ± 0.1 cm; Day 6, 5.30 ± 0.06 cm). Figure 2 also demonstrates that peppermint oil integrated with Tragacanth gum exhibited relatively insignificant inhibition on *C. musae* (TGPOCM: 0.47 ± 0.06 cm on day 1 and 0.87 ± 0.06 cm on day 6). Likewise, peppermint oil integrated with Tragacanth gum had no inhibitory effect against *A. fumigatus* (TGPOAF: 0.54 ± 0.02 cm on day 1 and 0.76 ± 0.02 cm on day 6).

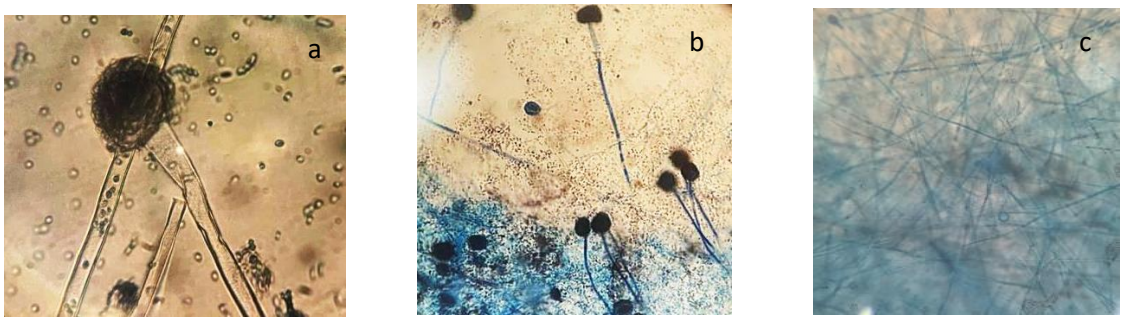


Fig. 1. a: Micrographs of *Aspergillus fumigatus*, b: *Aspergillus niger*, c: *Colletotrichum musae* grown on PDA.

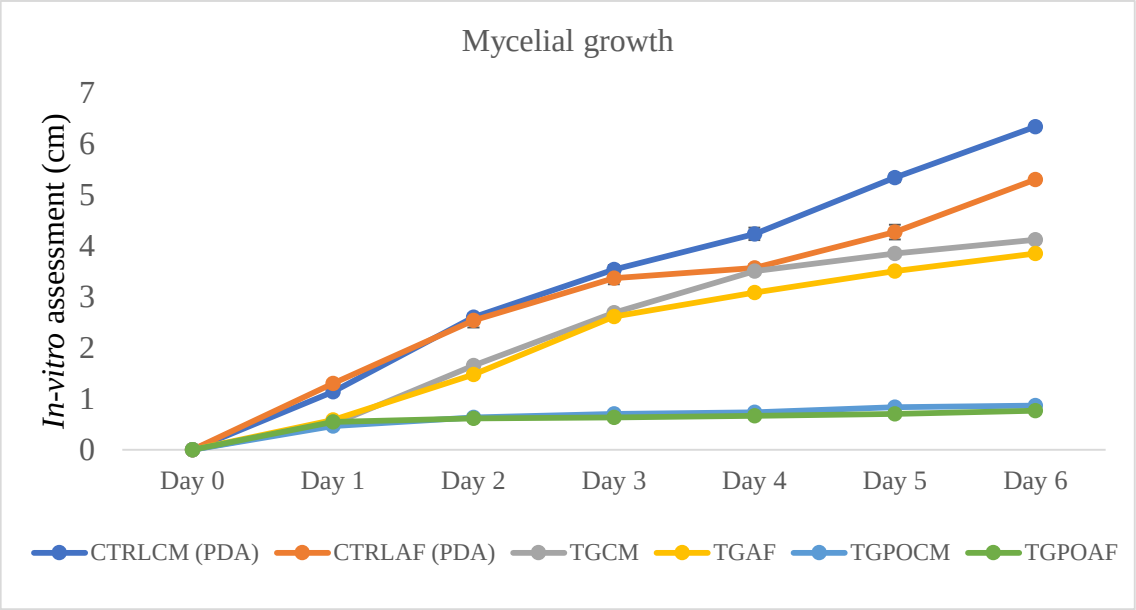
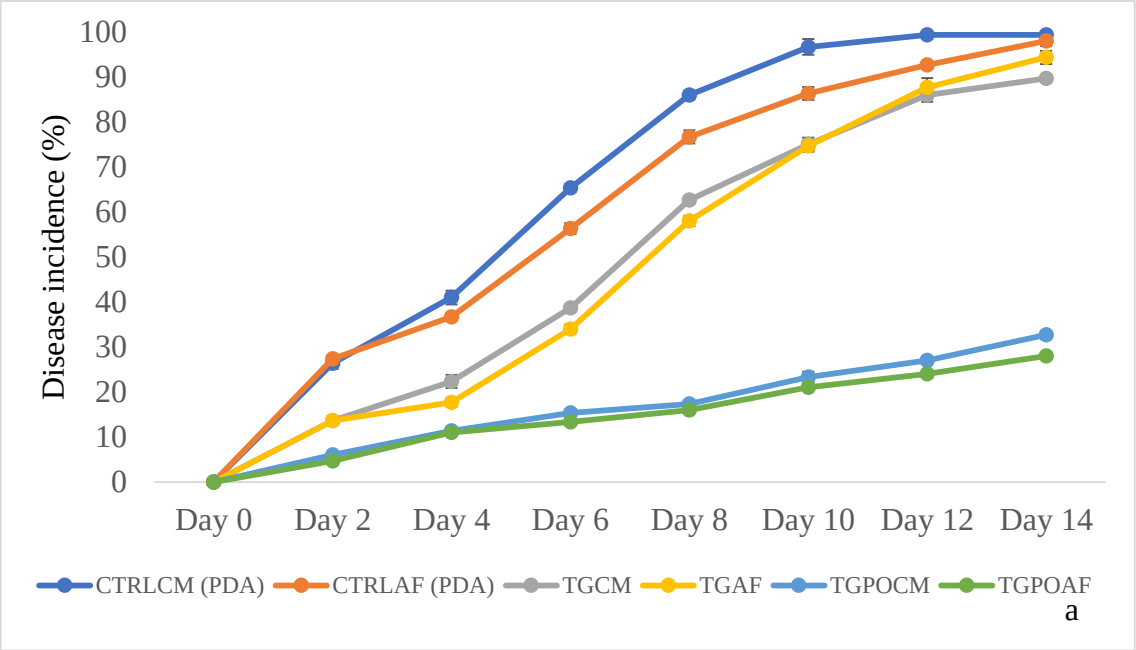


Fig. 2. *In-vitro* activities of peppermint oil incorporated with Tragacanth gum on *Colletotrichum musae* and *Aspergillus flavus*
TGCM: Tragacanth Gum on *Colletotrichum musae*
TGPOCM: Peppermint oil integrated with Tragacanth Gum on *Colletotrichum musae*
CTRLCM: Control solution on *Colletotrichum musae*
TGAF: Tragacanth Gum on *Aspergillus fumigatus*
TGPOAF: Peppermint oil integrated with Tragacanth Gum on *Aspergillus fumigatus*
CTRLAF: Control solution on *Aspergillus fumigatus*

In-vivo assessment of antifungal activity of peppermint oil integrated with tragacanth gum against *Colletotrichum musae* and *Aspergillus fumigatus*

This study, as illustrated in Figure 3a and 3b, showed the *in-vivo* evaluation of the test formulations, including Tragacanth gum alone (TGCM and TGAF), Peppermint oil integrated with Tragacanth gum (TGPOCM and TGPOAF), and control solutions (CTRLCM and CTRLAF), against *Colletotrichum musae* and *Aspergillus fumigatus*. The results demonstrated that the Peppermint oil integrated with Tragacanth gum exhibited significant antifungal activity, markedly reducing the incidence and severity of disease in banana fruits, as shown in Figs 3a and 3b. In contrast, the effects of TGCM and TGAF and the control solutions (CTRLCM and CTRLAF) were notably less effective in mitigating disease occurrence.

Figure 3a shows that Peppermint oil integrated with Tragacanth gum achieved the lowest disease incidences of 32.67±1.00 % for *C. musae* and 28±1.00 % for *A. fumigatus* after 14 days of incubation, with corresponding disease severities of 22.33±1.52 % and 30.67±1.53%, respectively as shown in Figure 3b. These inhibitory effects were significantly more pronounced than Tragacanth gum alone, which resulted in higher disease incidences of 64.33±1.52% for *C. musae* and 57.67±3.51% for *A. fumigatus*. The control solution exhibited the highest incidences at 84.33±2.51 % and 75.67±0.57 % for *C. musae* and *A. fumigatus*, respectively.



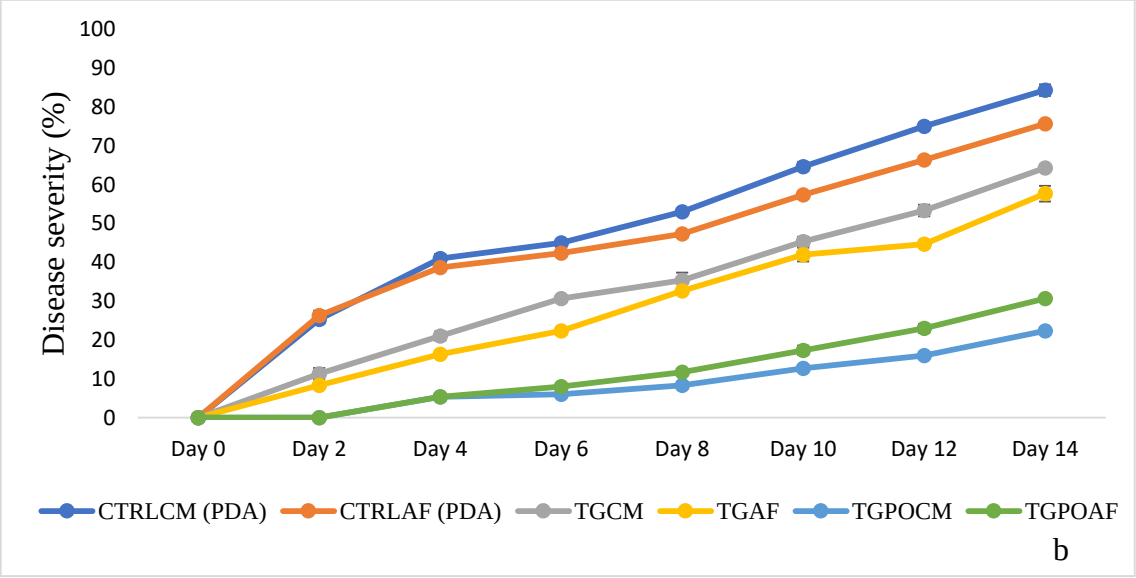


Fig. 3. *In-vivo* Evaluation of Peppermint Oil integrated with Tragacanth gum on *Colletotrichum musae* and *Aspergillus fumigatus*.

TGCM: Tragacanth Gum on *Colletotrichum musae*
TGPOCM: Peppermint oil integrated with Tragacanth Gum on *Colletotrichum musae*
CTRLCM: Control solution on *Colletotrichum musae*
TGAF: Tragacanth Gum on *Aspergillus fumigatus*
TGPOAF: Peppermint oil integrated with Tragacanth Gum on on *Aspergillus fumigatus*
CTRLAF: Control solution on *Aspergillus fumigatus*

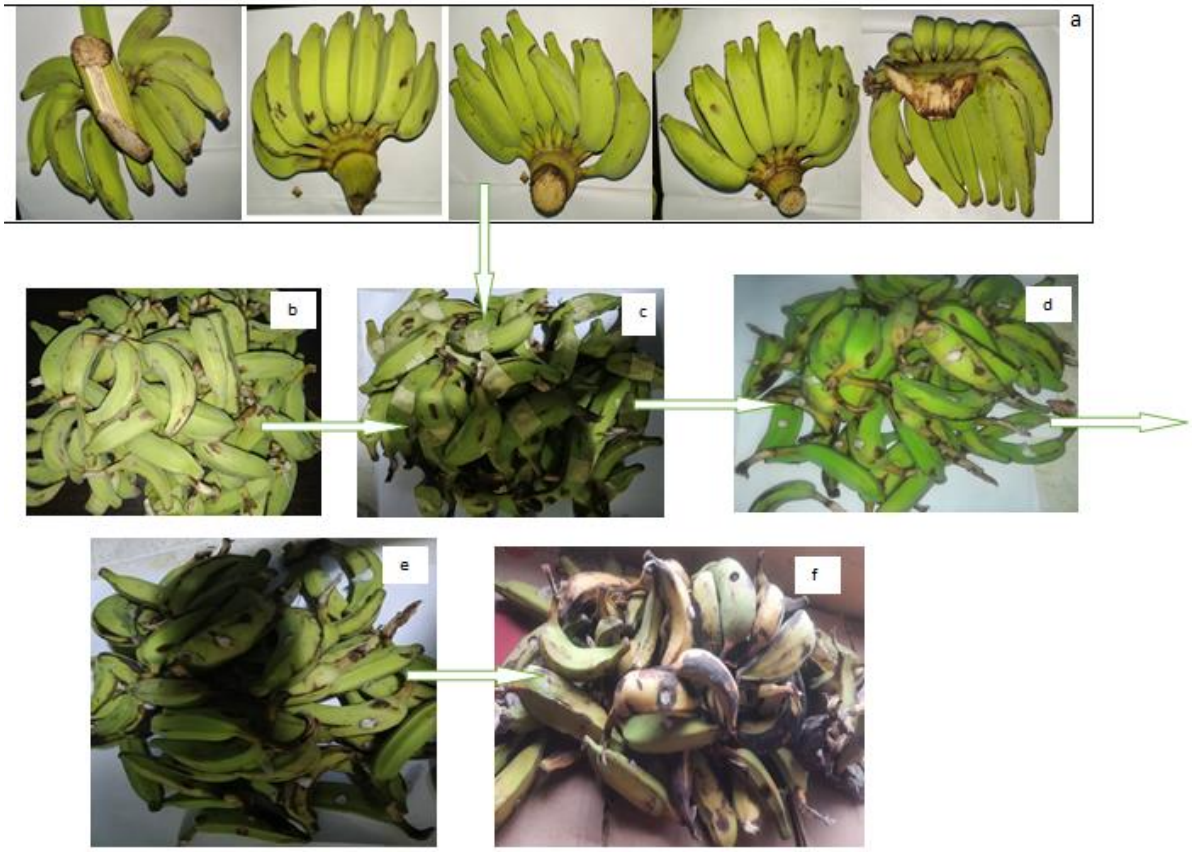


Fig. 4. Assessed fruits from disease incidence and severity evaluation.

a: Bunches of banana fruits to be processed for *in-vivo* assessment
b: Banana fruits after coating for *in-vivo* assessment
c: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 0
d: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 2
e: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 4
f: Banana fruits after inoculation with *Colletotrichum musae* and *Aspergillus fumigatus* at Day 14

Antioxidant Enzymatic Activities

Figure 5a depicts catalase (CAT) activity, showing a marked increase from day 0 to day 4, followed by a decline from day 4 to day 8 across all test agents: peppermint oil integrated with tragacanth gum (TGPOCM and TGPOAF), tragacanth gum alone (TGCM and TGAF), and the untreated control (CTRLCM and CTRLAF) for banana fruits infected with *Colletotrichum musae* and *Aspergillus fumigatus*. Banana fruits treated with TGPOCM and infected with *C. musae* demonstrated a CAT activity of 19.33 ± 0.57 units g^{-1} on Day 2, which slightly declined to 19.00 ± 1.0 units g^{-1} by Day 8. In the case of *A. fumigatus* inoculation, the CAT activity decreased from 22.33 ± 0.57 units g^{-1} on day 2 to 21.33 ± 1.16 units g^{-1} by day 8, demonstrating a more gradual decline in enzyme activity relative to other treatments. Conversely, TGCM-treated banana fruits inoculated with *C. musae* demonstrated a relative decline from 15.00 ±1 units g^{-1} on day 2 to 10.67 ± 1.53 units g^{-1} by day 8, whereas those inoculated with *A. fumigatus* experienced a decrease from 17.67 ± 0.56 units g^{-1} on day 2 to 13.67 ± 1.16 units g^{-1} by day 8. The untreated control samples exhibited the most significant reduction, with *C. musae*-inoculated banana fruits decreasing from 19.33 ± 0.57 units g^{-1} on day 2 to 19.00 ± 1 units g^{-1} by day 8, and *A. fumigatus*-inoculated fruits declining from 22.33 ± 0.57 units g^{-1} to 21.33 ± 1.16 units g^{-1} during the same timeframe. Notably, from days 2 to 4, all test agents demonstrated

a transient elevation in CAT activity. In Peppermint Oil integrated with Tragacanth gum, with inoculated *C. musae* (TGPOCM), there was an increase from 19.33 ± 0.57 units g^{-1} to 27 ± 1.0 units g^{-1}, but with inoculated *A. fumigatus* (TGPOAF) increased from 22.33 ± 0.57 units g^{-1} to 29.00 ± 1 units g^{-1}. In Tragacanath gum-treated fruits, inoculation with *C. musae* (TGCM) resulted in an increase from 15.00±1.00 units g^{-1} to 19.67 ± 1.16 units g^{-1}, whereas for *A. fumigatus* (TGAF), the activity rose from 17.67 ± 0.56 units g^{-1} to 24.00 ± 1.0 units g^{-1}. In the control group, *C. musae*-inoculated banana fruits (CTRLCM) exhibited a rise from 10.00 ± 1.00 units g^{-1} to 12.00 ± 1.0 units g^{-1}, whereas *A. fumigatus* (CTRLAF) samples rose from 13.00 ± 1.00 units g^{-1} at day 2 to 15.00 ± 1.00 units g^{-1} by day 4.

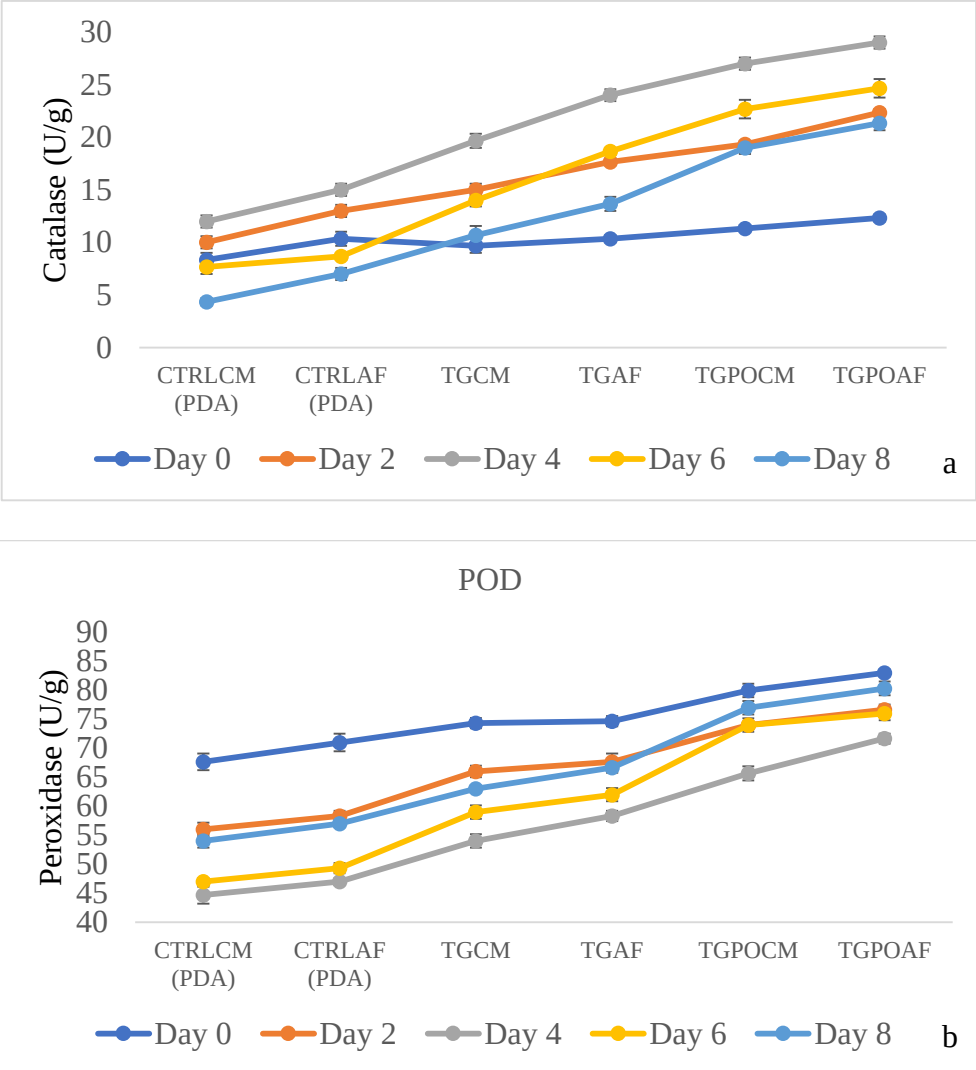


Fig. 5. Impact of the coating treatments on (a) Catalase (CAT) and (b) Peroxidase (POD) activity in banana fruits throughout an 8-day storage period.
POD: Peroxidase, CAT: Catalase
TGCM: Tragacanth Gum on *Colletotrichum musae*
TGPOCM: Peppermint oil integrated with Tragacanth Gum on *Colletotrichum musae*
CTRLCM: Control solution on *Colletotrichum musae*
TGAF: Tragacanth Gum on *Aspergillus fumigatus*
TGPOAF: Peppermint oil integrated with Tragacanth Gum on on *Aspergillus fumigatus*
CTRLAF: Control solution on *Aspergillus fumigatus*

Figure 5b illustrates the peroxidase (POD) activity, indicating a progressive decline from day 0 to day 6, followed by an increase from day 6 to day 8 across all groups, with Peppermint Oil integrated with Tragacanth gum-treated banana fruits exhibiting superior enzyme activity relative to the other treatments. In *C. musae*, the POD activity in TGPOCM-treated banana fruits diminished from 80.00 ± 2.00 unit's g^{-1} on day 0 to 77.00 ± 2.00 units g^{-1} by day 8, whereas *A. fumigatus* inoculation (TGPOAF) exhibited a decline from 83.00 ± 1.00 units g^{-1} to 80.30 ± 2.08 units g^{-1}. The Tragacanth gum alone-treated (TGCM) fruits inoculated with *C. musae* showed a reduction in POD activity from 74.33 ± 1.53 units g^{-1} on day 0 to 63.00 ± 1.00 units g^{-1} by day 5, while in *A. fumigatus* (TGAF), POD activity decreased from 74.67 ± 1.53 units g^{-1} to 66.67 ± 1.53 units g^{-1}. Control fruits had the lowest peroxidase activity, with *C. musae*-inoculated samples (CTRLCM) decreasing from 67.67 ± 2.52 units g^{-1} to 54.00 ± 2.00 units g^{-1}, and *A. fumigatus*-inoculated fruits declining from 71.00 ± 2.65 units g^{-1} to 57.00 ± 1.00 units g^{-1} over the same interval.

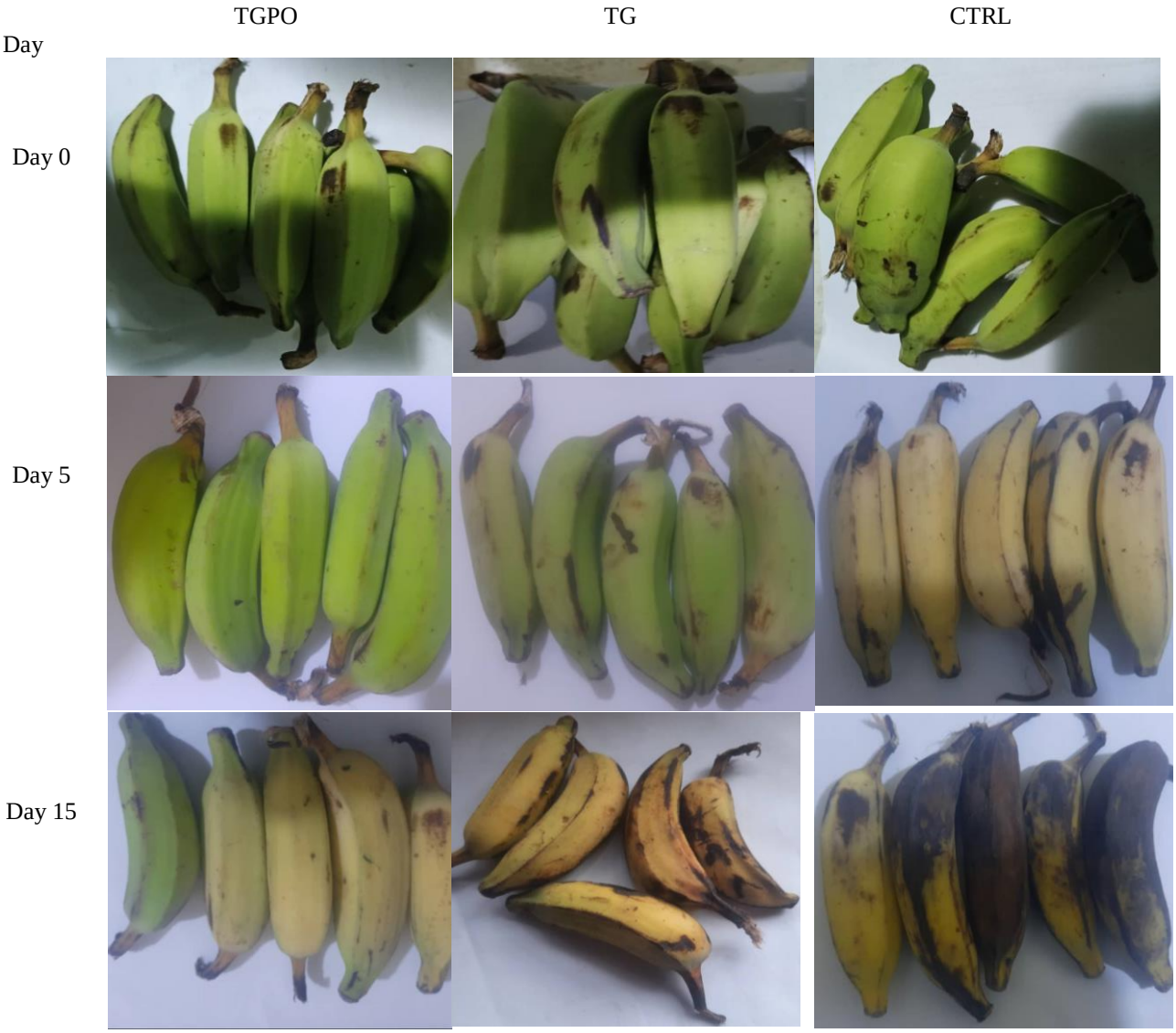


Fig. 6. Visual assessment of coated banana fruits stored for 15 days.
TG: Tragacanth Gum
TGPO: Peppermint oil integrated with Tragacanth Gum
CTRL: Control solution

Quality assessment

The control treatment (CTRL) had more noticeable effects on fruit appearance on day 15 of storage, as depicted in Figure 6. This figure shows the visual appearance of the fruits at day 0, day 5 and day 15.

It was observed based on Figure 7 and Figure 8 that there were significant differences in both pH and total soluble solids (TSS) across different treatment groups of banana fruits during storage. The pH measurements demonstrated that banana fruits treated with Tragacanth gum incorporated with peppermint oil (TGPO) maintained higher pH levels compared to both Tragacanth gum alone (TG) and fruits samples without any treatment (CTRL). Starting from similar baseline pH values (TG: 4.72 ± 0.049, TGPO: 4.44 ± 0.02, CTRL: 4.41 ± 0.04) on Day 5, the treatments showed divergent patterns over the 15-day storage period. By Day 15, TGPO-treated banana fruits maintained a pH of 5.29 ± 0.02, while TG-treated fruits increased to 6.44 ± 0.01, and untreated controls showed the most substantial increased to 6.94 ± 0.03.

Regarding TSS content (Fig. 8), all groups exhibited progressive increases throughout the storage period, but at different rates. TGPO-treated bananas showed the most moderate increase, from 12.33 ± 0.33 °Brix initially to 16.00 ± 0.58 °Brix by day 15. TG-treated fruits displayed an intermediate response, rising from 12.33±0.33 °Brix to 21.67±0.33 °Brix. The untreated control group demonstrated the most pronounced increase in TSS, climbing from 12.00 ± 0.00 °Brix to 25.00 ± 0.58 °Brix over the same period.

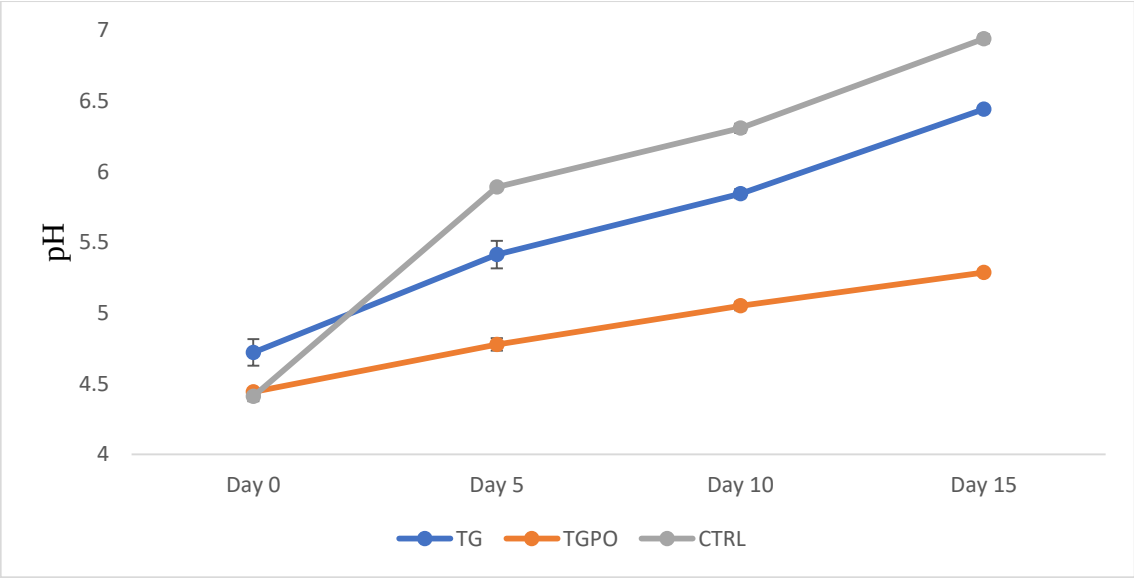


Fig. 7. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the pH of banana fruits during 15 days of storage.
TGPO: Peppermint oil integrated with Tragacanth gum, TG: Tragacanth gum, Untreated banana fruits (CTRL)

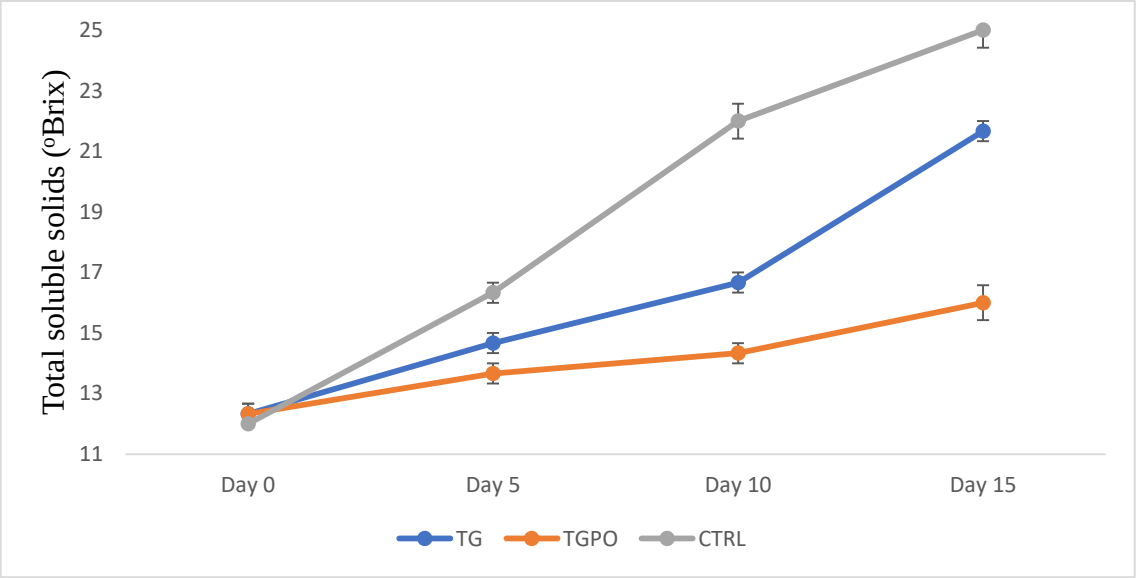


Fig. 8. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the total soluble solids of banana fruits during 15 days of storage.

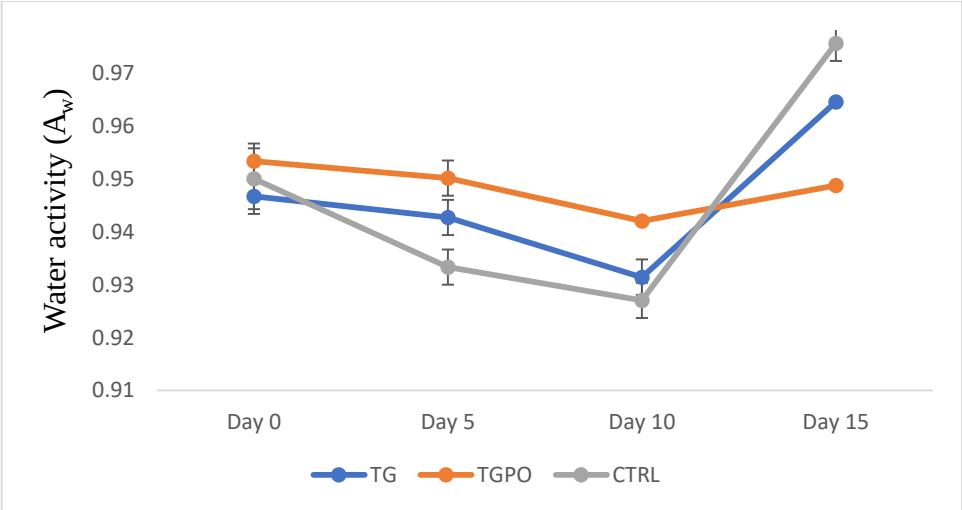


Fig. 9. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the water activity of banana fruits during 15 days of storage.

Figure 9 illustrates a trend in water activity characterized by a gradual decline from day 0 to day 10, followed by a subsequent rise from day 10 to day 15, observed across various treatments during a 15-day storage period. Banana fruits coated with a combination of Peppermint oil and Tragacanth gum (TGPO) exhibited a minimal reduction in water activity, starting at 0.9567 ± 0.06 on day 0, decreasing to 0.96 ± 0.06 on day 5, and further declining to 0.942 ± 0.04 on day 10. However, an increase in water activity was recorded on day 15, reaching 0.9467 ± 0.043. Similarly, fruits treated exclusively with Tragacanth gum (TG) displayed a comparable pattern, with water activity values decreasing from 0.95 ± 0.008 on Day 0 to 0.93 ± 0.03 on day 5 and 0.927 ± 0.05 on day 10, followed by an increase to 0.9756 ± 0.05 on day 15. Furthermore, untreated control fruits (CTRL) demonstrated the highest water activity levels, starting at 0.9467 ± 0.06 on Day 0, decreasing to 0.9427 ± 0.04 on day 5, and further dropping to 0.9314 ± 0.06 on day 10. A notable increase was observed by the end of the storage period, with water activity rising to 0.9645 ± 0.054 on day 15.

Figure 10 illustrates the decline in fruit firmness across banana fruits treated with TGPO, TG, and CTRL during the 15-day storage period. Firmness consistently decreased in all test samples, with TGPO-treated fruits retaining higher firmness values (day 0: 33.67 ± 0.6N; day 15: 24.52±0.58 N) compared to those treated with TG (day 0: 33.30 ± 0.08N; day 15: 17.38 ± 0.57N). Untreated fruits exhibited the steepest reduction in firmness, with values dropping from 34.20 ± 1.15N on day 0 to 11.33 ± 1.45 N by day 15.

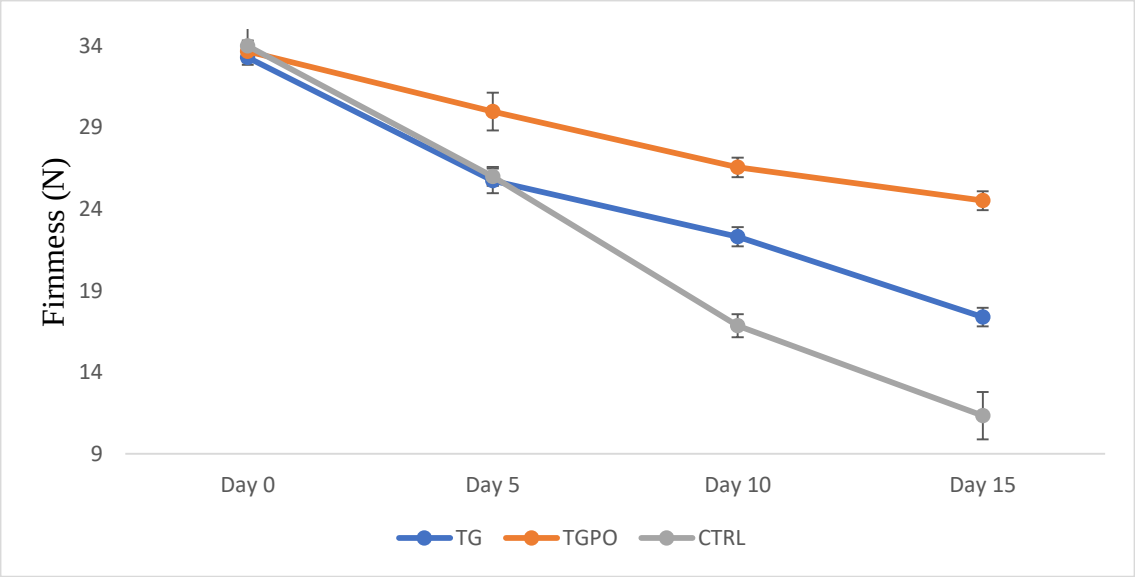


Fig. 10. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the firmness of banana fruits during 15 days of storage.

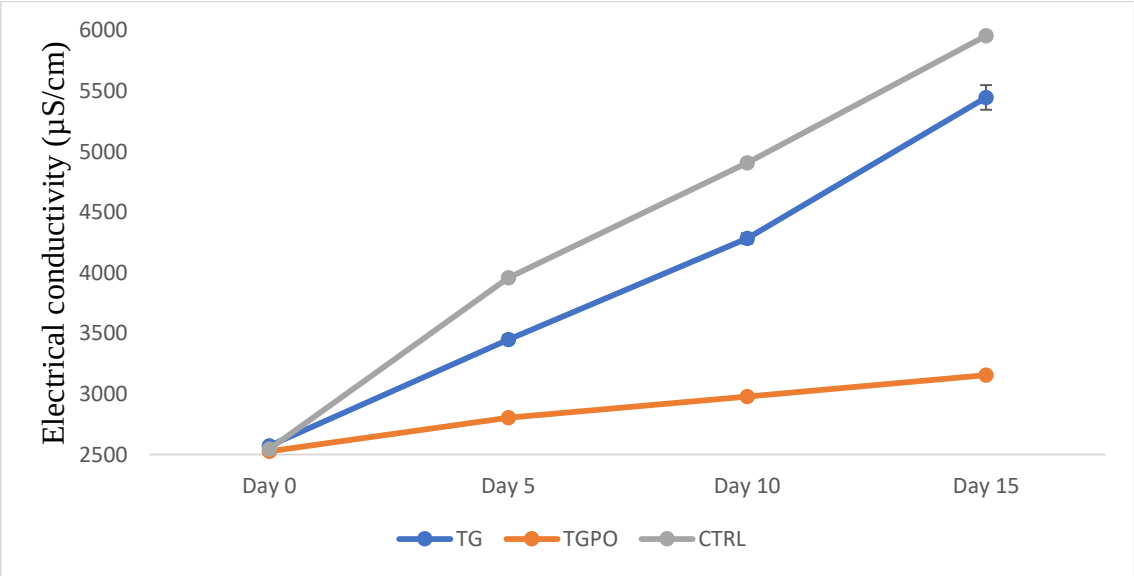


Fig. 11. The effect of Tragacanth gum incorporated with peppermint oil and Tragacanth gum alone on the electrical conductivity (EC) of banana fruits during 15 days of storage.

Figure 11 depicts variations in the electrical conductivity of banana fruits over a 15-day storage period. Banana fruits treated with TGPO displayed a gradual rise in electrical conductivity, starting at 2527.33 ± 59.61µS/cm on day 0 and reaching 3156.33 ± 23.60µS/cm by day 15. Similarly, fruits coated exclusively with TG showed an increase from 2572.33 ± 21.06 µS/cm on day 0 to 5447.67 ± 101.84 µS/cm on day 15. In comparison, the untreated fruits (CTRL) exhibited the most significant rise, from 2543.67 ± 1.33 µS/cm on Day 0 to 5956.67 ± 33.65 µS/cm by Day 15.

DISCUSSION

There is wide evidence about the contribution of fungal diseases to the inedibility of banana fruits over time due to the invasion of these organisms, and several methods to address these issues have been well established (Mairami, 2024; Kuyu & Tola, 2018). The use of synthetic preservatives and natural antimicrobial agents is well documented (Pandey & Negi, 2018). To the best of our knowledge, no research has been conducted on the use of antimicrobial agents like tragacanth gum incorporated with peppermint essential oil on fruits such as bananas (Surekha & Reddy, 2014; Teshome et al., 2022). Consequently, this study aims to determine the impact of tragacanth gum incorporated with peppermint oil on the post-harvest quality of banana fruit stored at room temperature.

This study confirmed that *Colletotrichum musae* and *Aspergillus fumigatus* are the key fungal pathogens responsible for banana fruit rot, as demonstrated through pathogenicity tests. Both fungi have also been identified by Ali et al. (2021) and Kuyu and Tola (2018) as significant contributors to banana fruit deterioration. *Colletotrichum musae*, closely related to *C. gloeosporioides*, is widely recognized as a major cause of anthracnose disease, resulting in severe postharvest decay. Likewise, *Aspergillus fumigatus*, similar to *A. niger*, is a common postharvest pathogen known for causing fruit rot and leading to significant economic losses (Matrose et al., 2021). It has been established that these fungi can penetrate fruit tissues, causing rot and affecting the quality and shelf life of fruits such as bananas (Murmu & Mishra, 2018). The presence of these pathogens has also been observed in fruits like mangoes, avocados, and strawberries (Eckert & Ogawa, 1985). Their virulent nature, particularly under warm and humid conditions, makes them highly problematic for fruit storage and transportation (Zakaria, 2021).

The results on the *in-vitro* effects demonstrated that the integration of Tragacanth gum with Peppermint oil was more effective at inhibiting *Colletotrichum musae* and *Aspergillus fumigatus* compared to using Tragacanth gum alone or leaving the fruits untreated. The study also showed that Peppermint oil played a key role in enhancing the antifungal activity of Tragacanth gum (TGPOCM and TGPOAF) against *C. musae* and *A. fumigatus*. These findings are supported by previous research, including studies by Pawar et al. (2024) and Vilaplana et al. (2018), which highlighted Peppermint oil's effectiveness in reducing postharvest fungal diseases in fruits like bananas.

Several studies have highlighted the effectiveness of edible coatings, such as tragacanth gum, in preserving the volatility of essential oils like peppermint oil, which plays a key role in controlling fungal pathogens in fruits and vegetables (Galus et al., 2020; Perumal et al., 2022). Ghayempour and Montazer (2019) and Godarzi et al. (2021) further reported that the incorporation of essential oils into tragacanth gum enhances the stability and delivery of their bioactive components, ensuring prolonged efficacy. Additionally, Abdi et al. (2024) and Felicia et al. (2024) confirmed that tragacanth gum significantly boosts the antifungal properties of essential oils like peppermint oil. These coatings adhere to fungal cell surfaces, creating a protective barrier that enables the controlled release of active compounds. This close interaction

with fungal membranes intensifies the oils' ability to disrupt cell walls and inhibit fungal growth.

The biofungicidal activity of peppermint oil in this study might be attributed to its phytoconstituents, as established in the literature. Bansod and Rai (2008) and Chaemsanit et al. (2018) indicated that peppermint oil, with main phytoconstituents such as menthol and menthone, is responsible for inhibiting *C. musae* and *A. fumigatus*, which cause diseases in crops like banana fruits. The integration of Tragacanth gum with Peppermint oil demonstrated a significant reduction in disease severity and incidence caused by *C. musae* and *A. fumigatus* when compared to the application of Tragacanth gum alone and the control treatments. This enhanced antifungal efficacy has been thoroughly supported by the works of de Oliveira et al. (2017), de Oliveira et al. (2023), and Gonçalves et al. (2021), which confirmed the active role of peppermint oil in mitigating the impact of these fungal pathogens, particularly in controlling both the severity and spread of infections associated with *C. musae* and *A. fumigatus*.

The controlled reduction of catalase (CAT) in banana fruits inoculated with *Colletotrichum musae* (TGPOCM) and *Aspergillus fumigatus* (TGPOAF), and treated with Tragacanth gum incorporated with Peppermint oil, as well as those treated only with Tragacanth gum (TGCM and TGAF), can be attributed to the modulating properties of Peppermint oil. These properties help protect the fruits from oxidative stress. Additionally, Tragacanth gum, integrated with Peppermint oil, forms a physical barrier against oxygen and moisture, which reduces the rate of enzymatic degradation and microbial contamination on the fruit surface (Pillai et al., 2024; Qu et al., 2020; Radev & Pashova, 2020; Saxena et al., 2020). Similar bioactivity of peppermint has been well documented in dragon fruit, strawberries, bananas, and mangoes (Chaemsanit et al., 2018; dos Passos Braga et al., 2019; Felicia et al., 2022).

The pH of TGPO, TG, and CTRL showed upward trends throughout the storage period, with the highest increase observed in CTRL, followed by TG, and the least in TGPO. Several studies have attributed this pH increase to the accumulation of solid matter and molecular breakdown resulting from cellular membrane deterioration. These changes contribute to the alteration of the fruits' mechanical, metabolic, and molecular characteristics over time. The enhanced cellular membrane stability in TGPO-treated banana fruits can be attributed to the integrated activities of peppermint oil and Tragacanth gum, which act as metabolic process regulators due to their antimicrobial properties and ability to form a protective barrier (Adekunle et al., 2021; Afedzi et al., 2022; Iacovino et al., 2024; Nasiri et al., 2018).

Analysis of sugar content in banana fruits after 15 days of storage revealed significant differences in total soluble solids (TSS) among the treatments. Both the TG (Tragacanth gum) and CTRL (control) groups exhibited increased TSS levels, suggesting that these treatments may have facilitated the retention or accumulation of soluble solids during ripening. The increase in TSS is primarily due to the natural ripening process in bananas, which involves the conversion of starch into sugar, leading to higher TSS. This process is driven by ethylene production, a key factor in the ripening of climacteric fruits like bananas, where the release of ethylene accelerates the conversion of starch into sugars, thus increasing TSS (Akkurt et al., 2024).

The lower TSS values in bananas treated with TGPO (Tragacanth gum incorporated with peppermint oil) suggest that TGPO influences the fruit's metabolic processes, particularly those related to sugar and carbohydrate metabolism (Felicia et al., 2022). The reduced TSS in TGPO-treated fruits could be attributed to the structural characteristics of the TGPO coating, which may alter gas exchange or moisture retention. The interaction between Tragacanth gum and peppermint oil may have slowed down the ripening process, possibly by modifying the fruit's respiration rate or limiting ethylene access to the fruit, thereby reducing the rate of starch conversion to sugar (Almeida et al., 2024; Shakil et al., 2023).

Zore et al. (2021) established that factors such as ethylene production significantly affect TSS increase during storage of fruits such as banana. Ethylene production, for instance, is a key trigger for the ripening process, and as bananas ripen, the release of ethylene further accelerates starch conversion into sugar. Additionally, Pamungkas et al. (2023) posited that coatings, such as Tragacanth gum combined with essential oils like peppermint oil, create a barrier that reduces moisture loss, inhibits fungal growth, and delays ripening, thus helping to limit TSS increases.

The effect of the coating treatments on banana fruits was evaluated during a 15-day storage period. The results indicate that control samples (CTRL) without treatment and those treated with Tragacanth gum alone (TG) exhibited a significantly greater increase in water activity compared to bananas treated with Tragacanth gum incorporated with peppermint oil (TGPO). This difference can be attributed to the interaction between bound and unbound water molecules, which plays a pivotal role in maintaining the microbial, structural, and chemical stability of the fruits (Rockland & Stewart, 1981). This study further established an initial decline in water activity (a_w), likely due to moisture loss. This reduction may be partly influenced by Tragacanth gum incorporated with Peppermint oil (TGPO) and Tragacanth alone (TG), both contributing to the decline (Barak et al., 2020). According to the literature, Tragacanth gum acts as a barrier to moisture evaporation. TGPO, which contains peppermint oil, shows slight variations in moisture retention compared to TG (Pamungkas et al., 2023; Zare-Bavani et al., 2024). After Day 10, a rise in a_w was observed, likely due to continued metabolic activity, carbohydrate breakdown releasing bound water, or coating degradation affecting moisture regulation (Almeida et al., 2024). The control group showed the highest aw on Day 15, suggesting the poorest moisture regulation. A study by Pamungkas et al. (2023) on carrageenan-based edible coatings incorporating peppermint essential oil on banana fruits supports our observation on the influence of edible coatings with essential oils. Other studies by Karnwal et al. (2025); Moreira et al. (2022); Soppelsa et al. (2023) also demonstrated the influence of edible coatings, such as tragacanth gum and essential oils like peppermint oil, in positively modulating water activity in fruits like apples, guavas, and bananas.

The impact of TG, TGPO, and CTRL on the tissue rigidity of banana fruits, as observed in this study, indicated that structural degradation was most pronounced in CTRL-treated fruits, followed by those treated with tragacanth gum alone (TG). In contrast, fruits treated with tragacanth gum incorporated with peppermint oil (TGPO) retained optimal firmness. The delayed softening observed in TGPO-treated fruits may be attributed to the antimicrobial properties of peppermint oil, which inhibit rapid cellular breakdown (Yousuf et al., 2021). Furthermore, the significant reduction in firmness in CTRL-treated fruits is likely due to increased activity of pectin-degrading enzymes, which are closely associated with fruit softening and accelerated metabolic processes (Saleh et al., 2019). However, the application of edible coatings such as tragacanth gum, especially when combined with essential oils like peppermint oil, appeared to inhibit water loss by moderating metabolic activity and reducing the production of pectin-degrading enzymes, thereby maintaining fruit firmness (Saleh et al., 2019). These findings align with the results reported by Saleh et al. (2019) in their study on pear fruits. It was also established in this study that the low electrical leakage in TGPO-treated banana fruit suggests enhanced cellular membrane preservation, as posited by Banti (2020). This potentially extends the preservation period by minimizing oxidative degradation.

CONCLUSION

This study established that integrating Tragacanth gum with Peppermint Oil (TGPO) offers a sustainable and eco-friendly solution for extending banana shelf life and maintaining quality during storage. TGPO-treated bananas showed enhanced firmness, better moisture retention, and reduced oxidative stress by preserving antioxidant enzyme activity. The coating also effectively controlled postharvest pathogens (*Colletotrichum musae* and *Aspergillus fumigatus*), highlighting its antimicrobial properties. By combining antioxidant defense with pathogen control, TGPO presents a natural, sustainable alternative to synthetic preservatives, addressing both quality preservation and environmental concerns.

Conflict of interest

The authors declare that there is no conflict of interest.

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