



Therapeutic and Protective Effect of Polyvinyl Alcohol Formulated with Clove Essential Oil as Ecofriendly Treatments to Control Gray Mold Disease of Apple Fruits During Storage

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ABSTRACT

To manage the gray mold disease on apple fruits, the impact of polyvinyl alcohol (PVA) combined with clove essential oil (CEo) was evaluated. With CEo at 1.0%, linear growth and spore germination of *B. cinerea* were completely suppressed. Formulation of CEo at 2.0% combined with a 10.0% PVA resulted in complete suppression of both linear growth and spore germination of tested fungus. All formulations were treated at zero time and 24 hours following inoculation, the incidence and severity of gray mold disease were dramatically reduced. When applied at zero time after inoculation, CEo at 2.0% combined with PVA at 10.0% resulted in a height reduction that lowered gray mold incidence and severity by 75.0 and 73.0%, respectively. When applied 48 hours after inoculation, none of the formulations had any effect on the gray mold disease. At various coating times prior to inoculation, namely 1.0, 12.0, and 24.0 hours, the protective effects of clove essential oil and PVA on apple fruit gray mold disease were investigated. The results showed that, over the course of the testing periods, every formulation considerably lowered the incidence and severity of gray mold disease. In all times, the height reduction was achieved by combining 2.0% CEo with 10.0% PVA, which decreased the incidence and severity of gray mold. However, statistical analysis showed that there were no appreciable variations in the protective effect of 1.0, 12.0, and 24 hours against apple fruit gray mold disease. The incidence and severity of gray mold disease were significantly reduced over the 60-day storage period by all formulations, including CEo at 1.0 and 2.0% combined with PVA at 10.0%, when administered as protective treatments. The quality of the fruit was unaffected by any of the earlier formulations.

Keywords: Apple fruits, gray mold, postharvest disease, disease incidence, disease severity, fruit quality

1. Introduction

Postharvest diseases in apples and pears can be caused by a variety of fungal infections (Sutton 2014). *Botrytis cinerea*, causes gray mold, primarily enter crops by wounds from insects and birds, as well as physical damage before or after harvest (Snowdon 1990 and Wenneker and Thomma, 2020 and Elshahawy *et al.*, 2023).

According to Dewey and Grant-Downton (2016) and Hua *et al.* (2018), *Botrytis cinerea* is widely cited as the main cause of notable losses in the production of commercially relevant horticultural plants due to its unlimited host range, several assault modes, and capacity to survive in harsh settings.

Botrytis cinerea grows on damaged tissues, causing tissue death and secreting poisons and enzymes associated with reactive oxygen species (Petrash, *et al.*, 2019). Botrytis species are thought to have caused damage worth tens to hundreds of billions of dollars worldwide (Hua *et al.*, 2018) because of their ability to affect all stages of production and acquire a resistance to current fungicides (Dean *et al.*, 2012 and Romanazzi *et al.*, 2016). Furthermore, environmental changes lead to rapid mutations and the introduction of new strains, posing a threat to the global food supply's security (Sundstrom, *et al.*, 2014). High temperatures and humidity cause Botrytis species to develop and sporulate more readily in some

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ecozones (Sehajpal and Singh, 2014; Fininsa *et al.*, 2015). *Botrytis cinerea* affects around 200 plant hosts. It is widely dispersed throughout many regions and affects plant species like apples (*Malus domestica*) and strawberries (*Fragaria × ananassa*) (Elad *et al.*, 2016 and Bui *et al.*, 2019).

This disease is very aggressive because of its tendency to mutate and develop new strains. Choquer *et al.* (2007 and Elshahawy *et al.*, 2023) report that populations of *B. cinerea* have developed strain-dependent infection strategies and have partially but successfully adapted to a variety of host plants.

Essential oils (EOs) are volatile, sweet-smelling, aromatic liquid oils that are extracted supercritically or hydrodistillation-wise from flavors and plants. With antioxidant and antibacterial qualities, they are readily accessible in bioactive combinations (Taghavi *et al.* 2018; Mari *et al.*, 2016; Nazzaro *et al.*, 2017; Prakash *et al.*, 2015 Danh *et al.*, 2021).

One of the effective and practical ways is to apply essential oils (EOs) as antifungal agents. According to Tisserand and Young (2013) and Perumal *et al.* (2016), essential oils (EOs) are mixtures of hundreds of naturally occurring volatile plant-based compounds, mainly oxygenated derivatives of monoterpenes and sesquiterpenes.

Several EOs have demonstrated strong anti-fungal efficacy (Rabari, *et al.*, 2018). Since the antifungal activity of EOs may be primarily linked to their fundamental components as well as the interaction influence of major and minor components, pathogen resistance to a wide spectrum of chemicals in EOs is improbable. Many EOs are commonly regarded as safe (GRAS) and biodegradable, as well as being biodegradable. Several essential oils (EOs) have been shown to effectively prevent anthracnose on mango fruits of various varieties (Sefu *et al.*, 2015 and Danh *et al.*, 2021). Peppermint oil has been shown to be effective on Tommy Atkins variety (de Oliveira *et al.*, 2017); orange, lemon, and mustard oil on Zabdia variety (Abd-ALLA and Haggag 2013); lemongrass (Duamkhmanee 2008); basil oil on Willard variety (Karunanayake *et al.*, 2020; 2013); thyme, clove, and cinnamon oil on Banganapalli and Totapuri varieties (Perumal *et al.*, 2017). According to Abd-El-Kareem *et al.* (2022), chitosan at 8.0 g/L and thyme or nerol at 1.5% decreased the incidence and severity of green, blue mold, and sour rot diseases.

Polyvinyl alcohol (PVA) is a biocompatible and biodegradable polymer with excellent mechanical, optical, physical, and film-forming properties in addition to strong chemical resistance, according to Zanela *et al.* (2018). Effective research has been done on the potential use of PVA and its composites in food packaging systems (Youssef *et al.*, 2019). Because of its safe and nontoxic profile, it has been designated as generally recognized as safe (GRAS), meaning that it can be utilized to make edible films (Keller and Heckman, 2018).

The introduction of bioactive substances such phenolic compounds in food packaging could have a significant positive impact (Andrade *et al.*, 2021). Gallic acid is one phenolic compound that dissolves in water and has been associated with potent antibacterial and antioxidant properties. It has been demonstrated that PVA films with gallic acid enhance their antioxidant properties (Awad *et al.*, 2017). Therefore, it is expected that adding an extract rich in gallic acid to PVA coatings will assist delay the ripening of bananas based on the research that is now available. It has been found that the leaf extract of *Ficus auriculata* is high in gallic acid and exhibits strong antioxidant activity (Baite *et al.*, 2021).

This suggests that the extract could be used to increase the shelf life of fresh food. However, no studies have been conducted on the use of *F. auriculata* leaf extract to prolong fruit shelf life. PVA is an environmentally benign synthetic polymer that is hydrophilic, nontoxic, biocompatible, and biodegradable (He *et al.*, 2019 and Candéo *et al.*, 2020). PVA is commonly utilized as a hot and cold water soluble film for food packaging, detergents, pharmaceuticals, and agricultural chemicals, among other packaging uses. Additionally, the FDA has cleared it for close contact with food items.

A request for approval to use PVA as a component of an edible film that dissolves in water and contains ingredients for dry food was recently submitted to the FDA (Keller and Heckman, 2018; GRAS Notice No. 676, 2018). PVA films have a high tensile strength, are translucent, and are flexible. They are also well-suited as an aroma and oxygen barrier. Numerous publications claim that the formation of interpenetrated polymer networks in mix films consisting of starch and PVA enhanced the mechanical and water barrier properties of the composite films, offering several advantages over pure starch films (Cano *et al.*, 2015).

Furthermore, according to Sapper *et al.* (2021), depending on the distribution and content of carvacrol in the films, different polyvinyl alcohol (PVA) coating formulations with starch and carvacrol as the active agent when applied to Golden Delicious apples demonstrated a highly effective disease

control against the growth of both black and green mold. Furthermore, according to Baite *et al.* (2022), *Ficus auriculata* leaf extract high in gallic acid an antioxidant was mixed with poly (vinyl alcohol) (PVA) and used as a coating to stop green bananas from ripening too quickly.

The objective of this study is to extend the shelf life of apples and manage the growth of gray mold by using PVA and clove essential oil as straightforward, affordable coating treatments.

2. Materials and Methods

Apple fruits

The National Research Center station provided the mature apple fruits c.v. Anna used in the experiment. They were selected based on the absence of fungal diseases and their homogeneity in size, color, and shape.

Fungal isolate

One virulent isolate of *Botrytis cinerea* (Accession number: ON1498639.1) the cause of gray mold, of apple fruits were obtained from our previous study (Elshahawy *et al.*, 2023) Plant Pathology. Department, National Research Centre, Egypt(NRC). Isolate was maintained on potato dextrose agar PDA for further study.

In vitro, trails

Testing of various concentrations of clove essential oils on linear growth and spore germinations of *Botrytis cinerea*

On PDA medium at $25 \pm 2^\circ\text{C}$, the effects of clove essential oil (CEo) at 0.0,0.25,0.50,0.75 and 1.0% against the mycelial growth and spore germination of *Botrytis cinerea* were evaluated. To sterilize the PDA medium, it was diluted into 100 ml portions and placed in 250 ml Erlenmyer flasks. The flasks were then autoclaved at 121°C for 15 minutes.

Final concentrations were prepared separately, added to PDA medium right before it hardened, and then gradually mixed in 0.1% Tween 80 (Sigma) to increase solubility in order to get the final concentrations. The media in each flask was first broken up in a sterile, 9-cm-diameter Petri plate before it solidified. Ten-day-old *Botrytis cinerea* cultures were utilized to inoculate individual plates. A temperature of $25 \pm \text{C}$ was maintained for the cultures. The linear mycelial development of the fungus was measured when the control plates were fully grown and the average growth diameter was established. For every treatment, five replica plates were used.

Regarding spore germination, the technique outlined by Yan *et al.* (2021) was used to examine the impact of previous concentrations of clove essential oil (CEo) against *Botrytis cinerea* spore germination. A 20 ml test tube was filled with 10 ml of potato dextrose broth (PDB) and sterilized at 121°C for 20 minutes. To acquire the preceding concentrations, each clove concentration was added to PDB. Next, a gentle mixture with 0.1% Tween 80 (Sigma) was made. One milliliter of *B. cinerea* spore suspension (106 spores/ml) was added to test tubes, and they were then incubated for twenty-four hours on a rotating shaker at $20 \pm 2^\circ\text{C}$. Microscopical analyses of the percentage of germinated spores were performed. Five duplicates of each treatment were used in the experiment.

Testing of polyvinyl alcohol formulated with clove essential oils on linear growth and spore germination of *Botrytis cinerea*

The effects of clove essential oil (CEo) at 0.0,0.25,0.5,0.75,1.0 and 2.0% were examined when combined with 10% polyvinyl alcohol to examine the spore germination and linear growth of *Botrytis cinerea*. To obtain the final concentrations, previous concentrations combined with 10% PVA were made separately, added to the PDA medium just before it solidified, and then progressively mixed in 0.1% Tween 80 (Sigma) to improve solubility. Before the medium in each flask solidified, it was first broken up in a sterile, 9-cm-diameter Petri plate. To inoculate individual plates, cultures of *Botrytis cinerea* that were ten days old were used. For the cultures, a temperature of $25 \pm \text{C}$ was maintained.

The linear mycelial development of the fungus was measured when the control plates were fully grown and the average growth diameter was established. For every treatment, five plates were utilized as duplicates.

As for spore germinations, the effect of previous concentrations of clove essential formulated with PVA against spore germination of *Botrytis cinerea* was carried out as mentioned before described.

***In vivo* trails**

Therapeutic effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease at different coating times

Inoculum preparation

The standard inoculum for these assays was prepared by cultivating the pure isolate of *Botrytis cinerea* on PDA plates for 10 days at 20±2°C. For each plate, 10 mL of sterilized distilled water was brushed over the surface of the culture to create an isolate spore suspension. After that, the spore suspensions were passed through muslin filters. The spore suspension concentration was lowered to about 10⁶ spore/mL using a hemocytometer slide.

Inoculation of apple fruits

In order to examine the potential therapeutic benefits of clove essential oil at concentrations of 0.0, 0.50, 0.75, 1.0 and 2.0% in conjunction with 10% PVA, apple fruits cultivated under the cultivar Anna were exposed to an *in vivo* evaluation. The fruit was repeatedly washed in sterile water following a two-minute application of 70% ethanol to its surface. Using a sterile scalpel, fake wounds were created on the fruits. After air drying, injured fruits were injected with a *Botrytis cinerea* spore suspension containing 10⁶ spores/ml.

After inoculation, the fruits were individually coated with the aforementioned concentrations at varying durations (0.0, 24.0, and 48.0 hours) and allowed to air dry. A control group of fruits was employed, which had only been inoculated with *Botrytis cinerea*. To enable evaluation, five fruits were placed in each of the four carton boxes used for each treatment, which held either treated or untreated (control) fruits. The boxes were then stored for 21 days at 20–2°C and 90–95% relative humidity. A frequent check was conducted on fruits for anthracnose infections. The severity and incidence of the diseases were measured and computed.

Disease incidence (%) = (number of diseased fruits/total fruits) × 100.

Disease severity (%) = weight of rotted parts/weight of fruit × 100.

Protective effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease of apple fruits at different coating times.

To investigate the protective impact of clove essential oil (CEo) at 0.0, 0.50, 0.75, 1.0 and 2.0% in conjunction with PVA at 10% on apple fruit gray mold disease, apples of the cultivar Anna were exposed to an *in vivo* evaluation. The fruit was repeatedly washed in sterile water after being exposed to 70% ethanol on its surface for two minutes at room temperature. A sterile scalpel was used to create fake incisions on the fruits. Individually injured fruits were coated with the aforementioned concentrations at several intervals prior to inoculation (1.0, 12.0, and 24.0 hours) and then left to air dry.

After air drying, fruits that had been treated or left untreated were inoculated with a *Botrytis cinerea* spore suspension containing 10⁶ spores/ml. As a control, a set of fruits that had only been inoculated with *Botrytis cinerea* were used. In order for the fruits to be evaluated, the four carton boxes used for each treatment, each holding five fruits, were filled with either treated or untreated (control) fruits. The boxes were then stored for 21 days at 20–2°C and 90–95% relative humidity. Fruits were routinely examined for anthracnose diseases. Calculations were made to determine the severity and occurrence of the diseases.

Long-term protective effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease of apple fruits during 60 days of storage

The effects of 0.0, 1.0 and 2.0% clove essential oil combined with 10% PVA were studied for a 60-day period to examine their protective effects against apple fruit gray mold disease. The fruit was wiped off with sterile water several times after being exposed to 70% ethanol for two minutes at room temperature. We used a sterilized scalpel to create fake wounds on the fruits. After being individually dipped in the earlier concentrations, injured fruits were allowed to air dry. *Botrytis cinerea* spore suspension containing 10⁶ spores/ml was applied to both treated and untreated fruits, and the fruits were allowed to air dry.

As a control, a set of fruits that had only been inoculated with *Botrytis cinerea* were used. To enable evaluation, five fruits were placed in each of the four carton boxes used for each treatment, which held five fruits each. The fruits were then stored for sixty days at 20–2°C and 90–95% relative humidity. Fruits were routinely examined for anthracnose diseases. Calculations were made to determine the severity and occurrence of the diseases.

Effect of polyvinyl alcohol formulated with clove essential oils on fruit quality of apple fruits after 60 days of storage without artificial infection

Apple fruit quality was examined for 60 days without fake infection by examining the effects of clove essential oil at 0.0, 1.0 and 2.0% in combination with PVA at 10%. After soaking the fruit's surface in 70% ethanol for two minutes at room temperature. After being individually dipped in the earlier concentrations, apple fruits were allowed to air dry.

Total soluble solids (TSS), fruit degradation, and fruit weight loss were measured in order to determine how the fruits influenced the mango fruit's quality.

Three carton boxes of 60 by 40 by 15 centimeters, each containing one layer weighing around 10 kg, made up each treatment. The experimental boxes were maintained at 20±2°C and 90% relative humidity for 21 days without the presence of any artificial diseases.

The effects of the studied treatments on apple fruits were evaluated using the following findings: -

Fruit weight loss percentage

Apple fruits weights were initially recorded for each treatment. Fruit weight loss percentage was then calculated by weighing the same fruits at the end of the cold storage duration using the following formula:

$$\text{Weight loss \%} = \frac{\text{Initial weight} - \text{Weight at end experiment}}{\text{Initial weight}} \times 100$$

Total soluble solids percentage (TSS)

Chen and Mellenthin (1981) state that a hand refractometer was used to determine the TSS% in orange fruit juice.

Fruit decay percentage

Fruits affected with either pathological or physiological decay were counted by visual and calculated as a percentage to the initial number of fruits per each treatment.

Statistical analysis

Tukey test for multiple comparison among means was utilized Neler *et al.* (1984).

3. Results

3.1. Impact of different concentrations of clove essential oils on linear growth and spore germinations of *Botrytis cinerea*

The study assessed the impact of clove essential oil at concentrations of 0.25, 0.25, 0.75, and 1.0% on the mycelial growth and spore germination of *Botrytis cinerea*. The results presented in Table (1) demonstrate that the linear growth and spore germinations of *Botrytis cinerea* were considerably inhibited by all tested concentrations of clove essential oil (CEo). With CEo at 1.0%, linear growth and spore germination were completely inhibited. The reduction in height was achieved with a CEo of 0.75%, resulting in a decrease of 80.0 and 87.2% in linear growth and spore germinations, respectively. The effects of other concentrations were moderate.

Table 1: Impact of different concentrations of clove essential oils on linear growth and spore germinations of *Botrytis cinerea*

Clove (EO) Concentration (%)	<i>Botrytis cinerea</i>			
	Linear growth (mm)	Reduction %	Spore germination	Reduction %
0.0	90.0a	00.0	94.0a	00.0
0.25	39.0b	66.7	36.0b	61.7
0.50	26.0c	71.1	22.0c	76.6
0.75	18.0d	80.0	12.0d	87.2
1.0	0.0e	100.0	0.0e	100.0

Figures with the same litter are not significantly different (P =0.05)

3.2. Impact of polyvinyl alcohol formulated with clove essential oils on linear growth and spore germination of *Botrytis cinerea*

The effects of clove essential oil at 0.0, 0.25, 0.5, 0.75, 1.0 and 2.0 % were studied in a mixed with 10% polyvinyl alcohol on *Botrytis cinerea* linear growth and spore germination. Results in Table (2 and Fig.1) shows that all formulations significantly decreased *Botrytis cinerea's* linear growth and spore germinations. With a formulation of CEO at 2.0% combined with PVA at 10.0%, linear growth and spore germinations were completely inhibited. The combination of CEO at 1.0% and PVA at 10.0% resulted in a reduction of the heights by 92.2 and 94.7 %, respectively, in terms of linear growth and spore germinations. Other formulations had a middling result.

Table 2: Effect of polyvinyl alcohol formulated with clove essential oils on linear growth and spore germination of *Botrytis cinerea*

Treatments (%)	<i>Botrytis cinerea</i>			
	Linear growth (mm)	Reduction %	Spore germination	Reduction %
PVA 10 + clove 0.0	90.0a	0.0	94.0a	00.0
PVA 10 + clove 0.25	44.0b	51.1	35.0b	62.8
PVA 10 + clove 0.50	31.0c	65.6	26.0c	72.3
PVA 10 + clove 0.75	14.0d	84.4	11.0d	88.3
PVA 10 + clove 1.0	7.0	92.2	5.0	94.7
PVA 10 + clove 2.0	0.0e	00.0	00.0e	100.0
Control (un treated)	90.0a	0.0	94.0a	00.0

Figures with the same litter are not significantly different (P =0.05)

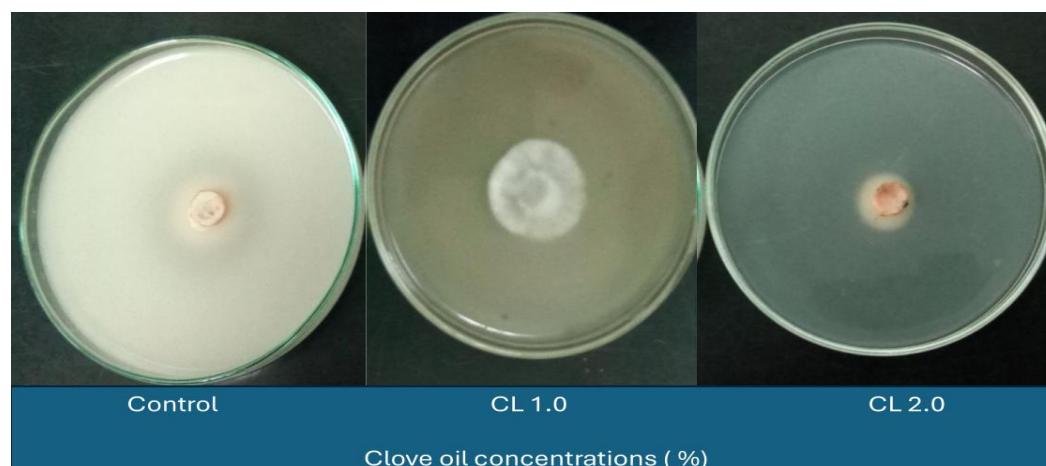


Fig. 1: Effect of polyvinyl alcohol formulated with clove essential oils on linear of *Botrytis cinerea*

3.3. Therapeutic effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease at different coating times

At various coating times after inoculation, i.e., 0.0, 24.0, and 48.0 hours, the clove essential oil at 0.0, 0.50, 1.0, and 2.0%, mixed with PVA at 10%, were applied to examine their therapeutic impact on gray mold illness of apple fruits. When applied at zero time and 24 hours after inoculation, all formulations considerably decreased the disease incidence and severity of gray mold disease, according to results in Table (3).

Gray mold incidence and severity were reduced by 75.0 and 73.0%, respectively, when CEo at 2.0% mixed with PVA at 10.0% was administered at zero time after inoculation. This resulted in the height reduction. CEO came next, at 1.0%, along with PVA at 10.0%. Applying any formulation 48 hours after inoculation, however, had no effect on the gray mold disease.

Table 3: Therapeutic effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease at different coating times.

Treatments (%)	Gray mold disease					
	Disease incidence			Disease severity		
	Coating time after fruit inoculation (hrs)					
	0.0	24	48	0.0	24	48
PVA 10 + clove 0.0	100.0a	100.0a	100.0	100.0a	100.0a	100.0a
PVA 10 + clove 0.50	70.0b	84.0b	100.0	65.0b	77.0b	100.0a
PVA 10 + clove 0.75	60.0c	72.0c	100.0	58.0c	72.0c	100.0a
PVA 10 + clove 1.0	35.0d	55.0d	100.0	35.0d	53.0d	100.0a
PVA 10 + clove 2.0	25.0e	45.0e	100.0	27.0e	47.0e	100.0a
Control (un treated)	100.0a	100.0a	100.0	100.0a	100.0a	100.0a

Figures with the same litter are not significantly different (P =0.05)

3.4. *In vivo* trails

3.4.1. Protective effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease of apple fruits at different coating times.

At various coating times prior to inoculation, namely 1.0, 12.0, and 24.0 hours, the protective effects of clove essential oil at concentrations of 0.0, 0.50, 0.75, 1.0 and 2.0% in conjunction with 10% PVA were evaluated *in vivo*. In all tested periods, the incidence and severity of gray mold disease were dramatically reduced by all formulations, according to the results shown in Table (4). The heights reduction of gray mold incidence and severity was achieved over 88.0 and 86.0%, respectively, by utilizing a combination of 2.0% CEo and 10.0% PVA over all the periods. PVA at 10.0% blended with CEo at 1.0% came next. Statistics, however, show that there are no appreciable variations across the protective times i.e. 1.0, 12.0, and 24 hours for apple fruit gray mold disease.

Table 4: Protective effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease of apple fruits at different coating times.

Treatments (%)	Gray mold disease					
	Disease incidence			Disease severity		
	Coating time before fruit inoculation(hrs)					
	1	12	24	1.0	12	24
PVA 10 + clove 0.0	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a
PVA 10 + clove 0.50	55.0b	53.0b	55.0b	52.0b	55.0b	54.0b
PVA 10 + clove 0.75	42.0c	40.0c	40.0c	41.0c	41.0c	43.0c
PVA 10 + clove 1.0	21.0d	25.0d	26.0d	22.0d	25.0d	24.0d
PVA 10 + clove 2.0	12.0e	12.0e	11.0e	14.0e	14.0e	12.0e
Control (un treated)	100.0a	100.0a	100.0a	100.0a	100.0a	100.0a

Figures with the same litter are not significantly different (P =0.05)

3.5. Long-term protective effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease of apple fruits during 60 days of storage

Over the course of 60 days, the effects of clove essential oil at 0.0, 1.0 and 2.0% in combination with PVA at 10% were investigated for their ability to protect apple fruits against the gray mold disease. The results presented in Table (5 and Fig. 2) demonstrate that during the course of the 60-day storage period, every formulation significantly decreased the incidence and severity of gray mold disease. The height reduction was achieved by combining 2.0% CEO with 10.0% PVA, which decreased the incidence and severity of gray mold by 82.0 and 85.0%, respectively. CEO came next, at 1.0%, mixed with PVA at 10.0%.

Table 5: Long-term protective effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease of apple fruits during 60 days of storage

Treatments (%)	Gray mold disease							
	Disease incidence				Disease severity			
	Days of storage							
	20	40	60	Efficacy	20	40	60	Efficacy
PVA 10 + clove 0.0	100.0a	100.0a	100.0a	0.0	100.0a	100.0	100.0a	000.0
PVA 10 + clove 1.0	16.0b	20.0b	28.0b	72.0	14.0b	18.0b	25.0b	75.0
PVA 10 + clove 2.0	11.0c	14.0c	18.0c	82.0	10.0c	13.0c	15.0c	85.0
Control (un treated)	100.0a	100.0a	100.0a	00.0	100.0a	100.0a	100.0a	00.0

Figures with the same litter are not significantly different (P =0.05)

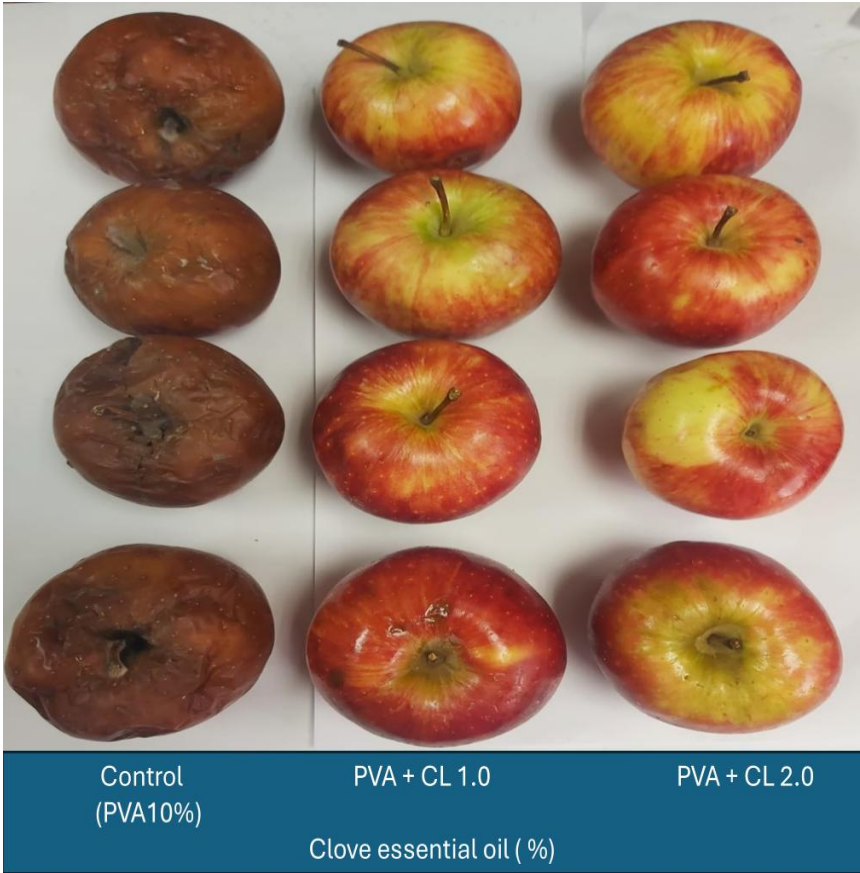


Fig. 2: Long-term protective effect of polyvinyl alcohol formulated with clove essential oils on gray mold disease of apple fruits during 60 days of storage

3.6. Effect of polyvinyl alcohol formulated with clove essential oils on fruit quality of apple fruits after 60 days of storage without artificial infection

The impact of 0.0, 1.0 and 2.0 clove essential oil combined with 10% PVA was evaluated to see how it affected the quality of the apple fruit throughout a 60-day period free from artificial infection. Fruits were evaluated for fruit weight loss, total soluble solids (TSS), and fruit decay in order to determine how these factors affected the quality of the apple fruit. All evaluated formulations had no adverse effects on the quality of the fruit, as shown by the results in Table (6). The weight reduction was less than 40% when PVA alone or in combination with CEo at 1.0 and 2.0% was used. The results show that PVA alone or in combination with CEo at 1.0 and 2.0% enhanced total soluble solids (TSS) by more than 21.4%. Reduced fruit degradation by 75.0 and 80.0%, respectively, when PVA at 10.0% combined with CEo at 1.0 and 2.0%

Table 6: Effect of polyvinyl alcohol formulated with clove essential oils on fruit quality of apple fruits after 60 days of storage without artificial infection

Treatments (%)	Fruit quality		
	Weight loss (%)	Total soluble solids(TSS)	Fruit decay percentage
PVA 10 + clove 0.0	12.0b	17.0a	15.0b
PVA 10 + clove 1.0	12.0b	17.0a	5.0c
PVA 10 + clove 2.0	11.0b	18.0a	4.0c
Control (un treated)	20.0a	14.0b	20.0a

Figures with the same letter are not significantly different (P=0.5)

4. Discussion

In apples and pears, a number of fungal infections can result in postharvest diseases (Sutton 2014). *Botrytis cinerea*, causes gray mold, primarily enter crops by wounds from insects and birds, as well as physical damage before or after harvest (Snowdon 1990 and Wenneker and Thomma, 2020 and Elshahawy *et al.*, 2023). Populations of *B. cinerea* have developed strain-dependent infection strategies and have partially but successfully adapted to a variety of host plants, according to Choquer *et al.* (2007).

Essential oils (EOs) are volatile, aromatic, and sweet-smelling liquid oils that are extracted supercritically or hydrodistillation-wise from flavors and plants. They are readily accessible in bioactive combinations with antibacterial and antioxidant qualities (Taghavi *et al.*, 2018; Mari *et al.*, 2016; Nazzaro *et al.*, 2017; Prakash *et al.*, 2015 and Danh *et al.*, 2021).

Results in the present study revealed that clove essential oil (CEo) concentrations at all tested concentrations significantly inhibited *Botrytis cinerea* linear growth and spore germination. CEo at 1.0% effectively inhibited linear growth and spore germination. The formulation of 2.0% CEo combined with 10.0% PVA completely inhibited linear growth and spore germinations. The clove essential formulated with PVA were applied to study their therapeutic and protective effects on gray mold disease of apple fruits. Results indicated that all formulations significantly reduced the disease incidence and severity of gray mold disease when applied at zero time and 24 h after inoculation. The heights reduction was obtained with CEo at 2.0 % mixed with PVA at 10.0% when applied at zero time after inoculation which reduced gray mold incidence and severity by 75.0 and 73.0% respectively. Clove essential oil in combination with PVA were tested to study their protective effect on gray mold disease of apple fruits at different coating times before inoculation i.e.1.0, 12.0 and 24.0 hrs. Results indicated that all formulations i.e CEo at 1.0 and 2.0% mixed with PVA at 10.0% when applied as protective treatments significantly reduced the disease incidence and severity of gray mold disease during 60 days of storage. All previous formulation had no negative effect on fruit quality.

In this regard, multiple beneficial research have showed that the application of biological agents, such as essential oils, can effectively manage postharvest illness in various fruits(Feng *et al.*, 2007; Amiri *et al.*, 2008; Liu *et al.*, 2009).Essential oils (EOs) have been found to be an effective control measure for minimizing the environmental impact of fruit production (Burt, 2004; Bakkali *et al.*, 2008); however, the bulk of these studies were conducted under greenhouse settings (Lopez-Reyes *et al.*, 2010, 2013).Essential oils (EOs), such as aldehydes, phenols, and ketones, interact with pathogens in different ways depending on their molecular structure. These compounds effectively limit the growth of

infections. Furthermore, environmental factors high in fungicidal compounds, such as thymol, carvacrol, and *p*-anisaldehyde, have the most potent inhibitory effects on *Penicillium digitatum* (Daferera *et al.*, 2000) and *Colletotrichum gloeosporioides* (Barrera-Nacha *et al.*, 2008). EO has antifungal activity in vitro against a variety of postharvest fungi, including *Aspergillus* spp. (Tang *et al.*, 2018), *Penicillium* spp. (Xing *et al.*, 2016), *Alternaria* spp. (Chen *et al.*, 2016 a, b), *Colletotrichum* spp. (Bill *et al.*, 2016), and *Botrytis cinerea* (Banani *et al.*, 2018).

In general, EOs are tested for effectiveness through direct contact with the fruit, spraying, or dipping (Elshafie *et al.*, 2016). Furthermore, chitosan, the most malleable biopolymer, has antibacterial action against a variety of foodborne pathogens, raising interest as a possible preservative (Ganan *et al.*, 2009).

According to Abd-El-Kareem *et al.* (2022), attempts were made to test the effects of thyme, nerol, and chitosan against *Penicillium digitatum* mycelial growth and spore germination in vitro. The results demonstrated that using thyme and nerol at 1.5% as well as 8.0 g/L of chitosan completely suppressed the mycelial growth and spore germination of all fungi that were investigated. They added that in order to investigate their potential protection against green and blue molds as well as sour rot diseases of Washington navel orange fruits, thyme or nerol at a concentration of 1.5% and chitosan at 8.0 g/L were administered separately or in combination. The greatest inhibition, according to the results, was achieved when thyme or nerol at 1.5% was mixed with 8.0g/L of chitosan. This combination reduced the incidence and severity of green, blue mold, and sour rot diseases by more than 88.0 and 92.0%, respectively. Fruit quality was unaffected negatively by any of the studied procedures.

Polyvinyl alcohol (PVA) is a biocompatible and biodegradable polymer with exceptional mechanical, optical, physical, and film-forming qualities as well as good chemical resistance (Zanela *et al.* 2018).

The current study results showed that all formulations of clove essential oil mixed with 10% polyvinyl alcohol decreased the linear growth and spore germinations of *Botrytis cinerea*. Complete suppression of linear growth and spore germinations was achieved with formulation of CEo at 1.0% mixed with PVA at 10.0%. The clove essential formulated with PVA were applied to study their therapeutic and protective effects on gray mold disease of apple fruits. Results revealed that all formulations significantly decreased the disease severity of gray mold disease when applied at zero time and 24 h after inoculation. The heights reduction was obtained with CEo at 1.0 % mixed with PVA at 10.0% when applied at zero time after inoculation which reduced gray mold incidence and severity by 75.0 and 73.0% respectively. All formulations i.e CEo at 0.75 and 1.0% mixed with PVA at 10.0% when applied as protective treatments decreased the disease incidence and severity of gray mold disease during 60 days of storage. All previous formulation had no negative effect on fruit quality.

Youssef *et al.* (2019) report that PVA and its composites have been thoroughly researched for potential applications in food packaging systems. It has been designated as generally recognized as safe (GRAS) due to its nontoxic and safe profile, implying that it can be utilized to make edible films (Keller and Heckman, 2018).

Food packaging could greatly benefit from the use of bioactive ingredients such phenolic compounds (Andrade *et al.*, 2021). One phenolic molecule that dissolves in water and has been linked to strong antibacterial and antioxidant effects is gallic acid. Gallic acid-incorporated PVA films have been shown to improve their antioxidant qualities (Awad *et al.*, 2017). According to current research, adding a gallic acid-rich extract to PVA coatings is expected to help delay banana ripening. *Ficus auriculata* leaf extract has been shown to have good antioxidant properties and to be high in gallic acid (Baite *et al.*, 2021). This suggests that the extract could be added to fresh foods to increase their shelf life. Nonetheless, no research has been conducted on using *F. auriculata* leaf extract to keep fruits fresher for longer periods of time. He *et al.* (2019) describe PVA as a synthetic polymer that is hydrophilic, nontoxic, biocompatible, and biodegradable, making it an environmentally beneficial product.

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