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Impact of Gelatin, Clove Oil and Olive Oil on Storability and Blue Mold of Anna Apple Caused by *Penicillium expansum* Under Cold Storage Conditions

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Abstract

This experiment was carried out during the 2020 and 2021 seasons to evaluate the physiological and pathological effects of edible coating with gelatin 8%, clove oil 1%, and olive oil 1% separately, or gelatin incorporated with clove oil or olive oil, on maintaining quality and control blue mold development caused by *Penicillium expansum* on Anna apple fruits under cold storage conditions. *In vitro*, both oils were evaluated on linear growth of *Penicillium expansum* at concentrations ranging from 0.2 to 1%. Clove oil at 1% had a high efficacy at inhibiting the mycelial growth of *Penicillium expansum* with 77.8%. The results showed that all studied coating treatments were effective in controlling blue mold in Anna apple during cold storage for 4 weeks in artificial infection and 12 weeks in natural infection compared to control. Also, all coating treatments had a significant effect on delaying changes in fruit weight loss percentage, color values (L* and h°), hardness, respiration rate, TSS: acid ratio, total phenolic content (TPC), and activities of peroxidase (POD) and catalysis (CAT), especially coating by gelatin mixed with olive oil. Therefore, it could be recommended that coating Anna apple fruits by gelatin 8% mixed with (clove oil 1% or olive oil 1%) to improve their quality and storability during cold storage.

Keywords: Anna apple; Gelatin; Clove oil; Olive oil; *Penicillium expansum*; Cold storage; TPC; CAT; POD.

1. Introduction

Apple (*Malus domestica* Borkh) represents the third most important horticultural fruit in the world for human nutrition, after citrus and banana [1]. In Egypt, Anna apple cultivar (Red Hadassiya X Golden Delicious) considered the leading apple cultivars that of a low chilling requirement [2]. Apple, likes most of the other perishable fruits contains large of water, which cause a number of physiological and pathological disorders, and consequently causes a reduction in storage and shelf life [3].

Postharvest pathogens cause major losses in apple production. More than 90 fungal species have been described as causative agents of apple decay during storage [4]. Blue mold caused by *Penicillium expansum* is the most important postharvest disease of apples [5]. This disease causes shortage of shelf life and consequently reduces the economic value of apple. The use of synthetic fungicides is a primary method to control for this disease. However, concerns about public health and the development of resistant pathogens have increased the need to search for alternative methods [6].

A new approach to extend shelf life of fruits is the use of edible coatings of natural antimicrobial compounds. Numbers of studies have been performed for the benefits of coating applications on fruits and vegetables. These materials can help to prolong shelf life and color retention, reduce moisture loss, inhibit ethylene production [7], improve visual appearance, reduce shriveling and wilt while retaining biochemical properties [8], improve texture and color retention, cell membrane stability, and storage life [9] inhibit browning [10], and reduce weight loss and decay incidence [11].

Gelatin has a high molecular weight and is a water-soluble protein with a broad range of functional properties and applications including thickening, gelling, stabilizing, emulsifying, foaming, encapsulating and film-forming abilities [12]. It is obtained by chemical denaturation of skin or bone collagen of animals and is one of the most promising renewable resources for the production of bioplastic due to having relatively low price, high availability, fast biodegradability and good film-forming properties [13].

Like most biopolymer based films, pure gelatin films are brittle and lose their mechanical and barrier properties upon hydration [14]. The main functional properties of these hydrophilic polymers are also highly dependent on their water content and surrounding humidity. Therefore, plasticizers are added to improve the polymer flexibility by weakening the polymer chains within the intermolecular and intermolecular bonds [15]. In order to fix this defect in

gelatin films, a number of approaches have been studied such as combination with other compatible biopolymers, using hydrophobic compounds such as waxes, lipid and essential oil to produce emulsions or coating films [16-18]. The lipids are dispersed and homogenized in an aqueous solution of a biopolymer containing a suitable surfactant to form a stable emulsion, and then the resulting emulsion would be dried to obtain an emulsion film [19].

Olive oil is natural lipids which have also high antioxidant and probably antimicrobial properties due to presence of phenolic compounds such as oleorupin and resveratrol [20]. Moreover, olive oil has contained large amounts of bioactive substances [21], antioxidants [22, 23], antimicrobials [24]. Accordingly, they were incorporated with polylactic acid and methylcellulose films [25, 26] and had the effect of improving the inhibition properties of films against spoilage and pathogenic strains as it enhanced its antifungal activity against them especially *P. expansum* and *R. stolonifer*. The use of olive oil also had a beneficial impact on maintaining lower activities of cell wall deterioration and retention of apple and strawberry fruits quality under cold storage [27].

In the recent years, environmentally friendly essential oils have been extensively studied as a natural food and fruits preservatives due to their broad antimicrobial activities [28, 29]. Adding essential oil to biopolymer films is an interesting choice, as they are naturally hydrophobic and bacteriostatic materials due to their high content of terpenes and phenolic compounds. Clove essential oil contains enormous amounts of bioactive compounds, such as triterpenoids, sesquiterpenes, and tannins [30]. In addition, some studies reported that one of the main components, viz. is eugenol (4-allyl-2-methoxyphenol), which works actively as antifungal agent [31, 32]. Clove oil has been proven to be very effective against different fungal diseases and various pathogens and therefore to maintenance the fruit quality during storage. Arpit, *et al.* [33], found that coated papaya fruits with clove oil combined with Gum ghatti showed a reduction of weight loss, decay percentage, accumulation of TSS and sugar by diminishing the rate of respiration and metabolism. Clove oil reduces the growth, number, and viability of *P. expansum* spores 24 hours after the induction to germination, as well as the diameter of blue mold lesions in apples [34].

Moldão-Martins, et al. [35], reported that plasticizers including glycerol, sorbitol and polyethylene glycol are used to improve the efficiency of mechanical properties as well as barrier properties of edible films which directly affect the coating of fruits and vegetables such as film thickness, moisture content, water solubility and water absorption. Consistent with previous reports, Zapata, *et al.* [36] found that adding 20% (w/w) of glycerol to edible coating solutions containing 1% gum was sufficient to achieve a significant reduction in weight loss.

The objectives of this work were (1) to study the effect of olive and clove oil on some pathological properties of *Penicillium expansum*, (2) to evaluate the addition of these oils to a gelatin-based edible film to increase storability, delay ripening and maintain the quality of Anna apple fruits during cold storage and (3) to determine the effect of the optimal anti-fungal film on the quality parameters of Anna apple fruits in order to enhance the control of blue mold disease.

2. Materials and Methods

2.1. Apple Samples and Treatments

Mature Anna (*Malus domestica* Borkh) apple fruits were chosen with uniform size, shape, weight, color and free from visual symptoms of disease or mechanical damages which were harvested in June during both 2020 and 2021 seasons from a commercial orchard located at El Behaira Governorate, Egypt. Fruits were transported immediately to the laboratory of Fruit Handling Department, Horticulture Research Institute, Agricultural Research Center. The fruits were cleaned and divided into two groups, each group were 162 Kg of fruits (6 treatments X 3 replicates X 3 boxes X 3 Kg for each box).

The first group considered a natural infection and the second group considered an artificial infection by inoculating with *pencillium expansum* isolated. Six treatments were performed as follows:

T1. Control (uncoated)
T2. Gelatin 8%
T3. Clove oil 1%
T4. Olive oil 1%
T5. Gelatin 8% + Clove oil 1%
T6. Gelatin 8%+ Olive oil 1%

2.2. Clove and Olive Oils Coating Film Preparation

Clove (*Syzigium aromaticum* L.) oil and olive (*Olea europaea* L.) oil were obtained from the oil extraction unit of National Research Centre. Oils coating film were prepared by dissolved 1 ml oil in 2 ml of glycerin as a plasticizer according to Al-Hashimi, *et al.* [37], and then adding distilled water to a volume of 100 ml.

2.3. Gelatin Coating Film Preparation

Gelatin coating film was obtained and prepared according to Alparslan, *et al.* [38] by dissolving 8 g of gelatin powder in 50 ml distilled water (at room temperature) and the mixture was stirred until the gelatin completely dissolved and heating to 45 °C for 15 min, then adding 5 ml of glycerin and then supplemented up to 100 ml with distilled water.

2.4. Preparation of Gelatin Coating Mixed With Oils

The previously prepared gelatin coating were mixed with clove oil or olive oil coating previously prepared 1% and 8%, respectively, and stirred until the mixture became homogeneous [39] and then used to coat the fruits.

The fruits were immersed in film solutions for (2 min) then left for air drying at room temperature. After the treatment first group of fruits placed in carton boxes and stored at 5±1°C and 90% relative humidity for a total storage period of 12 weeks.

The second group of fruits, after coating was subjected for the artificial infection as the following:

The fruits allocated for artificial inoculation were surface sterilized by dipping in 70% alcohol for 2 min followed by dipping in 2% sodium hypochlorite for 10 seconds, then washed thoroughly with sterilized distilled water for 1 min [40]. Surface sterilized fruits were left for air drying at room temperature. Fruits were punctured using a stainless-steel rod with 2 mm diameter and to 2 mm depth, as one puncture for fruit at the middle of one fruit side. The treated fruits were allowed for air drying at room temperature under aseptic conditions, after 24 h the fruits were inoculated with a conidial suspension of *pencillium expensum* (5 × 104 spore/mL) for 30 seconds [41]. Control fruits were injured as above and treated with sterile distilled water containing Tween 20, then fruits placed in carton boxes and stored for 4 weeks in the same conditions.

2.5. Isolation and Obtain From the Pathogen

The fungi *Penicillium expansum* was isolated from naturally infected Anna apple fruits during storage of cold room in Behaira governorate as described by Taş and Karaca [42]. The infected apple was cut into small pieces and surface sterilized by dipping in 2% sodium hypochlorite for 2 min. The tissues were washed 3 times using sterile distilled water, cultured on Potato Dextrose Agar (PDA) and incubated at 25°C. The causal pathogen was then confirmed through Koch's postulates were performed to confirm the pathogenicity of each isolate. Sub-culturing was carried out at 7 day intervals and conidia from 7 day-old pure cultures were used for inoculations were selected from isolated collection at Department of postharvest diseases, Plant Pathology Research Institute, ARC, and Giza.

2.6. In Vitro Assay

Effect of clove oil and olive oil on linear growth of *pencillium expansum* was investigated by using poisoned food technique according to Abd-Alla and Haggag [43]; Tongfei, *et al.* [29]. Oils were tested *in vitro* at serial concentrations; 0.2, 0.4, 0.6, 0.8 and 1% into worm sterile PDA medium before medium solidification, 45-55°C, in aseptic conditions providing target concentration. Then petri plates were inoculated with 5 mm discs from periphery of 7day old cultures of such *pencillium expansum* isolate by placing fungal discs on solidified medium surface at the centre. Inoculated plain medium with fungus discs was used as a control. Three petri dishes were used as replications per each tested concentration and the control. Inoculated cultures were incubated at 25°C for 7days. Average linear growth for each replicate of such treatment was determined (cm). The chemical concentration that inhibits *pencillium expansum* linear growth ranges of tested concentrations was selected precisely according to preliminary tests.

2.7. Disease Assessment

2.7.1. Disease Incidence (%)

Determined for each replicate by relating decayed fruits with blue mould disease to the total number of fruits according to Moreira and Mio [40].

2.7.2. Disease Severity (%)

It was determined according to the equation described by Chen, *et al.* [44] as follows:

Disease severity % = ∑decayed area (%) of each fruit /No. of fruits of each replicate X 100

Where, decayed area (%) for each single fruit was determined as periods.

The disease severity was observed by evaluating the disease development of blue mold using the following scale: scale 0, lesion diameter = 0 mm (no decay); scale 1, 1 mm ≤ lesion diameter ≤ 10 mm; scale 2, 10 mm < lesion diameter ≤ 20 mm; scale 3, 20 mm < lesion diameter ≤ 40 mm; scale 4, lesion diameter > 40 mm.

2.7.3. Fruit Quality Assessments

Fruit quality was assessed at 2 weeks intervals from the beginning of cold storage (0 times) till the end of storage period (12 weeks) in both seasons of the study as the following:

2.8. Physical Properties

2.8.1. Fruit Weight Loss Percentage

Calculated as the difference between fruit weight at the beginning of storage and fruit weight at the inspection dates using the following equation:

Loss in fruit weight % = [(Initial weight – Weight at time of sampling)/Initial weight] X 100

2.8.2. Fruit Firmness

Determined as (kg/cm^2) by using fruit pressure tester mod. FT 327.

2.8.3. Fruit Color

Lightness and hue angle were estimated using Minolta Calorimeter (Minolta Co. Ltd.,Osaka, Japan) as described by McGire [45].

2.9. Physiological Properties

2.9.1. Respiration Rate (CO_2 Production)

Individual fruits from each treatment were weighed and placed in 2 liter jars at 20°C. The jars were sealed for 3 h with a cap and a rubber septum. The resulting O_2 and CO_2 samples of the head space were removed from the septum with a syringe and injected into Service Inst. Model 1450 C (Food Pack Gas Analyzer) to measure carbon dioxide production. Respiration rate was calculated as ml CO_2 /kg/h according to Varit and Songsin [46].

2.10. Chemical Properties

2.10.1. TSS/Acid Ratio

Total soluble solids percentage was determined in fruit juice using Digital refractometer PR32 (AtagoPalete ATago.CO .LTD. Japan); titratable acidity percentage was determined by titrating the juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator and expressed as gm of malic acid /100 ml juice according to AOAC [47] and then the TSS: TA ratio was calculated.

2.10.2. Total phenolic content (TPC)

TPC in juice was determined using the Folin–Ciocalteu method [48]. Total phenolic content was expressed as mg gallic acid equivalent in 100 mL of juice (mg gallic acid /100 ml juice).

2.10.3. Defense-Related Enzymes Activities

In the **Determination of peroxidase (POD) activity**: Using guaiacol as a substrate, was assayed by the method described by Zhang, *et al.* [49] in a reaction mixture (3 ml) containing 25 µl of enzyme extract, 2.78 mL of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 20 mM H_2O_2, and 0.1 ml of 20 mM guaiacol. An increase in POD activity at 470 nm, due to guaiacol oxidation, was recorded after 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 absorbance / min.

2.10.4. Determination of Catalase (CAT) Activity

It was analyzed according to the methods described by Wang, *et al.* [50]. The reaction mixture consisted of 2 ml sodium phosphate buffer (50 mmol/ L, pH 7.0), 0.5 ml H_2O_2 (40 mmol/ L), and 0.5 ml enzyme extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm and 25 °C. CAT specific activity was expressed as U/g FW.

2.11. Statistical Analysis

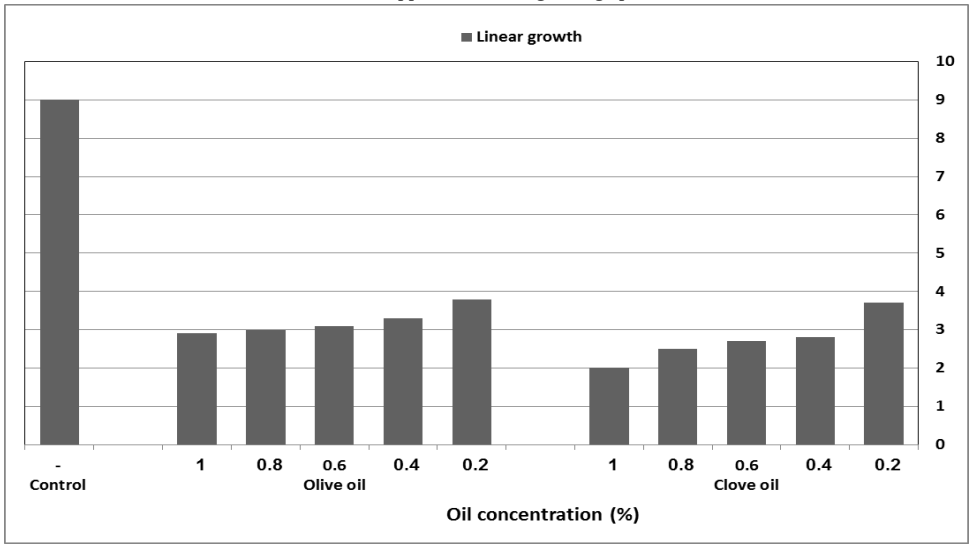
The data were arranged as a randomized complete design with three replicates. All data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran [51] and means were compared by Duncan's Multiple range test at the 5% level of probability in the two seasons of experimentation.

3. Results

3.1. Effect of Clove Oil and Olive Oil on Mycelial Growth of *Penicillium Expansum*

Figure 1 shows the effect of tested clove oil and olive oil *In vitro* on the linear growth of the pathogenic fungi isolated from Anna apple fruits. The results revealed that clove oil was highly effective in inhibiting the mycelial growth of *penicillium expansum* (77.8%) compared to olive oil (61%), and the percentage of mycelial growth inhibition increases gradually with increasing the concentration of oils.

Fig-1. Effect of clove oil and olive oil on linear growth of *penicillium expansum* caused blue mold in Anna apple fruits Effect of gelatin, clove oil and olive oil treatments on incidence of blue mold in Anna apple fruits during storage periods



Figures 2, 3, 4, and 5 revealed that coating Anna apple fruits with gelatin 8%, clove oil 1%, and olive oil 1% alone or gelatin combined with clove oil or olive oil, significantly reduced the incidence of blue mold compared to control during the two seasons of study. No appearance of blue mold was observed during the first two weeks of storage, either in all applied coating treatments or in untreated fruits (control). The prolonged storage period to 10 weeks at 5 ± 1 °C and 90% relative humidity showed the positive effect of different coating treatments in controlling blue mold. Coating with clove oil only and gelatin mixed with clove oil or olive oil treatment were successful in total control of blue mold for 6 weeks of cold storage as well as, gelatin plus olive oil continued until 8 weeks of cold storage. At 10 weeks of cold storage, coating the fruits with clove oil or gelatin mixed with clove oil was significantly more effective than other treatments in controlling disease incidence and severity.

Fig-2. Effect of gelatin, clove oil and olive oil on disease incidence (%) of Anna apple fruits naturally infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during season 2020

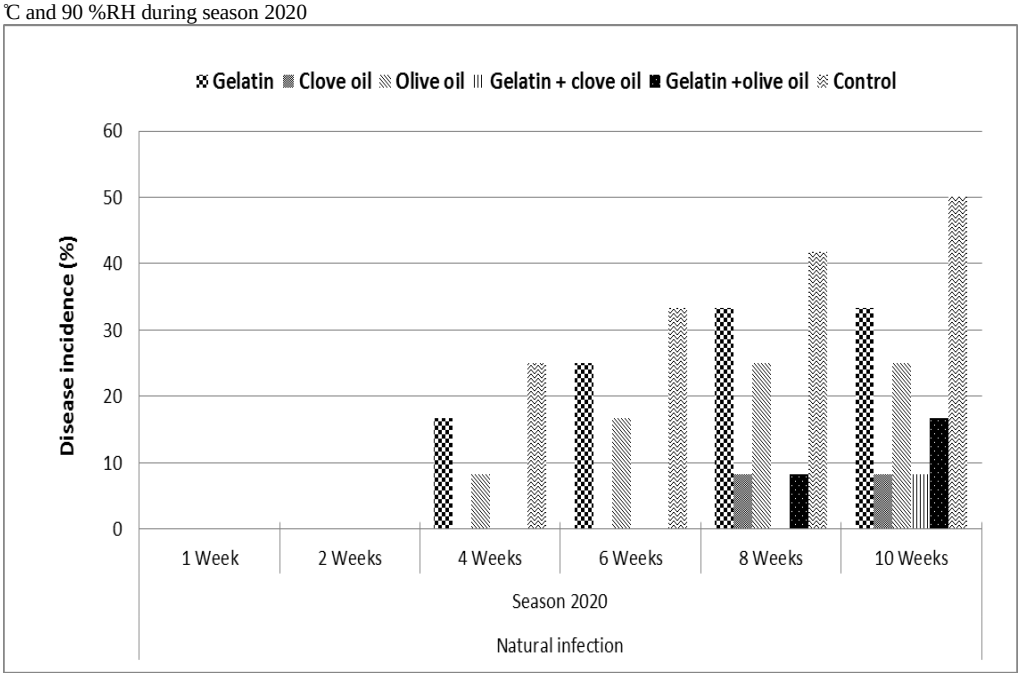


Fig-3. Effect of gelatin, clove oil and olive oil on disease incidence (%) of Anna apple fruits naturally infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during season 2021

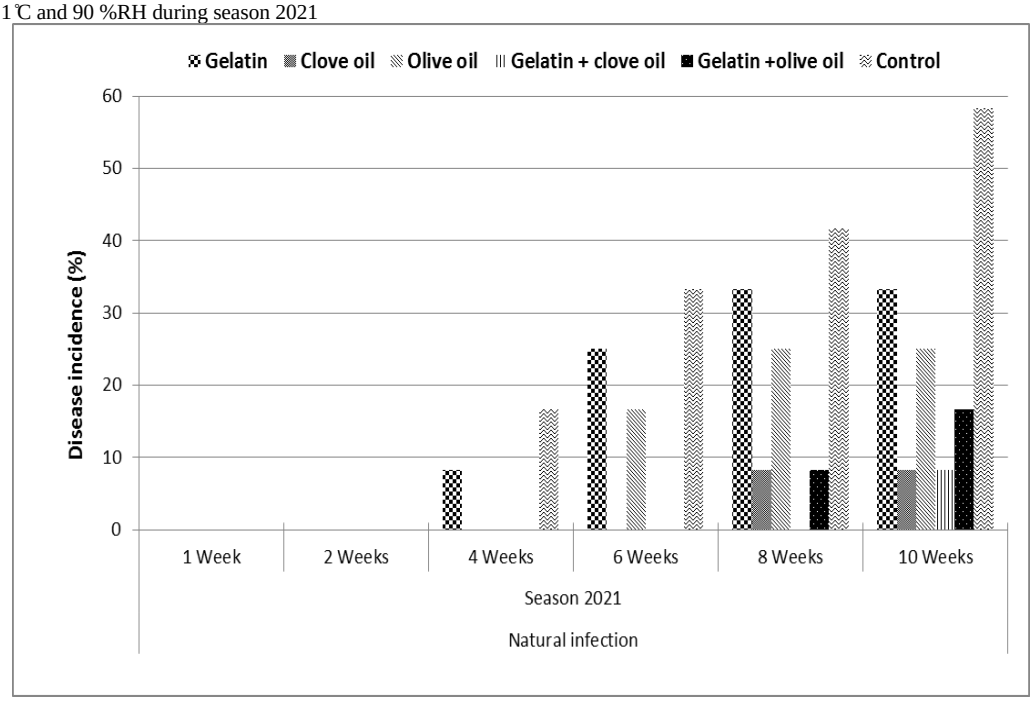


Fig-4. Effect of gelatin, clove oil and olive oil on disease severity (%) of Anna apple fruits naturally infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during season 2020

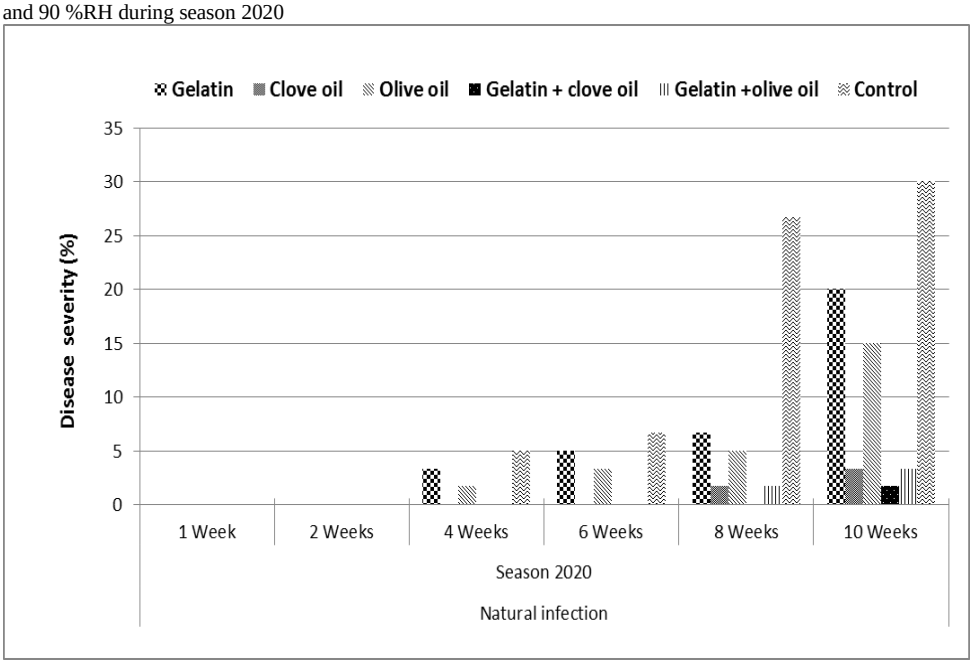
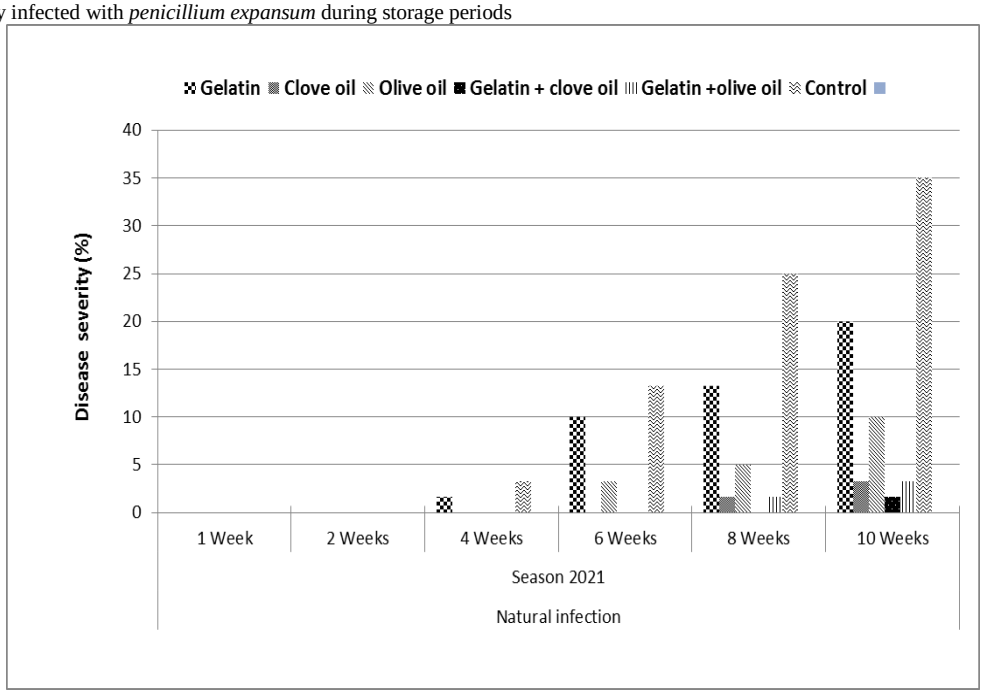


Fig-5. Effect of gelatin, clove oil and olive oil on disease severity (%) of Anna apple fruits naturally infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during season 2021 Effect of gelatin, clove oil and olive oil treatments on incidence of blue mold in Anna apple fruits artificially infected with *penicillium expansum* during storage periods



Prolonged cold storage of artificially inoculated Anna apple with *penicillium expansum* resulted significant reduction in disease incidence and more pronounced reduction in disease severity. It is clear from Figs 6 and 7 that all treatments and control resulted in controlling significantly blue mold disease and severity in the artificially infected fruits during cold storage for one week under cold storage conditions at 5 ± 1°C and 90% relative humidity in the both seasons. The incidence and severity percentage of the blue mold disease in the control was recorded 100% during the both seasons of study. At the first 2 weeks of cold storage, coating Anna apple fruits with gelatin mixed with clove oil or olive oil completely inhibited blue mold incidence compared to untreated fruits (control). Fig 8 show symptom expression related to incidence and severity of blue mold after 4 weeks of artificially inoculated and cold storage and effects of coating applied treatments in their disease controlling.

Fig-6. Effect of gelatin, clove oil and olive oil on disease incidence (%) of Anna apple fruits artificially infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

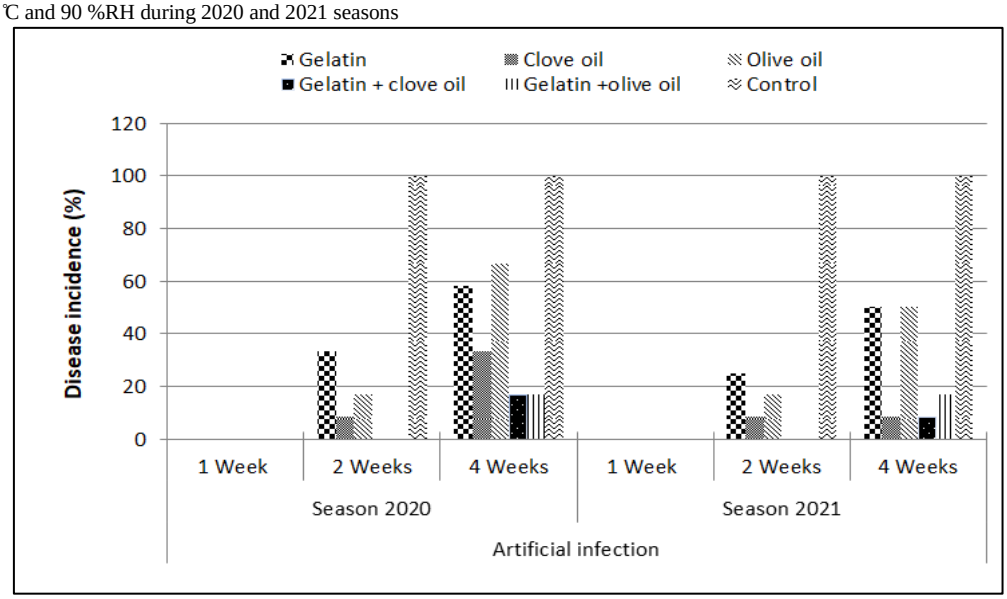


Fig-7. Effect of gelatin, clove oil and olive oil on disease severity (%) of Anna apple fruits artificially infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

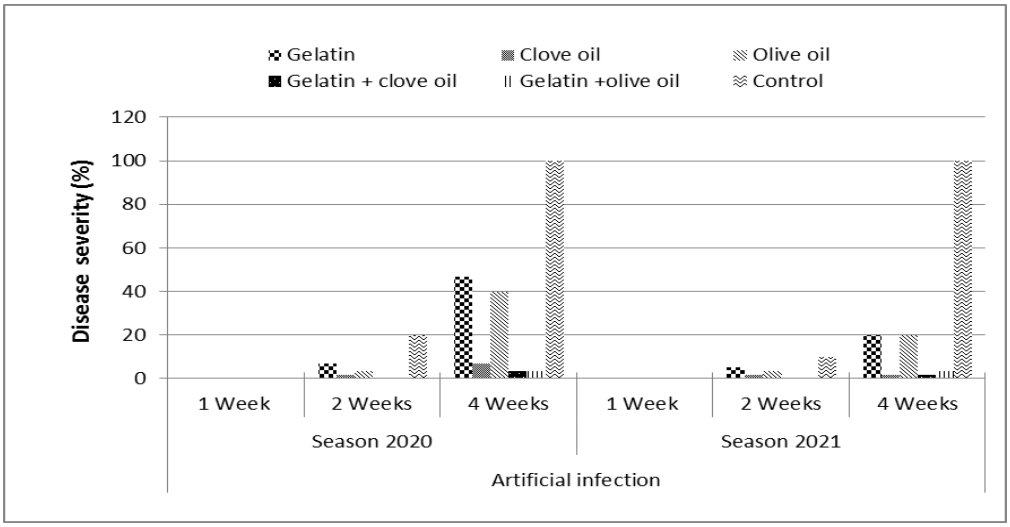
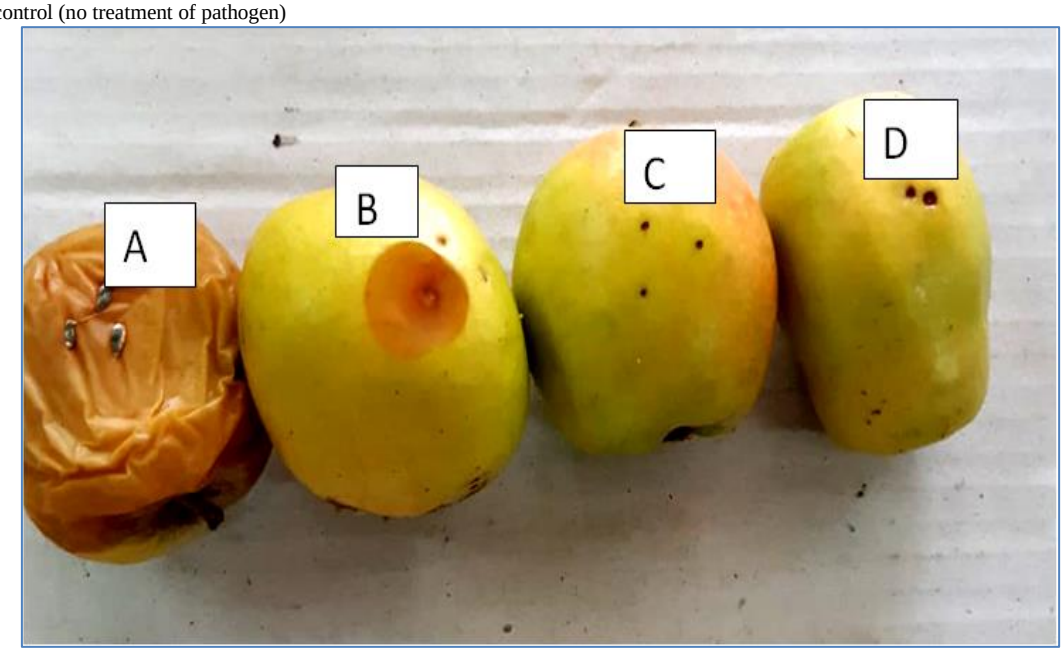


Fig-8. Results of control in blue mold of Anna apple fruits artificially infected with *penicillium expansum* during cold storage at 5± 1℃ and 90 %RH. (A) Untreated; (b) Coated with gelatin; (c) Coated with clove oil; (d) Coated with gelatin + olive oil at the 30th days of inoculation. The picture include, from the right: positive control treated by gelatin plus olive oil; followed by treatment with clove oil; then gelatin treatment and negative control (no treatment of pathogen)



3.2. Effect of Gelatin, Clove Oil and Olive Oil Treatments on Physical Properties of Anna Apple Fruits During Storage Periods

3.2.1. Fruit Weight Loss Percentage

Results in Table 1 show the effect of coating Anna apple fruits with gelatin 8%, clove oil 1%, and olive oil 1% alone or gelatin combined with clove oil or olive oil on fruit weight loss percentage under cold storage conditions during 2020 and 2021 seasons.

Fruit weight loss percentage increased gradually toward the end of storage period. All coating treatments decreased significantly weight loss% compared with untreated fruits during both seasons of this study. After 12 weeks of cold storage, fruits treated by gelatin combined with olive oil recorded the lowest value of weight loss percentage (8.44& 9.64%) in the two seasons, respectively. Whereas, the highest value of weight loss percentage was found in untreated fruit (13.79&15.74 %) during 2020 and 2021 seasons, respectively.

With regard to the effect of different coating treatments on the percentage of weight loss in fruits infected artificially with *pencillium expansum*, the results presented in Table 2 that fruit weight loss percentage was increased gradually toward the end of storage period during 2020 and 2021 seasons. All treatments decreased significantly weight loss% compared with untreated fruits during both seasons of this study. After 4 weeks of cold storage, fruits treated by gelatin combined with clove oil recorded the lowest value of weight loss percentage (5.3%) in the first season, while in the second season clove oil recorded the lowest value of weight loss percentage (5.9%). Whereas, the highest value of weight loss percentage was found in untreated fruit (14.7&10.7 %) during 2020 and 2021 seasons, respectively.

Table-1. Effect of gelatin, clove oil and olive oil on weight loss percentage of Anna apple fruits stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	0.00A	1.74A	3.57A	5.11A	6.94A	8.93A	13.79A
Gelatin 8%	0.00A	0.92B	2.54B	3.64B	5.20B	6.88B	10.68B
Clove oil 1%	0.00A	1.52A	3.38A	4.83A	6.85A	8.87A	13.31A
Olive oil 1%	0.00A	1.65A	3.51A	5.12A	6.78A	8.90A	13.54A
Gelatin + Clove oil	0.00A	0.70B	2.03C	2.88C	4.37C	5.86C	8.69C
Gelatin + Olive oil	0.00A	0.79B	1.76C	2.77C	4.09C	5.30D	8.44C
LSD at 5 %	**N.S.**	**0.3314**	**0.353**	**0.4191**	**0.3699**	**0.4289**	**0.4686**
2nd Season							
Control	0.00A	1.75A	3.72A	5.74A	8.08A	10.48A	15.74A
Gelatin 8%	0.00A	1.11BC	2.71C	3.74D	5.22D	6.79D	10.35D
Clove oil 1%	0.00A	1.34B	3.32B	4.75C	6.61C	8.63C	13.01C
Olive oil 1%	0.00A	1.72A	3.81A	5.23B	7.30B	9.32B	13.58B
Gelatin + Clove oil	0.00A	0.88A	2.53CD	3.24E	4.74E	6.28E	10.11D
Gelatin + Olive oil	0.00A	0.84C	2.19D	3.10E	4.49E	6.23E	9.64E
LSD at 5 %	**N.S.**	**0.3358**	**0.3707**	**0.4223**	**0.3821**	**0.4426**	**0.459**

The same letter(s) in each column are statistically insignificant at 5% level.

Table-2. Effect of gelatin, clove oil and olive oil on weight loss percentage of Anna apple fruits artificially infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)							
Treatments	**1st Season**				**2nd Season**			
	0	**1**	**2**	**4**	**0**	**1**	**2**	**4**
Control	0.00A	3.9A	7.1A	14.7A	0.00A	2.A	5.1A	10.7A
Gelatin 8%	0.00A	2.2C	5.0B	8.9B	0.00A	2.5A	5.1A	10.7A
Clove oil 1%	0.00A	2.2C	4.0C	7.7C	0.00A	2.2AB	3.4C	5.9C
Olive oil 1%	0.00A	2.7B	5.0B	5.7D	0.00A	2.4A	4.4B	7.5B
Gelatin + Clove oil	0.00A	2.5B	3.6D	5.3D	0.00A	2.6A	4.8A	7.4B
Gelatin + Olive oil	0.00A	2.4B	4.2C	6.9C	0.00A	2.1B	5.0A	7.9B
LSD at 5 %	**N.S.**	**0.5**	**0.326**	**0.388**	**N.S.**	**0.397**	**0.357**	**0.307**

The same letter(s) in each column are statistically insignificant at 5% level.

3.3. Firmness (kg/cm^2)

It is amply from Table 3 that the firmness decreased with progress of storage periods in the two seasons. All coating treatments decreased firmness deterioration of fruits compered to control fruits with significant differences

between all treatments in both seasons. After 12 weeks of storage, fruits coated with gelatin combined with olive oil had significantly higher in firmness fruits (4.30 and 4.33 kg/cm^2) in the two seasons, respectively.

Concerning the effect of different coating treatments on the firmness of artificially infected fruits with *pencillium expansum*, Table 4 showed that the firmness of fruits took the same direction as it decreased with the prolonged of storage periods during the two study seasons. All treatments succeeded in decreased firmness deterioration of fruits compared to the control in both seasons. After 4 weeks of storage, treatment of fruits with gelatin plus clove oil recorded the highest values (2.8 kg/cm^2) in the first season while coating with gelatin plus clove oil or olive oil recorded the highest values in fruit firmness (3 kg/cm^2) without significant between them in the second season.

Table-3. Effect of gelatin, clove oil and olive oil on firmness (kg/cm^2) of Anna apple fruits stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	6.67A	5.23C	4.50D	4.33D	4.07D	4.03CD	3.90C
Gelatin 8%	6.67A	6.13A	5.40AB	5.30A	4.50B	3.90D	3.70D
Clove oil 1%	6.67A	5.90AB	4.80C	4.60BC	4.30BC	4.10BC	4.00BC
Olive oil 1%	6.67A	5.23C	4.90C	4.50CD	4.20CD	4.00CD	3.90C
Gelatin + Clove oil	6.67A	5.70B	5.20B	4.80B	4.50B	4.20B	4.07B
Gelatin + Olive oil	6.67A	6.00A	5.60A	5.23A	4.90A	4.50A	4.30A
LSD at 5 %	**N.S.**	**0.2375**	**0.2266**	**0.2096**	**0.1911**	**0.1531**	**0.1462**
2nd Season							
Control	6.93A	5.30C	5.00D	4.90B	4.60AB	4.40B	4.00B
Gelatin 8%	6.93A	5.57B	5.50B	4.90B	4.67AB	4.43B	4.30A
Clove oil 1%	6.93A	4.50D	5.20C	5.00B	4.80A	4.50B	4.00B
Olive oil 1%	6.93A	4.50D	5.20C	5.00B	4.80A	4.50B	4.07B
Gelatin + Clove oil	6.93A	5.67B	5.30C	4.80B	4.50B	4.30B	4.20AB
Gelatin + Olive oil	6.93A	5.90A	5.80A	5.23A	4.80A	4.70A	4.33A
LSD at 5 %	**N.S.**	**0.2067**	**0.1652**	**0.2324**	**0.1979**	**0.1941**	**0.2138**

The same letter(s) in each column are statistically insignificant at 5% level.

Table-4. Effect of gelatin, clove oil and olive oil on firmness (kg/cm^2) of Anna apple fruits artificially infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)							
Treatments	**1st Season**				**2nd Season**			
	0	**1**	**2**	**4**	**0**	**1**	**2**	**4**
Control	6.67A	4.5A	3.5B	2.1B	6.67A	4.8A	4.0A	1.6C
Gelatin 8%	6.67A	4.8A	3.5B	2.2B	6.67A	4.8A	4.2A	2.5B
Clove oil 1%	6.67A	4.8A	3.8A	2.4AB	6.67A	4.5A	4.0A	2.5B
Olive oil 1%	6.67A	4.8A	3.1C	2.5A	6.67A	4.8A	4.2A	2.0C
Gelatin + Clove oil	6.67A	5.0A	4.0A	2.8A	6.67A	5.0A	4.5A	3.0A
Gelatin + Olive oil	6.67A	5.0A	4.2A	2.5A	6.67A	5.0A	4.5A	3.0A
LSD at 5 %	**N.S.**	**0.326**	**0.357**	**0.326**	**N.S.**	**0.326**	**0.547**	**0.412**

The same letter(s) in each column are statistically insignificant at 5% level.

3.4. Fruit color

3.4.1. Lightness (L*)

Results in Table 5, show that lightness (L*) of fruits was gradually decreased towards at the end of the storage period (after 12 weeks). At the end of storage period, fruits treated by gelatin combined with olive oil gave the highest values of L* (70.57 & 65.75) in the first and second seasons, respectively. On the other hand, control treatment exhibited the lowest values of L*(61.53 & 61.75) in the two seasons, respectively.

3.5. Hue Angle (h^o value)

Results in Table 6 show that hue angle (h^o) was decreased (increase density of red color) with the advance in cold storage periods. Significant differences between all treatments were observed in the two seasons. At the end of storage period, fruits treated by gelatin plus olive oil gave the lowest value of h^o (high density of red color) in the two seasons. On the other hand, the highest values were recorded with fruits treated by gelatin plus clove oil in first and second seasons, respectively.

Table-5. Effect of gelatin, clove oil and olive oil on lightness (L*) of Anna apple fruits stored at 5± 1 ℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	74.31A	63.00F	62.89F	62.87e	62.70F	62.17F	61.53F
Gelatin 8%	74.31A	67.80D	66.65D	66.56C	66.29D	65.82D	65.18D
Clove oil 1%	74.31A	69.98C	69.63C	68.07b	67.62C	67.58C	67.21C
Olive oil 1%	74.31A	72.54B	70.36B	72.04a	70.06B	69.68B	69.59B
Gelatin + Clove oil	74.31A	66.38E	66.20E	66.06d	65.06E	64.75E	63.35E
Gelatin + Olive oil	74.31A	73.86A	72.45A	71.92A	71.81A	71.41A	70.57A
LSD at 5 %	**N.S.**	**0.5892**	**0.3269**	**0.3488**	**0.3176**	**0.3966**	**0.3774**
2nd Season							
Control	70.18A	65.38E	65.27D	64.57E	64.21E	63.46E	61.75D
Gelatin 8%	70.18A	68.06BC	67.24B	66.85B	66.79B	66.24B	64.75B
Clove oil 1%	70.18A	68.34B	66.44C	65.42D	65.37D	65.35C	65.33A
Olive oil 1%	70.18A	67.64C	67.48B	66.18C	66.17C	66.05B	65.32A
Gelatin + Clove oil	70.18A	66.54D	66.49C	66.37C	66.24C	64.58D	63.70C
Gelatin + Olive oil	70.18A	69.13A	68.17A	67.84A	67.33A	67.15A	65.44A
LSD at 5 %	**N.S.**	**0.5393**	**0.2934**	**0.3358**	**0.3101**	**0.3618**	**0.389**

The same letter(s) in each column are statistically insignificant at 5% level.

Table-6. Effect of gelatin, clove oil and olive oil on Hue angle (hº value) of Anna apple fruits stored at 5± 1 ℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	80.29A	78.35C	76.28C	73.12E	67.19D	64.97C	62.54BC
Gelatin 8%	80.29A	78.15C	75.52D	73.87D	68.33C	64.17D	63.01B
Clove oil 1%	80.29A	78.91B	78.54A	78.12A	67.05D	63.20E	60.84D
Olive oil 1%	80.29A	79.19B	77.61B	74.38D	69.53B	66.44B	61.99C
Gelatin + Clove oil	80.29A	79.85A	77.11B	76.29B	67.54D	64.13D	60.63D
Gelatin + Olive oil	80.29A	79.80A	75.30D	75.16C	73.65A	67.48A	65.63A
LSD at 5 %	**N.S.**	**0.6423**	**0.6605**	**0.676**	**0.6469**	**0.5337**	**0.5943**
2nd Season							
Control	82.66A	80.72E	77.98D	76.41D	76.65E	72.67D	73.40D
Gelatin 8%	82.66A	81.43D	79.85C	79.20C	77.50C	72.77D	74.27C
Clove oil 1%	82.66A	83.51C	82.75B	82.17B	82.08B	81.55B	75.92B
Olive oil 1%	82.66A	84.86B	82.58B	82.40B	82.20B	81.80B	75.41B
Gelatin + Clove oil	82.66A	79.13F	75.56E	75.02E	74.36D	73.88C	72.96D
Gelatin + Olive oil	82.66A	86.42A	85.42A	85.21A	85.11A	84.13A	77.02A
LSD at 5 %	**N.S.**	**0.6494**	**0.612**	**0.5619**	**0.617**	**0.6029**	**0.5119**

The same letter(s) in each column are statistically insignificant at 5% level.

3.6. Effect of Gelatin, Clove Oil and Olive Oil Treatments on Physiological Properties of Anna Apple Fruits during Storage Periods

3.6.1. Respiration Rate (ml CO_2 /kg/h)

Results in Table 7 indicate that respiration rate was increased gradually toward the end of storage periods. All coating treatments significantly decreased respiration rate compared with untreated fruits in the both seasons. After 12 weeks of cold storage, fruits coated with gelatin plus olive oil recorded the lowest value of respiration rate (14.32 & 13.47 ml CO_2 /kg/h) in the two seasons, respectively. Whereas, the highest value of respiration rate was found in untreated fruit (15.17&14.94 ml CO_2 /kg/h) during 2020 and 2021 seasons, respectively.

Table-7. Effect of gelatin, clove oil and olive oil on respiration rate (ml CO_2 /kg/h) of Anna apple fruits stored at 5± 1 ℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	12.16A	11.98A	13.06B	13.15C	14.00B	14.95A	15.17A
Gelatin 8%	12.16A	10.36E	12.09E	13.50B	13.47D	14.01B	14.67C
Clove oil 1%	12.16A	10.82D	13.42A	13.64A	13.78C	14.86A	14.91B
Olive oil 1%	12.16A	11.72B	12.69D	13.05D	13.51D	14.00B	14.44D
Gelatin + Clove oil	12.16A	11.59C	11.97E	13.13CD	14.22A	14.90A	15.25A
Gelatin + Olive oil	12.16A	11.53C	12.84C	12.87E	13.50D	13.68C	14.32D
LSD at 5 %	**N.S.**	**0.109**	**0.1452**	**0.0944**	**0.1218**	**0.113**	**0.156**
2nd Season							
Control	12.28A	11.26D	12.93A	13.06A	13.67A	14.25A	14.94A
Gelatin 8%	12.28A	10.17F	11.94C	12.98AB	13.16D	13.37D	13.55C
Clove oil 1%	12.28A	11.71A	12.80A	13.00A	13.27C	13.46C	14.92A
Olive oil 1%	12.28A	11.55B	11.78D	12.88C	12.90E	13.13E	14.20B
Gelatin + Clove oil	12.28A	10.91E	11.48E	12.91BC	13.44B	13.76B	14.14B
Gelatin + Olive oil	12.28A	11.34C	12.55B	12.75D	12.93E	13.03F	13.47C
LSD at 5 %	**N.S.**	**0.077**	**0.1334**	**0.0844**	**0.0731**	**0.0689**	**0.1004**

The same letter(s) in each column are statistically insignificant at 5% level.

3.7. Effect of Gelatin, Clove Oil and Olive Oil Treatments on Chemical Properties of Anna Apple Fruits during Storage Periods

3.7.1. Total Soluble Solids/ Acidity Ratio (TSS/ Acid Ratio)

Results in Table 8 cleared that TSS/ acid ratio of fruits gradually increased with the advance in cold storage. Significant differences between the treatments were obtained during storage periods at the most cases in the two seasons. After 12 weeks of storage, the highest percentages of TSS/ acid ratio (82.34& 72.16) were obtained by gelatin combined with olive oil treatment in the two seasons, respectively. On the other hand, control exhibited the lowest values of TSS/ acid ratio (55.38 and 46.15) in the first and second seasons, respectively.

Table-8. Effect of gelatin, clove oil and olive oil on TSS/acid ratio of Anna apple fruits stored at 5± 1 ℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	28.46A	32.81C	37.30E	40.15E	42.24E	49.36E	55.38E
Gelatin 8%	28.46A	39.68A	40.50C	44.15C	46.31D	54.65C	71.43B
Clove oil 1%	28.46A	33.96B	37.34E	44.04C	47.63C	53.64D	59.12D
Olive oil 1%	28.46A	33.82B	38.30D	42.69D	47.04C	50.00E	55.42E
Gelatin + Clove oil	28.46A	39.35A	41.47B	47.86B	55.85B	59.92B	67.73C
Gelatin + Olive oil	28.46A	39.67A	44.54A	53.34A	67.67A	76.49A	82.34A
LSD at 5 %	**N.S.**	**0.7349**	**0.6399**	**0.6564**	**0.6871**	**0.6743**	**0.704**
2nd Season							
Control	28.85A	31.00E	32.48F	37.76E	39.59E	44.05F	46.15E
Gelatin 8%	28.85A	33.46D	35.76D	38.60D	43.59C	48.76C	54.74C
Clove oil 1%	28.85A	34.63C	38.31C	39.36C	41.70D	45.46E	51.38D
Olive oil 1%	28.85A	33.37D	34.77E	39.88C	43.00C	47.11D	54.31C
Gelatin + Clove oil	28.85A	40.26B	41.23B	45.23B	47.28B	50.12B	63.79B
Gelatin + Olive oil	28.85A	42.95A	47.20A	52.01A	54.87A	63.52A	72.16A
LSD at 5 %	**N.S.**	**0.7268**	**0.6912**	**0.6627**	**0.6806**	**0.645**	**0.7353**

The same letter(s) in each column are statistically insignificant at 5% level.

3.8. Total Phenolic Content (TPC)

As shown in Table 9, total phenolic content in Anna apple fruits decreased by the elongation of storage periods. All coating treatments significantly reduced the decreasing rate of total phenolic content in Anna apple fruits during cold storage. After 12 weeks of storage periods, the highest values of total phenolic content (1.171 and 1.221 mg GAE/100g FW) were recorded by coating with gelatin plus clove oil treatment in the both seasons, respectively. On the other hand, control treatment exhibited least values (1.051 and 1.101 mg GAE/100g FW) in the first and second seasons, respectively.

As for the effect of different coating treatments on the total phenolic content in artificially infected fruits with *pencillium expansum*, it can be noticed that total phenolic content decreased towards the end of storage periods with significant different between all treatments in the most cases (Table 10) . After 4 weeks of storage, the highest significant values of total phenolic content recorded by coating with gelatin plus clove oil and gelatin plus olive oil treatments in the both seasons. On the contrary, control recorded least values (1.260 and 1.250 mg GAE/100g FW) in the first and second seasons, respectively.

Table-9. Effect of gelatin, clove oil and olive oil on total phenolic content (mg GAE/100g FW) of Anna apple fruits stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	1.250A	1.221A	1.171A	1.151C	1.110B	1.100A	1.051B
Gelatin 8%	1.250A	1.230A	1.199A	1.179B	1.160AB	1.149A	1.091AB
Clove oil 1%	1.250A	1.231A	1.200A	1.190AB	1.161AB	1.150A	1.101AB
Olive oil 1%	1.250A	1.230A	1.200A	1.191AB	1.181A	1.161A	1.120AB
Gelatin + Clove oil	1.250A	1.247A	1.217A	1.199A	1.189A	1.168A	1.171A
Gelatin + Olive oil	1.250A	1.247A	1.220A	1.210A	1.200A	1.169A	1.159A
LSD at 5 %	**N.S.**	**0.077**	**0.0944**	**0.0826**	**0.0645**	**0.0975**	**0.8261**
2nd Season							
Control	1.310A	1.271A	1.221A	1.171D	1.161B	1.150A	1.101B
Gelatin 8%	1.310A	1.280A	1.249A	1.229C	1.210AB	1.199A	1.141AB
Clove oil 1%	1.310A	1.281A	1.250A	1.240B	1.211AB	1.200A	1.151AB
Olive oil 1%	1.310A	1.280A	1.250A	1.241B	1.231AB	1.211A	1.170AB
Gelatin + Clove oil	1.310A	1.297A	1.267A	1.249AB	1.239AB	1.218A	1.221A
Gelatin + Olive oil	1.310A	1.297A	1.270A	1.260A	1.250A	1.219A	1.209A
LSD at 5 %	**N.S.**	**0.0808**	**0.071**	**0.1062**	**0.0793**	**0.099**	**0.0895**

The same letter(s) in each column are statistically insignificant at 5% level.

Table-10. Effect of gelatin, clove oil and olive oil on total phenolic content (mg GAE/100g FW) of Anna apple fruits artificially infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)					
Treatments	**1st Season**			**2nd Season**		
	1	**2**	**4**	**1**	**2**	**4**
Control	1.350C	1.330C	1.260C	1.350B	1.330AB	1.250C
Gelatin 8%	1.400A	1.390A	1.290B	1.390A	1.350A	1.300AB
Clove oil 1%	1.371B	1.370B	1.290B	1.380A	1.287C	1.280B
Olive oil 1%	1.351C	1.328C	1.299B	1.340B	1.327B	1.300AB
Gelatin + Clove oil	1.339C	1.329C	1.320A	1.340B	1.330AB	1.310AB
Gelatin + Olive oil	1.371B	1.354B	1.330A	1.380A	1.350A	1.320A
LSD at 5 %	**0.0172**	**0.0189**	**0.0181**	**0.0244**	**0.0211**	**0.0197**

The same letter(s) in each column are statistically insignificant at 5% level.

3.9. Peroxidase (POD) Activity

As shown in Table 11, the activity of peroxidase in Anna apple fruits increased gradually throughout the storage periods. There was a significant difference between all studied treatments in its effect on activity of peroxidase during the two seasons of study as they increased the rate of peroxidase activity during storage. Fruits coated by gelatin plus olive oil treatment recorded the highest values of POD activity (0.648 and 0.759 U/g FW) in the two seasons respectively, while the control treatment had the lowest values (0.525 and 0.636 U/g FW) in the both seasons, respectively.

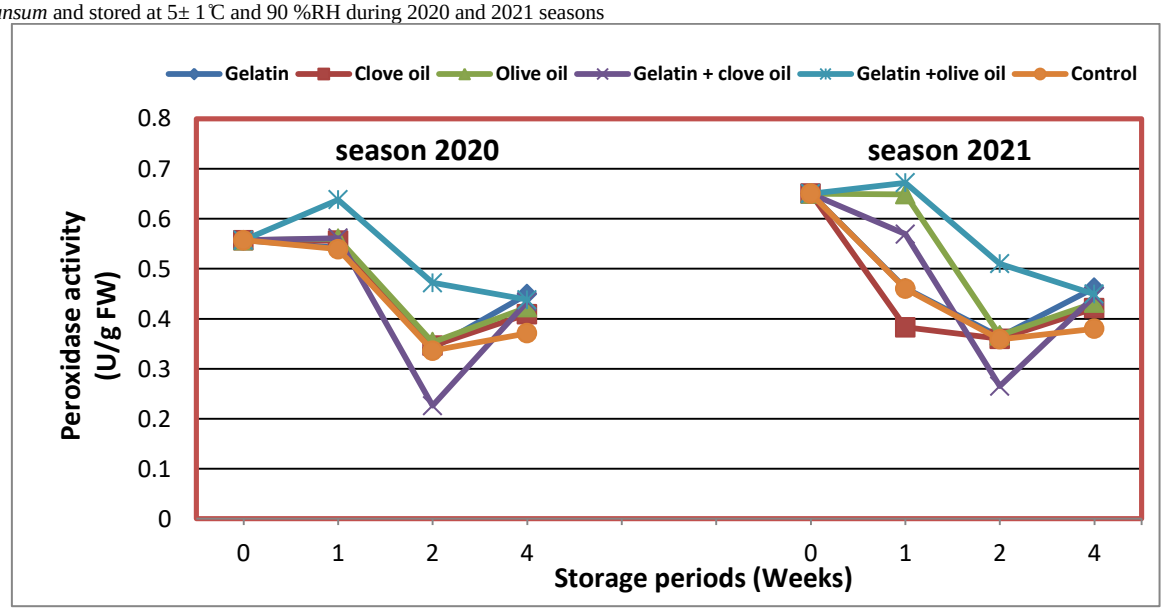
The activity of POD in Anna apple fruits artificially infected with *penicillium expansum* in treated and control fruit decreased and reached their peaks at two weeks of storage, followed by a gradual increase until the end of storage during both seasons of study (Fig 9). At the end of storage period, the highest value of POD activity was recorded in fruits coated with gelatin plus olive oil treatment (0.526 and 0.570 U/g FW) in the two seasons, respectively while the control treatment recorded the lowest value of POD activity (0.445 and 0.454 U/g FW) in 2020 and 2021 seasons, respectively.

Table-11. Effect of gelatin, clove oil and olive oil on peroxidase activity (U/g FW) of Anna apple fruits stored at 5± 1 ℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	0.323A	0.335CD	0.358D	0.370D	0.379E	0.452C	0.525C
Gelatin 8%	0.323A	0.363B	0.448A	0.460A	0.461C	0.541B	0.564B
Clove oil 1%	0.323A	0.359BC	0.382C	0.408C	0.420D	0.554B	0.555B
Olive oil 1%	0.323A	0.366B	0.422B	431B	0.559A	0.538B	0.571B
Gelatin + Clove oil	0.323A	0.331D	0.343D	0.433B	0.444CD	0.560B	0.568B
Gelatin + Olive oil	0.323A	0.448A	0.459A	0.471A	0.509B	0.637A	0.648A
LSD at 5 %	**N.S.**	**0.02436**	**0.01964**	**0.0225**	**0.02555**	**0.02724**	**0.02245**
2nd Season							
Control	0.434A	0.446BC	0.469CD	0.481D	0.490E	0.563C	0.636C
Gelatin 8%	0.434A	0.474BC	0.559AB	0.571AB	0.572C	652B	0.675B
Clove oil 1%	0.434A	0.470BC	0.493C	0.519C	0.531D	0.665B	0.666BC
Olive oil 1%	0.434A	0.477B	0.533B	0.542BC	0.620B	0.649B	0.682B
Gelatin + Clove oil	0.434A	0.442C	0.454D	0.544BC	0.555C	0.671B	0.679B
Gelatin + Olive oil	0.434A	0.559A	0.570A	0.582A	0.670A	0.748A	0.759A
LSD at 5 %	**N.S.**	**0.02984**	**0.02883**	**0.0278**	**0.02375**	**0.02496**	**0.03176**

The same letter(s) in each column are statistically insignificant at 5% level.

Fig-9. Effect of gelatin, clove oil and olive oil on peroxidase activity (U/g FW) of Anna apple fruits artificially infected with *penicillium expansum* and stored at 5± 1 ℃ and 90 %RH during 2020 and 2021 seasons



3.10. Catalase (CAT) Activity

The results in Table 12 indicated that there was a gradual increase in the catalysis activity with increasing storage periods during the two seasons. At the end of storage periods, the highest significant value of CAT activity was obtained in fruits coated with gelatin plus olive oil treatment in the first season and the second season, while the control fruits recorded the lowest significant value of CAT activity during the two seasons.

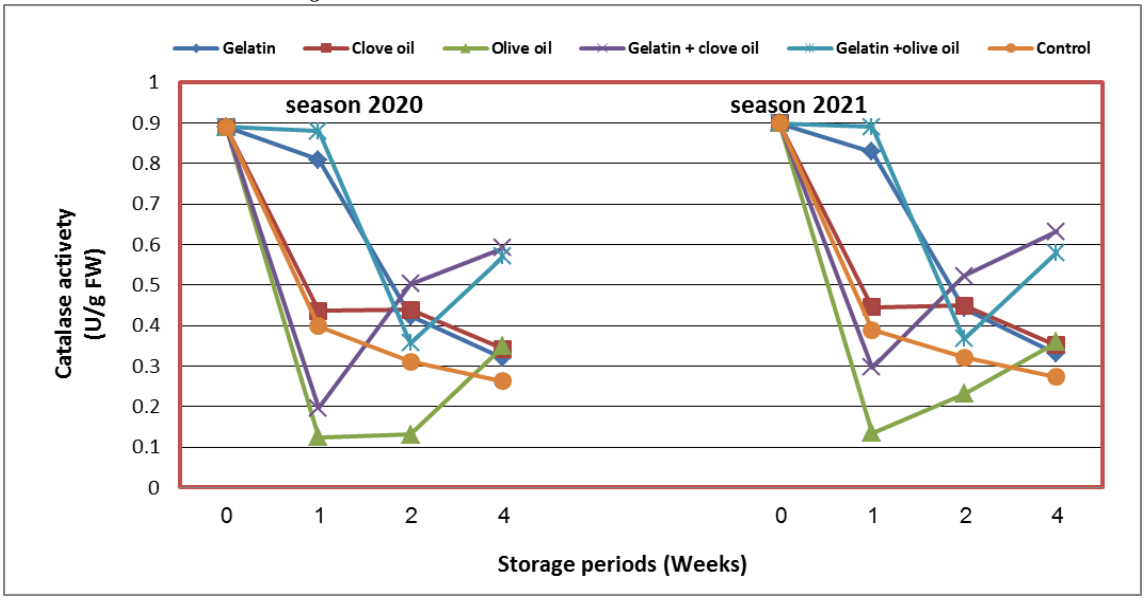
Significant effects of different coating treatments on CAT activity in Anna apple fruits artificially infected with *penicillium expansumin* were noticed in the two seasons of study (Fig 10). After 4 weeks of storage, coating treatment with gelatin plus olive oil had the highest value of catalysis activity (0.674 and 0.684 U/g FW) during the two seasons, respectively. On the other hand, control treatment recorded the lowest value of CAT activity (0.374 and 0.406 U/g FW) in both seasons, respectively.

Table-12. Effect of gelatin, clove oil and olive oil on catalase activity (U/g FW) of Anna apple fruits stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons

	Storage periods (Weeks)						
Treatments	**0**	**2**	**4**	**6**	**8**	**10**	**12**
1st Season							
Control	0.112A	0.124CD	0.147CD	0.159D	0.168E	0.241C	0.314C
Gelatin 8%	0.112A	0.152B	0.237AB	0.249AB	0.250C	0.330B	0.353B
Clove oil 1%	0.112A	0.148BC	0.171C	0.197C	0.209D	0.343B	0.344B
Olive oil 1%	0.112A	0.155B	0.211B	0.220BC	0.348A	0.327B	0.360B
Gelatin + Clove oil	0.112A	0.120D	0.132D	0.222BC	0.233CD	0.349B	0.357B
Gelatin + Olive oil	0.112A	0.237A	0.248A	0.260A	0.298B	0.426A	0.437A
LSD at 5 %	**N.S.**	**0.02669**	**0.02934**	**0.03223**	**0.02635**	**0.02788**	**0.02305**
2nd Season							
Control	0.115A	0.139C	0.185BC	0.209BC	0.227DE	0.273D	0.419D
Gelatin 8%	0.115A	0.195B	0.265A	0.289A	0.291C	0.451C	0.497BC
Clove oil 1%	0.115A	0.187B	0.133D	0.185C	0.209E	0.477BC	0.479C
Olive oil 1%	0.115A	0.201B	0.213B	0.231B	0.487A	0.445C	0.511B
Gelatin + Clove oil	0.115A	0.131C	0.155CD	0.235B	0.257D	0.489B	0.505BC
Gelatin + Olive oil	0.115A	0.265A	0.287A	0.311A	0.387B	0.643A	0.665A
LSD at 5 %	**N.S.**	**0.03053**	**0.03139**	**0.0284**	**0.03139**	**0.03067**	**0.02804**

The same letter(s) in each column are statistically insignificant at 5% level.

Fig-10. Effect of gelatin, clove oil and olive oil on catalase activity (U/g FW) of Anna apple fruits artificially infected with *penicillium expansum* and stored at 5± 1℃ and 90 %RH during 2020 and 2021 seasons



4. Discussions

This study research investigated whether coating Anna apple fruits by gelatin 8%, clove oil 1%, and olive oil 1% alone or gelatin 8 % combined with clove oil % or olive oil 1 % enhance the bio-control efficacy of against *penicillium expansum* both *in vitro* and *in vivo*. In vitro, the reduction in liner growth of *penicillium expansum* was positive correlated to the type and concentrations of oils used in study. The clove oil % had significantly high efficacy against *penicillium expansum*. This finding could be explained by the clove oil containing antioxidant properties. Al-Hashimi, *et al.* [37], found that the incorporation of clove oil at 1- 3% to the edible films presented the highest antioxidant activity and antimicrobial activity on several microorganisms. This result agrees with Vieira, *et al.* [34]. Chuying, *et al.* [41] reported that clove oil effectively control the disease incidence of blue mold decaying the fruits by reduces the growth, number, viability of *P. expansum* spores and the diameter of blue mold lesions, as well as motivating the host-defense responses and suppressing the malondialdehyde (MDA) accumulation with enhancing the activities and gene expressions of defense related enzymes. Coating Anna apple fruits with gelatin mixed with clove oil or olive oil completely inhibited blue mold incidence compared to untreated fruits in natural and artificial infection could be due to the coating mode of action in modifying the atmosphere and reducing moisture loss and surface wounding as well as reducing a variety of diseases [52]. Schirra, *et al.* [53]; Al-Rawashdeh and Karajeh [54] confirmed that postharvest treatment is very important because it can eliminate blue mold infection by removing spores from the surface of the fruit and directly acting on viability and/or stimulating fruit defense mechanisms in the outer layers of the epicarp that reduce pathogen growth and development.

The quality of fruit is determined by the combination of different physical, chemical, and physiological characteristics, a high quality fruits are firm, colored and without any defects. In this study, coating Anna apple fruits by gelatin 8 % combined with clove oil % or olive oil 1 % was more effective than coating with gelatin 8%, clove oil 1%, and olive oil 1% alone on delay changes related to ripening, such as softening, color changes, respiration rate, acidity changes and decrease in weight. This is in a line with Al-Rawashdeh and Karajeh [54] who reported that a combination of post-harvest methods could be more effective and consistent than the use of one method alone.

Fruit weight loss is mainly associated with respiration and moisture evaporation through the fruit skin Woods [55]. In this study, the loss of water in control fruit increased gradually during storage (Table 1). Weight loss in coated fruit remained at a significantly low level compared to the control fruit at all-time points examined. This may be attributed to that coating fruits by gelatin combined with oil provides a partial barrier to the movement of moisture on the surface of fruits thereby minimizing moisture loss during storage. It has been reported similar results by Nandane, *et al.* [56]; Alparslan, *et al.* [38]; Yousef, *et al.* [57]; Salehi [58].

All coating treatments showed a good result with respect to the retention of fruit firmness. These results are in a harmony with those obtained by Amal, *et al.* [59]; Aitboulahsen, *et al.* [39]; EL-Gioushy and Baiea [60]; Yousef, *et al.* [57]. After 12 weeks of storage, fruits coated with gelatin combined with olive oil showed higher firmness, which may be due to the effect of this film in delaying the ripening changes of Anna apple fruits during cold storage. Fruit coating film form a partial barrier for oxygen and carbon dioxide (low oxygen retention and high concentrations of carbon dioxide), this reduces the activities of pectin-esterase and polygalactonase enzymes that cause the decomposition of the insoluble proto-pectins in the fruit wall to the more soluble pectin acid and pectin which occurs during fruit ripening. Thus, allows the firmness of fruits and vegetables to be preserved during storage [61].

Color skin of apple fruits relates with the natural water-soluble pigments which is connected with ripening stage of the fruit and mainly used as an indicator of quality [62]. Color evaluation of Anna apple fruits affected by coating and storage periods as shown in (Tables 5, 6) were evaluated based on the lightness (L*) and hue angle (hº). At the end of storage period, the lowest and highest colour were related to control and gelain enriched with olive oil samples, respectively. Lightness of coated fruits were higher than those of control samples and changed at a much slower rate. The coated fruits were much more glossy and brighter than the control, and thus it can be recognized that coating has a beneficial effect on the reduction of color changes in apple. This is agreed with the findings of Meighani, *et al.* [63] who mentioned that the reason for higher colour in coated fruits may be explained with the reduced losses of anthocyanins. The edible film coating reduce the decrease of skin pigments, probably due to changes in the fruit internal atmosphere. The presence of CO_2 in the storage atmosphere is an important factor in preventing pigments degradation [64].

Respiration is a major factor contributing to the postharvest loss that provides energy for plant biochemical processes, and accelerates plant ripening and senescence [65]. In this study, respiration rates decreased in all applied treatments and control fruit after a week of storage, and then gradually increased during storage periods, reaching a maximum at 12 weeks of storage. The results indicated that coating Anna fruits with gelatin and olive oil is more effective than other treatments in inhibiting respiration rates during 12 weeks of storage at 5±1°C. This result are in agreement with previous reports [52, 59, 60] suggesting the beneficial effects of coating gelatin film with oils in reducing respiration rates through creating a gas barrier around the fruits and thus creating a modified atmosphere around them and slows down respiration during cold storage. A high respiration rate is generally detrimental to the maintenance of high fruit quality. Decreasing the respiration rate leads to extending the shelf life and retaining the produce quality [66].

The total soluble solid (TSS) increased slightly during storage probably due to the water loss, activity of hydrolytic enzymes, or the decrease in respiration rate and conversion of sugars in CO_2 and H_2O during the storage period and the hydrolysis of fruit starch is completed [67]. Titratable acidity (TA) estimates the organic acid content of fruit which generally decreases during postharvest storage due to the use of organic acids as substrates for respiratory metabolism [68]. In general, TA decreased and TSS increased during storage for both control and coated fruits, which may be due to the influence of these values of acid and sugars concentrations by fruit weight loss factor some extent during storage, which affects on TSS/TA ratio. These results are in agree with Naweto, *et al.* [4]; Eleryan and EL-Metwally [52]. After 12 weeks of storage, the highest and lowest amounts of TSS/ TA ratio were related to gelatin combined with olive oil and control samples, respectively. The increase in TSS/TA ratio during storage was attributed to higher decrease in TA in comparison with the TSS [63].

All fruit samples showed a decrease in phenolic compounds concentration until the end of storage periods in naturally and artificially infected fruits during the two seasons of study. This might be due to the activity of peroxidase and polyphenol oxidase enzymes [69-71]. Fruits treated with edible coatings had a higher amount of total phenolic, indicating the positive effect of gelatin and clove oil or olive oil coating as antioxidants and inhibitors of phenolic degradation during storage. Our findings are in agreement with those reported by Gülçin, *et al.* [72], who reported that coated fruit exhibited a lower rate of decrease in total phenolic content values compared with uncoated fruit, probably due to lower permeability of oxygen and thereby lower activity of enzymes [73]. Previous studies also, demonstrated that the loss of phenolic compounds could be restricted by coating in various fruits such as strawberries coated with gelatin incorporated with *Mentha pulegium* essential oil [39] and Samany date palm coated with gelatin in combination with lemon grass oil [60].

The defense-related enzymes such as CAT and POD are very important enzymes in protecting the cell from oxidative damage by reactive oxygen species, through preventing build-up of excessive H_2O_2 [74]. Oxidative stress from excess oxygen species (ROS) has been associated with the appearance of membrane in fruits [75]. The toxicity of ROS is due to their reactions with numerous cell components causing a cascade of oxidative reactions and the

consequent inactivation of enzymes including SOD, CAT and POD [76], which is thought to be a major mechanism of resistance to senescence [77, 78]. Our results suggest that the activities of these enzymes, to some extent, were induced by treatments. These results are in accordance with previous studies, which reported that coating treatments may be an effective method to induce the activities of CAT and POD to enhance the oxidation resistance of strawberries fruit [79]. It worth noting that, the resistance to blue mold of Anna apple fruits caused by *P. italicum* in the natural and artificial infections exhibited in the present study was accompanied by a prominent activation of the two enzyme activities mentioned above in the coated fruit. This is consistent with previous reports regarding the gene expressions of defense-related enzymes in fruits subjected to fungal infections such as 'Valencia' orange fruit [80] and 'Shogun' mandarins coated with Aloe Vera gel [81], in apples coated with thyme and savory essential oil [82], as well as in citrus fruit coated with clove essential oil [41], who reported that coating treatments to be effective in controlling various postharvest fungal diseases by activating defense-related enzymes that have important functions in plant defense responses to pathogen attacks and other physiological stresses [83] including chilling, salinity, drought, heavy metals, etc.

5. Conclusions

In conclusion, the results suggested that postharvest physiological and pathological responses, quality parameters and bioactive compounds of Anna apple fruits during cold storage, are affected by coating treatments. It also suggested that clove oil was highly effective in inhibiting the mycelial growth of *penicillium expansum* compared to olive oil *In vitro* test. Coating fruits by gelatin mixed with both oils was significantly more effective than other treatments in controlling blue mold disease incidence and severity. The result also showed that coating treatments significantly maintained in reducing fruits weight loss, color changes and respiration rate, and enhancing fruit firmness, TSS/ acid ratio, total phenolic and defense-related enzymes activities (CAT and POD) in naturally and artificially infected fruits with *pencillium expansum* during storage especially gelatin combined with clove oil or gelatin combined with olive oil.

Conflict of Interest

The Authors Declared That Present Study Was Performed in Absence of Any Conflict of Interest

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