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Effect of Some Preharvest Treatments on Growth, Fruit Quality and Storability of Strawberry (*Fragaria × ananassa* Duch.) cv. "Rubygem" Grown under Plastic House Conditions

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Abstract

This research was conducted in a greenhouse at a private orchard in the village of Bawamarda in the Iraqi province of Sulaimani to determine the effects of spraying the leaves of strawberry plants with 5% pomegranate peel extracts (PPE), 5% rosemary leaf extracts (RLE), 3% potassium metabisulfite (KMS), 3 mmol·L^{-1} salicylic acid (SA), with their combinations and 0.15% pristine fungicide on strawberry growth and yield. In addition, the qualitative changes in fruits during storage in the cold room at 2°C with a relative humidity of 85–95% were measured. Thereafter, by traditional and molecular methods, the types of fungi on strawberry fruits were identified. Spraying with 5% PPE + 3mmol·L^{-1} SA caused a significant increase in the number of leaves·plant^{-1}, chlorophyll intensity in the leaf, and yield using 5% RLE + 3% KMS (T10) registered the highest records and maintained berry firmness before and after storage of strawberry fruit compared to the other treatments. The maximum contents of total sugar after storage and ascorbic acid before storage were also detected with (T10). Spraying strawberry plants with 5% PPE + 5% RLE significantly increased TSS before storage, ascorbic acid after storage, and TTA% before and after storage of strawberry fruits. The lowest value of 5.29% was obtained from foliar application of pristine on fresh weight loss of strawberry fruits, while the highest value of this trait was obtained under untreated conditions 9.94%. After storage, the only isolate *Botrytis cinerea* Pers (1794) was found on the strawberry fruits using morphologically and molecularly.

Keywords: plant extracts, quality of fruits, cold storage, fungi.

1. Introduction

Strawberry (*Fragaria × ananassa* Duch.) is a herbaceous perennial plant having a soft and delicious exotic taste that belongs to the family Rosaceae. It is growing in the most arable areas of both Southern and Northern Hemisphere [1]. Strawberries are excellent source of vitamins, potassium, fiber, sugars, and widely appreciated for their bright red color, juicy texture, sweetness. Besides their attractive color and taste [2], as compared to other berry fruits, it is also a good source of ascorbic acid and other antioxidant compounds such as phenolics and flavonoids [3]. In recent *years,* strawberry is becoming the favored food in the diets of millions of people worldwide and cultivated at least in 74 countries, and growing areas increase year after year.

The area occupied by the strawberry in the world is about 395,844 hectares and the total

annual production is estimated as 922, 3815 tons in 2017 [4].

Strawberry is a non-climacteric fruit, one of the most perishable fruits, although have a short postharvest life with rapid spoilage, reflecting high susceptibility to mechanical injury, excessive texture softening, physiological disorders and infection through several pathogens during transport, storage and processing [5].

The pre and postharvest practices are aimed at slowing the respiration rate and water loss, maintaining fruit firmness, and minimizing the growth of pathogens.

Subsequently, the development and use of alternative postharvest control options, involving biological agents or natural plant extracts, have become important since it is perceived as being environmentally safer and more acceptable to the general public health [6]. Therefore, to reduce damage and losses in strawberry production, various conservation techniques have been conducted, such as cooling, natural substances, edible covering and nutrition, which are increasingly being used as alternatives [7].

Fungal disease after harvest is one of the main factors limiting the marketing and shelf life of strawberry fruits, which also results in severe economic losses worldwide [8].

The reason for its relatively short harvest period requires the producer to immediately sell its production, which clearly compromises its reduced price due to its large volume sales [9]. Nowadays, the post-harvest life of strawberries may be prolonged by natural substances, such as rosemary extract (*Rosmarinus officinalis* L.).

Consequently, it is edible, inexpensive, environmentally friendly, antioxidant, antimicrobial, and anti-inflammatory, and has health benefits. It may extend the shelf life of strawberries by reducing gas exchange, loss of moisture, respiration rate and oxidative reaction by providing a barrier to the hazards [10, 11]. Also, pomegranate peel which has medicinal qualities such as antibacterial, anti-fungal, antiviral, and antioxidant, have been investigated in recent years [12].

Optimum storage conditions for strawberry fruit are (0°C and 85-90% relative humidity - RH). In such conditions, strawberries may be stored for 7 to 10 days [13].

There are a few scientific studies on the strawberry fruits about the storage and decay control in Kurdistan Region-Iraq.

The important aims of this study are to investigate the effects of foliar spraying, with potassium metabisulfite (KMS), salicylic acid (SA) and two plant extracts, on vegetative growth, yield, quality, prolonging storability of strawberry fruits, to observe the qualitative changes in fruits during storage and isolation and identification of associated fungi during storage by morphological and molecular methods.

2. Material and Method

Location of the field experiment. This study was conducted during the growing season 2018 - 2019 in a plastic house located in Bawamarda, Sulaimani Governorate, Kurdistan Region, Iraq at 794 m above sea level with latitude 35°35 46 north and longitude 45°17 47 east.

Plastic house trails. A plastic house with a length of 45 m, width of 9 m and a height of 3 m, with a total area of 405 m^2, was used for this study. It was divided into three blocks, with three replicates; each replicate was divided into twelve plots, resulting in a total number of 36 plots. The area of each experimental unit was 4 m^2.

Each experimental plot included 20 rows of 2 m length and 2 m width with 0.40 m between rows, and 0.2 m within rows between individual plants. The total number of plants by each plot was 40. Rubygem strawberry (*Fragaria* x *ananassa* Duch.) cultivar was used in this experiment as shown in Fig. 1. Plants were planted in October 28, 2018, and the soil was covered with black polyvinyl chloride (PVC) to prevent weed growing's.

The field was irrigated with a drip system as needed, which was approximately at every 2 days. Cultural practices such as hoeing, weed control, pest control, and chemical fertilization were carried out annually for all treatments during the fall and spring seasons.

This experiment included twelve treatments on strawberry plants under plastic house conditions (Table 1).

Spray materials were used before flowering on March 14. Fruits were harvested early in the morning (4:30 - 5:00) every 2-3 days, from April 20 to June 15, 2019. Fruits showing surface defects, and damage were discarded.

The cold chamber sterilized by 3 $mL \cdot L^{-1}$ 36% formalin and 2% sodium hypochlorite.

After harvesting, fruits were placed in shad at room temperature for short period before transferring to the cold room at the Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani.

All treated fruits were stored for 20 days in a cold room at 2 °C and 85 - 90% RH, with continuous monitoring.

Figure 1. Strawberry plants cv. Rubygem grew under the greenhouse (Original)

Plant materials and chemicals used in the study

Extract preparation from rosemary leaves and pomegranate peel. The rosemary leaves, and pomegranate Salakhani Var. were collected from Sulaimani public garden at Sulaimani, and pomegranate fruit from Halabja governorates, from December 2018 to January 2019. The samples were washed thoroughly 2-3 times with running tap water to eliminate the excess of impurities, then disinfected by immersion in a 2% sodium hypochlorite solution for 30 seconds, and rinsed twice with distilled water to remove residual hypochlorite [14].

Afterwards they were dried in the oven at 40°C for 48 hours. The operation was repeated until a constant weight was obtained. The dried material was stored in an airtight glass container for further use, and stored in plastic containers under dry condition for extraction [15]. Preparation of samples were done by aqueous extraction [16].

The reagents used in the study are: Salicylic acid (99.9%, Alfa Aesar Company, Germany), and potassium metabisulphite (99.9%, Brupaks Company, England). Pristine fungicide 38%WG was used. It is a large spectrum fungicide with active components Boscalid 25.2% and Pyraclostrobin 12.8%, produced by BASF Corporation (the United States and Italy), with Registration Number of the Ministry of Agriculture Iraq (1421), Supplement and Distribution Dabana Company for Limited Modern Agriculture. **The treatments used in the experiment are presented in Table 1.**

Characteristics

1 - Vegetative growth and yield parameters**.** The vegetative growth was performed under plastic house condition, The number of leaves·plant^{-1} was recorded for five plants from 40 plants per plot [17]. The leaf area (cm^2) was measured using a Placom Digital Planimeter (Kolzumi Placom Planimeter Inc., Model KP-90/Japan) [18]. The leaf chlorophyll intensity (SPAD units) was measured using a hand-held SPAD-502 (Portable chlorophyll meter) [19], and fruit yield g·plant^{-1} was performed by harvesting fruits was after they reached the ripening stage, and then the total fruit yields were taken from each plot divided by an average number of plants per experimental unit.

2 - Quantitative and qualitative parameters of fruits. The qualitative and quantitative parameters were measured in two different periods (at the time of harvest and after storage for 20 days in a cold room) and they are represented by fruit weight (g), and fruit firmness (Newton), which was determined by a Texture Analyzer (TA) (Brookfield Engineering Labs INC./U.S.A) as described by Horwitz (2002) for strawberry fruits [20].

3 - Chemical parameters of fruits. The chemical parameters were measured twice at harvest time and after storage for 20 days in a cold room included. The following parameters were measured: Total Titratable Acidity (TTA%) was calculated by titration with NaOH and phenolphthalein index [21], Total Soluble Solids (TSS%) were measured using a Portable Hand Refractometer Erma Japan [21], the pH of the strawberry juice samples was determined using a microprocessor pH meter (model-pH 211-HNA Com. Italy) [22], total sugars (%) according to Joslyn (1970) [23]. Ascorbic acid (mg/100g fresh weight) according to Elgailani et al. (2017) [24], and anthocyanins (mg/100g fresh weight) according to Ranganna (2011) [25].

4 - After storage the fruits in cold chamber for 20 days, three critical parameters

including; fresh weight loss (%) [26], disease incidence% and disease severity% [27] were recorded.

The attack severity was recorded according to an empirical scale with six degrees (0: No symptoms on the fruits, 1: 1–20%, Fruit surface infected%, 2: 21–40% Fruit surface infected%, 3: 41–60% Fruit surface infected%, 4: 61–80% Fruit surface infected%, 5: >81% Fruit surface infected% as described by [28].

Isolation of fungus from decaying naturally infected strawberry fruits of "Rubygem" cultivar during the storage period was performed by directly plating the inner fallen tissue [29].

Table 1. The treatments used in the experiment

No.	Treatments
T1	Control (distilled water)
T2	Pomegranate peel extracts (5%)
T3	Rosemary leaf extracts (5%)
T4	Salicylic acid (3 mmol·L^{-1}) (SA)
T5	Potassium metabisulphite (3%) (KMS)
T6	Pomegranate peel extracts (5%) + rosemary plant extracts (5%)
T7	Pomegranate peel extracts (5%) + salicylic acid (3 mmol·L^{-1})
T8	Pomegranate peel extracts (5%) + potassium metabisulphite (3%)
T9	Rosemary leaf extracts (5%) + salicylic acid (3 mmol·L^{-1}).
T10	Rosemary leaf extracts (5%) + potassium metabisulphite (3%)
T11	Salicylic acid (3 mmol·L^{-1}) + potassium metabisulphite (3%)
T12	Pristine fungicide (0.15%)

Identification of the isolated fungi. The fungi were identified by using two methods: morphologically and molecularly. Morphological characteristics of *Botrytis cinerea* Pers (1794) depend on the colony's morphology, color, and growth habits, in addition to the appearance of the colony. The pure, isolated fungi were identified and compared with the documented key provided by Elad et al. (2004) [30].

To examine hyphal growth, Motic microscope Model 1802 LED, and Microscope Digital Camera (AmScope, 18MP USB 3.0, Model MU1803) for images captures, were used.

Molecular identification**.** In order to confirm the accuracy of morphological identification, all isolated species were subjected to molecular analysis with fungus-specific universal primers ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [31]. Sodium Dodecyl Sulfate (SDS) method was used for genomic DNA extraction of fungi, following the methodology previously described by [32]. The genomic DNA of the fungi were used for PCR amplification. All amplification reactions were carried out in volumes of 50 µl containing 20 µl master mix, 4 µl of each pimer, 10 µl DNA template, and 12 µL of nuclease free water. PCR was carried out using the following condition: initial denaturation at 94°C for 5 min; 37 cycles of denaturation (94°C for 10 min., 1 cycle), 37 cycles at a melting temperature of (95°C for 1 min.), annealing (55°C for 1 min.), and extension (72°C for 2 min.); and a final extension step at (72°C for 10 min., 1 cycle) [33]. The quality and quantity of DNA extract was checked by Nanodrop spectrophotometer (Nano Plus/ Maan LB., Sweden). PCR products were detected in 1% agarose stained with ethidium bromide gels in 1×TAE buffer, the electrodes of the chamber were connected to the power supply and run was achieved at 84 V for 90 min (RUNVIEW-S, CS CLEAVER Scientific Ltd/ the UK), finally, the bands in the gel were visualized by the gel documentation system (ENDUROTM GDS Touch, LABNET). PCR amplicons were purified by the Addbio Qucik Gel Extraction Kit and sequenced by Sanger sequencing method performed by Macrogen (South Korea).

The obtained nucleotide sequences were trimmed using SnapGene ® software (GSL Biotech, Version 3.2.1, available at snapgene.com), and compared with those already stored in the National Center for Biotechnology and Information (NCBI) sequence database, using of the Basic Local Alignment Search Tool (BLAST).

Experimental design and statistical analysis. The experimental design was a randomly complete block design (RCBD) with

three replicates. The analysis of variation (ANOVA) and Duncan's multiple range test (P≤ 0.05) were used to compare the means [34]. Data were analyzed using XLSTAT [35].

3. Results and Discusions

Effect of some treatments on vegetative growth and yield of strawberry grown under plastic house conditions. Results shown in Table 2 indicate that the highest value of leaf area 190.53 cm^2 was obtained in control (T1) treatment, while the lowest value 138.91 cm^2 was shown when pomegranate peel extracts 5% + 3% KMS (T8) were used. The same table shows that the highest number of leaves·$plant^{-1}$, fruit yield (g·$plant^{-1}$), and leaf chlorophyll intensity (SPAD unit) of cv. Rubygem strawberry, was noticed when the pomegranate peel extracts 5%+ salicylic acid 3 mmol·L^{-1} was used, Thus in T7 are reported the highest mean values 27.83 leaves·$plant^{-1}$, 590.74 g·$plant^{-1}$, and 57.27 SPAD units, respectively. The lowest values were 19.75 leaves·$plant^{-1}$, which corresponds to the rosemary plant extracts 5%+ salicylic acid 3 mmol·L^{-1} (T9) treatments, and 490.36 g·$plant^{-1}$ and 47.77 SPAD units, which corresponds to the pristine fungicide 0.15% (T12), successively (Table 2).

Table 2 Effect of some treatments on growth and yield of strawberry grown under plastic house conditions

Treatments †	Leaf area cm^2	Number of leaves·$plant^{-1}$	Leaf chlorophyll intensity SPAD unit	Yield g·$plant^{-1}$
T1	190.53a *	23.75abc	56.20ab	520.13ab
T2	146.7b	22.16bc	55.26abc	550.09ab
T3	164.10ab	26.83a	47.80e	500.72ab
T4	146.47b	21.66bc	51.29d	560.95ab
T5	143.91b	24.83ab	53.00bcd	570.37ab
T6	154.22b	24.50ab	52.39cd	540.80ab
T7	140.13b	27.83a	57.27a	590.74a
T8	138.91b	23.75abc	55.80abc	560.11ab
T9	142.95b	19.750c	54.39abcd	500.93ab
T10	149.21b	27.00a	56.44ab	590.43a
T11	148.71b	25.00ab	53.77abcd	560.40ab
T12	162.46ab	23.50abc	47.77 e	490.36b

† T1: Control (Distill Water), T2: Pomegranate peel extracts (5%), T3: Rosemary plant extracts (5%), T4: Salicylic acid (3 mmol·L^{-1}), T5: Potassium Metabisulphite (3%), T6: Pomegranate peel extracts (5%) + Rosemary plant extracts (5%), T7: Pomegranate peel extracts (5%) + Salicylic acid (3 mmol·L^{-1}), T8: Pomegranate peel extracts (5%) + Potassium Metabisulphite (3%), T9: Rosemary plant extracts (5%) + Salicylic acid (3 mmol·$L^{-1\ 1}$), T10: Rosemary plant extracts (5%) + Potassium Metabisulphite (3%), T11: Salicylic acid (3 mmol·L^{-1}) + Potassium Metabisulphite (3%), and T12: Pristine Fungicide 0.15%
*In a column, means followed by the same letter are not significantly different according to Duncan's multiple range tests at (p≤ 0.05).

Leaf area gives a fairly good idea of the photosynthetic capacity of the plant. Positive association was found between leaf area and total yield of fruits, meaning that with leaf area increased the sugar content and TSS of the fruits [36]. Recent reports also suggest that SA has a role to play in photosynthesis as it influences chloroplast and leaf carotenoid, chlorophyll content, stomatal closure, and enzymatic activities. Even under the availability of low concentrations of SA, photosynthesis and carbon dioxide assimilation were improved dramatically [37]. Furthermore, when plants were sprayed with lower SA concentrations, fruit yields improved significantly [38].

Our results are in accordance with the findings in which the availability of used plant extracts and SA improved the most studied parameters as displayed in Table 2.

Effect of different treatments on some physical properties of strawberry fruits grown under plastic house conditions and stored for 20 days in cold room. Before storage under control conditions (T1), the lowest value of fresh weight was of 13.69 g while in the same storage conditions, this value increased by using the different treatments in which the maximum fresh weight was of 17.70 g (T4) followed by the values of 17.48 g in T9 and 17.24 g respectively in T6 (Table 3). After storage, as it was expected in all treatments conditions the fresh weight decreased compared to the weight before storage. The same phenomena were observed in control conditions after storage for fresh weight, as the lowest value 12.43 g again was observed in T1, while the highest values in T9 (16.14g), T4 (15.90g) and T11 (15.86 g). No considerable change of firmness was detected between control and T4 conditions

before storage as same value were observed 4.90 Newton, while the T10 conditions improved the firmness under these particular conditions as the highest value were recorded was of 6.03 N·(T10) followed by 5.77 N in T7 conditions. After storing the materials under cold conditions, T10 treatments achieved the superiority in maintaining the firmness compared with other treatments.

For this treatment (T10) the maximum firmness value was eported, 4.27 N respectively (Table 3).

Table 3. The effect of some treatments on some physical characteristics of strawberry fruits grown under plastic house condition (before storage), and stored at 2°C and 85-90% RH for 20 days (after storage)

Treatments †	Fresh weight (g)		Firmness (Newton)	
	Before storage	After storage	Before storage	After storage
T1	13.69e *	12.43e	4.90bc *	4.16ab
T2	14.83cde	13.77cde	4.68c	3.52ab
T3	15.08cde	13.83bcde	5.09bc	4.10ab
T4	17.70a	15.90a	4.90bc	3.94ab
T5	14.30de	13.25de	5.32abc	3.74ab
T6	17.24ab	15.76a	5.02bc	3.44ab
T7	16.43abc	15.33ab	5.77ab	3.94ab
T8	15.26bcde	14.58abcd	5.35abc	4.11ab
T9	17.48a	16.14a	4.73c	3.39b
T10	16.37abc	15.27abc	6.03a	4.27a
T11	16.69abc	15.86a	5.30abc	3.80ab
T12	16.18abcd	15.53a	5.05bc	3.77ab

*In a column, means followed by the same letter are not significantly different according to Duncan's multiple range tests at ($p \leq 0.05$).

Fruit firmness is one of the most important traits after harvest for strawberry growers, consignors, and consumers. Firmer fruits have a greater potential to withstand transport and are less likely to break down [39]. The softening is one of the most characteristic developmental events of the fruit ripening process. In soft fruits, such as strawberries, the melting texture characteristic of ripe fruits is highly appreciated by consumers. Nevertheless, it poses a major problem for strawberry producers, determining the short postharvest shelf life of this fruit and limiting its storage and postharvest transport [40]. Softening of the fruits during post-harvest storage may be related to changes in the pectic materials, cementing cell walls. The insoluble protopectin may break down during storage and it will get converted into soluble proteins [41]. These results are consistent with those obtained by Gonchikari (2020) who found that leafy use of SA 200 ppm + potassium silicate 0.2% was found to improve fruit quality and firmness during the storage period of mango fruit [42].

Effect of some treatments on some chemical properties of strawberry fruits grown under plastic house conditions and stored for 20 days in cold room. The data relating to the total soluble solids and the total sugar of strawberry fruits affected by various treatments of plant extracts with chemicals are given in Table 4. The data indicated that the TSS and total sugar increased significantly in all treatments in which fruits were stored for 20 days, compared to the harvest time when TSS% was between 7.40 (T8) and 9.10 (T6). The total sugar range between 3.31% (T9) and 5.26% (T2), but the maximum percentage of TSS and total sugar values after storage were 12.45% (T8) and 6.07% (T10), respectively, while the minimum value of 10.08% (T7) and 4.54% (T6), respectively. Increased TSS% and total sugars % may be due to the treatments applied to T8 and T10 experimental variants. The results presented in Table 4 show that the foliar application of various treatments on strawberries significantly reduced the quality of fruits in terms of total titratable acidity (TTA%) and pH, compared to the harvesting time in which TTA% and pH varied between 0.70% and 0.92% and 3.11 and 3.74, respectively. According to the results of our study, T8 and T1 treatments after storage treatment significantly improved the quality parameters of the fruit, with the highest values of TTA (0.93%) and pH (4.44), respectively. The smallest values of the same parameters are 0.76% for TTA and 4.19 for pH, recorded in T4. Also, the data presented in Table 4 reveals that the leaf spray with plant extracts and chemicals had significantly reduced the ascorbic acid, and anthocyanins of the strawberry fruit during 20 days of storage. Among

the various treatments, the maximum content of ascorbic acid of 43.53 mg/100g fresh weight is reported in T6, and anthocyanins of 53.94 mg/100g fresh weight is reported in T9, respectively, while the minimum contents of ascorbic acid of 30.98 mg/100g fresh weight (FW) is reported in T11, and anthocyanins of 24.95 mg/100g fresh weight) is reported in T2.

This study showed a mean ascorbic acid content reported at harvest, which varies from 36.47 mg/100g to 66.18 mg/100g as shown in Table 4. This study also showed an average anthocyanins content at harvest, which vary from 38.57 mg/100g fresh weight to 58.93 mg/100g fresh weight as shown in Table 4. In the end of storage, both ascorbic acid and anthocyanins values decreased as mentioned formerly.

The increment in total soluble solid up to 20 days could be caused by the conversion of reserved starch and or other polysaccharides to the soluble form of sugar.

The values of TSS obtained as consequence of the different treatments are within the range set forth by Roudeillac and Trajkovski [43] who give values of between 7 and 12% as acceptable for strawberries. Acidity is an useful organoleptic quality when determining strawberry flavor, and sweetness intensity, and it is the primary factor contributing to an overall liking for the consumer [44]. TTA is directly related to the concentration of dominant organic acid that might be utilized slowly in respiration, which is an important parameter in maintaining fruit quality [45]. Strawberry fruits are rich in phenolic compounds such as anthocyanins [46]. Strawberry fruit storage at the ambient temperature caused a significant loss of the anthocyanin content, and loss of anthocyanins can be due to their degradation in plant tissues by enzyme systems such as glycosidases, polyphenol oxidases, and peroxidases [47].

Ascorbic acid is rather unstable and therefore it is also an indicator of the freshness of the fruit [48]. Among the vitamins, ascorbic acid is less stable and very sensitive to degradation during the storage and processing, after harvest [49]. Results similar to those reported in our study were found by Khan et al. [50] with strawberry storage in air at 2^{o}C. Pérez et al. [51] and Sanz et al. [49] who studied strawberry cultivars ”Dorit” and ”Selva” had mentioned the loss of ascorbic acid at the end of the storage, in terns in which ”Dorit” showed a greater decrease compared to ”Selva”.

Effect of some treatments on fresh weight loss, %. Fig. 1 shows fresh weight loss (%) of strawberry fruits which were stored for 20 days in a cold room. There were significant changes in fruit weight loss. The highest percentage of 9.64% weight loss was recorded in T1, while the lowest of 5.29% is reported in T12. Weight loss in fruits and vegetables during storage is due to water exchange between the inner and outer atmospheres as the rate of transpiration is accelerated by cellular degradation [52]. The results reported in our study are similar to those of Martínez-Romero *et al*. [53] who found that the highest weight loss was achieved in the end of the storage period. They assume that the higher microbial destruction of control strawberries, not treated with fungicides, would lead to tissue disruption and be responsible for the reported higher weight loss. In this respect, they also assume that the physiological role of fungicide in declining metabolism in strawberry tissue could be suggested. Chemical fungicides are the most commonly used means to control gray mold on strawberry fruits, but the repeated use of synthetic fungicides can develop fungal resistance and be harmful to consumer health. However, the residue and toxicity concerns may limit their use [54]. Furthermore, weight loss of approximately 10% was observed after 21 days at 0^{o}C [55], meanwhile Sanford *et al*. [56] reported 5.3% and 7.6% weight loss mean values when blueberries were stored for 14 days at 0^{o}C, or 5^{o}C, respectively. Gonchikari *et al*. [42] found that the foliar spray with salicylic acid + potassium silicate improves fruit quality and reduces physiological weight loss during the storage period of *Mangifera indica* L. fruit cv. Alphonsoin. The application of rosemary plant extract at highest concentration of 6% decreased the fresh weight loss (%) and decay (%) on strawberry fruits cv. Rubygem stored for 30 days in a cold room [57].

Effect of some treatments on incidence (%) and severity (%) of disease produced by *Botrytis cinerea* Pers (1794) on stored strawberry fruits. The impacts of some treatments on disease severity (%) and disease incidence (%) of gray mold produced by *Botrytis cinerea* Pers (1794) on strawberry fruits cv. Rubygem stored at 2 °C and 85-90% RH for 20 days were shown in Fig. 2.

According to the analysis, the treatment T9 caused the maximum reductions of appearing the studied isolate on strawberry fruits as the disease incidence was only 1.72 % followed by the treatments T8 and T12 with values of 1.78% and 1.83% respectively. The fungicide used in T12 had great influence on the severity of disease, in this case only 0.71% incidence was reported.

Table 4. Effect of some treatments on some chemicals properties of strawberries cultivated under plastic house (before storage), and stored at 2°C and 85-90% RH for 20 days (after storage)

Treatments †	TSS%		Total sugar%		TTA %		pH		Ascorbic acid mg/100 g fresh weight		Anthocyanin mg/100 g fresh weight	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
T1	9.03a	10.6cd	3.37c	5.34ab	0.76ab	0.83ab	3.47ab	4.45a	44.1efg	38.6abc	44.04de*	34.8cd
T2	7.83ab	10.8cd	5.26a	5.31ab	0.78ab	0.81ab	3.11b	4.40ab	57.2abc	41.7a	38.5e	24.9e
T3	8.43ab	10.6cd	5.16a	5.24ab	0.72b	0.86ab	3.16b	4.24cd	62.0ab	41.7a	50.0abcd	35.3cd
T4	8.97a	10.5cd	4.35ab	5.46ab	0.79ab	0.76b	3.37ab	4.18d	36.4g	31.7c	58.9a	42.3bc
T5	8.60ab	11.0bc	3.55bc	5.32ab	0.86ab	0.91a	3.58a	4.26cd	55.2bcd	38.4abc	51.3abcd	28.0de
T6	9.10a	11.8ab	4.84a	4.54b	0.86ab	0.91a	3.36ab	4.25cd	49.4cdef	43.5a	48.9bcd	42.0bc
T7	8.80ab	10.0d	3.51bc	4.60b	0.72 b	0.84ab	3.34ab	4.22cd	53.7bcde	41.9a	56.0ab	44.6b
T8	7.40b	12.4a	3.38c	5.39ab	0.92a	0.93a	3.13b	4.30bc	46.0defg	37.8abc	43.9de	40.8bc
T9	8.70ab	10.5cd	3.31c	5.63ab	0.83ab	0.84ab	3.14b	4.33bc	58.5abc	40.5ab	54.9abc	53.9a
T10	8.83a	10.7cd	4.57a	6.07a	0.75ab	0.88ab	3.37ab	4.27cd	66.1a	37.6abc	48.6bcd	40.61 bc
T11	8.27ab	10.5cd	5.07a	5.25ab	0.84ab	0.85ab	3.33ab	4.24cd	41.1fg	30.9c	44.9de	25.8e
T12	8.80ab	11.7ab	5.13a	5.33ab	0.70b	0.90a	3.74a	4.30bc	52.1bcde	32.3bc	45.4cde	36.02cd

*In a row, means followed by the same letter are not significantly different according to Duncan's multiple range tests at (p≤ 0.05).

Beside using the fungicide, T11 also caused lowering the severity of the disease (90 %). These results are in line with those obtained by Sunil [58], who carried out pre-and post-harvest applications of SA (treatment of young strawberry plants, then during the fruit development stage and later treatment of harvested fruits), effectively controlled decay and increased fruit durability against having disease.

The application of salicylate derivatives induced resistance to *Botrytis cinerea* Pers (1794) spoilage. A preharvest treatment with SA decreased postharvest disease caused by *Botrytis cinerea* Pers (1794), this probably due to

increasing levels of phenolic compounds and the antioxidant activity [39]. On cherry fruits, pomegranate peel extracts, foliar administered, reduced the infection with *Botrytis cinerea* Pers (1794), by 95.6% while in our investigation the administration of T8, let to the value of 1.78 % indicating the effectiveness of using this treatment [59].

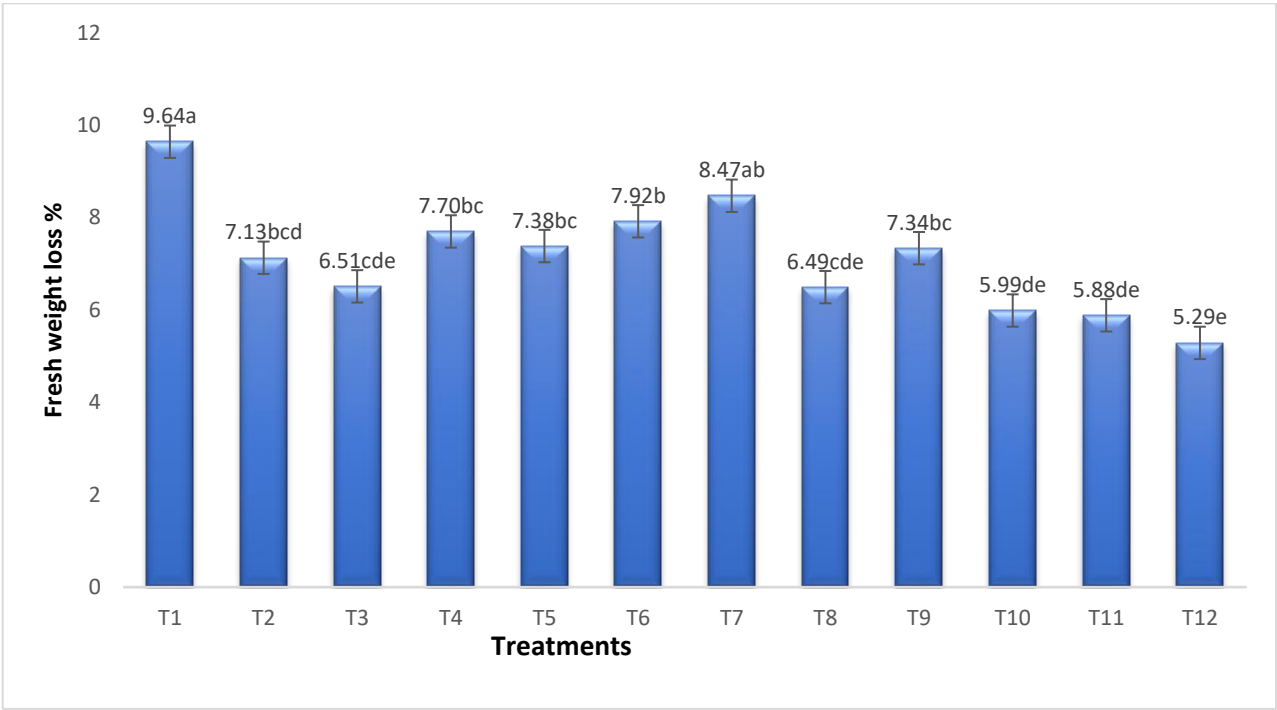

Figure 1. Effect of some treatments on fresh weight loss (%) of strawberry fruits cv. Rubygem stored at 2°C and 85-90% RH for 20 days

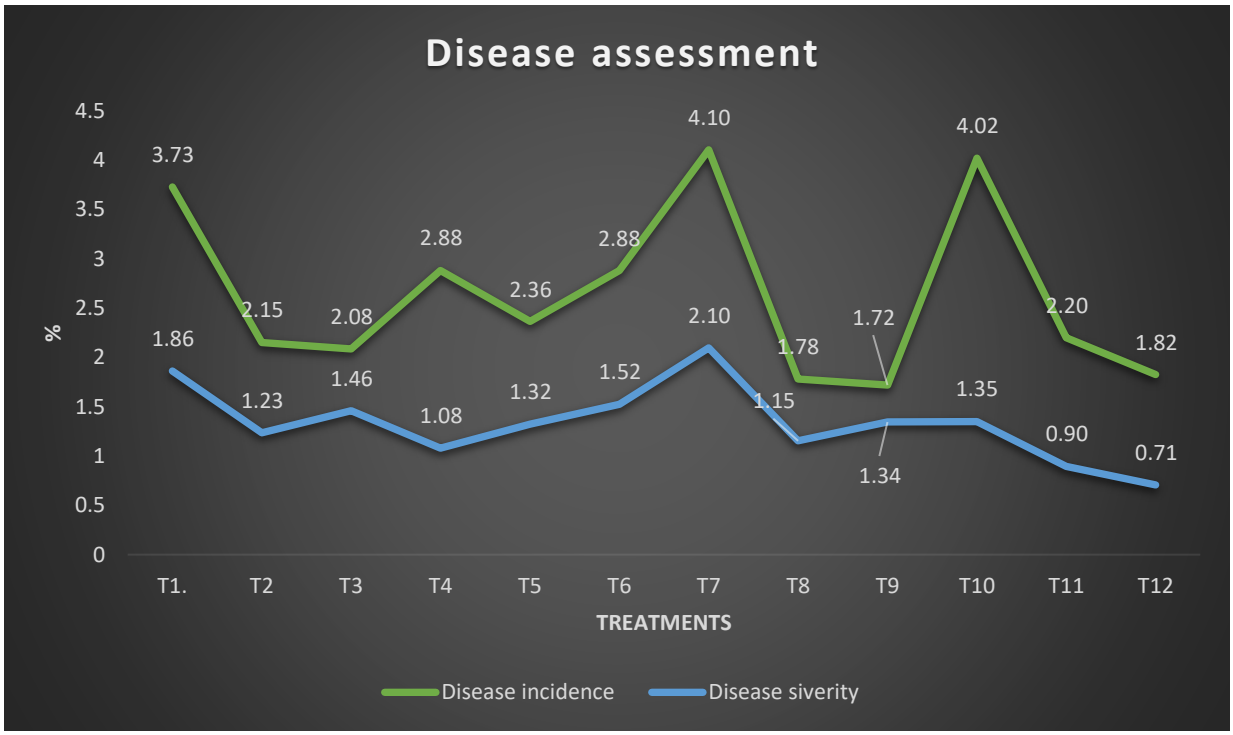

Figure 2. Effect of some treatments on disease incidence (%) and disease severity (%) of gray mold produced by *Botrytis cinerea* Pers (1794), in strawberry fruits cv. Rubygem stored at 2 °C and 85-90% RH for 20 days

Isolation and identification of *Botrytis cinerea* on stored strawberry fruits. In the present study, one fungus was isolated from strawberry fruits during the storage which was identified as *Botrytis cinerea* Pers (1794), according to the characteristics of colony during microscopic observation (Fig. 3-A, and conidia Fig. 3-B). These results are consistent with the findings of Leyronas *et al*. [60] . Postharvest losses caused by *Botrytis cinerea* Pers (1794) and other fungi are particularly severe because they may include accumulated costs of harvesting, packaging, cooling, and transport, while markets can also be depressed as a result of consumer dissatisfaction. *Botrytis cinerea* Pers (1794) infection can develop in the field and can also cause postharvest decay or remain latent until storage.

Spore germination and infection are most rapid at temperatures of 22-25 °C. *Botrytis cinerea* Pers (1794) is also active at relatively low temperature, however, it can cause considerable damage to flowers and plants stored at temperatures ranging within the interval 0 °C – 10 °C. Thus, in cold storage, it leads to the development of gray mold symptoms, and this disease spreads rapidly among fruits in the same packaging [61].

Figure 3. Images of *Botrytis cinerea* Pers (1794) colonies, plate 1:7 days old, plate 2:12 days old showed the seclerotia) grown on PDA (A), and conidia (400 x) (B) (Original)

Molecular based Identification. The molecular techniques using PCR applied in order to identify the *Botrytis cinerea* Pers (1794) variety showed that the sequence of the extracted isolates had the highest similarity 97% with the sequence from the database. The PCR product of the fungi ITS region on gel electrophoresis appears as having 540 bp band size (Fig. 4).

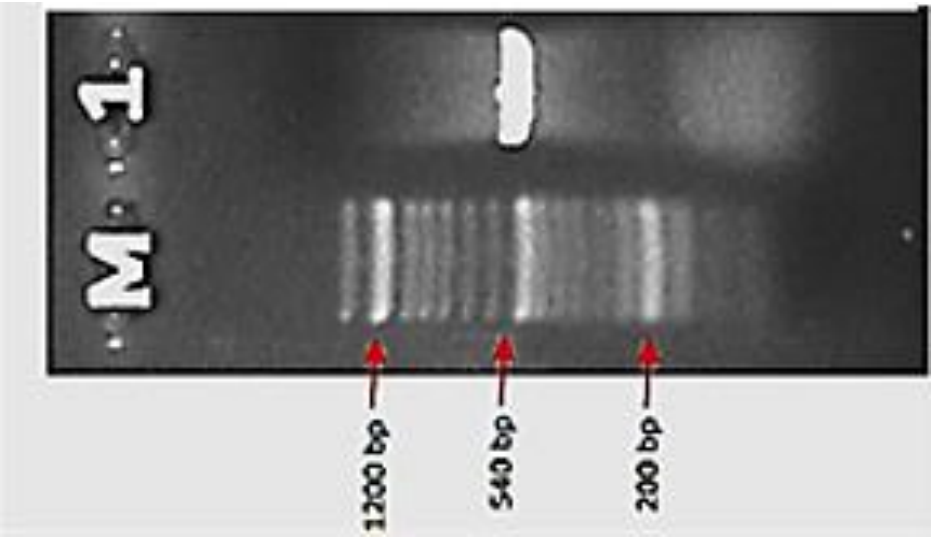

Figure 4. Agarose gel showing PCR product (540bp) of ITS1 and ITS4 DNA of *Botrytis cinerea* Pers (1794), (M) DNA Lader (100 bp) (Original)

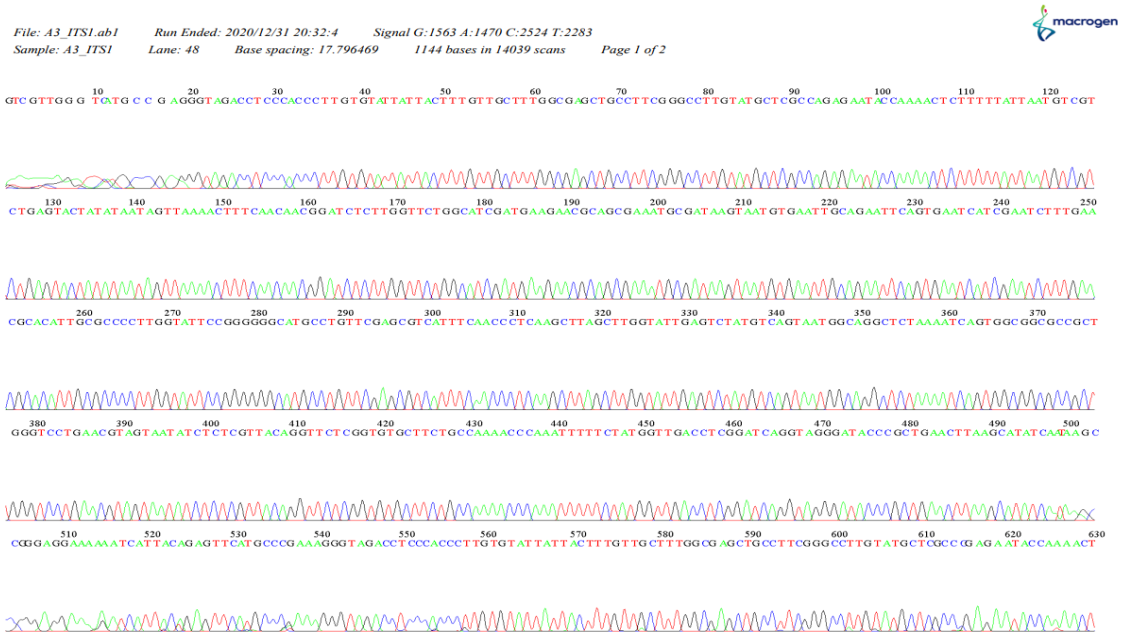

Figure 5. Partial sequence of *Botrytis cinerea* Pers (1794), internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2 (Original)

The partial sequence of *Botrytis cinerea* Pers (1794), that are shown in Fig. 5. These results are in accordance with the results concerning *Botrytis cinerea* Pers (1794) reported by Behr et al. [62].

4. Conclusions

The treatment combination of pomegranate peel extracts + salicylic acid had a significant effect on leaf number, leaf chlorophyll intensity, and yield of strawberry cv. Rubygem. The spray application of SA has a significant effect on the physical property of strawberry fruit's weight. Due to the combination of rosemary plant extracts + KMS with 20 days storage, high properties of firmness are recorded.

The maximum TSS (%) and total sugar (%) were obtained when pomegranate peel extracts + rosemary plant extracts were used in under plastic house conditions. However, in conditions of storage for 20 days, the maximum value of TSS is reported when pomegranate peel extracts + KMS treatment is applied, and the maximum value of total sugar when SA + KMS is applied.

Total soluble solids and total sugar (%) were increased with advanced cold storage, regardless of the used treatments. The spray application with rosemary plant extracts + SA and Pristine treatments were the most effective treatment in reducing diseases incidence, severity, and fresh weight loss in the cold room.

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