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Potassium Sorbate Postharvest Treatment to Control *Penicillium* spp. Molds and Maintain Quality of 'Wonderful' Pomegranates and 'Anna' Apples during Cold Storage

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Abstract

This study was carried out for two successive seasons 2020 and 2021 in order to evaluate the efficiency dipping of Wonderful pomegranate and Anna apple fruits in potassium sorbate (K-sorb.) at 3% / 2 min to maintain fruit quality and control postharvest diseases caused by *Penicillium* spp. (*P. italicum* and *P. sp.*) during cold storage. Fruits were stored at 5°C and 85-90% RH for 75 days. The reduction in linear growth was correlated to the increase in potassium sorbate concentrations. Potassium sorbet at 0.06% concentration gave the maximum inhibition of the linear growth of *Penicillium* spp. Also, the application of potassium sorbate showed the best results in reducing the deterioration of various physical and chemical characteristics of 'Wonderful' pomegranate and 'Anna' apple fruits during cold storage and extending its storability. Potassium sorbate treatments mitigated the incidence of fruit decay and successfully decreased fruit weight loss and increased SSC: TA ratio, the total phenolic content, activities of peroxidase and catalase enzymes as relative to the control treatment.

Keywords: Pomegranate; Apple; Potassium sorbate; *Penicillium sp.; Penicillium expansum;* Cold storage; Quality; TPC; POD; CAT.

1. Introduction

Penicillium spp. is one of the most important postharvest pathogens of fruit worldwide. Among postharvest diseases, blue mold rot, caused by *Penicillium* spp., is a serious postharvest disease and accounts for up to 20–50% of fruit decay [1]. It can lead to significant economic losses during storage, which can also impact fruit destined for processing due to the production of carcinogenic mycotoxin patulin. Patulin has been reported to have mutagenic, carcinogenic and teratogenic properties but this is still a matter of debate [2]. However, there is a need to provide more biocompatible fungicides which are safe in the environment, non-toxic to humans and animals and are rapidly biodegradable.

Potassium sorbate (K-sorb.) is common food additives with potent antimicrobial activity [3]. Potassium sorbate that applied after and before harvest controlled a variety of postharvest pathogens, keeping the fruits quality and reducing decay during cold storage [4-10] with a potential as a commercially feasible treatment. Moreover, it has a low order of toxicity to workers and the environment, is inexpensive, readily available, exempt from residue tolerances [11].

Significant advantages of potassium sorbate over the commonly used sodium bicarbonate, which similarly improved fungicide performance, are the relatively low salt concentration of potassium sorbate, the absence of sodium and its lower pH, so disposal of used potassium sorbate solutions would raise fewer regulatory issues [12]. Sorbates are the best characterized of all food antimicrobials as to their spectrum of action. They inhibit certain bacteria and food-related yeasts and mold species. Sorbates are common food preservatives for many applications and its spectrum of activity includes *Penicillium* spp.

The mode of action of sorbate could be through the alteration of the morphological structure of the cell, genetic changes, cell membrane alterations, inhibition of cell transport processes and inhibition of enzymes involved in metabolism of transport functions [13]. Potassium sorbate inhibited respiration of fruits and protected fruit skin from decay, thus delaying deterioration and keeping quality by slowing down the metabolism activity and physiological disorders [14]. According to Lopez, *et al.* [15]; Flores Fidelis, *et al.* [16], films containing a minimum inhibitory concentration of 0.3% potassium sorbate inhibited the growth of *Penicillium* spp.

Therefore, the objective of this study was to evaluate using of potassium sorbate postharvest treatments as natural alternatives to maintain 'Wonderful' pomegranates and 'Anna' apples quality and prolong the cold storage periods as long as possible by inhibition fungal diseases.

2. Materials and Methods

2.1. Fruits

Fruits of pomegranate (*Punica Granatum* L. cv. Wonderful) and apple (*Malus domestica* Borkh cv. Anna) were obtained from a private orchard located at El Behaira Governorate, Egypt during both seasons 2020 and 2021. The fruits were chosen with uniform size, shape, weight, color and free from visual symptoms of disease or mechanical damages. Fruit were immediately transported to the laboratory of Fruit Handling Department, Horticulture Research Institute, Agricultural Research Center. Pomegranate and apple fruits were cleaned and divided into four treatments; each treatment was content (3 replicates X 3 boxes X 3 Kg for each box).

2.2. Treatments

T1. Control fruits (untreated).
T2. Dipping fruits in K-sorb. at 3%.
T3. Artificial inoculation by pathogenic fungi isolated.
T4. Dipping fruits in K- sorb. at 3% + Artificial inoculation pathogenic fungi isolated.

The fruits were immersed in solutions for (2 min) then left for air drying at room temperature. After the treatment fruits placed in carton boxes and stored at 5°C and 85-90% relative humidity for a total storage period of 75 days.

2.3. Isolation and Identification of the Pathogen

Fungi of *Penicillium* spp.; *P italicum* and *P. sp.* were isolated from naturally infected fruits. These fungi were purified and 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 7d intervals and conidia from 7d old pure cultures were used for inoculations were selected from isolated collection at Department of postharvest diseases, Plant Pathology Research Institute, ARC, and Giza.

2.4. Inoculation of Fruits with Isolated Fungi

Fruits allocated for artificial inoculation were surface sterilized by dipping in 70% alcohol/2 min followed by dipping in 2% sodium hypochlorite/10 sec, then washed thoroughly with sterilized distilled water for 1 min [17], and were left for drying in air at room temperature. Fruits were punctured using a stainless steel rod with 2 mm diameter and 2 mm depth, as one puncture for fruit at the middle of one fruit side, then inoculated with a conidial suspension of P italicum and P. expansum (5 × 104 spore/mL) for 30 sec [18]. After inoculation fruits with pathogens treated with potassium sorbate, and then placed in carton boxes and stored for 75 days in the same conditions. Disease incidence and severity percentage were assessed during cold storage each 15 days from the beginning until the end of storage period.

2.4.1. Linear Growth

Potassium sorbate was tested in vitro on linear growth of the pathogenic fungi according to EL-Eryan and EL-Metwally [19].

2.4.2. Disease Incidence (%)

Determined for each replicate by relating decayed fruits with blue mold disease to the total number of fruits according to Moreira and Mio [17].

2.4.3. Disease Severity (%)

It was determined according to the equation described by Chen, *et al.* [20] as follows:

2.5. Fruit Quality

Fruit quality was assessed at 15 days intervals from the beginning of cold storage (0 times) till the end of storage period (75 days) in both seasons of the study as the following:

2.5.1. Loss in Fruit Weight Percentage

It was determined according to the following equation:

$$\text{Loss in fruit weight } \% = \frac{\text{Initial fruit weight} - \text{Weight at sampling date}}{\text{Initial fruit weight}} \times 100$$

2.5.2. SSC/TA Ratio

Soluble solids content (SSC) was determined in fruit juice using Digital refractometer PR32 (AtagoPalete ATago.CO .LTD. Japan); titratable acidity (TA) percentage was determined by titrating the juice against 0.1 N sodium hydroxide using phenolphthalein as an indicator according to A.O.A.C. [21] and then the SSC: TA ratio was calculated.

2.5.3. Total Phenolic Content (TPC)

TPC in juice was determined using the Folin–Ciocalteu method according to Meighani, *et al.* [22].

2.5.4. Peroxidase (POD) Enzyme Activity

It was assayed by the method described by Zhang, *et al.* [23].

2.5.5. Catalase (CAT) Enzyme Activity

It was analyzed according to the methods described by Wang, *et al.* [24].

2.5.6. Statistical Analysis

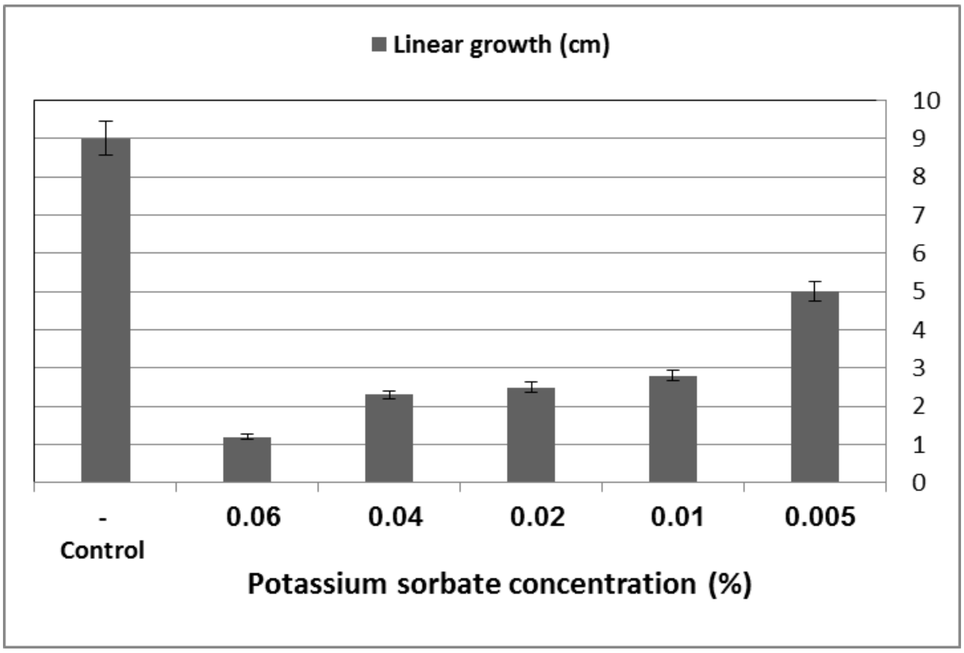
Data of the both seasons of study were arranged as a randomized complete design with three replicates. All data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran [25] and means were compared by Duncan's Multiple range test at 5% level of probability.

3. Results

3.1. Effect of Potassium Sorbet on Linear Growth of *Penicillium* spp. Isolated From 'Wonderful' Pomegranate and 'Anna' Apple Fruits

In vitro test, potassium sorbet affected on linear growth of *Penicillium* spp.; *P sp.* and *P. expansum* isolated from 'Wonderful' pomegranate and 'Anna' apple fruits, respectively. It was noticed that the reduction in linear growth of *Penicillium* spp. were correlated to the increase in potassium sorbate concentrations above 0.005 % (Fig.1). The lowest significant inhibition of mycelia growth of *Penicillium* spp. was recorded by potassium sorbet treatment at 0.06 % concentration.

Fig-1. Effect of potassium sorbet on linear growth of *Penicillium* spp. isolated from 'Wonderful' pomegranate and 'Anna' apple fruits



3.2. Effect of Potassium Sorbate on 'Wonderful' Pomegranate Fruits

3.2.1. Disease Incidence and Severity Percentage

In both seasons, prolonging storage period to 75 days the postharvest treatment of 'Wonderful' pomegranate fruits with potassium sorbet resulted in significant control of blue mold disease in naturally infected fruits, with a significant difference in the incidence and severity percentage in both treated fruits and untreated fruits (control) (Tables 1-2).

After 15 days of storage, the disease incidence and severity percentage in fruits artificially inoculated with *Penicillium sp.* was 100% in both seasons.

At 30 days of storage, the disease incidence percentage in control fruits was 25 and 18 % during the first and second seasons, respectively while the disease incidence percentage in fruits treated with K- sorb. + inoculated with *Penicillium sp.* was 8 % in the first season of the study.

At the end of storage period (75 days), the disease incidence in control fruits was 50 and 55 % during the first and second seasons, respectively. While, in fruits treated with K- sorb. the disease incidence was 8 % in the first season of the study.

Table-1. Effect of potassium sorbate treatment on blue mold disease incidence percentage of pomegranate (cv. Wonderful) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.00A	0.00B	25.00B	34.00A	42.00A	50.00A
K-sorb.	0.00A	0.00B	0.00D	0.00B	0.00B	8.00C
Penicillium sp.	0.00A	100.0A	100.0A	-	-	-
K- sorb. + *Penicillium sp.*	0.00A	0.00B	8.00C	-	-	-
Season 2021						
Control	0.00A	0.00B	18.00B	35.00A	43.00A	55.00A
K-sorb.	0.00A	0.00B	0.00C	0.00B	0.00B	0.00B
Penicillium sp.	0.00A	100.0A	100.0A	-	-	-
K- sorb. + *Penicillium sp.*	0.00A	0.00B	-	-	-	-

Table-2. Effect of potassium sorbate treatment on blue mold disease severity percentage of pomegranate (cv. Wonderful) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.00A	0.0B	25.0B	25.00A	40.0A	50.00A
K-sorb.	0.00A	0.0B	0.00C	0.00B	0.00B	10.00B
Penicillium sp.	0.00A	25.0A	100.0A	-	-	-
K- sorb. + *Penicillium sp.*	0.00A	0.0B	25.00B	-	-	-
Season 2021						
Control	0.00A	0.00B	25.0B	32.0A	45.0A	50.0A
K-sorb.	0.00A	0.00B	0.00C	0.00B	0.00B	0.00B
Penicillium sp.	0.00A	100.0 A	100.0A	-	-	-
K- sorb. + *Penicillium sp.*	0.00A	0.00B	0.00C	-	-	-

3.2.2. Physical Properties

3.2.2.1. Weight Loss Percentage

Data shown in Table 3 indicated that weight loss percentage increased gradually and significantly with extending cold storage periods with significant differences between them during two seasons.

After 30 days of storage, the least significant of weight loss percentage obtained in fruits treated with K- sorb. + inoculated with *Penicillium sp.*and K- sorb. treatment in the first and second seasons, respectively. On the other hand, the highest value of weight loss percentage (4.70% and 4.60%) obtained in fruits inoculated with *Penicillium sp.*in the both seasons, respectively.

After 75 days of storage, the least value of weight loss percentage (10.39 and 10.85%) obtained in fruits treated with K- sorb. in the two seasons, respectively. On the other hand, the highest value of weight loss percentage (11.17% and 12.57%) obtained in control fruits in the both seasons, respectively.

Table-3. Effect of potassium sorbate treatment on weight loss % of pomegranate (cv. Wonderful) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.00A	2.40B	3.88A	6.50A	10.38A	11.17A
K-sorb.	0.00A	1.20C	2.53B	4.29B	8.08B	10.39B
Penicillium sp.	0.00A	3.10A	4.70A	-	-	-
K- sorb. + *Penicillium sp.*	0.00A	1.00D	2.40B	-	-	-
Season 2021						
Control	0.00A	2.00B	4.07A	6.86A	11.90A	12.57A
K-sorb.	0.00A	1.30C	2.60B	4.07B	8.43B	10.85B
Penicillium sp.	0.00A	2.50A	4.60A	-	-	-
K- sorb. + *Penicillium sp.*	0.00A	1.40C	2.70B	-	-	-

3.2.3. Chemical Properties

3.2.3.1. Soluble Solid Content (SSC): Titratable Acidity (TA) Ratio

It is clear from the tabulated data in Table 4 that SSC: TA ratio increased with the advance in cold storage period.

After 30 days of storage, the highest values of SSC: TA ratio (10.34 and 9.74) recorded in fruits treated with K-sorb. during the first and second seasons, respectively. However, the least values of SSC: TA ratio were found in fruits inoculated with *Penicillium sp.* in the two seasons, respectively.

After 75 days of storage, the highest values of SSC: TA ratio (12.58 and 11.96) also recorded in fruits treated with K- sorb. during the first and second seasons, respectively. However, the least values of SSC: TA ratio (10.32 and 10) were noted in control fruits in the both seasons, respectively.

Table-4. Effect of potassium sorbate treatment on SSC: TA ratio of pomegranate (cv. Wonderful) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	**15**	**30**	**45**	**60**	**75**
	Season 2020					
Control	8.29A	9.11B	10.33A	10.04B	10.06B	10.32B
K-sorb.	8.29A	9.15A	10.34A	11.58A	12.08A	12.58A
Penicillium sp.	8.29A	8.57C	9.49C	-	-	-
K- sorb. + *Penicillium sp.*	8.29A	8.57C	9.63B	-	-	-
	Season 2021					
Control	8.04A	9.15B	9.34B	9.80B	9.91B	10.00B
K-sorb.	8.04A	9.18A	9.74A	10.54A	10.75A	11.96A
Penicillium sp.	8.04A	8.32C	9.28C	-	-	-
K- sorb. + *Penicillium sp.*	8.04A	8.33C	9.33B	-	-	-

3.2.3.2. Total Phenolic Content (TPC)

As shown in Table 5, total phenolic content decreased significantly by the extending the storage periods. Total phenolic content was significant during the both seasons in the most cases.

After 30 days of storage, the highest values of total phenolic content were recorded in fruits treated with K-sorb. in the both seasons. On the other side, the least values of total phenolic content were found in fruits inoculated with *Penicillium sp.* during the two seasons.

After 75 days of storage, the highest values of total phenolic content were recorded in fruits treated with K-sorb. in the two seasons. While, the least values of total phenolic content were found in control fruits during the both seasons.

Table-5. Effect of potassium sorbate treatment on total phenolic content of pomegranate (cv. Wonderful) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	**15**	**30**	**45**	**60**	**75**
Season 2020						
Control	0.530A	0.470A	0.450A	0.431B	0.410A	0.400A
K-sorb.	0.530A	0.490A	0.472A	0.440A	0.421A	0.410A
Penicillium sp.	0.530A	0.325B	0.320B	-	-	-
K- sorb. + *Penicillium sp.*	0.530A	0.346B	0.333B	-	-	-
Season 2021						
Control	0.540A	0.456A	0.448A	0.446A	0.445A	0.444A
K-sorb.	0.540A	0.452A	0.449A	0.447A	0.446A	0.445A
Penicillium sp.	0.540A	0.334AB	0.323B	-	-	-
K- sorb. + *Penicillium sp.*	0.540A	0.455A	0.453A	-	-	-

3.2.3.3. Peroxidase Activity

Regarding the changes in peroxidase activity during cold storage period, the results noted that there was decrease the peroxidase activity until the 15 days of storage then increased up to 75 days in two seasons (Table 6).

After 30 days of storage period, the highest values were recorded in fruits treated by K-sorb. in the first and second seasons. On the contrary, fruits inoculated with *Penicillium sp.* and treated by K- sorb. + inoculated with *Penicillium sp.* exhibited the least value of peroxidase activity in both seasons.

At the end of storage periods (75 days), the highest values were recorded in fruits treated by K-sorb. in the both seasons, while untreated fruits (control) recorded the least value of peroxidase activity in the both seasons.

Table-6. Effect of potassium sorbate treatment on peroxidase activity of pomegranate (cv. Wonderful) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.119A	0.022C	0.125B	0.191B	0.224B	0.239B
K-sorb.	0.119A	0.107A	0.184A	0.225A	0.308A	0.334A
Penicillium sp.	0.119A	0.077B	0.094C	-	-	-
K- sorb. + *Penicillium sp.*	0.119A	0.069B	0.102C	-	-	-
Season 2021						
Control	0.121A	0.027C	0.123B	0.195B	0.228B	0.245B
K-sorb.	0.121A	0.118A	0.191A	0.306A	0.324A	0.391A
Penicillium sp.	0.121A	0.082B	0.112B	-	-	-
K- sorb. + *Penicillium sp.*	0.121A	0.086B	0.107B	-	-	-

3.2.3.4. Catalase Activity

Data in Table 7 shows the changes in catalase activity during cold storage periods; it decreased until the 15 days of storage then increased up to 75 days of storage in two seasons, with significant difference in the effect on catalase activity in the most cases.

After 30 days of storage period, the highest values were recorded in fruits inoculated with *Penicillium sp.* and fruits treated with K-sorb. treatment in the first and second seasons, respectively. On the contrary, control exhibited the least value of catalase activity in both seasons.

At the end of storage periods (75 days), the highest values were recorded in fruits treated by K-sorb. in the both seasons, while the control recorded the least value of catalase activity in the both seasons.

Table-7. Effect of potassium sorbate treatment on catalase activity of pomegranate (cv. Wonderful) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.191A	0.055A	0.056B	0.140A	0.140A	0.143B
K-sorb.	0.191A	0.055A	0.094AB	0.141A	0.143A	0.172A
Penicillium sp.	0.191A	0.007B	0.120A	-	-	-
K- sorb. + *Penicillium sp.*	0.191A	0.054A	0.116A	-	-	-
Season 2021						
Control	0.192A	0.055BC	0.058B	0.072B	0.166A	0.170B
K-sorb.	0.192A	0.126A	0.140A	0.166A	0.170A	0.194A
Penicillium sp.	0.192A	0.008D	0.057B	-	-	-
K- sorb. + *Penicillium sp.*	0.192A	0.073B	0.135A	-	-	-

3.3. Effect of Potassium Sorbate Treatment on ‘Anna’ Apple Fruits

3.3.1. Disease Incidence and Severity Percentage

Postharvest treatment of ‘Anna’ apple fruits with potassium sorbet during 2020 and 2021 seasons resulted in significant control of blue mold disease in naturally infected fruits during cold storage at 5^{o}C and 85- 90%RH for 75 days, with a significant difference in the incidence and severity percentage in both treated fruits and untreated fruits (control) (Tables 8-9).

After 30 days of storage, the incidence percentage of disease in fruits artificially inoculated with *Penicillium expansum* was 100%, about 12% in control fruits and 5% in fruits treated with K- sorb. + inoculated with *Penicillium expansum*.

At the end of storage periods (75 days), control fruits recorded incidence percentage 50 % during 2020 and 2021 seasons, while the fruit treated by K-sorb. recorded 12% in the first season and don’t record any incidence in the second season.

Table-8. Effect of potassium sorbate treatment on blue mold disease incidence percentage of apple (cv. Anna) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.00A	0.00B	25.00B	33.30A	41.70A	50.00A
K-sorb.	0.00A	0.00B	0.00D	0.00B	0.00B	8.30B
Penicillium expansum	0.00A	100.0A	100.0A	-	-	-
K- sorb. + *Penicillium expansum*	0.00A	0.00B	8.30C	-	-	-
Season 2021						
Control	0.00A	0.00B	16.70B	33.30A	41.70A	50.00A
K-sorb.	0.00A	0.00B	0.00C	0.00B	0.00B	0.00B
Penicillium expansum	0.00A	100.0A	100.0A	-	-	-
K- sorb. + *Penicillium expansum*	0.00A	0.00B	-	-	-	-

Table-9. Effect of potassium sorbate treatment on blue mold disease severity percentage of apple (cv. Anna) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.00A	0.00B	6.30B	11.10 A	11.50A	12.50A
K-sorb.	0.00A	0.00B	0.00B	0.00B	0.00B	1.00B
Penicillium expansum	0.00A	5.00A	100.0A	-	-	-
K- sorb. + *Penicillium expansum*	0.00A	0.00B	0.4C	-	-	-
Season 2021						
Control	0.00A	0.00B	4.20B	8.30A	10.20A	25.00A
K-sorb.	0.00A	0.00B	0.00C	0.00B	0.00B	0.00B
Penicillium expansum	0.00A	10.00A	100.0A	-	-	-
K- sorb. + *Penicillium expansum*	0.00A	0.00B	-	-	-	-

3.3.2. Physical Properties

3.3.2.1. Weight Loss Percentage

Data shows in Table 10 indicated that weight loss percentage increased gradually and significantly with extending cold storage periods with significant differences between them during two seasons.

After 30 days of storage, the least significant of weight loss percentage (2.63 and 3.14%) was obtained in fruits treated with K- sorb. in the first and second seasons, respectively. On the other hand, the highest values of weight loss percentage (6.50% and 10.70%) were obtained by fruits treated with K- sorb. plus inoculated with *Penicillium expansum* and fruits inoculated by *Penicillium expansum* in the first and second seasons, respectively.

After 75 days of storage, the least value of (6.83 and 7.61%) weight loss percentage was obtained in fruits treated with K- sorb. in the two seasons, respectively. On the other hand, the highest values of weight loss percentage (8.93% and 10.48%) were obtained in control fruits in the both seasons, respectively.

Table -10. Effect of potassium sorbate treatment on weight loss % of apple (cv. Anna) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.00A	1.74C	3.57C	5.11A	6.94A	8.93A
K-sorb.	0.00B	0.95D	2.63D	3.60B	5.17B	6.83B
Penicillium expansum	0.00B	5.00A	5.70B	-	-	-
K- sorb. + *Penicillium expansum*	0.00B	4.10B	6.50A	-	-	-
Season 2021						
Control	0.00B	1.75C	3.72C	5.74A	8.08A	10.48A
K-sorb.	0.00B	1.29D	3.14D	4.21B	5.82B	7.61B
Penicillium expansum	0.00B	5.10A	10.70A	-	-	-
K- sorb. + *Penicillium expansum*	0.00B	4.40B	6.80B	-	-	-

3.3.3. Chemical Properties

3.3.3.1. Soluble Solid Content (SSC): Titratable Acidity (TA) Ratio

It is clear from the tabulated data in Table 11 that SSC: TA ratio increased with the advance in cold storage period. There were significant differences in the effect on SSC: TA ratio in the most cases.

After 30 days of storage, the highest values (40.81 and 41.33) recorded in fruits treated with K- sorb. in the first and second seasons. However, the least values of SSC: TA ratio (34.38 and 32.88) found in fruits inoculated with *Penicillium expansum* in the two seasons.

After 75 days of storage, the highest values (42.61 and 48.86) were recorded in fruits treated with K- sorb. in the first and second seasons, respectively. However, the least values (40.31 and 37.36) of SSC: TA ratio was noted in control fruits in the two seasons, respectively.

Table-11. Effect of potassium sorbate treatment on SSC: TA ratio of apple (cv. Anna) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	26.04A	33.66B	37.74B	37.09B	38.25B	40.31B
K-sorb.	26.04A	37.09A	40.81A	37.27A	38.44A	42.61A
Penicillium expansum	26.04A	31.32C	34.38D	-	-	-
K- sorb. + *Penicillium expansum*	26.04A	33.64B	36.62C	-	-	-
Season 2021						
Control	28.06 A	33.83B	34.95B	34.51B	38.17B	37.36C
K-sorb.	28.06 A	39.78A	41.33A	39.49A	42.05A	48.86A
Penicillium expansum	28.06 A	31.72C	32.88D	-	-	-
K- sorb. + *Penicillium expansum*	28.06A	33.73B	34.64C	-	-	-

3.3.3.2. Total Phenolic Content (TPC)

Result tabulated in Table 12 indicated that total phenolic content decreased significantly with advanced the storage periods. Total phenolic content was significant in both seasons in the most cases.

After 30 days of storage, the highest values of total phenolic content were recorded in fruits treated with K-sorb. during the both seasons. On the contrary, the least values of total phenolic content were found in fruits inoculated with *Penicillium expansum* during the two seasons.

After 75 days of storage, the highest values of total phenolic content were recorded in fruits treated with K-sorb. in the two seasons. On the other hand, the least values of total phenolic content were found in control fruits during the both seasons.

Table -12. Effect of potassium sorbate treatment on total phenolic content of apple (cv. Anna) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	230.0A	223.00B	221.00B	199.00B	198.00B	180.00B
K-sorb.	230.0A	225.00A	224.50A	200.00A	213.00A	181.00A
Penicillium expansum	230.0A	219.00D	195.00C	-	-	-
K- sorb. + *Penicillium expansum*	230.0A	222.00C	221.00B	-	-	-
Season 2021						
Control	234.0A	229.00B	230.00B	185.00B	182.00B	178.00B
K-sorb.	234.0A	230.00A	232.00A	198.00A	200.00A	190.00A
Penicillium expansum	234.0A	221.00D	215.00D	-	-	-
K- sorb. + *Penicillium expansum*	234.0A	225.00C	222.00C	-	-	-

3.3.3.3. Peroxidase Activity

The results in Table 13 noted that peroxidase activity was increasing gradually with advanced the storage periods in the two seasons. There were significant differences in the effect on peroxidase activity in the most cases.

After 30 days of storage period, the highest values (0.377and 0.382) were recorded in fruits treated by K-sorb. in the first and second seasons, respectively. On the contrary, fruits treated by K- sorb. + Penicillium expansum and inoculated with Penicillium expansum exhibited the least value of peroxidase activity in both seasons, respectively.

At the end of storage periods (after 75 days), the highest values were recorded in fruits treated by K-sorb. during the both seasons of this study, while the control fruit recorded the least value of peroxidase activity in the both seasons.

Table -13. Effect of potassium sorbate treatment on peroxidase activity of apple (cv. Anna) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.343A	0.351A	0.368AB	0.380A	0.396A	0.401A
K-sorb.	0.343A	0.355A	0.377A	0.389A	0.400A	0.412A
Penicillium expansum	0.343A	0.325A	0.324B	-	-	-
K- sorb. + *Penicillium expansum*	0.343A	0.348A	0.272A	-	-	-
Season 2021						
Control	0.341A	0.355A	0.371A	0.387A	0.399A	0.411A
K-sorb.	0.341A	0.364A	0.382A	0.391A	0.415A	0.421A
Penicillium expansum	0.341A	0.310C	0.319C	-	-	-
K- sorb. + *Penicillium expansum*	0.341A	0.334B	0.340B	-	-	-

3.3.3.4. Catalase Activity

Data in Table 14 indicates that the changes in catalase activity increase with advanced the storage period in the two seasons with significant differences in the effect on catalase activity in the most cases.

After 30 days of storage period, the highest values were recorded in fruits treated by K-sorb. in the both seasons. On the contrary, fruits treated with K-sorb. + Penicillium expansum exhibited the least value of catalase activity in two seasons.

At the end of storage periods (75 days), the highest values recorded in fruits treated with K-sorb. in the both seasons. Control fruits recorded the least value of catalase activity in the both seasons.

Table-14. Effect of potassium sorbate treatment on catalase activity of apple (cv. Anna) fruits stored at 5℃ and 85- 90 %RH during 2020 and 2021 seasons

Treatments	Storage periods (Days)					
	0-time	15	30	45	60	75
Season 2020						
Control	0.115A	0.152A	0.167A	0.194A	0.212B	0.220B
K-sorb.	0.115A	0.160A	0.189A	0.202A	0.242A	0.255A
Penicillium expansum	0.115A	0.093C	0.158A	-	-	-
K- sorb. + *Penicillium expansum*	0.115A	0.123B	0.132A	-	-	-
Season 2021						
Control	0.119A	0.180B	0.187A	0.198B	0.206B	0.242B
K-sorb.	0.119A	0.210A	0.240A	0.299A	0.320A	0.380A
Penicillium expansum	0.119A	0.151C	0.190A	-	-	-
K- sorb. + *Penicillium expansum*	0.119A	0.130D	0.139B	-	-	-

4. Discussion

Fungal spores and latent infections of *Penicillium* spp. usually appear on the fruits either on the surface or in the first few cell layers under the peel of the fruit [26]. Post-harvest treatments for fruits are of great importance, because they can eliminate the fungus infection by removing spores from the surface of the fruit, directly working on its vitality and/or inducing defense mechanisms in the outer layers of the fruit, thus reducing the growth and development of pathogens [27].

This experiment showed that potassium sorbate is the most effective in reducing disease severity and incidence in both pomegranate and apple fruits during the two seasons of the study. These results are in agreement with the work of Feliziani, *et al.* [7]; Ozgur, *et al.* [8] who reported that application of potassium sorbate after and before harvest controlled a variety of postharvest pathogens, improvement the quality of fruits and reduces decay during cold storage. The reduction in linear growth was correlated to the increase in potassium sorbate (K-sorb.) concentrations. This result is in agreement with the finding of Latifa, *et al.* [28] on citrus and EL-Eryan and EL-Metwally [19] on pear who reported complete inhibition of mycelia growth of *P. itelicum* which was generally associated with complete inhibition of sporulation by organic acids and salts. The obtained results are in accordance with those reported by El-Mougy, *et al.* [29] sorbate may help in control *P. italicum* infection on navel oranges fruits *in vivo*.

This may be due to the role of potassium sorbate in inhibiting spore germination, germ tube elongation and mycelial growth of several pathogens such as filamentous fungi, yeasts, and a selected number of bacterial strains in cheese, bread, fruits, vegetables, jams, and meat products [16, 30]. According to Lopez, *et al.* [15], films containing a minimum inhibitory concentration of 0.3% potassium sorbate inhibited the growth of *Candida* spp., *Penicillium* spp., *S. aureus* and *Salmonella* spp. The mode of action of sorbate could be through the alteration of the morphological structure of the cell, genetic changes, cell membrane alterations, inhibition of cell transport processes and inhibition of enzymes involved in metabolism of transport functions. However, inhibition of microorganisms by sorbates varies, depending on species and strain differences, extent of contamination, type and composition of the substrate, concentration and pH of sorbate, water activity, presence of other additives, temperature of storage and type of packaging [31]. Moreover, Palou, *et al.* [6] confirmed that potassium sorbate is food additives which have inhibitory action against fruit postharvest pathogens. It had an effective control effect on citrus green mold and sour rot, particularly when fruits were immersed in solutions of these salts.

It was obvious that potassium sorbate used as postharvest treatments were capable of reducing the deterioration of various physical and chemical characteristics of 'Wonderful' pomegranates and 'Anna' apples during cold storage and extending its storability. In our study, this treatment was applied to mitigate the incidence of both pomegranate and apple fruit decay and succeeded in maintaining the chemical and physical properties such as reduced fruit weight loss, increased SSC/TA ratio and total phenolic content, besides the activities of peroxidase and catalase enzymes compared to the control treatment. The results of the present study were in line with Youssef, *et al.* [32]; Cerioni, *et al.* [33]; Parra, *et al.* [34]; Youssef, *et al.* [35]; Cerioni, *et al.* [36]; Abd El-Khalek [37].

The inhibitory effect of sorbate salts on postharvest pathogens is probably due to the reduction of fungal cell turgor pressure that results in collapse and shrinkage of hyphae and spores and consequent inability of fungi. This is agree with Abdel Wahab and Rashid [38] who reported that, coating Navel orange fruits with potassium sorbate in wax or sorbate alone may protect fruit skin from decay and thus delaying dehydration and changes loss in the firmness and improve quality where firmness loss is one of the main factors limiting quality and postharvest shelf life of fruits.

Potassium sorbate protected fruit skin from decay, inhibited respiration of fruits during the storage period and subsequent decline production of acids by slowing down the metabolism activity and physiological disorders [14]. Besides, it has been Strydum and Loubser [39] reported that using potassium sorbate or the other sources of potassium leads to acceleration of TSS accumulation and reduction of titrated acidity in grapes. So, these results illustrate the reason for increasing SSC/AT ratio.

Fruits treated with potassium sorbate had a higher amount of total phenolic, indicating the positive effect of this treatment as inhibitors of phenolic degradation during storage. Our findings are in agreement with those reported by Molaei, *et al.* [40], who reported that pomegranate fruits (cv. Malas e Saveh) treated with potassium sorbate exhibited a higher content of arils' phenols.

Oxidative damage and resulted ROS accumulation throughout the plants' cell are common cross talk between different unfavorable abiotic conditions such as chilling stress. In this regard, plants have efficient enzymatic and non-enzymatic strategies for ROS scavenging [41]. POD and CAT are two of the most important antioxidant enzymes in the plant cell, acts as a component of the first line defense system against ROS. Accordingly, activities of these enzymes were evaluated at the present work as important antioxidant enzymes. The results showed that POD and CAT activity in pomegranate and apple juice increased in both the potassium sorbate treated and control fruit, but in treated fruit was significantly higher than the control and reached its highest value at the end of the storage period during the both seasons of study. This is consistent with Molaei, *et al.* [40], who reported that potassium sorbate treatment increased the activity of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO) in pomegranate fruits during cold storage at 4°C for 90 days. This may explain the reason for enhancing the quality of pomegranate and apple fruits treated with potassium sorbate, as its effect on increasing the activities of antioxidant enzymes (POD and CAT) in the fruit, which delays ripening.

Furthermore, increased POD and CAT activity was observed by extending storage time and this is in agreement with Susaj, *et al.* [42], who found that the levels of peroxidase (POD) and catalase (CAT) increased significantly during the cold storage period of three apple cultivars 'Red Delicious', 'Golden Delicious' and 'Granny Smith'. Also, these findings are confirmed recently by Molaei, *et al.* [40]; Fatma, *et al.* [43].

5. Conclusions

Post-harvest treatment of 'Wonderful' pomegranates and 'Anna' apples with potassium sorbate is very promising for controlling molds caused by *Penicillium* spp. and maintaining its quality and extending its life during cold storage. It is an easy-to-apply, nonpolluting and inexpensive tool that can be suitable for packinghouses or organic markets or export markets.

Conflict of Interest

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

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