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Black Seed Oil Applications for the Preservation of Postharvest Quality of ‘Wonderful’ Pomegranate Under Modified Atmosphere Packaging

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Abstract

This study was conducted to determine the effects of black seed oil (0.1% and 0.5%) applications with and without modified atmosphere packaging (MAP), on the postharvest quality of pomegranate cv. ‘Wonderful’. Fruit samples were stored at 6.5±1 ºC with 90-95% relative humidity for 150 days and quality analysis done at 30-day intervals. Furthermore, after each storage period, fruits were removed and kept at 20 ºC for 7 days to simulate a period of shelf-life. MAP alone or in combination with black seed oil application found to have a significant influence on the maintaining of fruit weight. Percent reduction in the fruit weight 150 days after storage (DAS) was 4.7% and 8.8% for black seed oil (0.5%)+MAP and control+MAP applications, respectively, where it was 18.9% for the control without MAP. The juice content of pomegranate fruits was 31.4% at harvest and it decreased to 21.9% on the control treatment in 150 days. Furthermore, juice content of the fruits with control+MAP and propolis+MAP were determined as 25.8%, and 28.1%, respectively, at 150 DAS. Applications of 0.5% black seed oil especially when combined with MAP, have found to be effective in preventing weight loss, preventing juice content, controlling gray mould development and decelerate the occurrence of chilling injury.

Keywords: chilling injury, gray mould, juice content, visual quality, weight loss

1. Introduction

Pomegranate fruit is traditionally known to have beneficial effects on human health and many scientific works during 21th century confirmed the traditional usage of the pomegranate fruit for medical purposes (Aviram et al., 2000; Lansky et al., 2005; Jurenka, 2008). Results of those studies caused a tremendous increase in the consumption of pomegranate fruit throughout the world. Pomegranates are reported to be originated around central Asia (Holland et al., 2009). However; pomegranate tree can be grown in a wide range of climatic conditions. Harvesting period of pomegranate fruits extends from March to May in southern hemisphere and from August to November in northern hemisphere and the year-round demand is being met by postharvest storage (Kahramanoğlu & Usanmaz, 2016). On the other hand, pomegranate fruit is oversensitive to storage conditions mainly because of fruit decay, weight loss and chilling injury (Elyatem & Kader, 1984; Opara et al., 2008). Inappropriate conditions cause huge losses on fruit weight especially in long term storage. Weight loss also cause hardening and browning of the rind and reduce the attractiveness of fruits (Caleb et al., 2012; Kazemi et al., 2013). Chilling injury is also an important postharvest problem for pomegranate fruits which reduce marketability of fruits. Main symptoms of chilling injury are necrotic pitting on the skin, pale colour of the arils, the brown discoloration of the skin and white segments separating the arils (Elyatem & Kader, 1984). Decay (mainly caused by *Botrytis cinerea* “gray mould”) is another important problem on pomegranate fruits during storage (Wilson & Ogawa, 1979). The losses due to decay may reach up to 30% of harvested pomegranates if there is not any control measure (Tedford et al., 2018).

Modified atmosphere packaging (MAP) is a nano-technological way of improving postharvest life of fruits and vegetables. Oxygen is the main cause of damages on the fruit and vegetable crops while crops require oxygen to do respiration. The MAP technology substitutes the air inside the package with a protective gas mix (reduced oxygen and increased carbon dioxide and/or nitrogen) (Caleb et al., 2012; Farber et al., 2003). It is reported to be effective for increasing the storage duration of pomegranate fruits up to 3 months while reducing weight loss and chilling injury; and preventing decay (Al-Mughrabi, 1995; Kader, 1995; Mirdehghan et al., 2006; Palou et al., 2007). Relative humidity (RH) and temperature are the two main elements of a successful postharvest storage life of pomegranate fruits. However, the three main causes of deterioration on harvested pomegranates; weight loss, chilling injury and decay are being differently influenced by temperature and relative humidity. Temperatures below 5 ºC enhance chilling injury while temperatures above 5 ºC increase weight loss and enhance fruit decay (Opara et al., 2008; Kazemi et al., 2013). Moreover, RH below 90% increases weight loss and RH above 90% enhance fruit decay. Numerous studies carried about the favourable conditions for storing of pomegranate fruits and they suggest 5-7 ºC and RH over 90% for the reduction of weight loss (Elyatem & Kader, 1984; Opara et al., 2008; Fawole et al., 2013; Kazemi et al., 2013). Current knowledge makes it possible to store pomegranates up to 3-4 months, which is not enough to fill the gap in the global market. Palou et al. (2007) suggested that using fludioxonil prevents the development of fruit mould. On the other hand, there is an increase in the consciousness levels of human beings about food safety and according to author's knowledge; there is not enough study about the effects of natural chemicals on the postharvest quality of pomegranates. Black seed oil reported to have a positive influence on the postharvest life of some other fruits but is not tested for pomegranates (Hanafi & Hatem, 1991; Maghoub et al., 2013). Therefore; the aim of present work was to assess the influence of black seed oil with MAP and without MAP on the postharvest quality of pomegranate cv. 'Wonderful'. Studies continued for 5 months with monthly measures on quality attributes. At each month, quality attributes were also determined after 7-days shelf life at 20 ºC.

2. Materials and Methods

2.1 Treatments

This study carried out with 'Wonderful' cultivar pomegranate fruits which had harvested in mid-October from a 7-year old orchard located in Güzelyurt in northern part of Cyprus in 2016. Fruits were harvested by hand at commercial maturity (red color, total soluble solids around 17% and titratable acidity around 1.80%). Harvested fruits were immediately transferred to Alnar Narcılık Ltd. by a ventilated truck and fruits were firstly categorized according to EU standards and only "Extra" category fruits were taken into experiments. Samples were selected for uniformity in size and colour. The size of the selected fruits is "8" for boxes of 40 × 30 cm dimensions. Fruits with signs of sunburn, cracks, disease and mechanical damage were discarded.

Black seed oil is a product of *Nigella Sativa* plant which is native to Asia. The black seed oil of present study is belonging to the Pelmur Ltd. with a brand name of Biotama. The black seed oil is obtained by the cold-press of black cumin seeds. The purchased black seed oil was 100% pure and was dissolved in ethanol by adding 90 mL of 70% ethanol to 10 mL of the black seed oil and agitating for 1 day. The 0.1% and 0.5% black seed oil were prepared by making a dilution of the final solution with pure water instead of ethanol. Preparation of final extracts is done with pure water to make it practicable and utilizable in real applications.

The modified atmosphere packaging bags of present study are obtained from the Dekatrend Ltd. located in Bursa, Turkey with a brand of Trendlife®. The packaging material used was low-density polyethylene film. The semi-permeable character of the film is based on the activity of several intelligent molecules placed inside film. The material is designed to regulate inner air composition with around 9-15% CO_2 and 6-10% O_2. The material also has the ability to leave out excessive ethylene gas and relative humidity.

2.2 Methods

Pomegranates were subjected to the following treatments: (1) dipping in pure water (control treatment); (2) dipping in black seed oil (0.1%); (3) dipping in black seed oil (0.5%); (4) dipping in pure water + MAP (control treatment); (5) dipping in black seed oil (0.1%) + MAP; (6) dipping in black seed oil (0.5%) + MAP. Dipping of fruits is done for 30 s into prepared solutions as stated by Palou et al. (2007). Each treatment contained 3 replications of totally 40 fruits. Half of the treated fruits were stored in modified atmosphere packaging bags and other half stored without MAP film treatment. Fruits were stored at 5.5-7.5 ºC and 90-95% relative humidity (as suggested by Fawole et al., 2013 and Kazemi et al., 2013) for 150 days. The fruits were stored according to split plot design in cold storage. The two main factors of present study were: (1) modified atmosphere packaging (with/without) and (2) storage duration. All fruits were numbered before the experiment and fruit weights were measured and recorded.

After each storage period (30-day intervals), 2 out of 4 fruits were measured instantly and rest were removed and kept at 20 ºC for 7 days to simulate a period of shelf-life and then analyzed. Digital balance (sensitive to 0.01 g) was used to measure fruit weight. Final fruit weight is subtracted from the harvest weight and the result is divided to the harvest weight to determine the weight loss (%). Juice yield was determined by pressing the half-cut fruits with an automatic squeezing machine. Pomegranate juice was then passed through 1 mm stainless steel sieves to remove seeds and pulp. Then the juice content (%) was calculated by dividing the juice content to the harvest weight of the fruits, not to the final weight. Total soluble solid contents were measured with a hand refractometer (Turoni tr®). Observation of gray mould is done according to 0-4 scale; (0) refers no infection; (1) mould at only crown; (2) mould covers less than 25% of the rind; (3) mould covers 25-50% of the rind and (4) mould covers more than 50% of the rind. Visual analysis was carried out by characterizing the external appearance with the scores from 0 to 5. Score of 5 represents the fruits with less than 1% of dents, absence of blemishes, and absence of pest and disease damage; 4 represents the fruits with 1-10% of blemished and/or dry; 3 represents the fruits with 11-30% of dry and/or slight blemishes; 2 represents the fruits with 31-50% of dry and/or had moderate blemishes; 1 represents the fruits with 51-60% dry and/or severe blemishes; and 0 represents the fruits with more than 60% of dry and/or very severe blemishes (Silva et al. 2015). Chilling injury scores were given according to the following scale: (0) none visible; (1) slight (≤ 25%); (2) moderate (26-50%); and (3) severe (> 51%).

The data of the experiments was subjected to analysis of variance (ANOVA) with postharvest treatment and storage condition as factors using SPSS 20.0 software. Mean separations was done by using Duncan's multiple range test at $P \leq 0.05$.

3. Results

Weight loss generally increases with storage duration and gives direct information about the storage quality of fruits. The weight loss of untreated pomegranate fruits was 7.9% at 30 days after storage (DAS) where the weight loss of fruits treated with black seed oil (0.5%) + MAP was only 1.4% (Table 1). At the 30 DAS, all treatments were found to be effective in controlling weight loss but the lowest weight loss obtained from the black seed oil applications combined with MAP. During storage, weight loss increased as expected and it reached to 18.9% at untreated control fruits at 150 DAS. At the same time, the weight loss of the fruits treated with 0.5% black seed oil application without MAP; and 0.5% black seed oil application with MAP were 12.1% and 4.7%, respectively.

Table 1. Changes in the weight loss (%) of pomegranate fruits during cold storage, exposed to black seed oil and MAP treatments

Treatments	Weight loss (%)					
	30 DAS	**+7 SL**	**90 DAS**	**+7 SL**	**150 DAS**	**+7 SL**
Control	7.9 a	12.8 a	13.3 a	18.6 a	18.9 a	24.4 a
Black seed oil (0.1)	4.9 b	5.0 b	7.1 b	11.5 b	13.6 b	17.6 b
Black seed oil (0.5)	3.4 c	4.4 bc	6.9 b	11.0 b	12.1 b	15.4 bc
Control + MAP	3.3 c	5.2 bc	6.3 c	12.6 b	8.8 c	18.5 b
Black seed oil (0.1) + MAP	1.9 d	3.6 cd	4.0 d	6.9 c	5.4 d	12.3 d
Black seed oil (0.5) + MAP	1.4 d	3.0 d	4.0 d	7.2 c	4.7 d	11.0 d

Note. DAS: Days After Storage; SL: Shelf Life. Values followed by the same letter or letters within same column are not significantly different at a 5% level (Duncan's multiple range test).

The results about the high efficacy of MAP on the prevention of weight loss are in agreement with previous studies (Kader, 1995; Artes, 2000; Selcuk & Erkan, 2016). When comparing the applications with MAP and without MAP, a significant difference obtained and MAP application found to reduce the weight loss. However, scientific and commercial acceptance of weight loss is around 5% and MAP alone could not provide such preservation after 90 days of storage. Therefore, the results showed that the combination of MAP with black seed oil (either 0.1% or 0.5%) could provide an acceptable control on the weight loss even after 150 days of storage. On the other hand, an important result obtained from this study which is; when fruits are taken out from MAP and put into shelf life, the weight loss increases rapidly. MAP is a dynamic process of changing gaseous composition inside the package (Elyatam & Kader, 1984; Farber et al., 2003). MAP has a pressure on fruits by preventing their respiration and while the respiration decreases, weight loss decreases too. However, when the bags are opened and fruits escape from this situation, they start to respire more and lose weight rapidly (Selcuk & Erkan, 2016). The juice content of fruits was determined as 31.4% at harvest. As expected, juice content decreased during storage duration but MAP was found to protect juice content (Table 2). After 30 days of storage, juice content of the untreated control fruits was measured as 27.9% and second lowest juice content was measured from 0.1% black seed oil application without MAP as 28.9%. The highest juice content was measured from 0.5% black seed oil application with MAP at 30 DAS. Shelf life simulation was found to reduce the juice contents of the fruits as in weight. The juice content of the fruits continued to decrease during storage. After 150 days of storage, the juice content of untreated fruits was measured as 21.9% (lowest) and all other applications found to reduce losses in the juice content of the fruits. Highest juice content was measured from the 0.5% black seed oil application with MAP as 28.5% which is higher than the juice content of control fruits at 30th day after storage. On the other hand, MAP application was determined very effective and juice content of control fruits with MAP was measured as 25.8% after 150 days of storage. After 30 days of storage, visual quality of all treatments, except untreated control, had no significant difference with respect to the harvest quality (Table 3).

Table 2. Changes in the juice content (%) of pomegranate fruits during cold storage, exposed to black seed oil and MAP treatments

Treatments	**Juice content (%)***					
	30 DAS	**+7 SL**	**90 DAS**	**+7 SL**	**150 DAS**	**+7 SL**
At first day of harvest	31.4 a	31.4 a	31.4 a	31.4 a	31.4 a	31.4 a
Control	27.9 d	27.1 d	25.6 d	24.7 d	21.9 e	19.6 d
Black seed oil (0.1)	28.9 c	28.2 c	28.3 c	28.1 c	25.1 d	23.9 c
Black seed oil (0.5)	29.6 c	28.6 c	28.8 c	28.8 b	25.6 d	24.0 c
Control + MAP	30.5 b	29.4 b	28.4 c	28.3 c	25.8 d	24.0 c
Black seed oil (0.1) + MAP	30.1 b	30.3 b	29.3 b	28.9 b	26.7 c	25.8 b
Black seed oil (0.5) + MAP	30.7 b	29.6 b	29.6 b	28.1 c	28.5 b	26.8 b

Note. DAS: Days After Storage; SL: Shelf Life. * Juice percentage was determined by dividing the juice contents to the harvest weight of the fruits, not to the final weight. Values followed by the same letter or letters within same column are not significantly different at a 5% level (Duncan's multiple range test).

Table 3. Changes in the visual quality scores of pomegranate fruits during cold storage, exposed to black seed oil and MAP treatments

Treatments	**Visual quality scores (0-5 scale)**					
	30 DAS	**+7 SL**	**90 DAS**	**+7 SL**	**150 DAS**	**+7 SL**
At first day of harvest	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a	5.00 a
Control	4.83 ab	4.40 b	3.67 c	3.33 e	1.80 e	1.33 e
Black seed oil (0.1)	5.00 a	5.00 a	4.67 b	3.67 d	3.00 d	2.67 d
Black seed oil (0.5)	5.00 a	5.00 a	4.83 ab	3.67 d	3.20 d	2.67 d
Control + MAP	5.00 a	5.00 a	4.67 b	4.00 c	4.00 c	3.00 c
Black seed oil (0.1) + MAP	5.00 a	5.00 a	5.00 a	4.33 b	4.60 b	3.67 b
Black seed oil (0.5) + MAP	5.00 a	5.00 a	5.00 a	4.33 b	4.60 b	3.67 b

Note. DAS: Days After Storage; SL: Shelf Life. Values followed by the same letter or letters within same column are not significantly different at a 5% level (Duncan's multiple range test). Visual quality scores: 5 refers less than 1% of dents, absence of blemishes, and absence of pests and disease; 4 refers fruits with 1-10% of blemishes and/or dry; 3 refers the fruits with 11-30% of dry and/or had minor blemishes; 2 refers the fruits with 31-50% of dry and/or had moderate blemishes; 1 refers the fruits with 51-60% of dry and/or had severe blemishes; and 0 refers dry, very severe blemishes or widespread attack by pests and diseases.

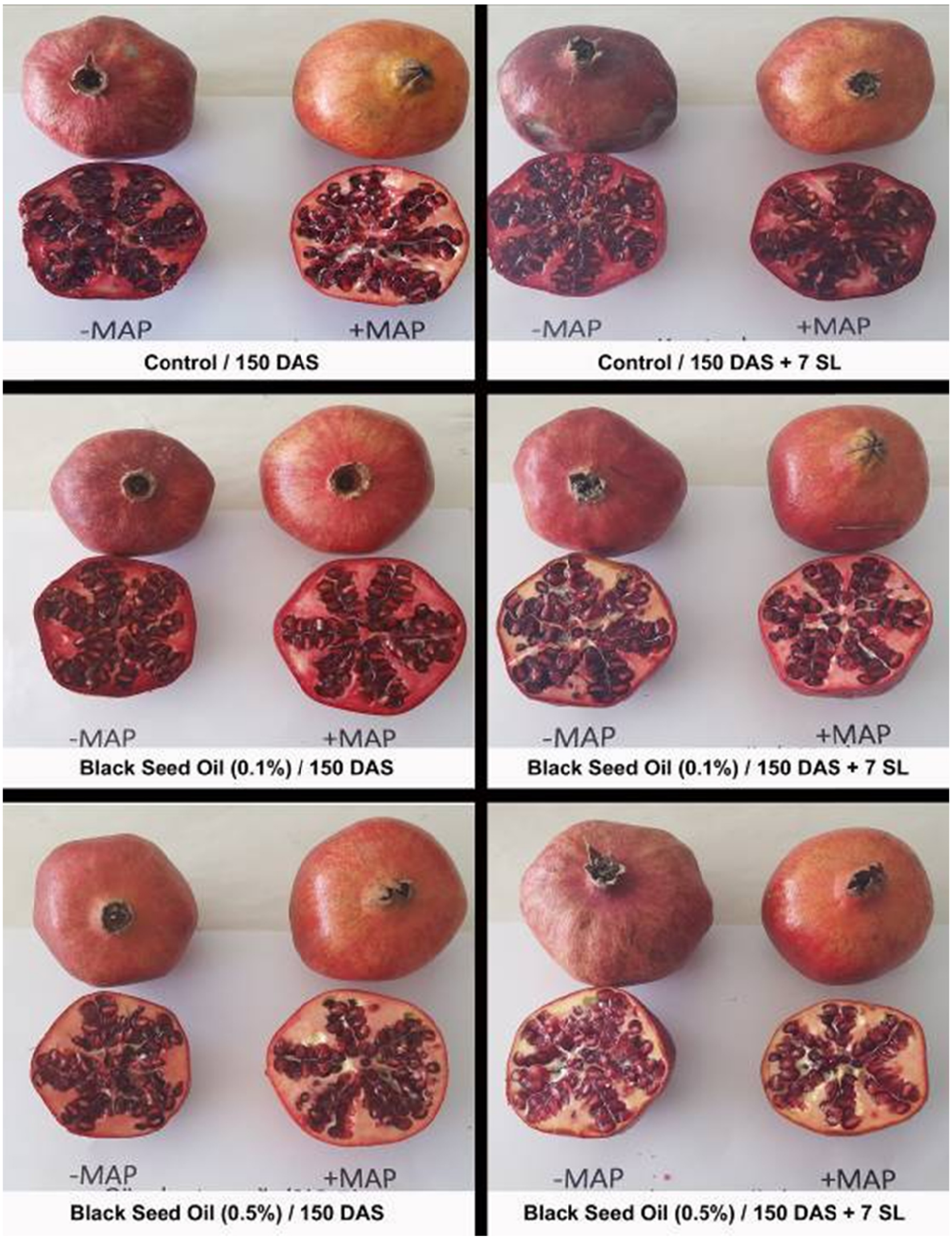

Figure 1. External and internal appearances of pomegranate fruits after 150 days of storage at 6.5±1 ºC with 90-95% relative humidity

After 90 days of storage, both doses of black seed oil with MAP had an excellent maintenance on the quality where no change had been observed. Other treatments without MAP had lower visual quality scores. Chilling injury mainly occurs after exposure to temperatures below 5 ºC for longer than 4 weeks. However, the chilling injury symptoms become more noticeable when transferred to ambient temperature after storage (Elyatem & Kader, 1984). First symptoms noticed at 90^{th} days of storage but 0.5% black seed oil application with or without MAP were found to prevent chilling injury (Table 4). On the other hand, chilling injury symptoms increased when the fruits were taken into shelf life as described by Elyatem and Kader (1984). After 150 days of storage, chilling injury scores of the control fruits without MAP was found to be 1.40 and at the same time, the chilling injury scores of control fruits with MAP were noted as 0.60 which is more than half of the untreated control. The best results obtained from the both black seed oil applications with MAP which was 0.40. Previously, Nerya et al. (2006) reported that MAP reduces the incidence and severity of chilling injury in pomegranates.

Table 4. Changes in the chilling injury scores of pomegranate fruits during cold storage, exposed to black seed oil and MAP treatments

Treatments	Chilling injury scores (0-3 scale)					
	30 DAS	**+7 SL**	**90 DAS**	**+7 SL**	**150 DAS**	**+7 SL**
At first day of harvest	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Control	0.00 a	0.00 a	0.50 c	0.67 b	1.40 d	1.67 e
Black seed oil (0.1)	0.00 a	0.00 a	0.17 ab	0.33 b	0.60 c	1.33 d
Black seed oil (0.5)	0.00 a	0.00 a	0.00 a	0.33 b	0.60 c	1.00 d
Control + MAP	0.00 a	0.00 a	0.17 ab	0.67 b	0.60 c	1.33 d
Black seed oil (0.1) + MAP	0.00 a	0.00 a	0.00 a	0.33 b	0.40 b	0.33 b
Black seed oil (0.5) + MAP	0.00 a	0.00 a	0.00 a	0.33 b	0.40 b	0.67 bc

Note. DAS: Days After Storage; SL: Shelf Life. Values followed by the same letter or letters within same column are not significantly different at a 5% level (Duncan's multiple range test). Chilling injury scores: 0 refers none visible; 1 refers slight (≤ 25%); 2 refers moderate (26-50%); and 3 refers severe (> 51%).

Gray mould (*Botrytis cinerea*) is one of the most important problems reducing postharvest quality and marketability of the produce. The most favourable conditions for the development of gray mould are 5-10 ºC and relative humidity higher than 90%. But, for the prevention of chilling injury and weight loss, pomegranates should be stored at 6-7 ºC with 90-95% relative humidity (Fawole et al., 2013 and Kazemi et al., 2013). Moreover, these storage conditions increase susceptibility to decay. Gray mould started after 30 days of storage and increased during storage and shelf life periods in present study (Table 5.).

Table 5. Changes in the Gray mould decay scores of pomegranate fruits during cold storage, exposed to black seed oil and MAP treatments

Treatments	Gray mould decay scores (0-3 scale)					
	30 DAS	**+7 SL**	**90 DAS**	**+7 SL**	**150 DAS**	**+7 SL**
At first day of harvest	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Control	0.83 d	1.00 e	1.33 d	1.67 e	2.60 e	3.00 d
Black seed oil (0.1)	0.50 c	0.75 c	1.00 d	1.33 d	1.60 d	1.67 c
Black seed oil (0.5)	0.33 b	0.50 b	0.67 bc	0.67 b	1.20 c	1.67 c
Control + MAP	1.00 d	1.50 d	1.00 d	0.67 b	1.00 c	1.33 c
Black seed oil (0.1) + MAP	0.50 c	0.75 c	0.50 b	1.00 c	0.40 b	0.67 b
Black seed oil (0.5) + MAP	0.17 b	0.50 b	0.33 b	1.00 c	0.60 b	0.67 b

Note. DAS: Days After Storage; SL: Shelf Life. Values followed by the same letter or letters within same column are not significantly different at a 5% level (Tukeys (HSD) multiple range test). Gray mould decay scores: 0 refers no infection; 1 refers mould at only crown; 2 refers mould covers less than 25% of the rind; 3 refers mould covers 25-50% of the rind and 4 refers mould covers more than 50% of the rind.

After 150 days of storage, highest gray mould score obtained from the untreated control fruits with 2.60 score which refers around 25-50% mould. All applications were found to be effective in controlling gray mould development but the efficacy increased when applications combined with MAP. The best results obtained from the two doses of black seed oil applications with MAP where the scores were 0.40 and 0.60 (referring mould at only crown). Significant reduction in the development of gray mould has been previously reported for fruits stored in modified atmosphere packaging (Nanda et al., 2001; Maghoub et al., 2013). On the other hand, all applications were found to have a considerable effect on the development of gray mould. Efficacy of black seed oil was previously reported by Hanafi and Hatem (1991), and Maghoub et al. (2013) on the Escherichia coli in laboratory conditions. Results indicated that black seed oil might be used as an alternative for the control of gray mould development on pomegranate fruit.

4. Conclusions

Findings of present study indicate that black seed oil application has considerable effects on the postharvest quality of pomegranates, especially when combined with modified atmosphere packaging. Application of both 0.1% and 0.5% black seed oil with MAP provides good control of weight loss, juice content, chilling injury and gray mould even after 150 days of storage and prolongs the storage duration of pomegranate fruits.

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