


Article

Effects of Putrescine Application on Peach Fruit during Storage

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Abstract: The peach industry faces serious economic losses because of the short “green” life of the fruit at postharvest. In the present study, we investigated the effects of putrescine (PUT) application on the quality characteristics, pattern of ripening, storage behaviour and shelf life of peach fruit during low-temperature storage. The aqueous solution of PUT (0, 1, 2 and 3 mM) was applied to the peach trees at three distinctive stages of fruit growth and development. The fruits, harvested at the commercial stage of maturity, were stored at 1 ± 1 °C and $90 \pm 2\%$ relative humidity for 6 weeks. The data for fruit firmness, total soluble solids (SSC), titratable acidity (TA), ascorbic acid (AsA) content, rate of ethylene production, chilling injury (CI) index and colour perception were collected at harvest and then on a weekly basis throughout the storage period. The results showed that spray application of PUT significantly reduced the incidence of CI and reduced the rates of fruit softening, loss in fruit weight, SSC, TA, AsA content and fading of skin colour during storage, regardless of the doses of PUT applied, or the time of application. However, the positive effects on the quality characteristics of peach fruit, including CI, were more pronounced with the higher doses of PUT, specifically when applied at 2 mM. In conclusion, CI in peach fruit may be substantially alleviated by the spray application of 1–3 mM PUT during fruit growth without compromising the quality of the fruit for up to 6 weeks in low-temperature storage.

Keywords: flesh firmness; fruit ripening; ethylene production; ascorbic acid; fruit colour

1. Introduction

Peach (*Prunus persica* L.) is considered one of the most important stone fruit and it has prodigious demands in the international market. It is one of the most important drupe fruits of Pakistan and it is cultivated on an area of around 14,700 ha that yields about 55,800 tons of fruit annually [1]. During the past decade, the production of peach fruit has gained perceptible momentum worldwide, mainly because of the availability of low-chill cultivars and improved postharvest management practices [2]. Baluchistan and the northern areas of Pakistan are considered peach hubs [3,4]. Despite the munificent increase in peach-growing area, a variety of issues associated with the yield potential and quality of peach fruit at harvest and during storage still need to be resolved systematically [2].

The ripening process of peach, like other climacteric fruits, is characterized by rapid increases in the rates of cellular respiration and ethylene production [5] that, in turn, cause substantial losses in the quality, including firmness and are, thus, responsible for the shorter postharvest shelf life of the fruit. This ripening behaviour of peach fruit epitomizes some serious constraints for efficient management practices such as postharvest handling, storage and transportation. In addition to

other strategies, pre-conditioning, intermittent warming, controlled atmosphere storage and the use of semi-permeable/biodegradable coatings are some of the common postharvest practices used to maintain the quality of peaches and extend their postharvest shelf life [6]. However, many of these practices are associated with undesirable traits of fruit quality such as chilling and/or CO₂ injury, production of ethanol and off-flavour taste due to fruit respiration under anaerobic conditions [7]. The postharvest quality of peach fruit is generally maintained by low-temperature storage. However, it causes chilling injury (CI) to the fruit [8], which further results in wooliness, internal browning and softening of the fruit and makes it more perishable and prone to decay [9]. In addition, the seasonality of many peach cultivars dictates their storage for an extended period of time [10].

Polyamines (PAs) are low-molecular-weight organic cations that are ubiquitous in all living beings. The involvement of PAs in the diverse physiological activities of plants and animals has been well established [11]. In plants, these organic molecules have been demonstrated to play essential roles in growth, development, proliferation and differentiation of cells and they are actively involved in the regulation of biotic/abiotic stress tolerance [12]. The exogenous application of PAs inhibits ethylene production/perception in fresh climacteric produce [13,14]. These molecules have been reported to slow down the ripening phenomenon, while maintaining the firmness of fruits during storage [7]. Through their active involvement in fruit ripening, PAs have been demonstrated to extend the postharvest life of many fruit species, including mango [15], apple [16], peach [17], apricot [18] and plum [7]. However, to the best of our knowledge, information on the use and effects of pre-harvest application of PAs on the quality and storage behaviour of peach fruit has not yet been systematically documented. Therefore, in the present study we aimed to investigate the effects of PUT—applied at certain critical stages of fruit growth—on the quality and postharvest life of the peach fruit cultivar Flordaking during low-temperature storage.

2. Materials and Methods

The selected orchard consisted of 220 trees planted on two acres of land, out of which thirty six peach trees (8-years-old) of the cultivar Flordaking (uniform size and shape)—grafted on Peshawar Local—were selected from a commercial orchard in Madrota Village (33°52'24" N, 72°21'44" E), Tehsil and Distt. Attock in the Punjab Province of Pakistan. The trees were selected on the basis of health and uniformity. The orchard was laid out in the square system with a plant-to-plant and row-to-row distance of 7 m and the plant rows were oriented in the north-south direction.

The aqueous solution of putrescine (PUT; 0, 1, 2 and 3 mM), with Tween-20 used as a surfactant, was applied onto the experimental peach trees till run-off on three stages of fruit growth and development, that is, cell division (20 March), pit hardening/lag phase (11 April) and cell enlargement (19 April), in the years 2011 and 2012. There was not any difference in the environmental data of the locality for both the years. The total numbers of twelve (12) peach trees from each experimental block were used in the experiment and three tree served as a treatment unit, replicated three times.

At the commercial stage of fruit maturity, 80 peach fruit, visibly uniform in size and shape and free from diseases/disorders/bruises and other physical damage, were harvested from each treatment unit including control and transported to the Post-harvest Laboratory at the Department of Horticulture, PMAS Arid Agriculture University, Rawalpindi, Pakistan. The fruit samples from each replication were divided into four equal lots, each containing 20 fruit. One lot was analysed for colour perception, rate of ethylene production, flesh firmness, soluble solids contents (SSC), titratable acidity (TA) and ascorbic acid (AsA) content at harvest. The other three lots were packed in corrugated boxes and stored at 1 ± 1 °C and $90 \pm 2\%$ relative humidity (RH) for 6 weeks. The incidence of fruit decay/CI was recorded for 4 weeks after the fruit were subjected to low-temperature storage, whereas weight loss, fruit firmness, rate of ethylene production, electrolytic leakage/membrane integrity and colour perception of peach fruit were evaluated on a weekly basis during storage.

2.1. Physiological Losses in Weight

The initial weight (W_1) of each box containing fruit samples from each replication was noted on the day of harvest (just prior to cold storage) and then subsequently on a weekly basis during storage. The physiological loss in fruit weight (PLW), expressed as the percentage of initial fruit weight, was calculated using the following formula:

$$\text{PLW (\%)} = (W_1 - W_2) \times 100/W_1$$

where, W_1 = initial weight and W_2 = weight of peach fruit after different intervals during storage.

2.2. Fruit Firmness

The flesh firmness was measured using the paped surface of peach fruit with a digital penetrometer, (Model BKD020; WEL, Willow Bank Electronics Ltd., Napier, New Zealand) fitted with an 8-mm plunger. The values were expressed in Newton (N) force, required to puncture the fruit flesh to a constant depth.

2.3. Fruit Color

The colour perception on the exterior sides along the equator of peach fruit was measured using a portable/hand-held chroma meter (CR-400; Konica Minolta 7 Sensing, Inc., Osaka, Japan) in Commission Internationale de L' Eclariage (CIE) units (L^* , a^* and b^*), as described by Shafiq and Singh [19]. Using the values of a^* and b^* , the chroma (C^*) and hue angle of the fruit colour were calculated [20].

2.4. Fruit Decay

The number of fruit showing lesions, rotting or surface softening was counted at the end of the storage period. The results were expressed as the percentage (%) of decayed fruit.

2.5. SSC, TA and AsA Content

SSC of the juice freshly extracted from the composite fruit samples in each replication ($n = 3$) were estimated with a portable refractometer (Model: FG-103, Chincan brand, Hangzhou Weiku Co. Ltd., Zhejiang, China) and the results were expressed as °Brix. TA of the juice was gauged using the standard method [21]. AsA content of the fresh peach juice was estimated using the method previously described by [22]. Pulp of peach (5 g) from ten fruits was ground using mortar and pestle with 5 mL of 1.0% HCl and the mixture was then centrifuged for ten minutes at 10,000 rpm. The supernatant was used for AsA estimation. Absorbance of the extract was noted at 243 nm by the spectrophotometer (Model sp3000 plus, Optima Japan).

2.6. Rate of Ethylene Production

The rate of ethylene production by the peach fruit was assayed at harvest and then on a weekly basis throughout the storage period. Three fruits from each replication were weighed together and maintained for 1 h in an airtight glass jar (head-space volume: 1 L) fitted with a rubber septum. After the incubation period, 1 mL of air was collected from the jar with a syringe through the rubber septum. The air sample was directly injected into the gas chromatograph (Agilent Technologies, 6890 N Network GC system, Palo Alto, CA, USA) fitted with a flame ionization detector. The thermal conditions of the column, injector and detector were maintained as described previously [23]. The rate of ethylene production by the peach fruit was reported as $\mu\text{mol kg}^{-1} \text{h}^{-1}$.

2.7. Electrolyte Leakage

The membrane integrity of skin discs from the peach fruit was estimated in terms of the relative rate of electrolyte leakage (EL), as described by Zheng et al. [24] with minor modifications. 15 skin-discs (8-mm diameter), excised from peach fruit ($n = 3$), were submerged in double de-ionized water (15 mL), vacuum infiltrated (30 min.) and shook for 2 h. Then, the initial electric conductance (EC_0) of the sample was noted. Following digestion of the sample at 95 °C for 30 min, the final electric conductance (EC_f) was noted. The percent EL was calculated by the following equation:

$$EL (\%) = (EC_0/EC_f) \times 100$$

2.8. CI (Internal Browning)

The degree of CI was assessed by visual observation of the extent of flesh browning on the flat surface of the mesocarp in the peach fruit by cutting the fruit from the middle (along the axial diameter) after four week of storage, as described by Wang et al. [25].

2.9. Economic Analysis

Economics of different spray treatments is determined by BCR (benefit cost ratio). More BCR means the treatment is more cost effective and benefiting the farmers. BCR was estimated by formula: $BCR = TR/TC$, where TR and TC represent total revenue from experimental fruits and total cost of using each spray treatment, respectively.

2.10. Statistical Analysis

The data collected using a completely randomized design were subjected to analysis of variance and all the statistical analyses were performed with Statistix 8.1 software [26]. Multiple comparisons among the mean values were performed with Duncan's multiple range test, with the error level set at 5% [27].

3. Results

3.1. PLW

The results confirmed a gradual but steady increase in the PLW of Flordaking peaches during the low-temperature storage for both the years under study (Table 1). The increase in weight loss was more pronounced during the storage period of 4–6 weeks. After 6 weeks of cold storage, the minimum PLWs (14.66 and 21.68) were observed in the peach fruit harvested from the trees treated with 2 mM PUT, whereas the highest PLWs (35.16 and 34.66) were recorded in the fruit harvested from the control trees, in the year 2011 and 2012, respectively. In general, all PUT treatments significantly reduced the rate of weight loss in the peach fruit during the 6 weeks in low-temperature storage, regardless of the concentration of PUT and time of spray application.

3.2. Fruit Firmness

The data collected during this study revealed that the peach fruit harvested from the PUT-treated trees were significantly firmer than those harvested from the untreated (control) trees at harvest (Table 1). In addition, the peach fruit from the PUT-treated trees maintained their flesh firmness during storage for significantly a longer period of time than those harvested from the control peach trees. At the end of the storage period (i.e., 6 weeks), the fruit harvested from the trees treated with 2 mM PUT exhibited 2.4- and 1.6-fold higher flesh firmness than those collected from control trees for 2011 and 2012, respectively. A rapid phase (the 1st phase) of softening in the peach fruit was observed during the 1st week of cold-storage, followed by a significantly slower rate of fruit softening (the 2nd phase) during 2–3 weeks of storage, regardless of the treatments used, including the control. The 3rd

phase of rapid loss in fruit firmness was observed during 3–6 weeks of cold storage (Table 1). At the end of cold storage, the peach fruit harvested from the PUT-treated trees maintained higher levels of flesh firmness, irrespective of the level/dose or time of PUT application. However, 2 mM PUT application proved to be significantly ($p \leq 0.05$) a better treatment for maintaining the flesh firmness of peach fruit, compared to control as well as other treatments.

3.3. Fruit Color

It is evident from Table 1 that the brightness (L^* value) of peach skin decreased as the storage period increased (temperature: 1 ± 1 °C; RH: $90 \pm 2\%$). The peach fruit treated with 2 mM PUT had significantly higher L^* values than those subjected to other treatments, including the control, irrespective of the time of application during both of the experimental repetitions (2011 and 2012). The peach fruit collected from the control (untreated) trees had the lowest value of skin brightness at harvest as well as during the 6 weeks of cold storage. Foliar application of PUT was found to be effective in maintaining the degree of red blush (a^* value) on the exterior of peach fruit during the low-temperature storage (Table 1). At the end of 6 weeks of storage, the development of red/pink blush on the surface of the peach fruit was more profound than that observed immediately at harvest, regardless of the treatments used. The fruit collected from the peach trees treated with 2 mM PUT significantly retained a lower level of red blush (a^* value) at the end of 6 weeks of storage, irrespective of the time of PUT application, for the years 2011 and 2012.

3.4. Proximate Quality Characteristics

The results of the present study revealed that PUT treatments significantly improved the quality attributes of Flordaking peaches, such as TA (Table 2) and AsA (Table 3), whereas the SSC of the fruit was not affected (Table 2).

The results confirmed a gradual but steady increase in the SSC of peach fruit till the end of storage (Table 2). The increase was more pronounced at the 4th week of cold storage. At the end of storage period, the PUT treatments significantly maintained the SSC of the fruit (Table 2). After the 6 weeks of cold storage, a significantly lower level of SSC was recorded in the fruits harvested from the trees that received 2 mM PUT (Table 2) than in those harvested from the untreated peach trees, irrespective of the time of PUT application.

The peach fruit exhibited, relatively, a linear and gradual decrease in TA, unlike SSC, as the storage period increased (Table 2), regardless of the PUT treatments. However, the fruit from the PUT-treated trees retained their TA for a longer period during low-temperature storage, regardless of the level and time of PUT application. It may be noted from the results (Table 2) that 2 mM PUT application produced peach fruit with the highest TA, whereas the fruit from the untreated (control) trees had the lowest TA. However, at the end of the storage period, the fruit ripening index (SSC:TA) was significantly higher in the fruit collected from the untreated trees, whereas the peach fruit from the trees treated with 2 mM PUT had the lowest SSC:TA value.

The results of the present study demonstrated that an extended period of cold storage resulted in a linear ($p \leq 0.001$) decrease in AsA content in peach fruit, regardless of the treatments used (Table 3). However, PUT application significantly delayed the rate of decrease in the AsA level of the fruit, regardless of the dose and time of PUT application. It may be noted that the fruit from the trees treated with 2 mM PUT had around 1.7-fold higher AsA content than those treated with water only (control) at the end of the low-temperature storage, irrespective of the time of PUT application, during both of the experimental years under study.

Table 1. Effects of exogenous application of putrescine on the physiological loss of weight, firmness and objective colour of peach skin during storage.

Storage Period	Putrescine Treatment	Weight Loss		Fruit Firmness		Brightness of Fruit-Skin Colour		Blush Colour of Fruit Skin	
(weeks)	(mM)	(%)		(N)		(L*)		(a*)	
		2011	2012	2011	2012	2011	2012	2011	2012
0	0	0.00 ± 00 ^O	0.00 ± 00 ^J	75.33 ± 1.91 ^{BCD}	97.43 ± 6.83 ^{A-D}	68.45 ± 0.31 ^{ABC}	72.47 ± 0.44 ^{ABC}	5.97 ± 0.45 ^J	4.14 ± 0.45 ^K
	1	0.00 ± 00 ^O	0.00 ± 00 ^J	87.70 ± 3.71 ^B	104.63 ± 5.44 ^{ABC}	69.55 ± 0.91 ^{AB}	72.66 ± 0.79 ^{ABC}	5.36 ± 0.24 ^J	4.10 ± 0.53 ^K
	2	0.00 ± 00 ^O	0.00 ± 00 ^J	109.07 ± 0.92 ^A	112.83 ± 9.31 ^A	69.34 ± 1.18 ^{AB}	73.63 ± 0.74 ^A	5.01 ± 0.26 ^J	4.15 ± 0.56 ^{JK}
	3	0.00 ± 00 ^O	0.00 ± 00 ^J	101.60 ± 2.96 ^A	110.27 ± 5.16 ^{AB}	68.94 ± 0.98 ^{AB}	73.81 ± 0.49 ^A	4.99 ± 0.49 ^J	4.04 ± 0.62 ^K
Day 0		0.00 ^G	0.00 ^F	93.43 ^A	106.29 ^A	69.07 ^A	73.14 ^A	5.33 ^E	4.11 ^F
1	0	8.52 ± 0.24 ^{KL}	6.0 ± 0.82 ^{HJ}	36.35 ± 2.11 ^{GHI}	75.67 ± 2.87 ^{EFG}	61.04 ± 2.25 ^{C-F}	70.58 ± 0.58 ^{A-D}	14.27 ± 0.37 ^{C-G}	5.52 ± 0.25 ^{H-K}
	1	5.87 ± 0.24 ^{MN}	5.02 ± 0.48 ^{IJ}	62.25 ± 2.94 ^{DE}	78.33 ± 2.04 ^{D-G}	69.01 ± 1.51 ^{AB}	72.32 ± 0.16 ^{ABC}	11.26 ± 0.70 ^{FG}	7.30 ± 0.57 ^{H-K}
	2	4.99 ± 0.17 ^N	2.77 ± 0.79 ^J	80.00 ± 5.00 ^{BC}	91.67 ± 6.05 ^{B-E}	70.00 ± 3.06 ^{AB}	73.42 ± 0.24 ^{AB}	6.20 ± 0.35 ^{IJ}	7.12 ± 0.36 ^{H-K}
	3	5.62 ± 0.27 ^{MN}	3.94 ± 0.33 ^{IJ}	66.35 ± 6.04 ^{CDE}	85.67 ± 2.86 ^{C-F}	69.73 ± 1.26 ^{AB}	72.79 ± 0.75 ^{ABC}	7.17 ± 0.20 ^{HJ}	4.54 ± 0.73 ^{IJK}
Week 1		6.25 ^F	4.45 ^E	61.24 ^B	82.83 ^B	67.44 ^{AB}	72.28 ^{AB}	9.73 ^D	6.12 ^E
2	0	13.27 ± 0.31 ^{GH}	17.06 ± 1.27 ^{DEF}	31.94 ± 3.18 ^{G-K}	45.67 ± 4.08 ^{H-K}	57.79 ± 1.07 ^{E-H}	70.16 ± 0.37 ^{A-D}	17.57 ± 0.45 ^{BCD}	14.26 ± 1.29 ^{DEF}
	1	9.73 ± 0.47 ^{JK}	11.97 ± 2.96 ^{FGH}	53.00 ± 0.45 ^{EF}	55.00 ± 5.81 ^{HI}	65.23 ± 1.54 ^{A-E}	71.76 ± 1.12 ^{ABC}	13.96 ± 0.21 ^{D-G}	8.08 ± 1.37 ^{H-K}
	2	7.23 ± 0.18 ^{LM}	6.29 ± 0.48 ^{HJ}	75.42 ± 1.02 ^{BCD}	65.67 ± 1.34 ^{FGH}	70.42 ± 0.47 ^A	72.33 ± 0.99 ^{ABC}	10.23 ± 0.61 ^{GHI}	8.64 ± 0.82 ^{G-J}
	3	9.59 ± 0.54 ^{JK}	9.88 ± 0.80 ^{GHI}	58.77 ± 0.30 ^E	65.00 ± 2.53 ^{GH}	69.52 ± 0.35 ^{AB}	71.65 ± 0.83 ^{ABC}	11.13 ± 0.69 ^{FGH}	8.18 ± 1.01 ^{H-K}
Week 2		9.95 ^E	11.30 ^D	54.78 ^C	57.83 ^C	65.74 ^{BC}	71.46 ^{AB}	13.22 ^C	9.79 ^D
3	0	18.74 ± 0.12 ^D	24.78 ± 1.97 ^{BC}	18.43 ± 0.90 ^{K-O}	30.33 ± 0.89 ^{JKL}	54.80 ± 0.49 ^{FGH}	69.84 ± 0.21 ^{A-D}	18.05 ± 0.78 ^{BC}	20.64 ± 0.83 ^{AB}
	1	11.91 ± 0.24 ^{HJ}	21.08 ± 1.26 ^{CDE}	28.45 ± 2.71 ^{H-L}	46.33 ± 1.87 ^{HJ}	63.42 ± 2.63 ^{A-E}	69.80 ± 0.30 ^{A-D}	14.66 ± 2.68 ^{B-F}	14.94 ± 0.32 ^{CDE}
	2	8.91 ± 0.08 ^{KL}	14.81 ± 1.41 ^{EFG}	44.02 ± 2.78 ^{FG}	43.00 ± 2.10 ^{IJK}	69.50 ± 1.91 ^{AB}	71.62 ± 0.87 ^{ABC}	10.73 ± 0.05 ^{F-H}	9.00 ± 0.70 ^{GHI}
	3	12.06 ± 0.13 ^{HI}	15.92 ± 0.67 ^{D-G}	39.90 ± 1.57 ^{FGH}	43.67 ± 1.87 ^{IJK}	67.32 ± 0.64 ^{A-D}	71.02 ± 0.54 ^{ABC}	11.48 ± 0.39 ^{FG}	9.87 ± 0.78 ^{FGH}
Week 3		12.90 ^D	19.15 ^C	32.70 ^D	40.83 ^D	64.11 ^{CD}	70.56 ^{BC}	13.73 ^C	13.61 ^C
4	0	23.26 ± 0.89 ^C	28.59 ± 1.81 ^{AB}	13.39 ± 3.14 ^{MNO}	27.00 ± 1.16 ^{JKL}	54.79 ± 0.86 ^{FGH}	64.86 ± 0.14 ^D	18.07 ± 0.26 ^{BC}	21.17 ± 0.65 ^{AB}
	1	13.23 ± 0.31 ^{GH}	24.10 ± 1.23 ^{BC}	23.36 ± 1.40 ^{I-N}	32.00 ± 1.16 ^{JKL}	63.98 ± 1.97 ^{A-E}	68.20 ± 0.95 ^{A-D}	14.65 ± 0.44 ^{B-F}	17.66 ± 2.01 ^{BCD}
	2	10.19 ± 0.26 ^{IJK}	19.35 ± 0.56 ^{CDE}	32.30 ± 1.68 ^{G-J}	43.33 ± 2.87 ^{IJK}	69.65 ± 0.65 ^{AB}	70.38 ± 0.14 ^{A-D}	11.37 ± 0.41 ^{FG}	9.76 ± 0.98 ^{GH}
	3	12.65 ± 0.32 ^{GH}	19.74 ± 2.20 ^{CDE}	28.20 ± 0.80 ^{H-L}	38.00 ± 3.63 ^{I-L}	68.00 ± 1.12 ^{A-D}	71.11 ± 0.75 ^{ABC}	12.08 ± 0.21 ^{EFG}	12.81 ± 0.08 ^{EFG}
Week 4		14.83 ^C	22.95 ^B	24.31 ^E	35.08 ^{DE}	63.76 ^{CD}	68.64 ^{CD}	14.04 ^C	15.35 ^{BC}
5	0	29.94 ± 0.84 ^B	29.72 ± 1.22 ^A	9.70 ± 0.66 ^{NO}	25.67 ± 0.34 ^{KL}	53.03 ± 1.15 ^{FGH}	64.83 ± 1.24 ^D	22.76 ± 0.74 ^A	21.56 ± 0.67 ^{AB}
	1	16.56 ± 0.33 ^E	25.61 ± 1.25 ^{AB}	15.50 ± 0.76 ^{L-O}	32.33 ± 1.87 ^{JKL}	62.48 ± 1.08 ^{B-F}	67.45 ± 0.22 ^{BCD}	16.40 ± 1.61 ^{BCD}	18.50 ± 0.46 ^{BCD}
	2	11.47 ± 0.88 ^{HJ}	19.75 ± 0.69 ^{CD}	24.33 ± 3.28 ^{I-M}	40.67 ± 0.67 ^{I-L}	68.45 ± 0.62 ^{ABC}	70.47 ± 2.07 ^{A-D}	13.65 ± 0.62 ^{D-G}	12.94 ± 0.34 ^{EFG}
	3	15.82 ± 0.35 ^{EF}	19.32 ± 2.43 ^{BC}	18.53 ± 3.76 ^{I-O}	36.33 ± 3.55 ^{I-L}	67.97 ± 0.94 ^{A-D}	70.37 ± 0.35 ^{A-D}	14.66 ± 0.59 ^{B-F}	15.24 ± 0.41 ^{CDE}
Week 5		18.44 ^B	23.60 ^B	17.02 ^F	33.75 ^{DE}	62.98 ^{CD}	68.28 ^{CD}	16.87 ^B	17.06 ^B

Table 1. Cont.

Storage Period	Putrescine Treatment	Weight Loss		Fruit Firmness		Brightness of Fruit-Skin Colour		Blush Colour of Fruit Skin	
6	0	35.16 ± 0.49 ^A	34.66 ± 2.42 ^A	8.73 ± 0.84 ^O	22.00 ± 0.58 ^L	51.83 ± 1.19 ^H	64.84 ± 2.42 ^D	26.00 ± 0.04 ^A	23.45 ± 0.41 ^A
	1	20.25 ± 0.40 ^D	29.12 ± 1.67 ^{AB}	13.23 ± 0.64 ^{MNO}	31.33 ± 1.87 ^{JKL}	60.54 ± 2.66 ^{D-G}	66.96 ± 0.91 ^{CD}	18.48 ± 0.52 ^B	20.97 ± 0.74 ^{AB}
	2	14.44 ± 0.32 ^{FG}	21.79 ± 1.12 ^{CD}	20.90 ± 0.32 ^{J-O}	34.67 ± 1.46 ^{I-L}	67.72 ± 1.27 ^{A-D}	69.54 ± 2.04 ^{A-D}	16.06 ± 0.24 ^{B-E}	18.19 ± 1.10 ^{BCD}
	3	19.55 ± 0.54 ^D	23.55 ± 0.60 ^{BC}	15.67 ± 1.43 ^{L-O}	36.33 ± 2.87 ^{I-L}	65.68 ± 0.93 ^{A-D}	68.96 ± 2.77 ^{A-D}	17.03 ± 0.35 ^{BCD}	18.77 ± 0.85 ^{BC}
Week 6		22.35 ^A	27.28 ^A	14.63 ^F	31.08 ^E	61.44 ^D	67.58 ^F	19.39 ^A	20.35 ^A
LSD (<i>p</i> < 0.05)		1.94	6.45	13.77	20.42	7.74	5.98	4.06	4.50
Conc.		**	**	**	**	**	**	**	**
Strg.		**	**	**	**	**	**	**	**
Conc. × Strg.		**	**	**	NS	**	NS	**	**

N = newton (force), mM = millimoles, Conc. = Concentration/dose, Strg. = storage period, NS = non-significant, ** = highly significant, the means within a column having same letters are statistically non-significant using the Least Significant Difference (LSD) Test.

Table 2. Effects of exogenous application of putrescine on the organoleptic properties of peach fruit during storage.

Storage Period (weeks)	Putrescine Treatment (mM)	SSC		TA		SSC:TA	
		(^o Brix)		(%)		(ratio)	
		2011	2012	2011	2012	2011	2012
0	0	9.32 ± 0.13 ^H	9.17 ± 0.08 ^E	0.93 ± 0.01 ^{D-G}	1.09 ± 0.02 ^{A-D}	9.99 ± 0.21 ^{F-N}	8.43 ± 0.09 ^{JK}
	1	9.35 ± 0.23 ^H	9.23 ± 0.37 ^E	1.10 ± 0.12 ^{A-D}	1.11 ± 0.01 ^{ABC}	8.53 ± 0.31 ^{L-O}	8.34 ± 0.40 ^{JK}
	2	9.53 ± 0.03 ^{FGH}	9.33 ± 0.20 ^E	1.22 ± 0.01 ^A	1.20 ± 0.03 ^A	7.82 ± 0.02 ^O	8.30 ± 0.37 ^{JK}
	3	9.45 ± 0.12 ^{GH}	9.40 ± 0.10 ^E	1.15 ± 0.01 ^{AB}	1.13 ± 0.03 ^{AB}	8.21 ± 0.21 ^{NO}	7.80 ± 0.21 ^K
Day 0		9.41 ^E	9.28 ^C	1.10 ^A	1.13 ^A	8.64 ^F	8.22 ^F
1	0	10.52 ± 0.31 ^{C-G}	10.33 ± 0.29 ^{A-E}	0.88 ± 0.03 ^{E-I}	0.96 ± 0.02 ^{C-H}	12.03 ± 0.75 ^{DEF}	10.82 ± 0.40 ^{E-K}
	1	9.73 ± 0.13 ^{E-H}	10.00 ± 0.17 ^{B-E}	1.01 ± 0.010 ^{B-F}	1.00 ± 0.06 ^{B-G}	9.62 ± 0.21 ^{I-O}	10.10 ± 0.73 ^{G-K}
	2	9.58 ± 0.22 ^{FGH}	9.33 ± 0.44 ^E	1.15 ± 0.010 ^{ABC}	1.06 ± 0.022 ^{A-D}	8.35 ± 0.06 ^{MNO}	8.80 ± 0.55 ^{JK}
	3	9.45 ± 0.15 ^{GH}	9.57 ± 0.23 ^{DE}	1.10 ± 0.14 ^{A-D}	1.01 ± 0.08 ^{B-G}	8.80 ± 0.86 ^{K-O}	9.60 ± 0.86 ^{IJK}
Week 1		9.82 ^{DE}	9.81 ^{BC}	1.03 ^{AB}	1.007 ^B	9.70 ^E	9.83 ^E

Table 2. Cont.

Storage Period	Putrescine Treatment	SSC		TA		SSC:TA	
2	0	10.65 ± 0.02 ^{C-F}	10.43 ± 0.29 ^{A-E}	0.79 ± 0.02 ^{G-K}	0.97 ± 0.00 ^{B-H}	13.57 ± 0.48 ^{CD}	10.72 ± 0.29 ^{E-K}
	1	9.99 ± 0.06 ^{D-H}	10.43 ± 0.93 ^{A-E}	0.96 ± 0.02 ^{DEF}	0.97 ± 0.02 ^{B-H}	10.37 ± 0.23 ^{F-M}	10.74 ± 1.04 ^{E-K}
	2	9.68 ± 0.26 ^{E-H}	10.00 ± 0.29 ^{B-E}	1.10 ± 0.03 ^{A-D}	1.04 ± 0.02 ^{A-E}	8.79 ± 0.29 ^{K-O}	9.60 ± 0.16 ^{IJK}
	3	9.71 ± 0.09 ^{E-H}	9.87 ± 0.19 ^{CDE}	1.02 ± 0.02 ^{B-F}	0.99 ± 0.02 ^{B-G}	9.48 ± 0.15 ^{I-O}	9.91 ± 0.41 ^{H-K}
Week 2		10.01 ^{CD}	10.18 ^B	0.97 ^{BC}	1.00 ^B	10.55 ^D	10.25 ^{DE}
3	0	11.29 ± 0.16 ^{ABC}	11.67 ± 0.42 ^{AB}	0.76 ± 0.01 ^{H-K}	0.93 ± 0.01 ^{D-I}	14.96 ± 0.26 ^{BC}	12.48 ± 0.36 ^{D-I}
	1	10.06 ± 0.11 ^{D-H}	10.73 ± 0.12 ^{A-E}	0.88 ± 0.018 ^{E-H}	0.96 ± 0.022 ^{C-H}	11.41 ± 0.09 ^{E-J}	11.20 ± 0.38 ^{E-J}
	2	9.76 ± 0.27 ^{E-H}	10.63 ± 0.23 ^{A-E}	1.05 ± 0.030 ^{A-E}	1.02 ± 0.015 ^{B-F}	9.32 ± 0.31 ^{J-O}	10.38 ± 0.08 ^{F-K}
	3	9.76 ± 0.11 ^{E-H}	10.53 ± 0.22 ^{A-E}	1.02 ± 0.00 ^{B-F}	0.98 ± 0.015 ^{B-G}	9.53 ± 0.27 ^{I-O}	10.72 ± 0.07 ^{E-K}
Week 3		10.21 ^{CD}	10.85 ^A	0.93 ^{CD}	0.98 ^B	11.30 ^{CD}	11.20 ^{CD}
4	0	11.28 ± 0.23 ^{ABC}	11.37 ± 0.19 ^{ABC}	0.71 ± 0.02 ^{IJK}	0.84 ± 0.03 ^{G-J}	16.04 ± 0.33 ^{AB}	13.48 ± 0.32 ^{CDE}
	1	10.29 ± 0.31 ^{C-H}	10.87 ± 0.03 ^{A-E}	0.90 ± 0.02 ^{E-H}	0.84 ± 0.00 ^{G-J}	11.50 ± 0.36 ^{D-I}	12.86 ± 0.04 ^{D-H}
	2	10.06 ± 0.16 ^{D-H}	10.62 ± 0.31 ^{A-E}	1.03 ± 0.009 ^{B-F}	0.97 ± 0.015 ^{B-H}	9.74 ± 0.23 ^{H-O}	10.92 ± 0.35 ^{E-J}
	3	10.09 ± 0.03 ^{D-H}	10.57 ± 0.29 ^{A-E}	1.02 ± 0.015 ^{B-F}	0.88 ± 0.022 ^{E-J}	9.85 ± 0.02 ^{G-O}	12.05 ± 0.44 ^{D-I}
Week 4		10.43 ^{BC}	10.89 ^A	0.91 ^{CD}	0.89 ^C	11.79 ^{BC}	12.33 ^C
5	0	11.90 ± 0.29 ^{AB}	12.00 ± 0.25 ^A	0.70 ± 0.01 ^{JK}	0.75 ± 0.03 ^{JKL}	17.18 ± 0.39 ^A	16.07 ± 1.02 ^{ABC}
	1	10.55 ± 0.11 ^{C-G}	11.23 ± 0.29 ^{A-D}	0.88 ± 0.03 ^{E-H}	0.77 ± 0.03 ^{I-L}	11.96 ± 0.47 ^{D-G}	14.67 ± 0.69 ^{BCD}
	2	10.19 ± 0.51 ^{C-H}	10.70 ± 0.36 ^{A-E}	1.01 ± 0.01 ^{B-F}	0.88 ± 0.01 ^{E-J}	10.12 ± 0.12 ^{F-N}	12.12 ± 0.48 ^{D-I}
	3	10.22 ± 0.20 ^{C-H}	10.73 ± 0.12 ^{A-E}	0.97 ± 0.02 ^{DEF}	0.81 ± 0.04 ^{H-K}	10.62 ± 0.71 ^{F-L}	13.26 ± 0.76 ^{C-F}
Week 5		10.72 ^{AB}	11.17 ^A	0.89 ^D	0.80 ^D	12.47 ^{AB}	14.03 ^B
6	0	12.26 ± 0.07 ^A	11.93 ± 0.23 ^A	0.69 ± 0.010 ^K	0.63 ± 0.022 ^L	17.84 ± 0.50 ^A	19.06 ± 0.52 ^A
	1	11.10 ± 0.27 ^{A-D}	11.37 ± 0.20 ^{ABC}	0.87 ± 0.023 ^{F-J}	0.65 ± 0.017 ^{KL}	12.84 ± 0.45 ^{DE}	17.57 ± 0.76 ^{AB}
	2	10.42 ± 0.30 ^{C-H}	11.12 ± 0.34 ^{A-D}	0.98 ± 0.010 ^{C-F}	0.89 ± 0.040 ^{E-J}	10.67 ± 0.18 ^{F-K}	12.51 ± 0.52 ^{D-I}
	3	10.82 ± 0.08 ^{B-E}	11.25 ± 0.28 ^{A-D}	0.92 ± 0.008 ^{E-H}	0.86 ± 0.037 ^{F-J}	11.77 ± 0.43 ^{D-H}	13.16 ± 0.78 ^{C-G}
Week 6		11.15 ^A	11.42 ^A	0.86 ^D	0.76 ^D	13.28 ^A	15.57 ^A
LSD ($p < 0.05$)		1.16	1.70	0.18	0.17	2.11	3.08
Conc.		**	**	**	**	**	**
Strg.		**	**	**	**	**	**
Conc. × Strg.		NS	NS	NS	NS	**	**

SSC = soluble solids content, TA = titratable acidity, mM = Millimoles, Conc. = concentration, Strg. = storage, NS = non-significant, ** = highly significant, the means within a column having same letters are statistically non-significant using the Least Significant Difference (LSD) Test.

Table 3. Effects of exogenous application of putrescine on ascorbic acid content, rate of ethylene production and the relative electrolyte leakage of the skin of peach fruit during storage.

Storage Period (weeks)	Putrescine Treatment (mM)	AsA Content		Rate of Ethylene Production		Relative Electrolyte Leakage	
		(mg/100 g, fw)		$(\mu\text{L Kg}^{-1} \text{h}^{-1})$		(%)	
		2011	2012	2011	2012	2011	2012
0	0	4.33 ± 0.29 ^{D-I}	4.90 ± 0.48 ^{C-F}	20.53 ± 0.78 ^{IJK}	7.34 ± 0.24 ^J	44.06 ± 0.18 ^{F-J}	34.92 ± 20.5 ^{C-H}
	1	4.97 ± 0.49 ^{A-F}	5.70 ± 0.15 ^{ABC}	16.65 ± 0.52 ^{LM}	7.42 ± 0.18 ^J	37.43 ± 1.56 ^{J-M}	32.70 ± 2.47 ^{E-H}
	2	6.17 ± 0.27 ^A	6.38 ± 0.20 ^A	14.15 ± 0.37 ^M	6.78 ± 0.28 ^J	29.66 ± 0.59 ^N	27.78 ± 0.37 ^H
	3	5.97 ± 0.29 ^{AB}	6.32 ± 0.16 ^{AB}	14.55 ± 0.25 ^M	6.69 ± 0.21 ^J	34.28 ± 1.70 ^{LMN}	31.37 ± 1.32 ^{FGH}
Day 0		5.36 ^A	5.83 ^A	16.47 ^E	7.06 ^F	36.36 ^E	31.69 ^D
1	0	3.93 ± 0.31 ^{F-K}	4.65 ± 0.00 ^{C-H}	25.77 ± 0.42 ^{DEF}	26.59 ± 0.41 ^E	49.53 ± 0.77 ^{EFG}	35.22 ± 8.56 ^{C-H}
	1	4.60 ± 0.15 ^{C-H}	4.60 ± 0.18 ^{C-H}	20.65 ± 0.14 ^{IJK}	21.88 ± 0.69 ^{FG}	40.39 ± 1.56 ^{H-M}	36.87 ± 0.74 ^{B-H}
	2	5.77 ± 0.03 ^{ABC}	6.28 ± 0.12 ^{AB}	18.29 ± 0.55 ^{KL}	18.10 ± 0.59 ^I	32.92 ± 0.31 ^{MN}	30.07 ± 3.45 ^{GH}
	3	5.50 ± 0.25 ^{A-D}	5.48 ± 0.31 ^{A-E}	19.34 ± 0.34 ^{JKL}	19.44 ± 0.30 ^{HI}	35.72 ± 1.08 ^{K-N}	31.63 ± 2.72 ^{FGH}
Week 1		4.95 ^{AB}	5.25 ^B	21.01 ^D	21.50 ^E	39.64 ^D	33.45 ^{CD}
2	0	3.63 ± 0.29 ^{G-K}	4.55 ± 0.05 ^{C-H}	30.17 ± 1.16 ^{BC}	30.39 ± 0.85 ^C	57.45 ± 0.41 ^{BCD}	46.33 ± 0.20 ^{A-F}
	1	4.53 ± 0.04 ^{C-H}	4.75 ± 0.22 ^{C-G}	23.96 ± 0.35 ^{F-I}	29.15 ± 0.45 ^{CD}	39.82 ± 2.27 ^{I-M}	38.83 ± 0.38 ^{A-H}
	2	5.37 ± 0.17 ^{A-D}	5.63 ± 0.34 ^{A-D}	19.49 ± 1.02 ^{JKL}	18.83 ± 0.61 ^I	35.64 ± 0.68 ^{K-N}	33.56 ± 1.64 ^{D-H}
	3	5.20 ± 0.00 ^{A-F}	4.98 ± 0.25 ^{B-F}	21.50 ± 0.68 ^{H-K}	20.17 ± 0.17 ^{GHI}	37.77 ± 1.00 ^{J-M}	37.52 ± 0.01 ^{A-H}
Week 2		4.68 ^{BC}	4.98 ^{BC}	23.78 ^C	24.63 ^D	42.67 ^C	39.10 ^{BC}
3	0	3.07 ± 0.10 ^{IJK}	4.53 ± 0.06 ^{C-H}	31.95 ± 0.43 ^{BC}	44.00 ± 0.58 ^A	59.54 ± 0.39 ^{ABC}	48.66 ± 6.29 ^{A-D}
	1	4.00 ± 0.51 ^{E-J}	4.40 ± 0.05 ^{C-H}	26.39 ± 0.41 ^{DEF}	30.81 ± 0.20 ^C	41.46 ± 1.13 ^{H-L}	47.08 ± 0.94 ^{A-E}
	2	5.27 ± 0.49 ^{A-E}	5.42 ± 0.47 ^{A-E}	21.50 ± 0.35 ^{H-K}	20.17 ± 0.44 ^{GHI}	38.74 ± 1.33 ^{J-M}	37.55 ± 1.25 ^{A-H}
	3	4.80 ± 0.19 ^{B-G}	4.50 ± 0.09 ^{C-H}	25.06 ± 0.46 ^{EFG}	23.35 ± 0.32 ^F	40.18 ± 2.25 ^{I-M}	36.85 ± 2.28 ^{B-H}
Week 3		4.28 ^{CD}	4.71 ^C	26.22 ^B	29.60 ^B	44.98 ^C	42.54 ^B

Table 3. Cont.

Storage Period (weeks)	Putrescine Treatment (mM)	AsA Content		Rate of Ethylene Production		Relative Electrolyte Leakage	
		(mg/100 g, fw)		$(\mu\text{L Kg}^{-1} \text{h}^{-1})$		(%)	
		2011	2012	2011	2012	2011	2012
4	0	2.97 ± 0.31 ^{JK}	3.35 ± 0.36 ^{HJ}	39.07 ± 0.87 ^A	39.36 ± 0.32 ^B	62.08 ± 1.55 ^{AB}	51.19 ± 0.78 ^{AB}
	1	3.63 ± 0.11 ^{G-K}	3.80 ± 0.10 ^{F-I}	28.76 ± 0.43 ^{CD}	30.25 ± 0.95 ^C	46.59 ± 2.06 ^{E-I}	45.71 ± 0.61 ^{A-F}
	2	4.73 ± 0.23 ^{B-H}	4.84 ± 0.34 ^{C-G}	25.17 ± 0.35 ^{EFG}	27.39 ± 0.85 ^{DE}	40.10 ± 2.26 ^{I-M}	38.39 ± 1.61 ^{A-H}
	3	4.50 ± 0.15 ^{C-H}	4.30 ± 0.50 ^{D-H}	28.45 ± 0.72 ^{CDE}	26.48 ± 0.52 ^E	43.01 ± 1.37 ^{G-K}	37.25 ± 2.32 ^{A-H}
Week 4		3.96 ^{DE}	4.07 ^D	30.36 ^A	30.87 ^A	47.95 ^B	43.14 ^B
5	0	3.03 ± 0.19 ^{JK}	2.90 ± 0.04 ^{IJ}	36.77 ± 1.12 ^A	39.26 ± 0.75 ^B	64.42 ± 1.24 ^{AB}	50.02 ± 2.05 ^{ABC}
	1	3.50 ± 0.00 ^{HK}	3.49 ± 0.60 ^{G-J}	24.92 ± 0.39 ^{FGH}	26.64 ± 0.36 ^E	50.98 ± 0.31 ^{DEF}	46.81 ± 1.49 ^{A-E}
	2	4.57 ± 0.20 ^{C-H}	4.13 ± 0.11 ^{E-I}	20.79 ± 0.59 ^{IJK}	21.26 ± 0.64 ^{FGH}	41.23 ± 2.52 ^{H-L}	40.46 ± 1.17 ^{A-H}
	3	4.40 ± 0.25 ^{D-H}	3.70 ± 0.28 ^{F-I}	21.71 ± 0.67 ^{G-K}	22.57 ± 0.43 ^F	46.98 ± 1.46 ^{E-I}	41.81 ± 1.20 ^{A-H}
Week 5		3.88 ^{DE}	3.55 ^{DE}	26.04 ^B	27.43 ^C	50.90 ^A	44.78 ^{AB}
6	0	2.70 ± 0.30 ^K	2.30 ± 0.31 ^J	33.09 ± 1.22 ^B	31.03 ± 0.56 ^C	66.43 ± 0.31 ^A	50.61 ± 5.00 ^{AB}
	1	3.53 ± 0.10 ^{G-K}	2.88 ± 0.03 ^{IJ}	21.84 ± 1.27 ^{G-J}	22.95 ± 0.58 ^F	53.86 ± 0.59 ^{CDE}	50.80 ± 1.14 ^{AB}
	2	4.47 ± 0.14 ^{D-H}	3.77 ± 0.11 ^{F-I}	18.85 ± 0.44 ^{JKL}	21.62 ± 0.38 ^{FGH}	40.51 ± 1.14 ^{H-L}	43.50 ± 3.22 ^{A-G}
	3	4.37 ± 0.07 ^{D-H}	3.80 ± 0.10 ^{F-I}	19.54 ± 0.24 ^{JKL}	21.85 ± 0.72 ^{FG}	47.79 ± 1.17 ^{E-H}	52.22 ± 2.76 ^A
Week 6		3.77 ^E	3.19 ^E	23.33 ^C	24.36 ^D	52.15 ^A	49.28 ^A
LSD ($p < 0.05$)		1.27	1.37	3.51	2.40	7.52	15.17
Conc.		**	**	**	**	**	**
Strg.		**	**	**	**	**	**
Conc. × Strg.		NS	NS	**	**	**	NS

mM = millimoles, fw = fresh weight, Conc. = concentration, Strg. = storage, NS = non-significant, ** = highly significant, the means within a column having same letters are statistically non-significant using the Least Significant Difference (LSD) Test.

3.5. Ethylene Biosynthesis

In the present study, an inverse relationship was observed between the spray application of PUT and the rate of ethylene production of peach fruit at harvest and during the entire period of cold-storage, irrespective of the time of PUT application (Table 3). During storage, PUT application significantly reduced the rate of ethylene production in the fruit, regardless of the dose and time of PUT application. Perceptibly, 2 mM PUT application proved to be more efficient in reducing the rate of production of this ripening hormone in peaches. Although all treatments significantly lowered the ethylene climacteric peak but the lowest climacteric peaks (1.6- and 2.2-fold lower than control, during 2011 and 2012, respectively) were observed in the fruit treated with 2 mM PUT treatment.

3.6. CI (Internal Browning) and EL

The foliar application of PUT significantly delayed the onset of CI in the fruit by 4 weeks during the low-temperature storage, regardless of the dose and time of PUT foliar spray for both years (Table 4). The highest CI index was observed in the fruit harvested from the control trees (0.23 and 0.45, respectively). Changes in the CI index of peach fruit were concomitant with those recorded for the EL from their skin discs (Table 3). In the present study, the lowest levels of EL from the peach skin were observed in the fruit collected from the trees treated with 2 mM PUT, irrespective of the time of spray application for the two consecutive years of experimentation.

Table 4. Effects of exogenous application of putrescine on the chilling injury index and the degree of disease/decay incidence in peach fruit during storage.

Putrescine (mM)	Chilling Injury Index		Disease and Decay Incidence (%)	
	2011	2012	2011	2012
0	0.23 ± 0.008 ^a	0.45 ± 0.003 ^a	51 ± 1.34 ^a	52 ± 0.089 ^a
1	0.07 ± 0.013 ^b	0.166 ± 0.002 ^b	25 ± 1.30 ^b	22 ± 0.00 ^b
2	0.04 ± 0.007 ^b	0.07 ± 0.003 ^c	13 ± 1.24 ^c	18 ± 0.67 ^c
3	0.06 ± 0.20 ^b	0.08 ± 0.00 ^c	25 ± 1.30 ^b	18 ± 0.34 ^c
LSD ($p < 0.05$)	0.06	0.01	6.04	2.61

mM = millimoles, $n = 3$ (replications), the means within a column having same letters are statistically non-significant using the Least Significant Difference (LSD) Test.

3.7. Disease and Decay Incidence

The pre-harvest application of PUT significantly reduced the rate of disease/decay occurrence in the peach fruit during low-temperature storage, regardless of the rate and time of PUT application (Table 4). During 2011, at the end of the storage period, the highest degree of disease/decay incidence (51%) was recorded in the fruit harvested from the untreated trees, whereas the foliar spray of PUT, applied at any stage of fruit growth/development, effectively reduced the occurrence of fruit disease/decay. In addition, 2 mM PUT application was found to be the most effective treatment in substantially reducing the rate of fruit disease/decay incidence in the peach cultivar Flordaking. While, during 2012, all the treatments were at par with each other in controlling the disease and decay index in stored peaches at low temperature (Table 4).

4. Discussion

In the present study, foliar application of PUT on peach trees resulted in significantly a slower rate of physiological loss of fruit weight, during low-temperature storage (Table 1). These results were consistent with those previously reported by Tareen et al. [28,29] for the same peach cultivar (Flordaking). Fresh produce such as fruits and vegetables maintain their functional, nutritional, textural and physicochemical properties during extended storage periods, provided that these commodities exhibit slower rates of moisture loss [30]. The natural or uncontrolled phenomena of respiration

and transpiration, along with the associated metabolic activities, are primarily responsible for the physiological loss of fruit weight during storage [31,32]. Probably because of its anti-senescence properties [33], PUT may have maintained the membrane stability or integrity of the epicuticular wax of peach fruit, thereby reducing the rate of moisture loss from the fruit during cold storage as demonstrated by Mirdehghan et al. [34] in pomegranate fruit.

Fruit softening is another important factor responsible for limiting the storage life of horticultural crops [35]. It is associated with deleterious changes in the metabolism of cell wall carbohydrates as well as the structural components of the cell wall [36]. These changes are triggered by the activities of hydrolytic enzymes [37]. In this study, maintenance of higher flesh firmness of the peach fruit in response to PUT application may be attributed to the reduced activities of the fruit-softening enzymes [18]. In this context, our results are consistent with those reported by Khan et al. [7], Valero et al. [38], Serrano et al. [13] and Abu-Kpawoh et al. [39] for various plum cultivars.

The cosmetic appearance of fresh produce is one of the most important quality factors that dictate the buying decision of consumers and lays down the foundation for maturity indexes in many horticultural crops. The ripening phenomenon in fresh produce is meticulously associated with the degradation of photosynthetic pigments coupled with the concomitant increases in the levels of phenolic pigments. Similarly, storage regime substantially influences the cosmetic appearance, including visible colour on the exterior. The slower changes in the brightness of colour (L^* ; Table 1) and red blush (a^* ; Table 1) on the surface of peach fruit after PUT application may be ascribed to the delay in chlorophyll senescence with the reduced rate of fruit ripening. The application of PAs has been a successful production practice to ensure optimum colour development on the fruit surface in many species such as lemon [40], apricot [41] and mango [15].

The results show that the PUT treatments significantly slowed down the increase in SSC, which can be ascribed to its effect in delaying the conversion of starch into simple sugars and other impacts of PUT such as decreasing the weight loss and ethylene biosynthesis, hence delaying the ripening process.

Gupta and Jawandha [42] demonstrated that malic, citric and quinic acids were the major organic acids found in peach fruit. These acids are converted to sugars as the storage period prolongs. It ultimately affects the qualitative and organoleptic characteristics of the fruit such as flavour and taste. Ripening increases the respiration rate, along with certain other metabolic activities, in climacteric fruits, causing an abrupt decrease in the levels of these organic acids [43]. Similar effects of different treatments were observed in case of SSC: TA (ratio) in the peach fruit during storage at low temperature. Slower and gradual increase in SSC and SSC: TA (ratio) in the fruit from the PUT-treated trees may be attributable to the antagonistic effects of PUT on ethylene biosynthesis (Table 3) that, in turn, affect sugar/acid metabolism during ripening. The exogenous application of PAs has been reported to show similar results in various stone fruits [7,44]. The lower values of SSC: TA ratio in the fruits from the PUT-treated trees may be explained by the slower metabolic changes in organic acids and carbohydrates (sugars). These findings were consistent with those previously reported by Mirdehghan et al. [34].

Yahia et al. [45] investigated the ripening-related dynamics of AsA and activities of ascorbate oxidase (AO) in tomato and bell pepper and reported a direct relationship between the two, suggesting the involvement of PAs in the biosynthesis and maintenance of AsA content. AsA is a well-known water-soluble vitamin which is sensitive to various environmental signals. It can readily be oxidized just by the brief exposure to oxygen, alkalinity, metals, heat and light. AO is believed to be the key enzyme involved in the oxidation process. The results of the present study revealed that the foliar application of PUT upregulated the biosynthesis of AsA in the fruit (Table 3), confirming the similar pattern of changes in AsA content previously reported by Yahia et al. [45] in tomato and bell pepper. Similarly, Mirdehghan et al. [34] reported higher levels of AsA content in pomegranate arils during storage in response to post-harvest application of PAs.

In the present study, the inhibition of ethylene biosynthesis by PUT application may indicate a stress response due to high amine concentrations, as reported by Flores [46]. The antagonistic effect of PAs (PUT, spermine and spermidine) on ethylene biosynthesis may be explained by their competition

for the same precursor [47]. The inhibitory effect of PUT on the enzymes responsible for ethylene production could be another reason for the decrease in the ethylene peak [48]. This antagonistic effect of PAs on ethylene production has also been reported in a variety of fruits such as apricot [41], peach [14], plum [13], kiwifruit [49] and plum [50].

In this study, the CI appeared in the form of flesh browning after 4 weeks of low temperature storage. Previously, Wang et al. [25] reported similar observations in peach fruit. Under the conditions that induce chilling disorders, the lipid fraction of the plasma membrane loses fluidity, which causes a series of changes in the permeability of cellular membrane [32]. Specifically, under thermal stress, cold acclimation has been shown to affect the fraction of unsaturated lipid content in pomegranate, suggesting its involvement in maintaining the integrity of the cell wall [51]. In response to the pre-harvest foliar applications of PUT, the skin membrane of the fruit maintained its integrity for a longer period of time during storage, as evidenced by reduced EL (Table 3) and the lower incidence of CI/flesh browning (Table 1), in the present study. This sort of involvement of PAs in reducing lipid peroxidation/EL has previously been reported in rice crop grown under saline stress conditions [52]. The lower incidence of CI/decay in Flordaking peaches (Table 4) after 4 weeks of cold storage, in response to the spray application of PUT may also be attributed to the increased activities of highly coordinated biomolecules or improved defence system against potential pathogens [53], as suggested by Poole and McLeod [54] in kiwi fruit, Yao and Tian [55] in sweet cherries and Haggag [56] in beans. This effect of putrescine on controlling the disease and decay incidence in peach has already been reported by Bal [57].

The economic analysis of the benefit cost ratio (BCR) of the present study (Table 5) demonstrated that the spray application of lower doses (1–2 mM) of PUT is a cost effective management strategy in peach production. Though the BCR for 2 mM PUT was higher compared to other PUT treatments (1 and 3 mM) and control, while the net profit fetched by 3 mM PUT was also higher than that fetched by the spray application of 1 mM or water (control).

Table 5. Economic analysis (benefit-cost ratio) of putrescine application in one acre of peach orchard.

Putrescine Treatment	Fixed Cost		Variable Cost		Total Cost		Yield/Acre		Percent Loss of Fruit Due to Decay or Disease		Marketable Fruit		Market Price		Total Return per Acre		Total Profit per Acre		Benefit-Cost Ratio	
	(mM)	(Rs.)	(Rs.)	(Rs.)	(Rs.)	(Rs.)	(Kg)	(Kg)	(%)	(%)	(Kg)	(Kg)	(Rs. Kg ⁻¹)	(Rs.)	(Rs.)	(Rs.)	(Rs.)	(Ratio)	(Ratio)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0	2000	2500	1000	1200	3000	3700	5399	4753	51	52	2646	2296	50	50	132,279	114,788	130,279	111,088	43.4	30.0
1	2000	2500	2200	2200	4200	4700	6001	5092	25	22	4500	3972	55	58	247,521	230,353	243,320	225,653	57.9	48.0
2	2000	2500	4400	4400	6400	6900	6896	5658	13	18	5999	4640	63	75	377,964	347,991	371,564	341,091	58.1	49.4
3	2000	2500	6600	6600	8600	9100	5755	5035	25	18	4316	4128	58	62	250,351	255,964	241,751	246,864	37.9	27.1

5. Conclusions

A single foliar spray of 1–3 mM PUT on peach trees cultivar Flordaking at cell division, pit hardening, and cell enlargement stage of fruit growth/development substantially improves the firmness of fruit flesh and development of red/pink blush on the fruit's exterior at harvest. The application of PUT significantly reduces the rate of ethylene production and delays the incidence of CI and fruit decay during low-temperature storage. Application of 2 mM PUT application was found to be more efficient in alleviating CI and maintaining the quality and improving the storage life of peach fruit during, for up to 6 weeks.

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References

1. FAO. FAO Statistical Database-Agriculture. 2016. Available online: <http://faostat3.fao.org> (accessed on 31 January 2019).
2. Irfan, A.; Abbasi, N.A.; Hafiz, I.A. Physiological response and quality attributes of peach fruit cv. Flordaking as affected by different treatments of calcium chloride, putrescine and salicylic acid. *Pak. J. Agric. Sci.* **2014**, *51*, 33–39.
3. Khalil, I.; Idrees, M.; Rabi, F.; Rehman, S.U.; Bostan, N. An investigation into the problems of peach growers in district swat. *Arpn J. Agric. Biol. Sci.* **2014**, *9*, 427–434.
4. Wasim, M.P. Trends, growth and variability of major fruits crops in Balochistan-Pakistan: 1989–2009. *Arpn J. Agric. Biol. Sci.* **2011**, *6*, 27–36.
5. Liguori, G.; Weksler, A.; Zutahi, Y.; Lurie, S.; Kosto, I. Effect of 1-methylcyclopropene on ripening of melting flesh peaches and nectarines. *Postharvest Biol. Technol.* **2004**, *31*, 263–268. [[CrossRef](#)]
6. Bakshi, P.; Masoodi, F.A. Use of intermittent warming to control chilling injury in peach during storage. In Proceedings of the VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics, Solan, India, 31 December 2005; *Acta Horticulturae* 696. pp. 523–526.
7. Khan, A.S.; Singh, Z.; Abbasi, N.A.; Swinny, E.E. Pre- or postharvest applications of putrescine and low temperature storage affect fruit ripening and quality of 'Angelino' plum. *J. Sci. Food Agric.* **2008**, *88*, 1686–1695. [[CrossRef](#)]
8. Bakshi, P.; Masoodi, F.A. Use of modified atmosphere and refrigeration to extend postharvest life of peach. Paper Presented at the 5th International Food Convention (IFCON-2003), Mysore, India, 5–8 December 2003.
9. González-Agüero, M.; Pavez, L.; Ibáñez, F.; Pacheco, I.; Campos-Vargas, R.; Meisel, L.A.; Orellana, A.; Retamales, J.; Silva, H.; González, M.; et al. Identification of woolliness response genes in peach fruit after post-harvest treatments. *J. Exp. Bot.* **2008**, *59*, 1973–1986. [[CrossRef](#)] [[PubMed](#)]
10. Lurie, S.; Crisosto, C.H. Chilling injury in peach and nectarine. *Postharvest Biol. Technol.* **2005**, *37*, 195–208. [[CrossRef](#)]
11. Abbasi, N.A.; Irfan, A.; Hafiz, I.A.; Khan, A.S. Application of polyamines in horticulture: A review. *Int. J. Biosci.* **2017**, *10*, 319–342.
12. Tang, W.; Newton, R.; Outhavong, V. Exogenously added polyamines recover browning tissues into normal callus cultures and improve plant regeneration in pine. *Physiol. Plant.* **2004**, *122*, 386–395. [[CrossRef](#)]
13. Serrano, M.; Martínez-Romero, D.; Guillén, F.; Valero, D. Effects of exogenous putrescine on improving shelf life of four plum cultivars. *Postharvest Biol. Technol.* **2003**, *30*, 259–271. [[CrossRef](#)]
14. Bregoli, A.M.; Scaramagli, S.; Costa, G.; Sabatini, E.; Ziosi, V.; Biondi, S.; Torrigiani, P. Peach (*Prunus persica*) fruit ripening: Aminoethoxyvinylglycine (AVG) and exogenous polyamines affect ethylene emission and flesh firmness. *Physiol. Plant.* **2002**, *114*, 472–481. [[CrossRef](#)] [[PubMed](#)]

15. Malik, A.; Singh, Z. Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *J. Hortic. Sci. Biotechnol.* **2005**, *80*, 363–369. [[CrossRef](#)]
16. Kramer, G.F.; Wang, C.Y.; Conway, W.S. Inhibition of Softening by Polyamine Application in ‘Golden Delicious’ and ‘McIntosh’ Apples. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 813. [[CrossRef](#)]
17. Martínez-Romero, D.; Valero, D.; Serrano, M.; Burló, F.; Carbonell-Barrachina, A.; Burgos, L.; Riquelme, F. Exogenous Polyamines and Gibberellic Acid Effects on Peach (*Prunus persica* L.) Storability Improvement. *J. Food Sci.* **2000**, *65*, 288–294.
18. Martínez-Romero, D.; Serrano, M.; Carbonell-Barrachina, A.; Burgos, L.; Riquelme, F.; Valero, D. Effects of Postharvest Putrescine Treatment on Extending Shelf Life and Reducing Mechanical Damage in Apricot. *J. Food Sci.* **2002**, *67*, 1706–1712. [[CrossRef](#)]
19. Shafiq, M.; Singh, Z. Pre-harvest spray application of phenylpropanoids influences accumulation of anthocyanin and flavonoids in ‘Cripps Pink’ apple skin. *Sci. Hortic.* **2018**, *233*, 141–148. [[CrossRef](#)]
20. McGuire, R.G. Reporting of objective color measurements. *HortScience* **1992**, *27*, 1254–1255. [[CrossRef](#)]
21. AOAC. *Official Methods of Analysis of AOAC International*, 19th ed.; AOAC International: Gaithersburg, MD, USA, 1990.
22. Hans, Y.S.H. *The Guide Book of Food Chemical Experiments*; Pekin Agricultural University Press: Pekin, China, 1992.
23. Whale, S.K.; Singh, Z. Endogenous ethylene and colour development in the skin of ‘Pink Lady’ apple. *J. Am. Soc. Hortic. Sci.* **2007**, *132*, 20–28. [[CrossRef](#)]
24. Zheng, X.; Tian, S.; Meng, X.; Li, B. Physiological and biochemical responses in peach fruit to oxalic acid treatment during storage at room temperature. *Food Chem.* **2007**, *104*, 156–162. [[CrossRef](#)]
25. Wang, L.; Chen, S.; Kong, W.; Li, S.; Archbold, D.D. Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. *Postharvest Biol. Technol.* **2006**, *41*, 244–251. [[CrossRef](#)]
26. Statistix. *User’s Manual, Analytical Software (version 8.1)*; Statistix: Tallahassee, FL, USA, 2003.
27. Steel, R.G.D.; Torrie, J.H.; Dickey, D.H. *Principles and Procedures of Statistics a Biometrical Approach*, 3rd ed.; The McGraw-Hill Companies Inc.: New York, NY, USA, 1997.
28. Tareen, M.J.; Abbasi, N.A.; Hafiz, I.A. Effect of salicylic acid treatments on storage life of peach fruits CV. ‘Flordaking’. *Pak. J. Bot.* **2012**, *44*, 119–124.
29. Tareen, M.J.; Abbasi, N.A.; Hafiz, I.A. Postharvest application of salicylic acid enhanced antioxidant enzyme activity and maintained quality of peach cv. ‘Flordaking’ fruit during storage. *Sci. Hortic.* **2012**, *142*, 221–228. [[CrossRef](#)]
30. Kissinger, M.; Tuvia-Alkalai, S.; Shalom, Y.; Fallik, E.; Elkind, Y.; Jenks, A.; Goodwin, M. Characterization of Physiological and Biochemical Factors Associated with Postharvest Water Loss in Ripe Pepper Fruit during Storage. *J. Am. Soc. Hortic. Sci.* **2005**, *130*. [[CrossRef](#)]
31. González-Aguilar, G.A.; Valenzuela-Soto, E.; Lizardi-Mendoza, J.; Goycoolea, F.; Martínez-Téllez, M.A.; Villegas-Ochoa, M.A.; Monroy-García, I.N.; Ayala-Zavala, J.F. Effect of chitosan coating in preventing deterioration and preserving the quality of fresh-cut papaya ‘Maradol’. *J. Sci. Food Agric.* **2009**, *89*, 15–23. [[CrossRef](#)]
32. Galindo, F.; Herppich, W.; Gekas, V.; Sjöholm, I. Factors Affecting Quality and Postharvest Properties of Vegetables: Integration of Water Relations and Metabolism. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 139–154. [[CrossRef](#)] [[PubMed](#)]
33. Schirra, M.; D’Hallewin, G. Storage performance of Fortune mandarins following hot water dips. *Postharvest Biol. Technol.* **1997**, *10*, 229–238. [[CrossRef](#)]
34. Mirdehghan, S.H.; Rahemi, M.; Castillo, S.; Martínez-Romero, D.; Serrano, M.; Valero, D. Pre-storage application of polyamines by pressure or immersion improves shelf-life of pomegranate stored at chilling temperature by increasing endogenous polyamine levels. *Postharvest Biol. Technol.* **2007**, *44*, 26–33. [[CrossRef](#)]
35. Brummell, D.A.; Harpster, M.H. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Mol. Biol.* **2001**, *47*, 311–340. [[CrossRef](#)] [[PubMed](#)]
36. Labavitch, M.J. Cell Wall Turnover in Plant Development. *Annu. Rev. Plant Physiol.* **2003**, *32*, 385–406. [[CrossRef](#)]
37. Payasi, A.; Nath Mishra, N.; Lucia Soares Chaves, A.; Singh, R. Biochemistry of fruit softening: An overview. *Physiol. Mol. Biol. Plants* **2009**, *15*, 103–113. [[CrossRef](#)]

38. Valero, D.; Martínez-Romero, D.; Serrano, M. The role of polyamines in the improvement of the shelf life of fruit. *Trends Food Sci. Technol.* **2002**, *13*, 228–234. [[CrossRef](#)]
39. Abu-Kpawoh, J.C.; Xi, Y.F.; Zhang, Y.Z.; Jin, Y.F. Polyamine Accumulation Following Hot-Water Dips Influences Chilling Injury and Decay in 'Friar' Plum Fruit. *J. Food Sci.* **2002**, *67*, 2649–2653. [[CrossRef](#)]
40. Valero, D.; Martínez-Romero, D.; Serrano, M.; Riquelme, F. Influence of Postharvest Treatment with Putrescine and Calcium on Endogenous Polyamines, Firmness, and Abscisic Acid in Lemon (Citrus lemon L. Burm Cv. Verna). *J. Agric. Food Chem.* **1998**, *46*, 2102–2109. [[CrossRef](#)]
41. Martínez-Romero, D.; Valero, D.; Riquelme, F.; Zuzunaga, M.; Serrano, M.; Burló, F.; Carbonell-Barrachina, A. Infiltration of putrescine into apricots helps handling and storage. *Acta Hort.* **2001**, *553*, 189–192. [[CrossRef](#)]
42. Gupta, N.; Jawandha, S. Effect of different packagings on quality of peaches during storage. *Hortflora Res. Spectr.* **2012**, *1*, 117–121.
43. Batista-Silva, W.; Nascimento, V.L.; Medeiros, D.B.; Nunes-Nesi, A.; Ribeiro, D.M.; Zsögön, A.; Araújo, W.L. Modifications in Organic Acid Profiles During Fruit Development and Ripening: Correlation or Causation? *Front. Plant Sci.* **2018**, *9*, 1689. [[CrossRef](#)]
44. Serrano, M.; Martínez-Romero, D.; Castillo, S.; Guillén, F.; Valero, D. Role of calcium and heat treatments in alleviating physiological changes induced by mechanical damage in plum. *Postharvest Biol. Technol.* **2004**, *34*, 155–167. [[CrossRef](#)]
45. Yahia, E.M.; Contreras-Padilla, M.; Gonzalez-Aguilar, G. Ascorbic Acid Content in Relation to Ascorbic Acid Oxidase Activity and Polyamine Content in Tomato and Bell Pepper Fruits During Development, Maturation and Senescence. *Lwt Food Sci. Technol.* **2001**, *34*, 452–457. [[CrossRef](#)]
46. Flores, H.E. Polyamines and plant stress. In *Stress Response in Plants: Adaptation and Acclimation Mechanisms*; Akscger, R.G., Cumming, J.R., Eds.; Wiley & Liss: New York, NY, USA, 1990; pp. 217–239.
47. Cohen, S.S. *A Guide to Polyamines*; Oxford University Press: New York, NY, USA, 1998.
48. Kakkar, R.K.; Rai, V.K. Plant polyamines in flowering and fruit ripening. *Phytochemistry* **1993**, *33*, 1281–1288. [[CrossRef](#)]
49. Petkou, I.T.; Pritsa, T.S.; Sfakiotakis, E.M. Effects of polyamines on ethylene production, respiration and ripening of kiwifruit. *J. Hortic. Sci. Biotechnol.* **2004**, *79*, 977–980. [[CrossRef](#)]
50. Khan, A.S.; Singh, Z.; Abbasi, N.A. Pre-storage putrescine application suppresses ethylene biosynthesis and retards fruit softening during low temperature storage in 'Angelino' plum. *Postharvest Biol. Technol.* **2007**, *46*, 36–46. [[CrossRef](#)]
51. Mirdehghan, S.; Rahemi, M.; Martínez-Romero, D.; Guillén, F.; Valverde, J.; Zapata, P.; Serrano, M.; Valero, D. Reduction of pomegranate chilling injury during storage after heat treatment: Role of polyamines. *Postharvest Biol. Technol.* **2007**, *44*, 19–25. [[CrossRef](#)]
52. Chattopadhyay, M.K.; Tiwari, B.S.; Chattopadhyay, G.; Bose, A.; Sengupta, D.N.; Ghosh, B. Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. *Physiol. Plant.* **2002**, *116*, 192–199. [[CrossRef](#)] [[PubMed](#)]
53. Lawton, K.A.; Friedrich, L.; Hunt, M.; Weymann, K.; Delaney, T.; Kessmann, H.; Staub, T.; Ryals, J. Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. *Plant J.* **1996**, *10*, 71–82. [[CrossRef](#)]
54. Poole, P.; McLeod, L. Development of resistance to picking wound entry Botrytis cinerea storage rots in kiwifruit. *N. Z. J. Crop Hortic. Sci.* **1994**, *22*, 387–392. [[CrossRef](#)]
55. Yao, H.; Tian, S. Effects of pre- and post-harvest application of salicylic acid or methyl jasmonate on inducing disease resistance of cherry fruit in storage. *Postharvest Biol. Technol.* **2005**, *35*, 253–262. [[CrossRef](#)]
56. Haggag, W.M. Polyamines: Induction and effect on rust disease control of bean. *Plant Pathol. Bull.* **2005**, *14*, 89–102.
57. Bal, E. Effects of exogenous polyamine and ultrasound treatment to improve peach storability. *Chil. J. Agric. Res.* **2013**, *73*, 435–440. [[CrossRef](#)]

