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ORIGINAL PAPER

Effect of exogenous fludioxonil postharvest treatment on physiological response, physico-chemical, textural, phytochemical and sensory characteristics of pomegranate fruit

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Abstract The study investigated the effects of fludioxonil (FLU) on the postharvest quality of pomegranate fruit (cv. Wonderful). Fruits were dipped in FLU concentrations (control, 150, 300 and 600 mg/L) and stored for 4 months at 5 °C and 90–95% relative humidity (RH) plus an additional 4 days at 20 °C and 65% RH. Effects of FLU were evaluated on physiological responses, quality and sensory attributes. Results showed that fruit weight loss and decay incidence were reduced by FLU treatment, with fruit treated with 600 mg/L concentration showing the best results. Fruit respiration rate was more influenced by storage duration than FLU concentration. The severity and occurrence of physiological disorders increased with storage duration but were more pronounced in fruits treated with FLU. Storage duration influenced fruit colour whereas aril colour was affected by FLU concentration. Untreated fruit showed better aril redness and chroma although treated fruit also had acceptable red colour. Chemical quality attributes of fruit juice were not significantly affected by FLU concentrations. Fruit treated with FLU had significantly ($p < 0.05$) higher total phenolic content during storage although lower ascorbic acid content was observed compared to untreated fruit. Treating fruit with FLU resulted in better sensory attributes with regards to crispness, juiciness and sweetness. Overall, this study showed that fruit treated with FLU at 600 mg/L had the best quality with respect to decay incidence, weight loss, total phenolics and sensory attributes.

Keywords Decay · Chilling injury · Phytochemical · Sensory properties · Bioyield · Hardness

Abbreviations

FLU	Fludioxonil
BMC	Benzimidazole carbamate
TBZ	Thiabendazole
US E.P.A	United States Environment Protection Agency
AI	Active ingredient
SE	Standard error
TA	Titratable acidity
TSS	Total soluble solids
MPA	Metaphosphoric acid
CI	Chilling injury
AA	Ascorbic acid
GAE	Gallic acid equivalent

Introduction

Pomegranate (*Punica granatum* L.) is a non-climacteric fruit and is an important horticultural crop in many tropical and subtropical regions of the world [1]. The edible portion of the fruit is comprised of arils that can be consumed fresh, as juice or used as flavouring and colouring agents [2, 3]. The fruit juice is high in sugars, vitamins, organic acids, polysaccharides, polyphenols and essential minerals [4]. Pomegranate fruit has recently captured consumer interest because of its reported beneficial health properties [5]. Several researchers have reported the chemo-preventative,

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anti-inflammatory and antibacterial properties of pomegranate fruit (and its derived products), which have been attributed to its high antioxidant capacity [3, 6, 7]. Beneficial properties of pomegranates against atherosclerosis, certain cancers and other degenerative diseases have also been reported [8, 9]. One of the challenges limiting the use of pomegranates is the limited postharvest life. When the fruit is stored under ambient conditions, storage life is limited to only a few weeks and therefore cold storage is recommended, with temperatures between 0 and 10 °C depending on cultivar [5]. At the same time, relative humidity during storage should be maintained at 90–95% to prevent desiccation of the skin because the fruit is very prone to moisture loss [5, 10]. However, high humidity promotes the growth of micro-organisms and enhances fruit decay [11]. Fruit decay is a major cause of postharvest loss during storage of pomegranate fruit. The major postharvest decay diseases during fruit storage include grey mould—caused by *Botrytis cinerea* (which develops under the recommended storage conditions of 5–8 °C and 90–95% RH), heart rot – caused by *Aspergillus niger* and *Alternaria* ssp., and Penicillium rot – caused by different species of *Penicillium*, including *P. digitatum* and *P. implicatum* [1, 12]. To reduce the incidence of fruit decay, fungicides are used both as preharvest and postharvest treatments of fruit. Preharvest fungicides include fenhexamid, azoxystrobin and tebuconazole [13] and postharvest fungicides include methyl 2-benzimidazole carbamate (BMC), thiabendazole (TBZ) and fludioxonil (FLU), among others [14].

A shift in interest to naturally occurring compounds as antimicrobial agents or as components for chemical synthesis of new active ingredients, together with the understanding of their structures and properties, has motivated the synthesis of new broad spectrum "natural mimetic" fungicides that have different mechanisms of action from previously registered ones [15, 16]. To cope with problems associated with resistance development to 'older fungicides' by current and emerging fungal pathogens, a number of novel fungicides have been developed for horticultural crops [15]. Among these is fludioxonil, a broad-spectrum fungicide with a different mode of action compared to older registered chemicals [13]. Fludioxonil, together with pyrimethanil and azoxystrobin were categorised as reduced-risk compounds by the United States Environment Protection Agency (US E.P.A) [17]. This indicates that they have more advantageous properties compared to old fungicides and have extensively been assessed for control of green mould in California [17]. Fludioxonil is a synthetic analogue of pyrrolnitrin belonging to the class of phenylpyrroles and is considered a reduced risk pesticide by the US E.P.A [11, 18]. Fludioxonil inhibits spore germination, germ tube elongation, and mycelium growth of *B. cinerea* and induces morphological alterations of germ tubes [18]. The mode of action is by inhibition of the transport-associated phosphorylation of glucose and the prevention of glycerol synthesis [19, 20]. Fludioxonil has been used as postharvest treatment for stone fruit, pome fruit, pomegranates, kiwi fruit, and citrus [20]. Developed in the mid-1900s to control *Botryrtis cinereas* in viticulture, it is also highly effective on a large number of pathogens, including *Botrytis* spp., *Penicillium* spp., *Monilia* spp., *Alternaria* spp, *Sclerotinia* ssp [11, 18]. Although the application of fludioxonil has been studied on pomegranate [11, 21], these studies majorly focused on control of fungal pathogens and decay incidence, as well as crop residue levels. There is limited information on the sole influence of FLU concentrations on the postharvest quality of pomegranate fruit, a factor that may affect the sensory and antioxidant property of fruit [22]. In addition, the different cultivars used from different climatic regions in previous studies may also influence the response of fruit to the chemical. Therefore, the aim of this study was to evaluate the effects of treating harvested whole pomegranate fruit with different concentrations of fludioxonil on the physiological response, physico-chemical and sensory quality attributes, and antioxidant properties.

Materials and methods

Plant material and chemicals

Pomegranate fruit (cv. Wonderful) were handpicked during commercial harvest from Heinrich F.R. Schaefer (HFR) farm in the Western Cape (33°44′26.185″S 18°44′41.193″E) in South Africa and transported in a well-ventilated vehicle to the Postharvest Technology and Research Laboratory at Stellenbosch University. Fruits were selected based on uniform size and colour, and absence of physical damage such as cracks, sunburn and bruises.

The fungicide was a commercial formulation of FLU (Scholar®, Syngenta, South Africa) containing 23% active ingredient (AI).

Treatments

Upon arrival at the laboratory, fruits were divided into four different treatment groups, each comprised of 108 fruits. Treatments were performed by dipping a batch of fruits in 15 L of scholar solution (AI. 23% fludioxonil). The four applied treatments were:

1. Control: immersion in water.
2. Immersion in 150 mg/L fludioxonil.
3. Immersion in 300 mg/L fludioxonil.
4. Immersion in 600 mg/L fludioxonil.

After immersion of each batch for 2 min, fruit surface was left to thoroughly dry at ambient room conditions (20 ± 2 °C and 65 ± 5% RH) for 12 h before transfer to cold storage (5 ± 0.7 °C and 95 ± 2% RH).

Packaging and storage

Fruits were packed inside commercial ventilated cartons (0.4 m long, 0.3 m wide and 0.133 m high) used for postharvest handling of pomegranate fruit and put in cold storage for 4 months. Each treatment comprised 108 fruits and packaged according to industry practice, each carton contained 12 fruits and a total of 9 cartons per treatment. Temperature and relative humidity within the cold room was recorded daily using Tiny Tag TV-4500 data loggers (Gemini Data Logger, Sussex, UK). At the end of cold storage, a batch of fruits (n = 10) from each treatment was placed under ambient storage (20–24 °C and 65–70% RH) for a 4-day period to simulate reasonable retail sale period. Fruits were then analysed for incidence of physiological disorders, physico-chemical, sensory and phytochemical properties after cold storage and shelf life. Ten fruits were randomly selected from 9 cartons at each storage interval. Data collected from ten randomly selected fruits were used for statistical analysis (n = 10). Measurement of all parameters was carried out on a monthly interval and results were presented as mean ± standard error (SE).

Fruit respiration rate and physiological disorders

Respiration rate

Fruit respiration was measured using a closed system as described by Caleb et al. [23]. In five replicates, two fruits of known weight were placed in a glass jar containing a rubber septum. The jar was sealed hermetically with vaseline to ensure a vacuum seal. Fruits were incubated for 2 h at 20 °C then gas composition inside each glass jar was measured using a calibrated O_2/CO_2 analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Carbon dioxide production was presented as mL CO_2 $kg^{-1}h^{-1}$ of five determinations.

Weight loss

Ten randomly selected fruits per treatment were used for this purpose. Fruits were weighed individually at monthly intervals during storage using an electronic scale (Mettler, Toledo, Switzerland, 0.0001 g accuracy). Cumulative weight loss of each fruit was calculated as:

$$W = [(W_o - W_i)/W_o] \times 100 \quad (1)$$

where W is the cumulative weight loss (%) of fruit; Wo is the weight (g) of fruit at the beginning of storage; Wi (g) is the weight of fruit at the storage time.

Decay incidence

Fruit decay incidence was visually assessed as total rots. Fruits with external decay appearance were counted and discarded. Percentage of discarded fruits was calculated using the formula:

$$\text{Fruit decay (\%)} = [(\text{Number of discarded fruit at each sampling date})/(\text{Total number of fruit})] \times 100 \quad (2)$$

Incidence of chilling injury, husk scald and aril browning

Indices and incidences of chilling injury and husk scald and index of aril browning were assessed monthly. The severity (index) of disorders were assessed using a four-level scale as described by Fawole and Opara [5]; where 0 = none (no symptom), 1 = traces (1–25%), 2 = slight (26–50%), 3 = moderate (51–75%) and 4 = severe (76–100%).

A physiological disorder index was calculated by multiplying the scores of severity by the number of affected fruits and dividing by the total number of assessed fruits [5, 24]:

$$\text{Disorder index} = \sum \text{value of scale}) \times (\text{number of fruit with the corresponding scale number})/(\text{total number of fruit}) \quad (3)$$

$$\text{Disorder incidence} = [(\text{Number of affected fruit})/(\text{Total number of fruit})] \times 100 \quad (4)$$

Physico-textural attributes

Whole fruit and aril colour

Colour parameters in CIELAB coordinates (L*, a*, b*) were measured using a Chroma meter (CR-400, Minolta Corp, Osaka, Japan). Ten fruits per treatment were used to monitor changes in external colour by measuring peel colour at two opposite spots on individual fruit, while aril colour was determined by placing the arils in a colourless glass Petri dish. Colour intensity or chroma (C*) and hue angle (h°) were calculated using the equations (5) and (6) [5, 25].

$$C* = \left(a*^2 + b*^2\right)^{1/2} \quad (5)$$

$$h^\circ = \arctan\left(b*/a*\right) \quad (6)$$

Furthermore, total colour difference (TCD) between the peel (external) and arils (internal) was calculated as;

$$TCD = \sqrt{(L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2} \quad (7)$$

where L^*_0, a^*_0 and b^*_0 are the colour parameters of the peel (reference value), while L*, a* and b* are the colour values of the aril [2].

Chemical quality attributes

Titratable acidity, total soluble solids and pH

Titratable acidity (TA) was measured by diluting 2 ml of fresh juice with 70 ml of distilled water and titrating with 0.1 M NaOH to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisua, Switzerland). The results were expressed as percentage of citric acid (% CA). Total soluble solids (TSS, °Brix) was measured using a digital refractometer (Atago, Tokyo, Japan) calibrated with distilled water. The pH values were determined at room temperature using a calibrated pH meter (Crison, Model 00924, Barcelona, Spain). BrimA, a criterion for consumer fruit juice acceptance was expressed as BrimA = TSS − k * TA, where k is the tongue's sensitivity index, which normally ranges from 2 to 10. A value of $k = 2$ was used to avoid a negative BrimA index [26]. As such, a high BrimA value would indicate high total soluble solids content thus fruit will be perceived as sweet with a slight sour taste, whereas a low BrimA value would mean high titratable acids hence the fruit would be perceived as extremely sour due to human tongue sensitivity. All measurements were made on ten individual fruit juice samples for each.

Phytochemical analyses

Ascorbic acid content

Ascorbic acid concentration was determined colorimetrically in triplicate using the method described by Barros et al. [27] with some modifications Fawole et al. [7]. Pomegranate juice (PJ) was extracted with 1% metaphosphoric acid (MPA) (0.5 mL of PJ to 14.5 mL of 1% MPA). Mixture was vortexed for 2 min and sonicated for 3 min in cold water before centrifugation (Merk, Eppendorf AG, Germany) at 4472 g force for 10 min at 4 °C. Approximately, 1 mL of the supernatant was mixed with 9 mL of 2, 6-dichlorophenolindophenol (dye), vortexed for 2 min and incubated in the dark for 10 min before the measurement read at absorbance 515 nm against a blank. Ascorbic acid content of each sample was calculated on the basis of the calibration curve of standard L-ascorbic acid. Results were expressed as milligram of ascorbic acid per a 100 mm of crude pomegranate juice (PJ) (mg AA/100 mL).

Total phenolic content

Folin–Ciocalteu (Folin C) method as described by Makkar et al. [28] was used to determine the total phenolic content in triplicate. Pomegranate juice (1 mL) was extracted with 50% methanol in a centrifuge tube. The mixture was sonicated in cold water for 10 min and thereafter centrifuged (Merk, Eppendorf AG, Germany) at 4472 g force for 15 min at 4 °C to prevent interference of particles during measurement of absorbance. About 50 µL of diluted aqueous methanolic juice extracts in the test tube was mixed with 450 µL of 50% methanol followed by the addition of Folin C reagent (500 µL) and 2.5 mL of sodium carbonate solution after 2 min. The mixture was vortexed and incubated in a dark chamber for 40 min at room temperature (20 °C) before measuring the absorbance at 725 nm using an UV–Visible spectrophotometer (Thermo Fisher Scientific, Madson, USA). The results were presented as mean of duplicate analyses and expressed as milligrams of gallic acid equivalent per 100 mL of crude pomegranate juice (mg GAE/ 100 mL).

Sensory attributes

Sensory evaluation was carried out using a trained panel of 6 members of the Postharvest Technology Research Group at Stellenbosch University who are familiar with the characteristic taste of pomegranate fruit and regular consumers [29]. Panelists received further orientation on pomegranate sensory attributes [30]. Sensory evaluation was carried out on arils (10 g) served at 21 °C on Petri dishes randomly coded [26]. The descriptive test required panelists to rate the intensity of the attributes on a scale of 0–4 (0 = none, 1 = slight, 2 = moderate, 3 = much, 4 = very much). Pomegranate of the 'Wonderful' cultivar is a sweet–sour cultivar and its acidic content contributes the taste of the cultivar. The sour taste is only undesirable when the sourness is extreme as can sometimes happen in some fruits. The descriptive attributes evaluated for the study included sweet taste, sour taste, crispness, astringency, off flavor, juiciness, grittiness and hardness. Sensory evaluation was not carried out beyond 3 months of storage due to decay and limited sample size.

Statistical analysis

Statistical analysis was carried out using Statistica software (Statistica version 14.0, StatSoft Inc., Tulsa, USA). Data were subjected to factorial analysis of variance

(ANOVA) at 95% confidence interval. Main effects (FLU concentration and storage duration) and their interaction effects (between concentration*storage duration) were also assessed. Post-hoc test (Duncan's Multiple Range Test) was used to test for statistical significance such that observed differences at $p < 0.05$ were considered significant.

Results and discussion

Physiological response

Fruit respiration rate

Results showed that fruit respiration rate was influenced mainly by storage duration ($p < 0.0001$), with increase in respiration rate between 1 and 3 months of storage followed by a decline in the last month regardless of FLU concentration (Fig. 1). The first month of storage showed differences in fruit respiration rate, with fruit treated with 150 and 600 mg/L FLU concentration showing the highest and lowest respiration rates, respectively. Thereafter, fruit respiration rate followed a similar trend with no significant difference among FLU concentrations as storage progressed (Fig. 1). Respiration of pomegranate fruit has been found to increase with advancement of storage [5, 31]. The initial increase in respiration could be due to increased fruit stress as a result of senescence and metabolic reactions as these phenomena have been reported to trigger respiration of pomegranate fruit [5]. The decline in respiration at the end of storage could possibly be due to excessive senescence, physiological disorders and cell death of the membrane due to reduced number of living cells [21]. Therefore, treating pomegranate fruit with fludioxonil did not significantly affect the respiration rate of stored fruit since the changes were mainly influenced by the duration of storage as confirmed by statistical analysis (FLU concentration: $p = 0.2760$; Duration: $p < 0.0001$).

Weight loss

Weight loss is considered as the main cause of loss of visual quality in horticultural produce as excessive transpiration leads to desiccation, shriveling and hastened senescence. In the present study, fruits lost weight over the entire storage period but the magnitude was dependent on FLU concentration of fludioxonil treatment as evidenced by the significant effects of concentration ($p < 0.0001$) and storage duration ($p < 0.0001$) (Fig. 2). After cold storage, fruit treated with FLU at 150 mg/L concentration showed the highest weight loss (26.8%) compared with those treated with 300 mg/L (20.9%) or 600 mg/L (21%) FLU (Fig. 2). In particular, fruit treated with 150 mg/L FLU concentration had higher weight loss (8.5, 15.8, 21.8 and 26.9%) after storage (1, 2, 3 and 4 months, respectively). Since weight loss is linked to respiration rate [5], the high weight loss could be explained by the high respiration rate that was observed for this treatment after the first and last month storage, all along the storage duration (Fig. 1).

Fruit decay

Fruit decay is a major cause of postharvest loss during storage of pomegranate fruit. Fruit decay increased with storage time. Low decay incidences were observed after

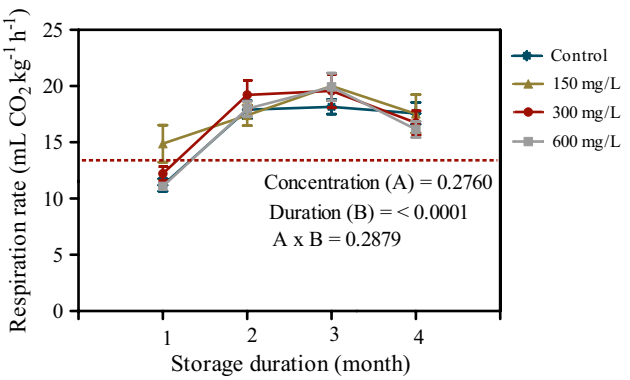

Fig. 1 Effect of fludioxonil concentration on respiration rate of pomegranate fruit during storage for 4 months at 5 °C and an additional 4 days at 20 °C. Each *data point* represents mean and *error bars* designate standard error (SE). ----Respiration rate at harvest. Numerical values for A and B are p-values. (Color figure online)

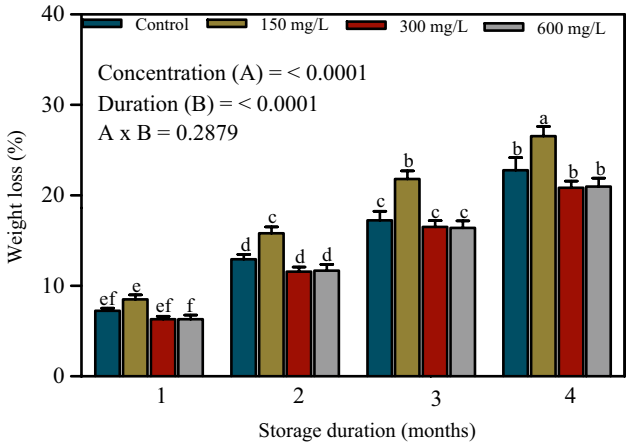

Fig. 2 Cumulative weight loss of pomegranate fruit treated with fludioxonil during storage for 4 months at 5 °C and an additional 4 days at 20 °C. Each *data point* represents mean and *error bars* designate standard error (SE). Numerical values for A and B are p-values

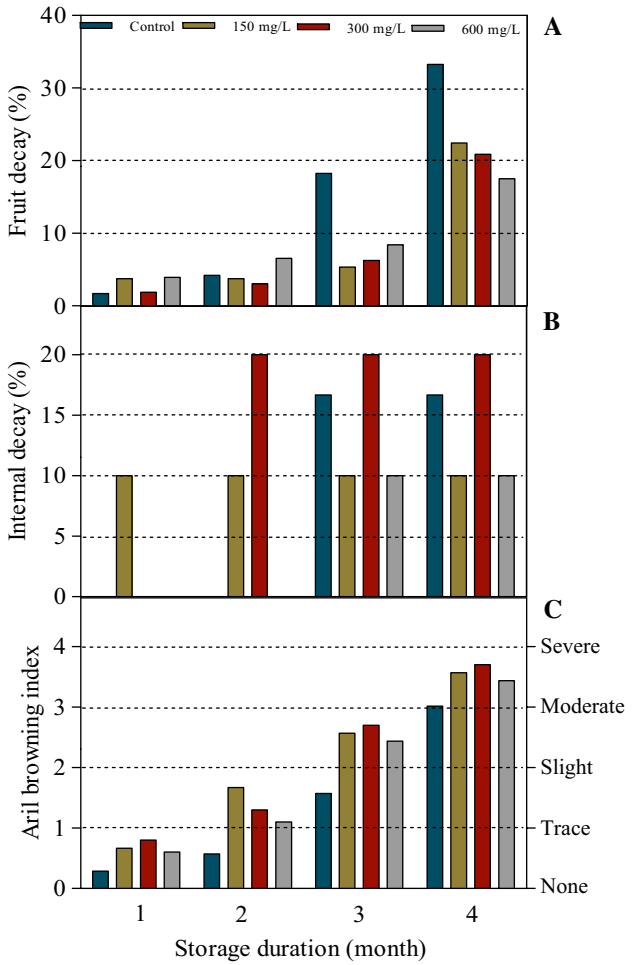

Fig. 3 Influence of fludioxonil concentration on physiological disorders on pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C. External decay (**a**), internal decay (**b**), aril browning (**c**)

the first 2 months of storage but more fruit were decayed as storage progressed (Fig. 3a). High decay incidence was observed for control fruits especially after 3 and 4 months of storage. It is also worth mentioning that percentage fruit decay seemed to decrease with FLU concentration at the end of the storage period (4 months), with 150, 300 and 600 mg/L having 10.8, 12.4 and 15.8% lower decay compared to control respectively (Fig. 3a). The decrease in decay among treated fruit is expected since fludioxonil is a fungicide with excellent protective and preventive activity on a large number of pathogens [21]. Fludioxonil is a reduced-risk fungicide that has a wide spectrum against many pathogens and has been used to control decay in a number of fruits such as citrus [32]. It has also been shown to be highly effective at low rates against large spectrum of fungi such as *Botrytis cinerea* and *Penicillium* spp [21]. Despite FLU reducing the decay in the fruit, complete control of decay was not achieved, possibly because FLU is non-systemic and is not able to move deep through the peel of the fruit [32].

Internal decay

The major internal decay disorder observed was heart rot, also known as black heart, caused by *Aspergillus niger* and *Alternaria* spp., which is characterised by a mass of black arils [33]. This is a preharvest disease that affects the postharvest quality of pomegranate fruit and from the results, it increased with storage of fruit during the study (Fig. 3b). Fruit treated with 150 mg/L FLU concentration developed internal decay earlier but maintained the same percentage decay throughout the storage duration whereas 300 mg/L and control fruit developed decay after storage for 2 months. The highest concentration (600 mg/L) developed decay latest and had the lowest percentage internal decay at the end of storage. No direct relationship can be drawn on the effect of fludioxonil on the internal decay of pomegranate fruit as some treatments had higher decay than untreated fruit although it could possibly be that fludioxonil reduced the growth and proliferation of the fungi that caused internal decay after long storage. The efficacy of FLU on internal decay could be questionable because despite being an excellent protective and preventative fungicide, its curative activity is decreased on old established latent infections because it is a non-systemic fungicide and also due to the difficulty of the solution in entering the crown of pomegranate fruit [21]. Therefore, being a preharvest condition [34], this could probably best be prevented by observing good agricultural practices and application of preharvest treatments.

Aril browning

Aril browning increased with progressive storage of fruit with untreated fruit consistently showing lower browning of arils compared to treated fruit throughout the storage duration (Fig. 3c). Aril browning has been associated with enzymatic oxidation and chilling injury in pomegranate fruit [35]. FLU is a non-systemic fungicide [21] and hence does not migrate deep into the fruit. Therefore, its protective effect could be more external with no direct effect on internal (aril) browning.

Chilling injury incidence and severity

Chilling injury (CI) developed from the first sampling date, with an increase in chilling injury percentage incidence as storage advanced indicating an increase in

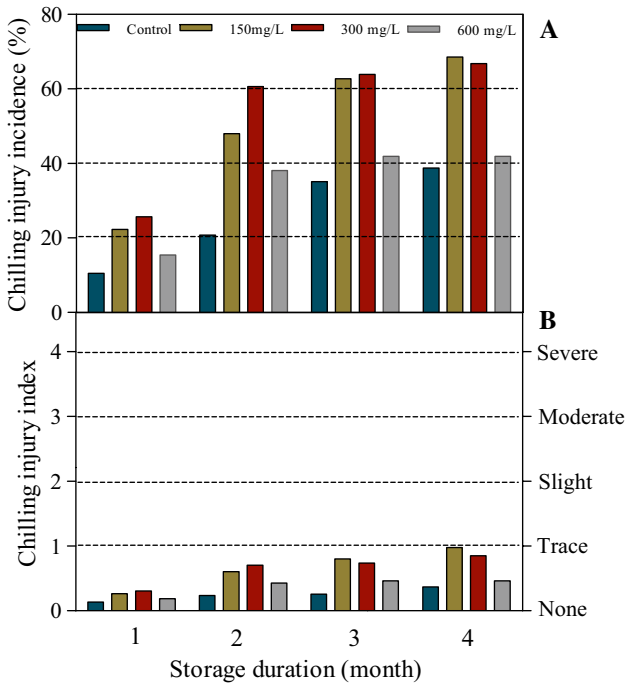

Fig. 4 Effect of fludioxonil on chilling injury incidence and index of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C

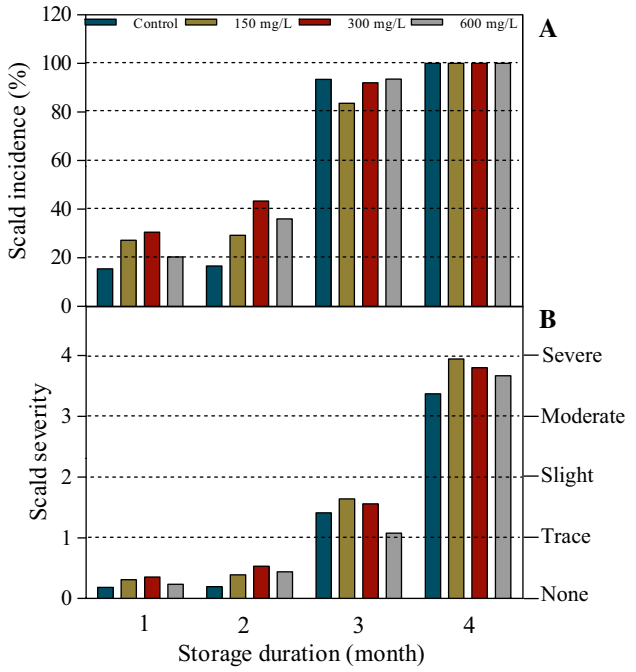

Fig. 5 Effect of fludioxonil on husk scald incidence and severity of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C

number of affected fruit with advancing storage period. Control fruits had a low incidence of CI while fruit treated with 150 and 300 mg/L FLU concentrations had high incidences throughout the storage period with incidences of 68.6 and 66.8% CI, respectively, at the end of storage (Fig. 4a). Despite the occurrence of chilling injury, the severity of the physiological disorder (index) remained below trace level for all concentrations throughout the storage period (Fig. 4b). During chilling conditions, there is a change in state of lipid cell membranes from liquid-crystalline to solid-gel state, which causes deleterious effects on the tissues [31, 36]. From the results, it is apparent that fludioxonil does not possess antioxidant properties that could have reduced chilling injury symptoms in treated samples. Despite its strong antifungal property [21], this study suggests that fludioxonil may indeed have no effect on the occurrence and or the severity of chilling injury on pomegranate fruit regardless of concentration. A similar observation was reported for citrus (oranges, lemon, grapefruit and clementine mandarins) treated with fludioxonil and imazalil at 20 °C [16].

Husk scald incidence and severity

Husk scald, which manifests as peel browning, together with weight loss and skin pitting are the main physiological disorders responsible for market downgrading in pomegranates [37]. Husk scald incidence increased with prolonged storage. Husk scald was observable after the first 2 months of storage albeit at low incidences, but the disorder greatly increased by the end of the storage trials (fourth month) (Fig. 5A). After the first 2 months, control fruit had the lowest husk scald incidence while fruit treated with 300 mg/L FLU concentration had the highest incidences (Fig. 5a). After 3 months, 150 mg/L concentration showed the lowest scalding but by the end of storage, regardless of treatment, all fruits had developed husk scald (Fig. 5a). Husk scald severity (index) was low for the first 2 months of storage with all treatments having below trace scalding (Fig. 5b). After 3 months of storage, scalding was between trace and slight with fruit treated with 600 mg/L concentration showing the lowest incidence. The last month of storage was prominently characterised by high scald severity between moderate and

severe although control fruit showed the lowest severity (Fig. 5b). This indicates that treating fruit with fludioxonil was not effective in alleviating husk scald in pomegranate fruit. This is in agreement with D'Aquino et al. [37] who reported no significant effect of fludioxonil on husk scald and overall appearance of pomegranate fruit (cv. Primosole) during 12 weeks of storage at 8 °C. Husk scald is as a result of enzymatic oxidation of the phenolic compounds in the fruit peel. Tissue browning is reported to be due to oxidation of phenolic compounds into quinone compounds under aerobic conditions by polyphenol oxidase. The quinones then undergo polymerization forming brown pigments thus leading to browning [38]. Zhang and Zhang [38] reported enzyme-mediated denaturation of skin tannins as the basis for pomegranate fruit browning. This was further buttressed by D'Aquino et al. [21] who observed that husk scald of 'Wonderful' pomegranate at 6 or 10 °C decreased and progressed at a slower rate when the tension of oxygen reduced, however, the incidence of scald increased after transfer of fruit to 20 °C in conventional atmosphere. The development of scald in all fruits at the end of storage (Fig. 5b) indicates the relevance of combining FLU treatment with other treatments that can reduce oxygen supply to the fruit during storage, for instance, physical treatments like wrapping, coatings and controlled atmosphere.

Fruit peel and aril colour parameters

Fruit peel colour

Colour of pomegranate is an important quality attribute that is fundamental for consumer preference [25]. Generally, peel redness (a*) decreased with storage for all concentrations, with significant differences observed in peel redness after 3 months of cold storage (Table 1). Changes in peel redness were influenced by storage duration ($p < 0.0001$). The decrease in redness could be the result of alteration of peel colour pigments due to senescence during storage and also peel browning as a result of physiological disorders [10]. Peel colour intensity (C*) followed a similar pattern with progressive storage, with significant effect

Table 1 Peel and aril colour parameters of pomegranate fruit treated with fludioxonil during storag e for 4 months at 5 °C and additional 4 days at 20 °C

Storage duration (month)	FLU Concentration (mg/L)	Peel			Aril			TCD
		a*	C*	h°	a*	C*	h°	
Harvest		42.5 ± 1.3	51.7 ± 1.0	34.7 ± 1.1	16.3 ± 0.7	18.1 ± 0.8	24.3 ± 1.0	46.2 ± 0.9
1	0 (Control)	38.2 ± 2.5^{ab}	49.1 ± 1.6^{a}	38.6 ± 2.8^{bcd}	20.2 ± 1.3^{abcd}	22.3 ± 1.4^{abc}	24.9 ± 1.2^{a}	40.3 ± 1.8^{abc}
	150	39.5 ± 1.4^{a}	48.5 ± 1.2^{a}	35.4 ± 1.4^{d}	18.3 ± 1.0^{bcdef}	20.2 ± 1.0^{abcdef}	23.3 ± 1.5^{ab}	43.3 ± 1.9^{a}
	300	37.4 ± 2.3^{ab}	46.7 ± 1.5^{ab}	36.5 ± 2.7 ^{cd}	20.2 ± 0.9^{abc}	21.6 ± 1.1^{abcde}	24.6 ± 1.6^{a}	38.3 ± 1.6^{abcde}
	600	38.3 ± 1.7^{ab}	46.7 ± 1.3^{ab}	35.1 ± 1.5^{d}	17.9 ± 0.9^{bcdef}	19.4 ± 1.1^{abcdef}	22.2 ± 0.9^{ab}	41.1 ± 1.4^{abc}
2	0 (Control)	36.1 ± 1.9^{ab}	47.4 ± 1.4^{ab}	39.9 ± 2.4^{bcd}	20.9 ± 1.7^{ab}	22.8 ± 1.9^{ab}	22.7 ± 0.8^{ab}	39.1 ± 2.3^{abcd}
	150	33.3 ± 1.4^{bcd}	44.9 ± 0.8^{bc}	41.1 ± 1.8^{abcd}	17.9 ± 0.9^{bcdef}	19.2 ± 1.0^{bcdef}	21.3 ± 0.6^{ab}	40.9 ± 1.4^{abc}
	300	35.3 ± 1.6^{abc}	47.3 ± 1.2^{ab}	41.5 ± 1.9^{abcd}	19.1 ± 0.8^{abcde}	21.0 ± 0.9^{abcde}	23.8 ± 0.9^{ab}	42.3 ± 2.1^{ab}
	600	33.8 ± 1.5^{bcd}	44.4 ± 1.1^{bc}	40.4 ± 1.7^{bcd}	17.1 ± 0.7^{def}	18.9 ± 0.8^{cdef}	23.9 ± 1.4^{ab}	38.2 ± 1.7^{abcde}
3	0 (Control)	34.9 ± 2.2^{abc}	47.9 ± 1.4^{ab}	43.1 ± 2.5^{ab}	20.6 ± 1.0^{ab}	22.2 ± 1.1^{abcd}	21.4 ± 0.5^{ab}	43.8 ± 1.7^{a}
	150	29.1 ± 1.0^{def}	38.2 ± 0.8^{ef}	41.9 ± 1.91^{abc}	15.3 ± 0.6^{f}	16.8 ± 0.6^{f}	23.5 ± 1.1^{ab}	35.4 ± 1.6^{cde}
	300	30.5 ± 1.5^{cdef}	41.8 ± 1.1^{cd}	43.9 ± 1.8^{ab}	16.9 ± 0.9^{ef}	18.4 ± 1.1^{ef}	21.3 ± 0.8^{ab}	36.9 ± 0.9^{bcde}
	600	30.9 ± 1.2^{cde}	42.5 ± 1.0^{cd}	43.6 ± 1.5^{ab}	17.1 ± 0.7^{cdef}	18.6 ± 0.8^{def}	21.9 ± 0.5^{ab}	43.6 ± 2.0^{a}
4	0 (Control)	28.5 ± 1.3^{ef}	40.3 ± 0.8^{def}	44.6 ± 2.1^{ab}	21.4 ± 1.1^{a}	22.9 ± 1.1^{a}	20.5 ± 0.5^{b}	33.1 ± 1.9^{ef}
	150	26.0 ± 1.1^{f}	35.2 ± 0.7^{f}	41.6 ± 2.1^{abcd}	18.5 ± 0.9^{abcde}	20.4 ± 1.1^{abcde}	24.3 ± 1.1^{a}	28.9 ± 2.3^{f}
	300	26.2 ± 1.0^{ef}	36.1 ± 0.9^{f}	42.7 ± 1.9^{abc}	19.6 ± 1.1^{abcde}	21.6 ± 1.1^{abcde}	24.0 ± 1.4^{ab}	29.8 ± 1.9^{f}
	600	25.9 ± 1.1^{f}	38.5 ± 0.9^{ef}	47.4 ± 1.5^{a}	17.1 ± 0.7^{cdef}	18.3 ± 0.9^{ef}	23.3 ± 1.2^{ab}	33.7 ± 1.6^{def}
Significance level	Concentration (A)	0.1570	<0.0001	0.6152	<0.0001	<0.0001	0.6146	0.1238
	Storage duration (B)	<0.0001	<0.0001	<0.0001	0.0483	0.0598	0.1932	<0.0001
	A x B	0.7878	0.0033	0.7304	0.6649	0.7566	0.0595	0.0052

Data presented as mean ± SE.

Different letters across concentration and storage duration for each attribute differ significantly ($p < 0.05$) according to Duncan's multiple range test.

SE standard error

of FLU concentration ($p < 0.0001$) and storage duration ($p < 0.0001$) (Table 1). It is, however, worth mentioning that after 3 months of storage, control fruit had the highest colour intensity (C* = 47.88) while fruit treated with 150 mg/L FLU concentration had the lowest values (C* = 38.23) (Table 1). Storage duration affected peel hue angle (h°) ($p < 0.0001$), which generally increased with fruit storage with no significant differences observed at all storage periods for all concentrations (Table 1). Again, this indicates loss of red colouration in fruit peel with storage duration possibly due to the development of physiological disorders such as husk scalding in fruits and degradation of anthocyanin pigments [10]. The findings in the current study corroborate the report by D'Aquino et al. [37] who found that treating 'Primosole' pomegranate fruit with FLU (at 600 mg/L) had no significant effects on the overall appearance after cold storage at 8 °C for 12 weeks.

Aril colour

Results showed a decrease in aril colour redness (a*) for all treatment concentrations after the first 3 months of storage followed by a slight increase with long storage. The effect of FLU concentration was significant ($p = < 0.0001$) with untreated fruit having highest aril a* from the second to the last month of storage (Table 1). The initial decrease in aril a* could be attributed to browning of arils due to alteration of red colour pigments [10]. A similar trend was observed for aril colour intensity (C*) with untreated fruit having significantly higher aril C* throughout the storage duration and significant effect of concentration ($p = < 0.0001$) (Table 1). The high C* in untreated fruit corresponds with the highest aril a* indicating greater anthocyanin biosynthesis in control compared to treated fruit. The high a* and C* in untreated fruit can be related to the lower aril browning that was observed for untreated fruit (Fig. 3c). However, although arils from treated fruit had lower C* and a* values, the aril colour was sufficient enough for consumption. Aril hue angle (h°) differed with storage of fruit with no significant differences observed among all concentrations at all storage times (Table 1). The total colour difference (TCD) indicates the colour disparity between the peel and aril and this was influenced by the storage duration. TCD decreased during storage however, no significant

Table 2 Chemical attributes and juice colour of pomegranate fruit treated with fludioxonil during storage for 4 months at 5 °C and additional 4 days at 20 °C

Storage duration (month)	FLU Concentration (mg/L)	pH	TA (% citric acid)	TSS (°Brix)	BrimA
Harvest		3.3 ± 0.0	1.7 ± 0.1	16.2 ± 0.2	12.8 ± 0.2
1	0 (Control)	2.9 ± 0.1^{h}	2.5 ± 0.3^{a}	14.8 ± 0.2^{d}	9.8 ± 0.6^{g}
	150	3.1 ± 0.1^{gh}	2.5 ± 0.2^{a}	15.2 ± 0.2^{bcd}	10.3 ± 0.3^{efg}
	300	3.2 ± 0.1^{fg}	2.1 ± 0.1^{bc}	15.8 ± 0.3^{ab}	11.6 ± 0.3^{bcd}
	600	3.4 ± 0.0^{cd}	2.0 ± 0.2^{bc}	16.0 ± 0.2^{ab}	11.9 ± 0.5^{bcd}
2	0 (Control)	3.3 ± 0.2^{def}	1.4 ± 0.1^{efg}	15.2 ± 0.4^{bcd}	12.8 ± 0.6^{ab}
	150	3.8 ± 0.1^{a}	1.4 ± 0.1^{efg}	16.1 ± 0.2^{a}	13.1 ± 0.3^{a}
	300	3.3 ± 0.0^{def}	1.3 ± 0.1^{ghi}	15.6 ± 0.2^{abc}	13.1 ± 0.4^{a}
	600	3.2 ± 0.0^{def}	1.4 ± 0.1^{fgh}	$15.3 \pm 0.2^{a–d}$	12.6 ± 0.2^{abc}
3	0 (Control)	3.5 ± 0.1^{bc}	2.2 ± 0.2^{ab}	14.7 ± 0.4^{d}	10.2 ± 0.6^{fg}
	150	3.2 ± 0.1^{ef}	1.7 ± 0.1^{def}	14.7 ± 0.2^{d}	11.4 ± 0.3^{cde}
	300	3.6 ± 0.0^{ab}	1.8 ± 0.0^{cde}	15.3 ± 0.2^{bcd}	11.8 ± 0.2^{bcd}
	600	3.7 ± 0.1^{a}	1.9 ± 0.1^{cd}	14.9 ± 0.2^{cd}	11.2 ± 0.4^{def}
4	0 (Control)	3.6 ± 0.0^{ab}	1.3 ± 0.0^{ghi}	14.6 ± 0.2^{d}	12.0 ± 0.2^{abcd}
	150	3.4 ± 0.1^{cde}	1.1 ± 0.1^{hi}	13.7 ± 0.4^{e}	11.3 ± 0.5^{def}
	300	3.4 ± 0.0^{cde}	0.9 ± 0.1^{i}	13.4 ± 0.3^{e}	11.5 ± 0.4^{cd}
	600	3.4 ± 0.0^{cde}	0.9 ± 0.1^{i}	13.3 ± 0.2^{e}	11.4 ± 0.3^{cde}
Significance level	Concentration (A)	0.1723	0.0004	0.664	0.0223
	Storage duration (B)	<0.0001	<0.0001	<0.0001	<0.0001
	A x B	<0.0001	0.1826	0.0001	0.0020

Data presented as mean ± SE

Different letters across concentration and storage duration for each property differ significantly ($p < 0.05$) according to Duncan's multiple range test

TA titratable acidity, *TSS* total soluble solids, *SE* standard error

differences were observed among concentrations during storage with the exception of month 3 (Table 1).

Chemical properties

pH, titratable acidity, total soluble solids and BrimA

There were slight fluctuations observed in pH during storage of fruit (Table 2), with a significant interaction ($p < 0.0001$) between FLU concentration and storage duration. With regard to TA, changes were driven by both storage duration ($p < 0.0001$) and FLU concentration ($p = 0.0004$) (Table 2). In general, TA decreased with prolonged storage duration except after month 3 where slight increases were observed probably due to the concentration of acids resulting from weight loss [5]. Organic acids (which mainly contribute to titratable acidity) have been reported to be the major substrates for respiration during storage of pomegranate fruit [5] hence the reduction in TA as storage advanced.

There was a general decrease in TSS during storage with no significant ($p = 0.6640$) effect of concentration on TSS values (Table 2). At the end of storage, no significant differences were observed among the treated fruit, which had lower TSS values than control fruit. In addition, a significant interaction ($p = 0.0001$) between concentration and storage duration was observed (Table 2). This indicates that storage duration had a prominent effect on TSS decrease, but FLU concentration seemed to amplify this influence of storage duration. A similar reduction in TSS during storage of pomegranate fruit has been previously reported [5, 24]. However with regard to the postharvest treatment of pomegranate fruit, Mirdehghan et al. [36] observed no changes in TSS during storage of pomegranate fruit treated with polyamines. In the current study, the observed reduction in TSS with prolonging storage duration could be attributed to the utilisation of sugars in some metabolic processes such as fruit respiration during storage [5]. The loss of volatile compounds, which may occur concomitantly with water loss, also play a role in TSS decrease.

BrimA is based on changes in soluble solids and titratable acidity and determines the characteristic taste and flavour of fruits [10]. BrimA was influenced by FLU concentration ($p = 0.0223$), storage duration ($p = < 0.0001$) and the interaction of the two factors ($p = 0.0020$) (Table 2) and generally increased after the first 2 months and thereafter decreased after the last 2 months of storage. The first 3 months of storage were characterised by lower BrimA in control fruit indicating lower sweet taste according to human tongue sensitivity. However, by the end of storage, control fruit had higher BrimA compared to treated fruit (Table 2).

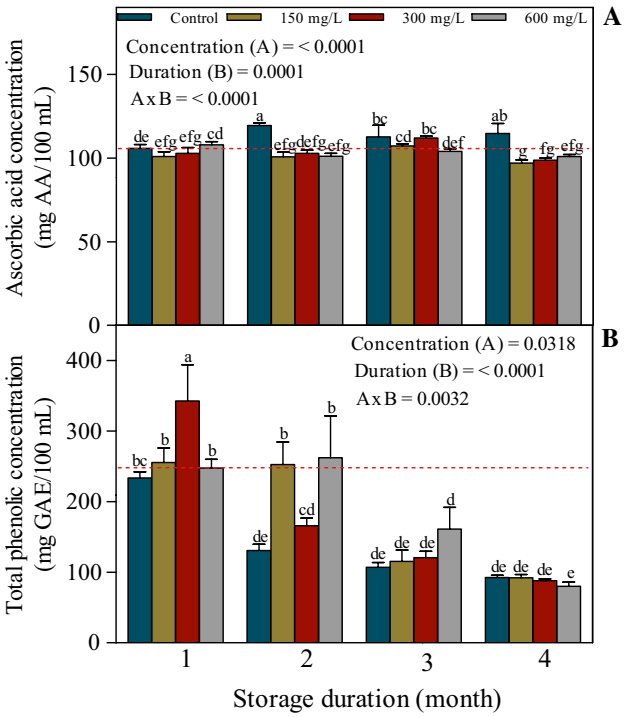

Fig. 6 Changes in ascorbic acid concentration (**a**) and total phenolic concentration (**b**) of pomegranate fruit during storage for 4 months at 5 °C and additional 4 days at 20 °C. Each bar represents mean and error bars denote standard error (SE) of the mean. Bars followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. *Red lines* Represents values at harvest. Numerical values for A and B are p-values. (Color figure online)

Phytochemicals

Ascorbic acid (AA) content

Ascorbic acid content decreased as storage progressed with significant differences observed among FLU concentrations and a significant interaction between FLU concentration and storage duration ($p < 0.0001$) (Fig. 6a). The decrease in ascorbic acid during storage could be attributed to conversion of ascorbic acid to dehydroascorbic acid, which decreases the active form of ascorbic acid [39]. Untreated fruit on the other hand consistently showed high AA content compared to treated fruit throughout the storage period (Fig. 6a) and this could be as a result of concentration due to weight loss. This seemed to highlight a negative influence of FLU treatment on AA content. Arendse et al. [39] also reported a decrease in AA content with progressive storage of 'Wonderful' pomegranate fruit stored at 5 and 7.5 °C for 5 months.

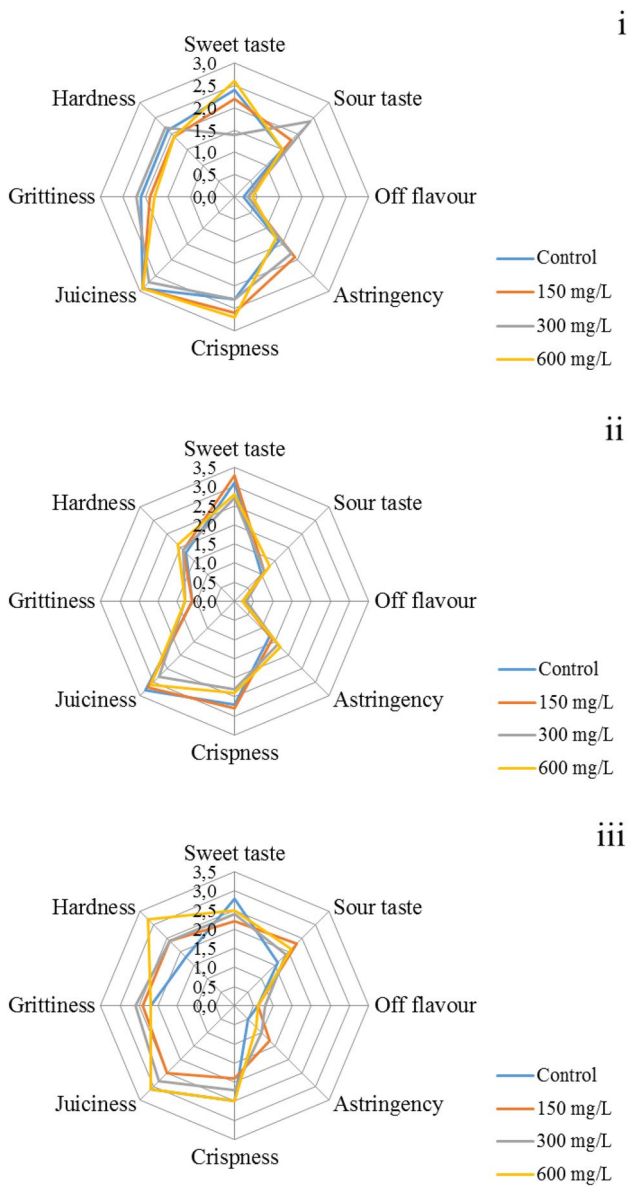

Fig. 7 Radar plot showing averaged sensory scores of pomegranate fruit treated with fludioxonil during storage for 3 months at 5 °C and additional 4 days at 20 °C. The plot represents storage at month 1 (**i**), month 2 (**ii**) and month 3 (**iii**)

Total phenolic content (TPC)

A significant reduction was observed in TPC during storage of pomegranate in this study with all FLU concentrations having less than 150 mg GAE/100 mL at the end of the storage duration (Fig. 6b). There was a significant interaction between FLU concentration and storage duration ($p=0.0032$) and fruits treated with FLU had higher TPC than untreated fruit for the first 3 months of storage (Fig. 6b). This showed that treating fruit with fungicide resulted in greater TPC than control for up to 3 months of storage. A similar decrease in phenolic content during storage of pomegranate fruit has been previously reported [5, 40]. The reduction in phenolic content during storage of pomegranate fruit can be attributed to alteration of phenolic compounds as a result of enzymatic activity [5].

Sensory attributes

The sensory analysis showed that sweet taste, crispness and juiciness was scored higher in fruit treated with 600 mg/L FLU concentration (Fig. 7a) while sour taste, grittiness and hardness were scored higher in control and 300 mg/L FLU treated fruit. Off flavour was generally low while astringency was more pronounced for 150 mg/L and 300 mg/L FLU concentration. Pomegranate fruit is characterised by high sweet taste, crispness and juiciness, moderate sour taste and astringency and absence of off-flavour. Therefore treating pomegranate fruit with the highest concentration (600 mg/L) of fludioxonil achieved the best sensory characteristics after storage for 1 month. No great differences were observed between samples after 2 months of storage (Fig. 7B). After 3 months of storage, 600 mg/L FLU treated fruit had high crispness, juiciness, hardness and sour taste and low off-flavour and grittiness while control had high sweet taste and 150 mg/L FLU had high astringency (Fig. 7c). The erratic evolution of grittiness could be attributed changes in fruit to physiology during storage. Untreated fruit had good sensory quality although 600 mg/L FLU concentration retained the best characteristics for most sensory attributes in terms of crispness, juiciness and low off-flavour.

Pearson's correlation analysis of physiological responses and disorders

Pearson's correlation showed that fruit weight loss increases with increased respiration and decay as indicated by the moderate positive correlation ($r=0.63$ and $r=0.77$ for respiration rate and weight loss respectively) (Table 3). Fruit weight loss had strong and positive correlations with fruit decay, chilling injury, scald and aril browning indicating the impact of such physiological disorders on fruit weight. Chilling injury correlated strongly with aril browning supporting findings by Elyatem & Kader [41], who reported that chilling injury affects fruit internal quality. Husk scald strongly associated with fruit decay (Table 3) suggesting that development of scald enhances susceptibility of fruit to decay possibly due to increased senescence. This is also true for internal decay, which had moderate positive correlations with respiration rate, weight loss, chilling injury, scald and aril browning.

Table 3 Pearson correlation coefficient matrix between assessed physiological disorders

Variables	Respiration rate	Weight loss	Fruit decay	CI severity	% CI incidence	Scald severity	% Scald incidence	Aril browning	Internal decay
Respiration rate	**1**	**0.632**	0.247	**0.589**	**0.707**	0.274	**0.562**	**0.509**	**0.595**
Weight loss		**1**	**0.766**	**0.690**	**0.733**	**0.864**	**0.860**	**0.912**	**0.566**
Fruit decay			**1**	0.267	0.348	**0.867**	**0.761**	**0.734**	0.489
CI severity				**1**	**0.970**	**0.573**	**0.573**	**0.741**	**0.577**
% CI incidence					**1**	**0.596**	**0.679**	**0.782**	**0.715**
Scald severity						**1**	**0.848**	**0.926**	**0.524**
% Scald incidence							**1**	**0.917**	**0.691**
Aril browning								**1**	**0.635**
Internal decay									**1**

Values of correlation coefficient >0.5 are highlighted in bold

Conclusions

Treating pomegranate fruit with FLU concentrations improved fruit attributes such as fruit decay, weight loss, fruit firmness and total phenolic content. The sensory attributes of FLU treated fruit were maintained especially when fruit were treated with 600 mg/L FLU concentration. However, the fungicide was not beneficial in improving parameters such as aril browning, chilling injury, scalding, aril redness and ascorbic acid content. Therefore further studies should be carried out on the use of hurdle technology where the fungicide may be used in combination with other technologies such as physical treatments to fully harness the potential of the chemical in postharvest technology.

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