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Comparative analysis of pomegranates harvested from an organic orchard and a conventional one irrigated with treated wastewater

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ABSTRACT

Purpose: Nowadays, the problem of water scarcity increased due to the increase of aridity, which enhances the use of non-conventional waters. The irrigation with treated wastewater was primarly developed. **Research method:** In the present research, we compared the quality of pomegranate fruits harvested from two '*Gabsi*' orchards managed according to two different cultural practices such as: organic (O) and conventional irrigated with treated wastewater (WW). We determine the soil and fruit characteristics. **Findings:** Results showed that the organic soil was richer in Fe, Mn and Mg; however, irrigated with WW was richer in Cu, Zn, and Cd with the absence of Ag and Ni in both soils. The analysis of fruits demonstrated that organic ones were richer in Cu, Zn and Mn. Mg content was similar in both fruits. Hg, Ni, Cd, and Fe were not present in fruits. The BCF of Cu and Zn were higher in organic fruits, contrary to Mg. Furthermore, the highest values of the SSC/TA ratio were recorded in organic fruits with no pathogenic contamination for both fruits. **Limitations:** No limitations were founded. **Originality/Value:** This study illustrates the possibility of treating wastewater in the irrigation of pomegranates without adverse effects.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient precious fruit tree. It is grown in many different geographical regions, including the Mediterranean basin. Due to its richness in various bioactive compounds, it is considered a medical remedy. Actual scientific findings confirm the traditional usage of the pomegranate (Holland & Bar-Ya'akov, 2014). Consequently, more attention was paid to this species, which manifests through the increased of cultivated areas in the world. Likewise, in Tunisia, the pomegranate orchards area was increased from 7898 ha in 2016, to 13257 ha in 2019 (DGPA, 2020). New orchards are now planted to increase production. Cultural practices were modified to actual conditions, particularly the irrigation was required to increase yield in arid and semiarid regions that are the most suitable regions for pomegranate. In view of climate change and water scarcity in these regions, the use of municipally treated wastewater was developed on various fruit orchards, among them pomegranate ones. This practice seems to be an effective solution, but the control of fruit quality is essential. Mainly, the determination of heavy metals accumulation is required. These compounds, even in trace amounts, showed a threat to human health. Heavy metals include micronutrients (Fe, Mn, Cu, Zn, Mo), which are essential for normal metabolic processes, but in excessive quantities, they become harmful. However, As, Hg, Pb, or Cd are also heavy metals even at low concentrations, they are harmful to humans and animals; they can be arranged in the following order: Hg > Cu > Zn > Ni > Pb > Cd > Cr > Sn > Fe > Mn > Al according to their toxicity (Wang et al. 2003; Pueyo et al. 2004; Filipiak-Szok et al. 2015). In this context, several studies were established to evaluate the quality of fruits irrigated with treated wastewater. Some of them were interested in the concentration of heavy metals. Some others were consecrated to bacteriological contamination and organoleptic characteristics. Furthermore, the irrigation of citrus trees by mixed waste and freshwater improved yield parameters, soluble solids, and Brix/acid ratio. Besides, fruits appeared healthy despite the increase of K, Fe, Mn, Zn, Ni, and Na in its peel and pulp (Galal, 2015). Moreover, some studies of the quality of commercialized fruit juice in markets were done. Anastácio et al. (2018) affirmed that the Mn and some heavy metals such as Ni and Pb content exceeded the limits imposed by international organizations, whereas Co, Cu, Zn, As, and Cd concentrations were below the acceptable limit. Also, they insisted on the reduction of metal concentrations in consumed juices. For vegetables, it was found a significant decrease in micronutrients in the edible part of crops from organic farming compared to conventional ones, which showed an increase in toxic metals. This increase remains under the accepted limits from human consumption (Hattab et al., 2019). Moreover, Riahi et al. (2009) noted that fruit soluble solids, pH, total titratable acidity and firmness of tomatoes were related to the production system, and cultivar. In another research on the quality of tomatoes irrigated with treated wastewater it was that treated wastewater was highly contaminated with total coliform and total bacterial count, which aggravated cross-contamination (Al Lahham et al., 2003). They suggested that tomatoes can be eaten after cooking. Also, a significant increase was recorded in fruit diameter by the use of treated wastewater irrigation treatments face to low values of soluble solids content. The pH remains unaffected by treatments. Despite its use as a medical remedy, pomegranate irrigated with treated wastewater was given less attention. Especially that authority allows irrigating with treated wastewater only for forages and trees. Indeed, few or no studies on the quality and the concentration of nutrients and heavy metals can be found.

Besides the conventional system and the use of treated wastewater, organic agriculture has been developed globally. Tunisia, like many other countries, works to increase organically cultivated areas. Fruit trees cultivated areas were 6550 ha in 2010 and 11243 ha in 2018 (ONAGRI, 2019). The pomegranate accounts among organic cultivated fruit species. The production of healthy fruits becomes a challenge for the next years.

Then, in the present study, we compare the quality of pomegranate harvested from two different orchards managed according to conventional system irrigated with treated waterwaste, and an organic system irrigated with freshwater. Organoleptic Characteristics, contamination assessment, micronutrients, and heavy metals contents were determined for a main cultivated variety 'Gabsi'.

MATERIALS AND METHODS

Area of Study and Orchards Characteristics

The present research was carried out in the Central East region of Tunisia in 2019. The climate is semi-arid. The average annual precipitation is 350mm. Two pomegranate orchards were selected. The first one is located in the 'Ouardanine' region, known for its significant fruit trees production. Tree are managed by a conventional system receiving the cultural practices and irrigated with treated wastewater (WW) for five years (date of tree plantation). The quality of water is regularly controlled. It is free from pathological agents. The average salinity is about 1.8 g/l. Irrigation was done following a seasonal calendar, and the use of WW deemed rich in required mineral elements, the use of fertilizers is reduced even absent. Trees were regularly controlled against pests by chemical treatments and traps. The second orchard is located in the 'Chott Mariem' region and certified as organic for more than five years. In this region pomegranate cultivation is a tradition. Trees that belong to the experimental station of the Regional Research Centre of Horticulture and Organic Agriculture are organically conducted. All cultural practices respect the organic rules. Trees are irrigated with freshwater (1g/l) according to seasonal schedule. For each orchard, 20 trees were used. In the present study, two types of soils and fruits were analyzed. Ones sampled from the organic orchards (O) and others from the conventional orchard irrigated with (WW).

Soil Analysis

From each orchard, five points were marked. Soil sampling was done from the horizon 20-45 cm main roots absorption zones. At each point three replicates were picked up. General properties such as: pH, electric conductivity (CE), and organic matter were determined. Micronutrient concentrations in soil were done using a Flame photometer (JENWAY®) for K, Na, Ca, and spectrophotometer (BIOBASE®) for the phosphorus. A 0.5 g of each air-dried soil sample was accurately weighed in a digestion tube. A mixture of acids (1:3 HCl: HNO_3) was added. The digestion tubes were then placed on the oven and heated for three h. All the digests were cooled and filtered through filter paper and diluted to 50 ml by distilled water. Each sample was kept for metal analysis. The instrument was calibrated using calibration blank and a series of working standard solutions of each metal to be analyzed. The digested samples were determined for the concentrations of heavy metals and nutrients (Fe, Zn Mn, Mg, Cu, Ni, Cd, and Hg) using flame atomic absorption spectrophotometer (Reeuwijik, 2002; Brown et al., 2003).

Fruit Sampling and Analysis

The fruits of nine trees of the different orchards were individually harvested. A subsample of 5 representative fruits of each tree was used to control the quality parameters. The measurement of weight and an equatorial perimeter was done to determine the average weight and size of the fruits.

Pomegranates were washed and rinsed twice with deionized water. Representative juices of the different pomegranate samples were obtained with a kitchen juicer, all in cleaning between samples. Total soluble solids content (SSC) of the juices in ◦Brix was assessed using a digital hand refractometer (ATAGO), the total acidity (TA) expressed in g L^{-1}, was measured by titration of 10 mL of juice with 0.1 N NaOH solution, and the pH was determined by pH-meter (JENWAY®). The sugar-to-acid ratio (SSC/TA) was estimated from recorded data to evaluate the management system's influence in this maturity index. This parameter is a determinant that is ultimately most closely related to consumer acceptance of fruits (Crisosto et al., 2006).

Micronutrients and heavy metals were determined using atomic absorption. After digestion with 1.5 mL of nitric acid and 2 mL of hydrogen peroxide during six h. Solutions were made up to 10 mL with ultrapure water. The resulting colorless solutions served in read the absorbance. The standard solutions, prepared at the same reagents and at the same ratios, were added for control. The concentrations of trace elements were estimated by atomic absorption spectrophotometer (Thermo-Scientific). Standard materials were included for assurance control. Average concentrations of metals were obtained from triplicate. The detection limit (LOD) of tested metals was 0.05 µg/g dry weights (Rangana, 2004).

Bioconcentration Factor Calculation

The Bioconcentration Factor (BFC) illustrates the capability of vegetal to accumulate heavy metals. It is defined as the ratio of any given element in plant tissue to that in the soil. In our case, we used the concentration in pomegranate fruit and soil (Hattab et al., 2019).

Quality Assurance Assessment

To guarantee the quality of fruit, it is necessary to make sure that this fruit is free from any risk (heavy metals or pathogens, for example) and presents good organoleptic quality attributes. The pomegranate characteristics data were judged according to the certified concentrations of different heavy metals allowed by OMS. Also, the quality acceptance by the consumers was assessed according to Crisosto et al. (2006).

Pathogenic Contamination

To assess total coliforms in pomegranate juice, we used the multiple tube fermentation technique according to Gaspard and Schwartzbrod (1993). The method was based on using a cationic detergent (1:1) and a solution of 55 % $ZnS0_4$. The quantification was done through a counting cell after culture

Statistics

Presented values are the means at least of three replicates. Analysis of Variance (ANOVA) was carried out using SPSS Statistics software (20). Significant differences between conventional and organic systems were performed using the Duncan's test at 5%. To compare fruit, characteristics and the effect of management systems, principal component analyses (PCA) were executed using the XLstat.

RESULTS

Data shows differences although, for the soil and the pomegranates harvested from two management orchards systems in some parameters.

Soil Characteristics

The determination of some parameters related to each orchard's soil characteristics showed a significant difference between O and WW (Table 1). The organic matter in the soil of the WW orchard was more critical than O one. The recorded pH given no significant differences, whereas the CEC showed high value in soil irrigated with treated wastewater. The presence of CaCo was noted but; the level is less than 3.00. Moreover, the content of P, K, Ca, and Na showed that they were more critical in WW soil.

Heavy Metals and Micronutrients Loads in Soils and Fruits

In Soils

Table 2 illustrates the concentration of some heavy metals and some micronutrients in the soil. Results showed that Cu and Cd contents were more critical in conventional orchard irrigated with treated wastewater than the organic orchard. The Cu content was 3.03 mg Kg^{-1} in the first face to 2.18 mg Kg^{-1} in the second one. Likewise, Cd content was about 0.015 and 0.013 respectively in O and WW. Hg and Ni were not defined in the soil for both orchards as far as Zn and Fe soil contents showed no significant differences. Whereas, Mn and Mg contents were more important in the O orchard's soil than the WW one. Mn values were range from single to double, such as 12.14 mg Kg^{-1} in O orchard and 6.29 mg Kg^{-1} in WW ones. Recorded Mg values were respectively 532.7 mg Kg^{-1} and 451.5 mg Kg^{-1} in O orchard and WW one.

In Fruits

Results indicated that some heavy metals and some micronutrients in pomegranate increased significantly in organic ones (Table 3). Indeed, Cu, Zn and, Mn pomegranates content were higher in organic ones compared to than those harvested from conventional orchard irrigated with wastewater. However, Hg, Ni, Cd, and Fe contents were not affected by the orchard management system. Pomegranates harvested from the organic orchard and conventional one irrigated with wastewater showed very low values. They are below 0.01µg L^{-1} for Hg, Ni and Cd, and below 0.01mg L^{-1} for iron. Mg fruit content was also influential in organic pomegranate but not statistically significantly different.

The Bioconcentration Factor

The estimation of bioconcentraion factor for the studied compounds reflects previous results (Table 4). Then the O orchard presented a great value compared to WW one, particularly for Cu, and Zn. The rest of the elements (Hg, Ni, Cd, Fe and Mn) didn't show differences between orchards. Inversely, the Mg results revealed that the most critical BCF was found in the WW orchard.

Table 1. Soil characteristics (Organic matter: OM in %, pH, conductivity: CEC in DSM^{-1}, active calcite: CaCo in $mgKg^{-1}$, % clay, % limon, % sand and content of some nutrients: P in ppm, K in $mgKg^{-1}$, Ca $mgKg^{-1}$ and Na $mgKg^{-1}$) of orchards conducted according to different management systems: organic (O) and conventional irrigated with wastewater (WW)

Parameters	O	WW
OM (g Kg^{-1})	1.8±0.20	2.72% ±0.79
pH	7.7 ±0.08	7.92 ±0.04
CEC (DSM^{-1})	180.16±12	414.5 ±2.12
CaCo (mg Kg^{-1})	<3.00	<3.00
% Clay	31.10 ±0.41	25.53 ±1.67
% limon	17.24 ±0.23	16.44 ±2.60
% sand	51.65 ±0.65	58.02 ±4.26
P (ppm)	9.23±0.11	13.34 ±0.199
K (ppm)	0.37±0.02	0.46 ±0.07
Ca (ppm)	2.10±0.10	2.98 ±0.21
Na (ppm)	0.20±0.01	0.22 ±0.02

Means are presented ± SE (n=15). Different letters within each column indicates significant difference between management systems according to Duncan's test ($P \leq 0.05$).

Table 2. Average values of some heavy metals and micronutrients concentration in soils for organic orchard (O) and conventional ones irrigated with treated wastewater (WW).

	Hg mg/Kg	Cu mg/ Kg	Zn mg/ Kg	Ni mg/ Kg	Cd mg/ Kg	Fe mg/ Kg	Mn mg/ Kg	Mg mg/ Kg
O	ND	2.18 ±0.16b	6.76 ±1.27a	ND	0.013 ±0.007b	2.51 ±0.27a	12.14 ±0.67a	532.7 ±1.69a
WW	ND	3.03 ±0.31a	8.22 ±1.49a	ND	0.015 ±0.000a	2.04 ±0.54b	6.29 ±1.42b	451.5 ±11.87b
Significance	ns	*	ns	ns	*	*	*	*

Means are presented ± SE. Different letters within each column indicating significant differences between management systems according to Duncan's test at $P \leq 0.05$ (ns: no significant difference, *: significant difference), ND: not detected.

Table 3. Average values of some heavy metals and micronutrients concentration in pomegranates harvested from an organic orchard (O) and from a conventional one irrigated with treated wastewater (WW)

	Hg µg/L	Cu mg/L	Zn mg/L	Ni µg/L	Cd µg/L	Fe mg/L	Mn mg/L	Mg mg/L
O	ND	0.6±0.09a	0.87±0.05a	ND	ND	ND	0.19±0.014a	4.26±0.94a
WW	ND	0.34±0.09b	0.64±0.007b	ND	ND	ND	0.075±0.007b	3.67±0.26a
Significance	ns	*	*	ns	ns	ns	*	ns

Means are presented ± SE. Different letters within each column indicating significant differences between management systems according to Duncan's test at $P \leq 0.05$ (ns: no significant difference, *: significant difference), ND: not detected.

Table 4. Bioconcentration factor (BCF) of some heavy metals in pomegranates harvested from an organic orchard (O) and conventional one irrigated with treated wastewater (WW)

	Hg	Cu	Zn	Ni	Cd	Fe	Mn	Mg
O	0.00a	0.27a	0.13a	0.00a	0.00a	0.00a	0.01a	0.007b
WW	0.00a	0.11b	0.079b	0.00a	0.00a	0.00a	0.01a	0.008a

Different letters within each column are indicating significant differences between management systems according to Duncan's test ($P \leq 0.05$).

Table 5. Pomegranate soluble solids concentration (SSC, in ◦Brix), pH, titratable acidity (TA in gL^{-1}) and SSC/TA ratio harvested from an organic orchard and a conventional one irrigated with treated wastewater

Treatments	SSC (°Brix)	pH	TA (gL^{-1})	SSC/TA
O	16.26 ±1.59 a	3.9 a	2.77±0.32 b	5.88±0.48 a
WW	15.70 ±0.79 b	4.05 a	4.7±0.70 a	3.19±0.44 b

Means are presented ± SE (n=10). Different letters within each column indicate significant differences between management systems according to Duncan's test ($P \leq 0.05$).

Table 6. Pomegranate total coliforms content harvested from an organic orchard, and a conventional one irrigated with treated wastewater

	Total coliforms
O	<1UFC/g
WW	<1UFC/g

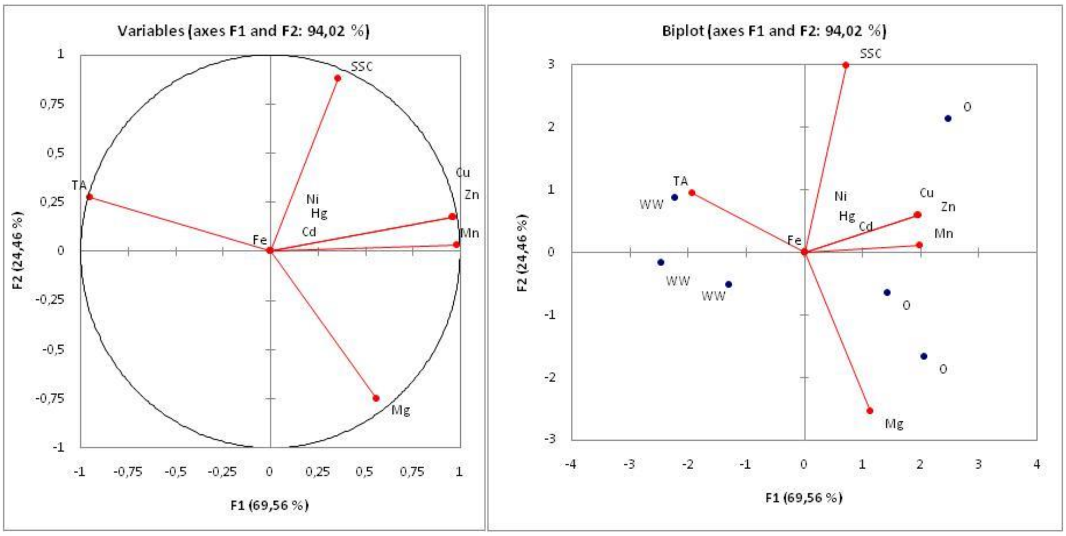

Fig. 1. PCA analysis of micro-nutrients heavy metals and organoleptic traits of pomegranates harvested from the organic orchard and conventional one irrigated with treated wastewater (WW, O: each point represents the mean of three measurements).

Organoleptic Characteristics

Results related to pomegranate quality are shown in Table 5. Significant differences in total soluble solids were found between those harvested from the organic orchard and those from the conventional one irrigated with wastewater. The SSC in organic fruits was higher than in conventional ones. It reached 16.26°Brix. No significant difference in pH, but organic fruits had low titratable acidity than conventional ones. This result is lead to an increase the SSC/TA ratio in the organic fruits. This parameter is considered one of the significant indicators of the taste, and flavor of the pomegranate fruit. At harvest, no difference between orchards was noted.

Pathogenic Contamination

The determination of total coliforms in pomegranates revealed no significant difference between those harvested from O orchard and WW one (Table 6). Indeed, the total counts were below 1 UFC/g.

Principal Component Analysis

Figure 1 summarizes the effect of orchard management on some studied parameters. Principal compound analysis (PCA) was individually realized for TA, SSC, micronutrients, and heavy metals in pomegranates harvested from organic and conventional orchards. Parameters were loaded into two axes with a total variance of 94.02%. The majority of studied parameters were selected by PC1 and so tabled by a larger proportion of variance (69.56%). The lower ratio of variable (24.46%) was presented by PC2. The variables distribution showed that PC1 was strongly and positively correlated with Cu, Zn, Mn, Mg, and SSC, and negatively with TA. The first group of parameters was higher in organic fruits compared to conventional ones inversely to titratable acidity. For Fe, Ni, Hg and Cd, the orchard management didn't affect them.

DISCUSSION

Our data highlighted the relationship between soil and fruits concentrations of some nutrients and heavy metals. The soil analysis showed differences between orchards. It was recorded that soil sampled from the conventional orchard was richer in mineral elements and heavy metals except for Fe, Mn, and Mg. This result confirms previous reports which affirmed that soils irrigated with treated wastewater presented a high content of mineral elements and heavy metals (Galal, 2015). Recent studies based on the comparison between organic and conventional soils showed that this last type was loaded in mineral elements and heavy metals (Hattab et al., 2019). Concerning the fruit content of micronutrients and heavy metals, results indicates that high values were obtained in organic ones. This is previously affirmed by Hattab et al. (2019) but for vegetables harvested from the organic and usual conventional production systems. They found that, in general, micronutrients concentrations increased significantly in crops from organic agriculture with respect to those from conventional farming. However, no significant differences were observed in some heavy metals (Cd, Cu, Zn, and Ni) for the lettuce, strawberry, and tomato crops over their cultivation mode. Moreover, it was noted that the absolute levels of micronutrients and the total micronutrient content were lower in conventional foods than in organic foods (Hunter et al., 2011). About, the effect of the use of treated wastewater, previous studies were interested in the comparison of the impact of waste and fresh water following a conventional production system. Notably, Galal (2015) affirmed that citrus fruits harvested from an orchard irrigated with mixed wastewater and freshwater appeared healthy despite the increase of K, Fe, Mn, Zn, Ni, and Na in its peel and pulp. Also, Al Lahham et al. (2007) indicated that tomato fruit irrigated with mixed fresh and wastewater showed an increased concentration of Fe, Cu, Ni, Mn, and Zn but no accumulation of Cd and Pb. According to international legislations concerning heavy metals in the foods our data do not exceed the allowed limits (FAO, 2016).

In overall, the concentration of studied micronutrients, and heavy metals, whether it be in soil or pomegranate, are lower than those recorded in several areas around the world. This result is true for both orchard management systems. For example, Anastácio et al. (2018) determinate the trace metals in fruit juices in the Portuguese market. They presented that As levels in four samples, Ni in thirteen samples, and Mn in all the twenty-one samples, were above the maximal permissible values specified by Decree-Law (FAO, 2016). They suggested reduction of metal concentrations in consumed juices. Moreover, Gan et al. (2017) conducted a field survey to investigate the concentrations of some heavy metals in vegetables, correspondingly cultivated soils, and irrigation waters. Due to high recorded values, they suggested strategies for clean agricultural production and healthy foods.

Comparing our results with those found in similar conditions but in vegetable crops data showed that fruit trees were less affected by production system management. Otherwise, the bioconcentration factor was more critical in vegetable crops than pomegranates. Lettuce, strawberry and tomato crops conducted following organic or conventional systems showed more micronutrients and heavy metals concentration than studied pomegranates. Although, for tomato harvested from crops irrigated with wastewater, the concentration of micronutrients and heavy metals were high. For this reason, some countries, like Tunisia, allowed the use of waste water only to irrigate trees or forages.

This part of the work draws well the effect of the orchard management system on fruit quality. We reported a significant impact on organoleptic characteristics. Particularly, soluble solids concentration (SSC), and titratable acidity (TA) surveys are those which indicate the potential fruit quality, according to Crisosto et al. (2006). The organic system management produced fruits with high SSC and low acidity compared to conventional ones irrigated with wastewater. This finding partially affirms those of Riahi et al. (2009). They compared the quality of some tomato cultivars conducted according to organic and conventional system. They found an increase of SSC in organic tomatoes for only one cultivar. Concerning the irrigation with wastewater, some works revealed some positive effects. In citrus orchards, the irrigation with mixed fresh and waste water increased SSC and decreased the titratable acidity without effect on pH (Galal, 2015). However, Al-Lahham et al. (2003) reported that the irrigation of tomato plants with wastewater decreased SSC in fruits.

CONCLUSION

In this research we investigated the potential role of preharvest factors on fruit quality attributes. In particular, the use of treated wastewater for irrigating pomegranate trees was evaluated. Fruit traits were compared with the most guarantee ones harvested from an organic orchard. Results seem reassuring for consumers because pomegranates harvested from the conventional orchard irrigated with treated wastewater contain some heavy metals, but values do not exceed the allowed limits. Also, these fruits showed a low SSC/TA ratio but free of bacterial contamination. So, the use of treated wastewater was allowed for a long time for irrigating fruit trees, but precautions are necessary to avoid contamination and low fruit quality.

Conflict of interest

The authors have no conflict of interest to report.

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