




## Article

# Bumblebee Pollination Enhances Yield and Flavor of Tomato in Gobi Desert Greenhouses

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**Abstract:** Bumblebee pollination is crucial to the production of tomato in protected cultivation. Both tomato yield and flavor play important roles in attracting attentions from growers and consumers. Compared with yield, much less work has been conducted to investigate whether and how pollination methods affect tomato flavor. In this study, the effects of bumblebee pollination, vibrator treatment, and plant growth regulator (PGR) treatment on tomato yield and flavor were tested in Gobi Desert greenhouses. Compared with vibrator or PGR treatments, bumblebee pollinated tomato had higher and more stable fruit set, heavier fruit weight, and more seed. We also found that the seed quantity positively correlated with fruit weight in both bumblebee pollinated, and vibrator treated tomato, but not in PGR treated tomato. Besides enhancing yield, bumblebee pollination improved tomato flavor. Bumblebee pollinated tomato fruits contained more fructose and glucose, but less sucrose, citric acid, and malic acid. Furthermore, the volatile organic compounds of bumblebee pollinated tomato were distinctive with vibrator or PGR treated tomato, and more consumer liking related compounds were identified in bumblebee pollinated tomato. Our findings provide new insights into the contributions of bee pollinator towards improving crop yield and quality, emphasizing the importance of bumblebee for tomato pollination.

**Keywords:** tomato; pollination; *Bombus lantschouensis*; fruit flavor; volatile organic compound



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## 1. Introduction

Tomato, *Lycopersicon esculentum*, is one of the most popular vegetables worldwide. China is the world's largest producer of tomatoes, both by area (hectares) and volume (tonnes) [1]. Protected cultivation, which can help growers overcome climate limitations and maximize profitable harvest, has been the most important cultivation systems in China [2]. In recent years, these protected cultivations, and tomatoes in particular, have expanded rapidly in Northwest China. However, pollination remains a limiting factor in greenhouse tomato cultivation.

Tomato flowers depend on pollination to produce seeds and fruits [3]. Studies have shown that tomato fruit-setting is highly correlated with pollen deposition on stigma, and limited pollination usually resulted poor fruit set [4]. Tomato is a buzz-pollinated crop, its flowers are self-compatible but the pollen is kept locked in non-dehiscent anthers, meaning that vibration is necessary to release high volumes of pollen for self-fertilization [5]. Under

protected cultivation conditions, where wind and wild insects are absent, commercially acceptable fruit set and quality are difficult to achieve without artificial pollination methods. Electric or manual vibrators were found to be an effective artificial pollination method in tomato greenhouses [4]. Compared to tomatoes without vibration treatment, vibrator-treated tomatoes ripened earlier and had greater yield. Plant growth regulator (PGR), which chemically induce fruit growth, are another widely used method in greenhouse tomato production [6,7]. However, both vibrator and PGR treatments have high labor costs and produce tomatoes with an unstable yield and quality.

The development of biological control and reduced use of chemical pesticides on crops made it possible to use bee pollinators in tomato greenhouses. Bee pollination is an efficient production technique in agriculture [8–11] and makes a huge economic contribution globally [12–15]. As the most widely used managed pollinator, several studies have explored the use of western honeybee, *Apis mellifera* L., in greenhouse tomato pollination, demonstrating improved yield and quality compared with greenhouses without honeybees [16–18]. However, studies have also showed that honeybee pollination efficiency in tomato greenhouses was variable and honeybee pollinated-tomato often couldn't meet the commercial production level [19–21]. In our previous study, we found that the innate dislike of tomato floral scent and unsuccessful food-collecting experience might explain the low foraging activity of honeybee in tomato greenhouse [22]. More studies are needed to understand and enhance honeybee foraging activity before honeybee colonies can be used for the pollination of greenhouse tomato.

Bumblebees are effective bee pollinators of tomato as, unlike honeybees, they are capable of buzz pollination to vibrate tomato anthers to release pollen grains and only one visit is sufficient for full tomato pollination [23]. Following the success of commercially rearing bumblebee colonies bumblebee pollination has become a standard practice in developed agricultural countries and regions [24]. Besides saving labor, many studies showed that bumblebee pollination can significantly improve tomato yield and quality [20,25–27].

Beyond yield and quality, fruit flavor is an increasingly important concern for growers, as more and more consumers have complained about the poor flavor of commercial tomato in recent years [28]. Tomato fruit flavor is determined by a complex of sugars, acids, and volatiles [29]. Many factors, such as variety and postharvest storage environment, can affect the flavor of tomato fruits [29–34]. Previous studies have demonstrated that bumblebee pollinated-tomatoes had a better flavor than PGR or vibrator-treated tomatoes [35] and consumers had higher willingness to pay for bumblebee pollinated tomatoes than PGR-treated tomato, despite the higher prices for bumblebee pollinated tomatoes [36]. However, it is still not very clear how bumblebee pollination enhances tomato flavor. Many studies only compared the fruit set, size, weight, or sugar and acids of tomato among different pollination methods [37], and few studies focus on the tomato fruit volatiles of different pollination methods.

The Gobi Desert spans 6.7 million hectares of Northwest China's Gansu province, which limits crop production opportunities and food security within this region. Agriculture in the region has increasingly taken advantage of solar energy to produce vegetables and fruits year-round in greenhouses, which now accounts for a large proportion of vegetables and fruits consumed in this region [38]. However, most tomato growers of this region, especially smallholders, still use PGR or vibrator to produce tomatoes, resulting in high labor costs, poor fruit quality and low profit. In this study, we examined the effects of bumblebee pollination (using reared colonies of the local species *Bombus lantschouensis*), vibrator and PGR treatment on tomato yield and flavor in Gobi Desert greenhouses of Northwest China. We determined how pollination by native bumblebee species affects tomato yield and tomato fruit flavor.

## 2. Materials and Methods

### 2.1. Study Site, Plants and Bees

The experiments were conducted from 26 September in 2020 to 25 February in 2021 in three solar greenhouses at Zhangye, Gansu, China, situated at 38.83° N 100.43° E, with an elevation of 1521 m. The experimental site was located in the Gobi Desert of the middle part of the Hexi Corridor in Northwest China and belongs to the arid mid-temperate zone. An average 2975 h annual sunshine duration, 6100 MJ m<sup>-2</sup> annual solar radiation and 140–174 annual frost-free days provide an efficient solar energy for protected cultivation. Solar-powered plastic greenhouse is the most popular cultivation system, which relies entirely on solar energy for heating, cooling and crop production.

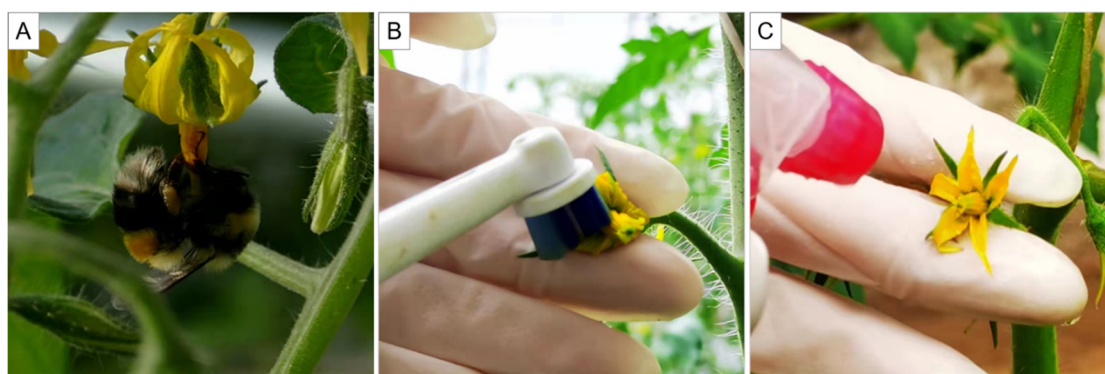
In the study, the three greenhouses used were of the same type, horticulture management and area (15 m × 100 m). The north, east, and west sides of the structure were built from rammed earth. The south side of the structure was a titled roof supported by a steel frame, and covered with two transparent polyethylene film layers (the light transmittance is 90%) with one of the layers could be opened and closed. During cold seasons (late autumn, winter and early spring), the roof was covered with straw mats at nights to reduce heat losses. An aisle, 0.5 m in width, was maintained along the north wall. The greenhouse was equipped with fly netting to prevent bees from flying out.

Tomatoes (*Lycopersicum esculentum* cv. 'Jingcai') were transplanted to the greenhouse on 4 September 2020. Tomatoes were planted in 67–68 rows of 26–30 plants each. All rows were perpendicular to the north wall, with a length of 14.5 m from north to south. Five inflorescences were retained on each plant to produce fruit; the inflorescences bloom sequentially from the bottom to the top of the plants. The peak blooming period of each inflorescence usually lasts for 6–8 days, and the blooming periods of adjacent inflorescences overlap slightly. The whole flowering period of the tomato plants lasted for ca. 75–80 days, beginning 25 September. During the flowering period, the temperature in the greenhouses was kept within 10–32 °C and the relative humidity was kept within 30–60%, conditions which were favorable for bee flight.

Colonies of the bumblebee *Bombus lantschouensis* used in the study were provided by the Institute of Apicultural Research, Chinese Academy of Agricultural Sciences. At the early bloom, a single bumblebee colony was placed in the middle of each greenhouse along the aisle in the north. The bumblebee colony consisted of one healthy queen, approximately 60 workers and with no males at the beginning of the pollination study. Two kilograms of sugar syrup (50% sugar content, *w/w*) was provided within the beehive.

### 2.2. Pollination Treatment

To analyze the influences of pollination method on tomato fruit quality, three pollination treatments, were conducted during the tomato blooming period of the second inflorescence: bumblebee pollination, vibrator treatment, and PGR treatment (Figure 1). In each greenhouse, 75–90 tomato plants were selected and labeled. Flower buds were marked in afternoon and newly opened flowers were checked in next morning. After flowers opened, one third of the bagged flowers were vibrated with an electric toothbrush as vibrator treatment. Specifically, the pedicel of tomato flower was touched by the electric toothbrush for 3 to 5 s. Another third of the bagged flowers were treated with a PGR spray of 200 ppm auxin 2-hydroxymethyl 4-chlorophenoxyacetic acid applied directly to the newly opened tomato flowers. Both the vibrator and PGR treatment were conducted in sunny mornings and each sampled flower was treated in every other day and treated twice during the blooming. After each vibration or spray treatment, the flowers were rebagged immediately to prevent visitation by bumblebees. The remaining third of flowers were uncovered and exposed to bees, forming the bumblebee pollination treatment. The pollination level of bumblebee pollinated flowers was checked every other day to confirm that each of the labeled flower has been visited by bumblebees.



**Figure 1.** Pollination treatments on greenhouse tomato. (A) Bumblebee *Bombus lantschouensis* pollination; (B) vibrator treatment; and (C) plant growth regulator treatment.

### 2.3. Measure of Yield

Tomatoes in our experiment were collected before commercial harvest. Fruit set (%), fruit weight (g), fruit diameter (mm), and fruit seed (number) were measured. During the blooming period, flowers of the second inflorescence were counted to examine fruit set. In each greenhouse, 25–30 plants from each pollination treatment were sampled for fruit-set. A total of 45–60 tomatoes from each pollination treatment was collected. Fruit weight was measured by electronic balance and fruit diameter was measured by vernier caliper. Then, tomatoes were cut in two and well-developed seeds in each tomato were counted.

### 2.4. Sample Preparation for Flavor Analysis

Sugar, organic acid, and volatile organic compound (VOC) were measured to compare the fruit flavor of tomato from different pollination treatments. The methods of sample preparation were adapted from a previous study by Zhou [39]. In each treatment, six to eight biological replicates, each consisting of three tomato fruits, were analyzed. For each treatment, tomato fruits were from flowers which opened at the same day and collected at the same time. The whole tomato fruits except fruit calyx and peduncle were wrapped with silver paper and flash frozen in liquid nitrogen (three fruits in each replicate). Then the wrapped and frozen tomato fruits were immediately smashed by hammer. The frozen and chopped material was homogenized into powder in a cryogenic mill (Mixer Mill MM 400, RETSCH, Stadt Haan, Germany), and stored at  $-80^{\circ}\text{C}$  until analysis.

#### 2.4.1. Sample Preparation for Sugar Analysis

A  $0.1 \times \text{g}$  sample of tomato powder was weighed into a 5 mL centrifuge tube and 1.9 mL L-arabinose aqueous solution ( $1 \text{ mg} \cdot \text{mL}^{-1}$ ) was added as internal standards. The mixture was ultrasonicated for 10 min, and the homogenized sample was centrifuged at  $13,000 \times \text{g}$  rpm for 10 min at  $4^{\circ}\text{C}$  on a high-speed centrifuge (5430R, Eppendorf, Hamburg, Germany). The clear supernatant was collected and filtered twice by a Millex-GP filter with a  $0.22 \mu\text{m}$  pore size polyethersulfone membrane (Aisimo corporation Co., Ltd., Shanghai, China). Then, 500  $\mu\text{L}$  of the filtrate was collected and mixed with 500  $\mu\text{L}$  of HPLC grade acetonitrile. The mixture was centrifuged at  $15,000 \times \text{g}$  rpm for 10 min and the supernatant was transferred into an amber vial (2 mL; Agilent Technologies, Palo Alto, CA, USA) for sugar analysis.

#### 2.4.2. Sample Preparation for Organic Acid Analysis

A  $0.2 \times \text{g}$  sample of tomato powder was weighed into a 5 mL centrifuge tube and 1.8 mL DL-lactic acid aqueous solution ( $0.5 \text{ mg} \cdot \text{mL}^{-1}$ ) was added as per internal standards. The mixture was ultrasonicated for 10 min, and the homogenized sample was centrifuged at  $13,000 \times \text{g}$  rpm for 10 min at  $4^{\circ}\text{C}$ . The clear supernatant was collected and filtered twice by a Millex-GP filter with a  $0.22 \mu\text{m}$  pore size polyethersulfone membrane (Aisimo



corporation Co., Ltd., Shanghai, China). Then, 50  $\mu\text{L}$  of the filtrate was collected and mixed with 950  $\mu\text{L}$  of pure water. Then, 20  $\mu\text{L}$  of the mixture was collected and mixed with 480  $\mu\text{L}$  of pure water and 500  $\mu\text{L}$  of HPLC grade acetonitrile. The mixture was centrifuged at  $15,000 \times g$  rpm for 10 min and the supernatant was transferred into an amber vial (2 mL; Agilent Technologies, Palo Alto, CA, USA) for organic acid analysis.

#### 2.4.3. Sample Preparation for VOC Analysis

A  $2 \times g$  sample of tomato powder was weighted into a 20 mL headspace bottle which contained  $0.6 \times g$  NaCl. An amount of 5  $\mu\text{L}$  of methanol solution of 2-nonanone ( $0.5 \mu\text{g} \cdot \text{g}^{-1}$ ) was added as per internal standard. The sample was pre-incubated at  $50^\circ\text{C}$  for 10 min. Then, a  $1.1 \text{ mm} \times 120 \mu\text{m}$  PDMS/DVB/CWR fiber was exposed to the headspace for 10 min of extraction at  $50^\circ\text{C}$ . The volatiles were extracted for 10 min and trapped on the fiber, then desorbed for 1 min at  $250^\circ\text{C}$  in the injection port of the GC/MS and the fiber was cleaned by exposing it for 15 min at  $250^\circ\text{C}$  in another injection port to prevent cross-contamination. The mode of injection was split at a ratio of 10:1 and the detection was performed automatically.

#### 2.5. Instrument Method for Flavor Analysis

The methods for flavor analysis were adapted from previous studies by Zhou and Rambla [39,40]. An ultra-high-performance liquid chromatography-triple quadrupole mass spectrometry (UPLC-QqQ-MS/MS, 1290–6420, Agilent Technologies, Santa Clara, CA, USA) was used for the detection of sugar and organic acid in tomato. Then, headspace solid-phase microextraction gas chromatography quadrupole time-of-flight mass spectrometry (HS-SPME-GC-QTOF-MS) was applied to compare the VOC emitted by tomato fruits of different pollination methods. The mass spectrometer was operated in the electron ionization (EI) mode at 70 eV and the mass analyzer was scanned in the range  $m/z$  30–600. The TOF mass resolution is 25,000 at  $m/z$  271.9867.

##### 2.5.1. UHPLC-QqQ-MS/MS for Sugar Analysis

An ACQUITY UPLC BEH Amide column ( $2.1 \times 100 \text{ mm}$ ,  $1.7 \mu\text{m}$ , Waters) was used for the separation of sugar. The temperature of the column oven was maintained at  $60^\circ\text{C}$ . The mobile phase A was water containing 5 mM ammonium formate. The mobile phase B was acetonitrile containing 5 mM ammonium formate. The UPLC separations were 10 min using the following scheme: (1) 0–2 min, 90% B; (2) 6 min, 80% B; and (3) 6.1 min, 90% B, 10 min 90% B. All the changes were linear, and the flow rate was set at  $0.2 \text{ mL min}^{-1}$ . The multiple reaction monitoring (MRM) parameters of each sugar including the precursor ion, the product ion, and collision energy (eV) were optimized with a gas temperature of  $200^\circ\text{C}$ , drying gas flow at 8 L/min, nebulizer pressure at 35 psi, and capillary voltage at  $-4000 \text{ V}$ . One major product ion for each sugar was selected for the subsequent analysis. The MRM parameters of sugars were as follows: glucose ( $179 \rightarrow 89$ ,  $-6 \text{ eV}$ ), fructose ( $179 \rightarrow 89$ ,  $-5 \text{ eV}$ ), sucrose ( $341.1 \rightarrow 179$ ,  $-5 \text{ eV}$ ), and L-Arabinose ( $149 \rightarrow 89.2$ ,  $-6 \text{ eV}$ ). System operation, data acquisition, and data analysis were performed using the Agilent MassHunter software.

##### 2.5.2. UHPLC-QqQ-MS/MS Condition for Organic Acid Detection

An ACQUITY UPLC HSS T3 column ( $2.1 \times 100 \text{ mm}$ ,  $1.8 \mu\text{m}$ , wa) was used for separation of organic acid. The temperature of the column oven was maintained at  $35^\circ\text{C}$ . The mobile phase A was water/formic acid (100: 0.1,  $v/v$ ). The mobile phase B was acetonitrile/formic acid (100: 0.1,  $v/v$ ). The UPLC separations were 8 min using the following scheme: (1) 0 min, 5% B; (2) 3 min, 45% B; (3) 3.5 min, 90% B; (4) 5 min, 90% B; (5) 5.1 min, 5% B; (6) 8 min 5% B. All the changes are linear, and the flow rate was set at  $0.2 \text{ mL min}^{-1}$ . The mass spectrometer was operated in a negative ion mode. The multiple reaction monitoring (MRM) parameters of organic acid, including the precursor ion, the product ion, and collision energy (eV), were optimized with a gas temperature of  $350^\circ\text{C}$ , drying gas flow at 8 L/min, nebulizer pressure at 35 psi, and capillary voltage at  $-3000 \text{ V}$ .

One major product ion for each organic acid was selected for the subsequent analysis. The MRM parameters of organic acids were as follows: citric acid (199→111, −9 eV), malic acid (133→115, −15 eV), and lactic acid (89→45, −5 eV). System operation, data acquisition, and data analysis were performed using the Agilent MassHunter software.

#### 2.5.3. HS-SPME-GC-QTOF-MS Condition for VOC Detection

An HP-5MS UI column (30 m × 0.25 mm × 0.25 µm, Agilent) was used to analyze the VOC of tomato fruits. Oven programming conditions were 40 °C for 3 min, 3 °C/min ramp until 60 °C, 6 °C/min ramp until 160 °C, 12 °C/min ramp until 260 °C and kept at 260 °C for 5 min. Helium was used as the quench gas with a 2.25 mL/min flow and nitrogen was used as the collision gas with a 1.5 mL/min flow.

#### 2.6. Statistical Analysis

First, the tomato fruit yield characteristics of different pollination treatments were compared with general linear model (GLM) using the software IBM SPSS 20 (Chicago, IL, USA), with ‘fruit set’, ‘fruit weight’, ‘fruit diameter’, and ‘seed quantity’ as the response variables; ‘pollination treatment’ as the fixed factor; and ‘greenhouse’ as the random factor. Duncan post-hoc pairwise comparisons were used to test the significant differences of these variables among the levels of pollination treatment. Shapiro–Wilk normality tests were used to test normality and Levene tests were used to test homoscedasticity. Fruit set data did not exhibit equal levels of variance across the different pollination treatments (Levene’s test,  $F_{2,218} = 13.092$ ,  $p < 0.001$ ), and were, therefore, arcsin transformed to meet the conditions for GLM.

For the sugar and organic acid data, GLMs followed by Duncan post-hoc method was used to compare the content of fructose, glucose and sucrose in tomato by different pollination treatments. The citric acid and malic acid data both had significantly different variances (Levene’s test, citric acid:  $F_{2,19} = 5.583$ ,  $p = 0.012$ ; malic acid:  $F_{2,19} = 7.300$ ,  $p = 0.004$ ) and failed to meet conditions for GLM after transformations; then, the non-parametric Kruskal–Wallis one-way ANOVA followed by the Dunn–Bonferroni post-hoc method was used to compare the medians of citric acid and malic acid content in tomato by different pollination treatments.

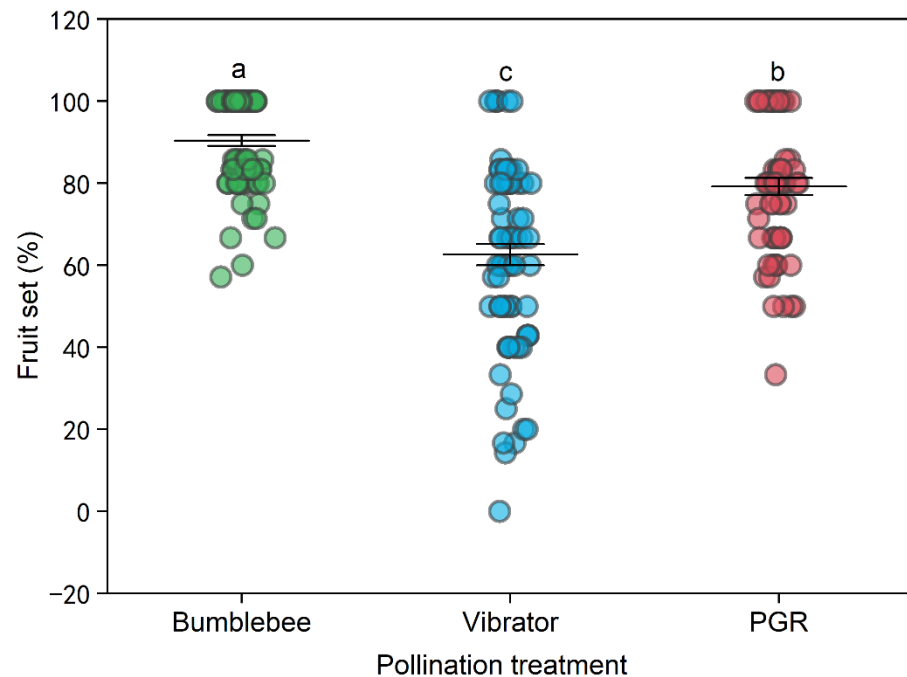
Finally, in order to highlight the differences among total VOCs in tomato fruits by different pollination treatments, orthogonal projections to latent structures discriminant analysis (OPLS-DA) was applied. In tomato, there are more than 400 volatile compounds detected [30]. However, not all of these compounds contribute to tomato fruit flavor, let alone consumer liking [41]. Compounds which make important contribution to flavor and consumer liking can be identified by consumer panel tests [42]. The consumer liking related VOCs were selected based on past research [28,39,41–43] and the composition of consumer liking related VOCs in tomato fruits by different pollination treatments was checked by hierarchical cluster analysis (HCA) with the distance metrics based on the Pearson correlation, and the normalized data was represented as heatmap. OPLS-DA was conducted by the software SIMCA P+ Version 15 (Sartorius Stedim Data Analytics AB, Umeå, Sweden) and HCA was conducted by OriginPro 2022 (Northampton, MA, USA). Before the VOCs data of tomato fruit samples were subjected to OPLS-DA and HCA, the content of each compound was calculated based on the comparison of its FID peak area and the internal standard 2-nonanone and the compounds not detected were assigned a value of 0.

### 3. Results

#### 3.1. Tomato Yield

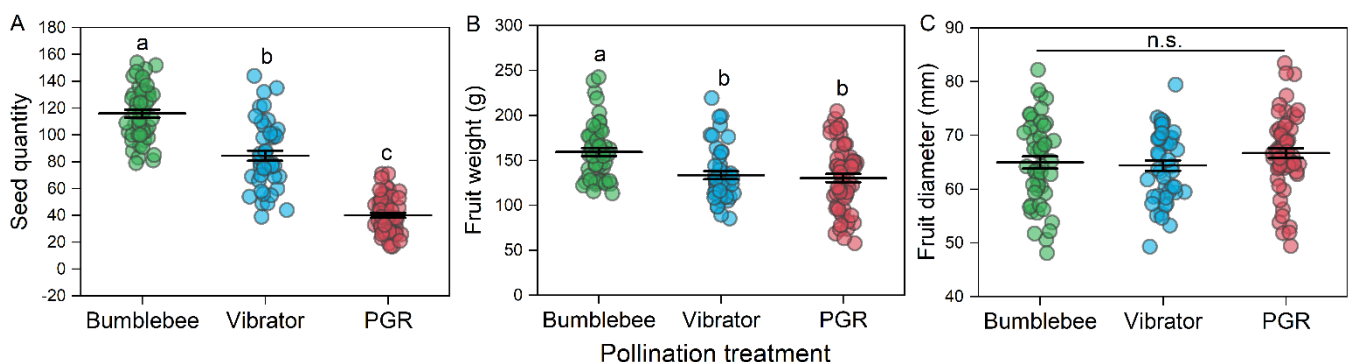
Tomato fruit set showed significant differences under different pollination treatments (Figure 2, GLM, pollination treatment:  $F_{2,216} = 44.983$ ,  $p < 0.001$ ). Greenhouse had no effect on the fruit set (GLM, greenhouse:  $F_{2,216} = 0.126$ ,  $p = 0.882$ ). Tomato flowers pollinated by bumblebees had the highest fruit set ( $90.4\% \pm 11.2\%$ ), followed by tomato flowers

treated with PGR ( $79.2\% \pm 16.8\%$ ) and vibrator ( $62.3\% \pm 22.8\%$ ). Tomatoes pollinated by bumblebees also had more stable fruit set (smaller CV, CV = 12.6%) than PGR (CV = 21.2%) and vibrator (36.5%).



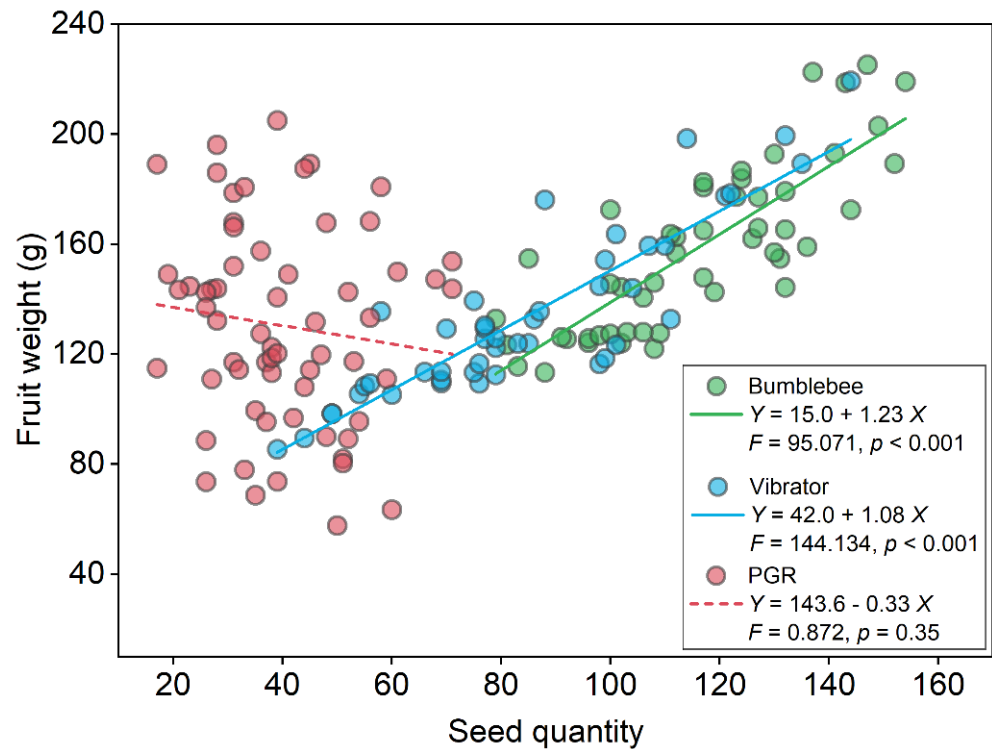
**Figure 2.** Fruit set of tomato by different pollination treatments. A total of 221 tomato plants from three greenhouses were observed for fruit set. Data are presented as the mean  $\pm$  95% confidence interval. A general linear model was used to compare the fruit set among different pollination treatments. Different letters indicate significant differences in fruit set based on the Duncan test  $\alpha = 0.05$ .

Significant differences were also found in seed quantity (Figure 3A, GLM, pollination treatment:  $F_{2152} = 215.5$ ,  $p < 0.001$ ) and fruit weight (Figure 3B, GLM, pollination treatment:  $F_{2152} = 11.370$ ,  $p < 0.001$ ), but not in fruit diameter (Figure 3C, GLM, pollination treatment:  $F_{2152} = 1.535$ ,  $p = 0.219$ ) under different pollination treatments. Bumblebee pollinated tomatoes had the largest seed quantity ( $115.9 \pm 19.8$ ), followed by tomatoes treated with vibrator ( $84.5 \pm 25.0$ ) and tomatoes treated with PGR had the fewest seeds ( $48.1 \pm 21.1$ ). Tomatoes pollinated by bumblebees also had a larger fruit weight ( $158.5 \pm 30.1$  g) than tomatoes treated with vibrator ( $133.5 \pm 30.9$  g) and PGR ( $130.3 \pm 36.4$  g).



**Figure 3.** The seed quantity (A), fruit weight (B), and fruit diameter (C) of tomato fruit by different pollination treatments. A total of 157 tomato fruits from three greenhouses were collected and measured. Data are presented as the mean  $\pm$  95% confidence interval. General linear models were used to compare the seed quantity, fruit weight, and fruit diameter of tomatoes by different pollination treatments. Different letters indicate significant differences in fruit set based on the Duncan test at  $\alpha = 0.05$ .

Positive correlations were found between seed quantity and fruit weight in tomatoes from the bumblebee pollination or vibrator treatments. However, seed quantity was unrelated with fruit weight in tomatoes from PGR treatment. (Figure 4). Like the tomato fruit set, the tomato seed quantity (GLM, greenhouse:  $F_{2152} = 0.138$ ,  $p = 0.872$ ), fruit weight (GLM, greenhouse:  $F_{2152} = 0.255$ ,  $p = 0.775$ ), and fruit diameter (GLM, greenhouse:  $F_{2152} = 1.880$ ,  $p = 0.156$ ) did not differ in different greenhouses.

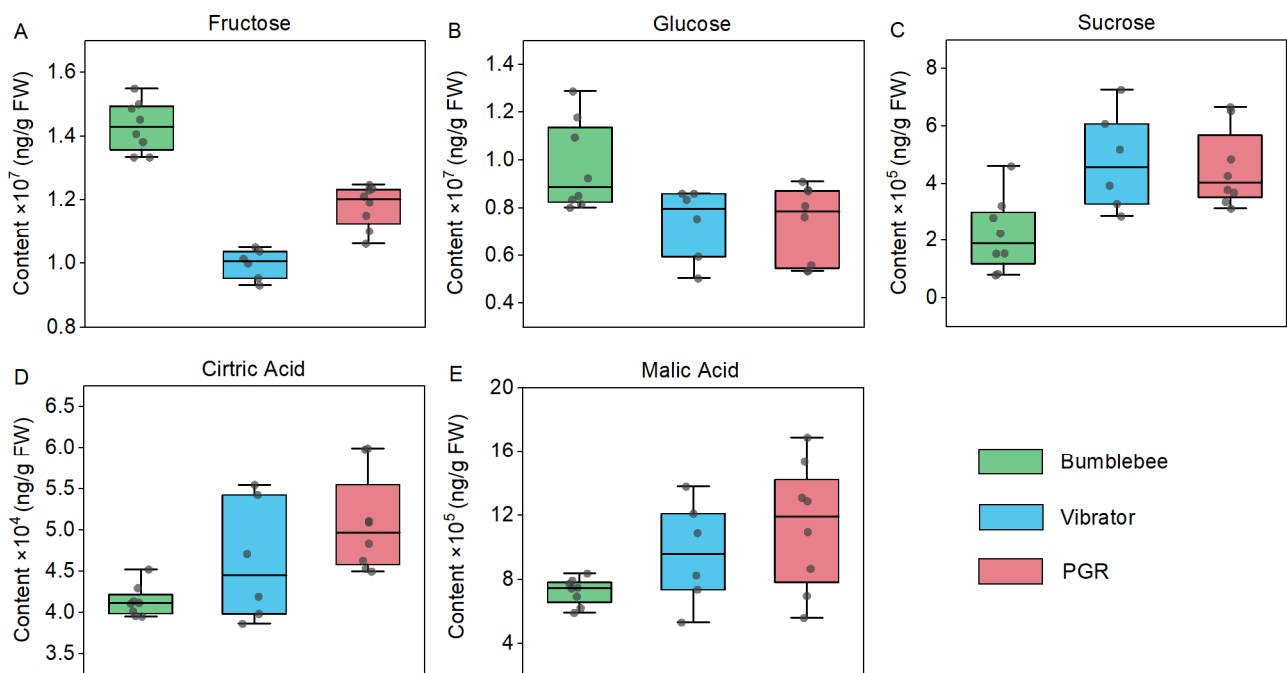


**Figure 4.** Fruit weight in relation to seed quantity of tomato fruit by different pollination treatments. Solid and dashed lines indicate significant and nonsignificant partial regressions, respectively.

### 3.2. Sugars and Organic Acids in Tomato

Significant differences were found in both sugars and organic acids content tomato fruits by different pollination treatments. Compared with vibrator and PGR treatments, tomato fruits pollinated by bumblebees contained more fructose (Figure 5A, GLM,  $F_{2,19} = 72.080$ ,  $p < 0.001$ ) and glucose (Figure 5B, GLM,  $F_{2,19} = 5.101$ ,  $p = 0.017$ ), but less sucrose (Figure 5C, GLM,  $F_{2,19} = 7.271$ ,  $p = 0.005$ ). Among the organic acids analyzed, bumblebee pollinated tomato fruits contained significantly less citric acid (Figure 5D, Kruskal–Wallis test:  $H = 9.000$ ,  $df = 2$ ,  $p = 0.011$ ) and malic acid (Figure 5E, Kruskal–Wallis test:  $H = 7.167$ ,  $df = 2$ ,  $p = 0.028$ ) than vibrator and PGR-treated tomato fruits.



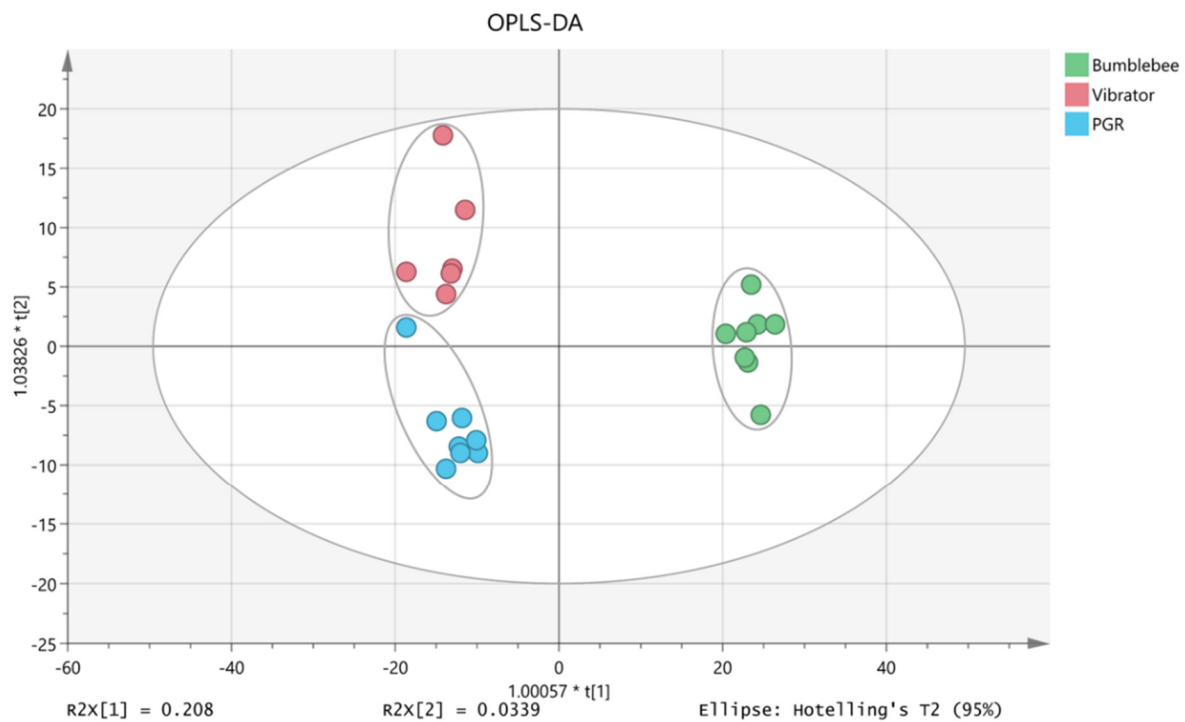


**Figure 5.** The content of fructose (A), glucose (B), sucrose (C), citric acid (D), and malic acid (E) in tomato fruit by different pollination treatments. A total of 66 tomato fruits were collected and each of three tomato fruits grouped into one biological replicate. Eight replicates were analyzed in treatments of ‘bumblebee’ and ‘PGR (plant growth regulator)’, and six replicates were analyzed in treatment of ‘vibrator’. Boxes indicate quartiles with the median marked as a horizontal line. General linear models followed by Duncan post-hoc method were used to compare the content of fructose, glucose, and sucrose, and non-parametric Kruskal–Wallis one-way ANOVA followed by the Dunn–Bonferroni post-hoc method was used to compare the content of citric acid and malic acid by different pollination treatments. Different letters indicate significant differences at  $\alpha = 0.05$ . ‘FW’ indicates fresh weight.

### 3.3. VOCs in Tomato

The volatile organic compounds from tomato fruit under different pollination treatment was analyzed by OPLS-DA. The distribution of samples on the score plot (Figure 6) clearly shows a separation between bumblebee pollination treatment and non-bumblebee pollination treatments, but also, to a lesser extent, between PGR treatment and vibrator treatment. This suggests that tomatoes pollinated by bumblebees produced the most distinctive volatile profiles in fruits.

A total of 21 compounds which had been reported to be positively correlated with consumer liking and 11 compounds negatively correlated with consumer liking were identified in this study (Table 1). To check the differences in those volatile compound profiles associated to each pollination treatment, HCA was applied. Based on the data set of consumer liking related compounds, an obvious clusters of different pollination treatments were found among different pollination methods (Figure S1). Tomato fruits from bumblebee pollination produced the greatest consumer liking related compound profiles among the three pollination treatments (Table 1).



**Figure 6.** Orthogonal projections to latent structures discriminant analysis (OPLS-DA) of the volatile organic compound profiles of tomato fruits by different pollination treatments.

**Table 1.** Consumer liking related volatile organic compounds from tomato fruit by different pollination methods.

Compounds Names	Formula	CAS	Concentration (ng g <sup>-1</sup> )			Consumer Liking Effect
			Bumblebee	Vibrator	PGR	
Hexanal *	C <sub>6</sub> H <sub>12</sub> O	66-25-1	484.19 ± 22.33a	296.66 ± 17.13b	251.94 ± 10.11b	Positive
Methyl salicylate *	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	119-36-8	80.77 ± 4.24a	39.69 ± 1.83b	57.02 ± 3.29ab	Positive
6-Methyl-5-hepten-2-one *	C <sub>8</sub> H <sub>14</sub> O	110-93-0	80.95 ± 2.62a	38.01 ± 2.68b	20.75 ± 0.89b	Positive
Phenylethyl alcohol *	C <sub>8</sub> H <sub>10</sub> O	60-12-8	12.62 ± 0.63a	5.68 ± 0.38b	3.78 ± 0.38b	Positive
1-Penten-3-one *	C <sub>5</sub> H <sub>8</sub> O	1629-58-9	10.85 ± 0.34a	5.30 ± 0.35c	7.86 ± 0.23b	Positive
2-Isobutylthiazole *	C <sub>7</sub> H <sub>11</sub> NS	18640-74-9	10.02 ± 0.38a	1.18 ± 0.24b	1.25 ± 0.17b	Positive
(E)-2-Octenal *	C <sub>8</sub> H <sub>14</sub> O	2548-87-0	6.10 ± 0.18a	3.54 ± 0.12b	2.71 ± 0.12b	Positive
1-Penten-3-ol *	C <sub>5</sub> H <sub>10</sub> O	616-25-1	4.47 ± 0.17a	1.92 ± 0.14b	1.54 ± 0.11b	Positive
Heptanal *	C <sub>7</sub> H <sub>14</sub> O	111-71-7	2.21 ± 0.23a	1.46 ± 0.13ab	0.97 ± 0.05b	Positive
Citral *	C <sub>10</sub> H <sub>16</sub> O	5392-40-5	2.01 ± 0.08a	1.01 ± 0.05b	0.86 ± 0.05b	Positive
β-Ionone *	C <sub>13</sub> H <sub>20</sub> O	14901-07-6	0.70 ± 0.02a	0.30 ± 0.04b	0.49 ± 0.03ab	Positive
Acetone *	C <sub>3</sub> H <sub>6</sub> O	67-64-1	0.58 ± 0.04a	0.27 ± 0.02b	0.25 ± 0.01b	Positive
(Z)-3-Hexen-1-ol	C <sub>6</sub> H <sub>12</sub> O	33467-74-2	1.86	n.d.	0.77	Positive
3-Pentanone	C <sub>5</sub> H <sub>10</sub> O	96-22-0	1.14 ± 0.14	0.31	0.80 ± 0.12	Positive
Butanoic acid, 3-methyl-	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	503-74-2	0.86 ± 0.05	0.66 ± 0.12	0.56 ± 0.09	Positive
β-Cyclocitral	C <sub>10</sub> H <sub>16</sub> O	432-25-7	0.62 ± 0.03	0.41 ± 0.02	0.49 ± 0.02	Positive
α-Phellandrene	C <sub>10</sub> H <sub>16</sub>	99-83-2	0.46 ± 0.03	0.15 ± 0.03	0.19 ± 0.04	Positive
α-Terpineol	C <sub>10</sub> H <sub>18</sub> O	98-55-5	0.19 ± 0.01	0.18 ± 0.02	0.19 ± 0.01	Positive
1-Hexanol	C <sub>6</sub> H <sub>14</sub> O	111-27-3	0.17 ± 0.01	0.11 ± 0.01	0.07 ± 0.01	Positive
(E)-2-Hexenal *	C <sub>6</sub> H <sub>10</sub> O	6728-26-3	1432.35 ± 57.81a	789.39 ± 48.14b	843.77 ± 23.20b	Negative
1-Butanol, 2-methyl- *	C <sub>5</sub> H <sub>12</sub> O	137-32-6	7.76 ± 0.61a	6.06 ± 0.56a	0.76 ± 0.21b	Negative
Benzeneacetaldehyde *	C <sub>8</sub> H <sub>8</sub> O	122-78-1	1.47 ± 0.06a	0.62 ± 0.06b	0.78 ± 0.03b	Negative
Benzaldehyde, 2-hydroxy- *	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	90-02-8	0.31 ± 0.02a	0.14 ± 0.01b	0.19 ± 0.01b	Negative
2,4-Decadienal, (E,E)- *	C <sub>10</sub> H <sub>16</sub> O	25152-84-5	1.04 ± 0.04a	0.42 ± 0.03b	0.49 ± 0.03b	Negative
2-Methoxy-phenol	C <sub>7</sub> H <sub>8</sub> O <sub>2</sub>	90-05-1	4.31 ± 0.19	2.24 ± 0.22	4.70 ± 0.44	Negative

Table 1. Cont.

Compounds Names	Formula	CAS	Concentration (ng g <sup>-1</sup> )			Consumer Liking Effect
			Bumblebee	Vibrator	PGR	
Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	97-53-0	0.68 ± 0.03	0.62 ± 0.05	0.70 ± 0.03	Negative
Disulfide, dimethyl	C <sub>2</sub> H <sub>6</sub> S <sub>2</sub>	624-92-0	0.19 ± 0.03	0.53 ± 0.10	0.08 ± 0.00	Negative
Furfural	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	98-01-1	0.04 ± 0.002	0.03 ± 0.01	0.03 ± 0.01	Negative
Ethyl acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	141-78-6	n.d.	0.08	0.24	Negative
Dimethyl sulfide	C <sub>2</sub> H <sub>6</sub> S	75-18-3	n.d.	0.02	0.02	Negative

One-way ANOVAs were used to analyze the compound concentration differences of tomato fruits by different pollination methods. Data are shown as average ± standard error and only average values are presented for compounds which detected in less than three samples. The 'n.d' indicates not detected. The '\*' indicates significant difference and different letters indicate significant differences among different pollination treatments based on the Duncan test at  $\alpha = 0.05$ . 'Positive' and 'Negative' indicate significant correlation with consumer liking scores based on consumer evaluation panels from references [28,39,41–43].

#### 4. Discussion

##### 4.1. Bumblebee Pollination Enhanced Tomato Yield

Bumblebees are efficient pollinators of greenhouse tomatoes, resulting in significantly improved tomato yield. In our study, tomatoes pollinated by bumblebees showed better production performance than tomatoes treated with vibrator or PGR, resulting in higher fruit set, heavier fruit weight, and higher seed numbers, but no significant increase in fruit size. These findings are in line with previous research on bee pollination on tomato, which has consistently demonstrated that buzz-pollinating bees, e.g., bumblebees significantly increased tomato fruit weight compared to no-pollination control, while auxin treatment, vibration, or non-buzz-pollinating bees did not significantly increase fruit weight [37].

We found that tomato flowers treated by PGR produced a slightly, but not significantly, larger fruits than buzz-pollination of bumblebee and vibrator. Some previous studies also found that tomato fruits from bumblebee pollination had a slightly but not significantly smaller size than PGR or vibrator treatments [44]. Normally, the fruit development of tomato is induced by fertilization, then the tomato ovary develops into a pericarp and the placental parenchyma fills the locular cavities with locular tissue, which encloses the seeds. Fruits can also develop without fertilization: parthenocarpic fruit-set occurs in unpollinated tomato ovaries when applied with plant growth regulator, mainly auxins and gibberellins [45–47]. Compared with bee-pollination, the size of fruit treated by PGR increased much faster [48]. However, the application of PGR on tomato needs extreme care, for deformed fruits often occur with unsuitable concentrations, which would lead to falling price. Furthermore, it has been reported that rates of petal and stigma retention on tomato fruits were increased significantly after PGR treatments, which could result higher occurrence of grey mold on the tomato fruits [6].

In contrast to fruit, seed development is strictly dependent on fertilization. Compared with tomato fruits from buzz-pollination, parthenocarpic tomato fruits contain fewer seed and less locular tissue. Usually, the weight of pollinated tomato fruits increases with seed quantity. In our study, only positive correlations between fruit weight and seed were found in tomato fruits by bumblebee and vibrator pollination, but not in fruits by PGR treatment. Although the use of PGR or vibrator treatment can improve fruit production compared to no pollination treatments in many crops [49,50], we found that more variations and fluctuations of fruit yield were observed in PGR and vibrator-treated tomato. Compared with manual pollination methods, bumblebee-pollinated tomato also had a more stable fruit yield between plants.

##### 4.2. Bumblebee Pollination Improved Tomato Flavor

Tomato fruits from bumblebee pollination had more consumer liking compounds than fruits from vibrator or PGR treatments. For most fruits, the contents of sugars and acids are the major factors in determining taste [28]. It was reported that the balance between sugar and acid contents affects tomato fruit taste, and high but balanced levels of sugar and acid

are desired by consumers [51]. Hogendoorn's study indicated that bee-pollinated tomato had more depth of flavor than wand-pollination and was more preferred in sensory pilot study, but they found no significant differences of the soluble solids and titratable acidity content in cherry tomatoes from bee and wand pollination [35]. In their study, a handheld refractometer was used to measure the soluble solid content and acid-base titration was used to measure the titratable acidity content. However, these methods are less accurate and do not detect the single compounds of sugar or organic acid in tomato, or levels of soluble solid or titratable acidity.

The main sugars in mature tomato are fructose and glucose with small amounts of sucrose. The major organic acids in tomato fruits are citric acid and malic acid, of which, citric acid is the most abundant acid while malic acid is only present 1/10 the level of citric acid [52]. In our study, a much more reliable technique-HPLC-QqQ-MS/MS was used to measure sugars and acids, and results showed that bumblebee pollinated-tomato fruits had much higher amount of fructose and glucose, but lower amount of sucrose and acids. Similar results were found in melon: melons from bumblebee or honeybee pollination had higher content of glucose and fructose, but lower content of sucrose than melons from PGR treatment [48].

The content of both sugars and acids in tomato are highly dependent on the fruit developmental stage and ripeness [31]. During the tomato fruit ripening stage, the fructose and glucose increase while the sucrose content declines [39]. Organic acids in tomato fruits can help to enhance the feeling of sweetness [42]. At the early stage of fruit development, the content of organic acids in tomatoes increased with time and decreased at the late stage of fruit development [31,53]. In our previous study, we found that fruit development could be accelerated by bumblebee pollination through adequate fertilization [54]. Although tomato fruits under different pollination treatments were from flowers opened at the same day and the fruits were collected at the same time, bumblebee pollination might accelerate the fruit development of tomato, resulting in higher contents of fructose and glucose, and lower amount of sucrose and acids than tomatoes from PGR or vibrator treatment. However, we only measured the sugar and acid content of mature fruits and didn't focus on the content dynamic in different fruit development stage. Further studies are needed to indicate whether pollination method would influence the changes of sugar and acid content during tomato fruit development.

In our study, we found bumblebee-pollinated tomato fruits had a different volatile profile compared to PGR or vibrator treated tomatoes. It was known that bumblebee pollinated tomato fruit had more jelly and fewer cavities than PGR-treated tomato [24] and VOCs were different in different fruit structures [55]. We assumed that the volatiles differences between bumblebee-pollinated tomato and non-bee-pollinated tomato were mediated through the tissue proportion in tomato fruits. In our study, only the volatiles of whole fruits from different pollination treatments were analyzed. Further work is required to analyze the effects of bumblebee pollination on tissue composition and the volatiles of different tissues in tomato. The ripeness of fruits from different pollination methods might also affect fruit volatiles. Like sugar and acid contents, the volatiles also change during fruit developmental stage and ripeness [56]. It is essential to analyze whether pollination methods affect the volatiles emitting dynamic during tomato fruit development.

When explaining the reason for the better flavor of bumblebee pollinated tomato, it is essential to analyze the composition of consumer liking related volatile compounds. In our study, we identified 21 compounds positively related with consumer liking and 11 compounds negatively with consumer liking. For the compounds which make positive contributions to consumer liking, bumblebee pollinated tomato fruits usually contained higher contents than PGR or vibrator treated tomatoes. However, we found that bumblebee pollinated tomatoes also contained more compounds which negatively related with consumer liking. Studies showed that many of the consumer liking related volatiles were metabolically linked [28]. The pollination method does influence the fertilization process and the initial fruit set of plants [54], but little is known about how these processes would

affect the metabolic synthesis process of fruit. Further studies are needed to test whether and how the pollination methods affect the metabolic synthesis of consumer liking related volatile compounds.

#### 4.3. Implications for Growers and Food Systems

Our findings have economic implications for both the affected growers and the wider food system. The clear improvements in yield, quality, and yield stability from bee-pollination compared to other methods demonstrate a clear potential to improve productivity and livelihoods of growers in the Gobi Desert region. Not only will greater yields improve economic output and livelihoods of these small farmers, but greater yield stability presents an opportunity to reduce the economic risks of unexpectedly poor harvests [8]. However, the use of bee pollination is presently uncommon in this region, despite being a well-established practice in other large economies [24]. Much of this is due to the farmers either not knowing about the availability or usefulness of managed bumblebee colonies, or not being able to purchase just one or two colonies to support their small holdings. Furthermore, as *B. terrestris* is not native to the region, it could pose a potential invasion risk should it escape and place pressure on native species [57,58]. At present, however, there is currently no commercial breeding of the local *B. lantschouensis*. Future research should, therefore, examine means to (1) develop *B. lantschouensis* for commercial rearing and (2) ensure that small holder farmers have access to these and other suitable managed pollinators.

## 5. Conclusions

Bumblebee pollination enhanced yield and improved flavor of tomatoes in Gobi Desert greenhouses compared with vibrator or PGR treatments. Our study explored aspects of not only crop yield, but also fruit quality, especially for the fruit chemistry of tomato from different pollination treatments. In our study, tomato pollinated by *B. lantschouensis* had higher and stable fruit yield and contained more consumer liking related volatile organic compounds. Future studies to explore the links between bee pollination and aspects of fruit quality to different actors in the food system would better capture the full range of benefits from pollination services to Chinese and international agriculture.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture12060795/s1>, Figure S1: Hierarchical cluster analysis of consumer liking related volatile organic compound profiles of tomato fruits by different pollination treatments.

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