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(Original Article)

Effect of Some Treatments on Seeds Number, Yield and Fruit Quality of Acidless Orange Tree "Succarri" (*Citrus sinensis* L.)

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Abstract

Seedless fruits are preferable for fresh consumption. The acidless orange cultivar "Succarri" contains a lot of seeds, reaching over 20 seeds/ fruit under normal conditions. The present investigation was conducted to assess the impact of three recommended treatments for reducing citrus seed formation, including Cover (net covering), InsRep (natural insect repellent based on hot pepper) and SulfurGA (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3), as well as their combinations as a trial to reduce seeds in "Succarri" orange fruits. Foliar applications were conducted three times at 25, 60, and 90% of opened flowers during the two successive growing seasons of 2021 and 2022. The results revealed that using SulfurGA alone or combined with InsRep resulted in the highest fruit weight, fruit number, and overall yield per tree throughout both seasons. The highest SSC were recorded with SulferGA and InsRep separately as well as their combination in 2021 and with SulferGA alone and SulferGA+ InsRep in 2022. The application of SulfurGA increased the percentage of incomplete seeds in both seasons and decreased the average number of seeds/ fruit from 20 to 18 in the first season and from 23 to 20 in the second one. The lowest average seed number in both seasons (around 16 seeds/ fruit) was recorded with Cover. Total and incomplete seed numbers per fruit are correlated to the viability percentage of pollen grains in treated flowers. The obtained results suggested a hypothesis that seed formation in "Succarri" oranges depends on self-pollination more than cross-pollination.

Keywords: *Seedless fruit، Cover، Insect repellent، Gibberellins، Copper sulfate*

Introduction

Citrus is the largest fruit crop cultivated in the world. FAOSTAT (2020) reports that sweet oranges (*Citrus sinensis* (L.) Osbeck) make up over half of the global citrus production. Sweet oranges are Egypt's most popular fruit crop, with an annual yield of more than 3.7 million tons (Egyptian Ministry of Agriculture and Land Reclamation, 2019). Low-acidity oranges are a well-known cultivar in Egypt and some Mediterranean areas. They are also known as "Vaniglia" in Italy,

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"Sucreña" in Spain (Seminara *et al*., 2023), and "Succarri" in Egypt. Despite its bland flavor, It is preferred to some people and children since it has extremely little acid and a very high sugar/acid ratio. Succarri is an early-season cultivar with medium-to-small fruit size. According to Galal and Fayed (2014), Fruits include many seeds under field conditions (about 20 seeds/ fruit), reducing their quality and commercial value. Therefore, it is necessary to look for strategies to reduce seed number in "Succarri" fruits, which make it more diserable to the consumers.

In General, there are two strategies to produce seedless fruit. The first strategy depends on generating new seedless varieties through traditional breeding or novel biotechnological techniques (Garcia Neves *et al*., 2018; Cimen *et al*., 2021; Wang *et al*., 2021). The second one is the application of some agronomic practices on seedy cultivated varieties.

Many breeding programs have been conducted on sweet oranges around the world to produce cultivars resistant to biotic and abiotic stress, increase production, and improve fruit quality for fresh consumption (such as flavor, seedlessness, peel and flesh color, and TSS: acid ratio), etc. Even though citrus breeding programs are an important strategy for improving cultivars, they facing several challenges, as summarised by (Vardi *et al*., 2008): (i) Citrus trees have a long juvenile period before they attain the flowering phase. (ii) They have a limited genetic base, which decreases the availability of alternative alleles that could be transferred to other lines to produce a particular phenotype. (iii) There is a lack of knowledge about the mode of inheritance of specific characteristics. (iv) The polygenic nature of many important characteristics limits breeding efforts. Because of these reasons, growers and researchers rely on horticultural practices and agronomic applications that can prevent or significantly reduce seed production. These applications depend on a) preventing cross-pollination by covering trees using anti-insect nets or by using insect-repellent products ((Pons *et al*., 1995; Otero and Rivas 2017) and b) inducing parthenocarpy and inhibiting fertilization by using preharvest treatments such as GA_3 and sulfur-based products (Gambetta *et al*., 2013; Bermejo *et al*., 2016, El-Shereif *et al*., 2017; Garmendia *et al*., 2019b).

According to Pons *et al*. (1995), keeping insects away could reduce pollination and, thus, the number of seeds per fruit. Since citrus pollen grains are heavy and sticky, cross-pollination happens mainly by insects, especially honey bees (Malerbo-Souza *et al*., 2003), while wind pollination is limited in citrus. Using net covering is effective in reducing the seeds number/fruit in self-incompatibility mandarin cultivars (Garmendia *et al*., 2019a). Insect repellents are also suggested for reducing the number of seeds in citrus fruit (Garmendia *et al*., 2019a). Pesticides and chemical repellents may harm bees or other beneficial insects. Therefore, many safe, natural ingredients have been utilized as insect repellents (Naumann *et al*., 1994; Salomon and Salomon, 2015).

On the other side, preventing natural pollination and fertilization could reduce fruit set and fruit size and decrease fruit yield and overall income (Chao, 2005; Malerbo-Souza *et al*., 2003; Garmendia *et al*., 2022). Plant growth regulators, including gibberellins, auxins, and cytokinins, have been used to encourage the production of parthenocarpic fruits in several plant species. Thus, high levels of endogenous auxin and gibberellin cause the formation of natural parthenocarpy fruits in tomato and citrus varieties (George *et al.*, 1984; Iglesias *et al.*, 2007; Talon *et al.*, 1992). Gravina *et al.* (2016) illustrated the role of GA_3 application at full bloom as (50 mg L^{-1}) in reducing the number of seedless fruit and yield of "Afourer" Mandarin while avoiding cross-pollination. Exogenous application of GA_3 during blooming enhances and may increase fruit set depending on the cultivar, as it increased fruit set in seedless "Clementine" mandarin but did not impact "Satsuma" mandarins (Talon *et al.*, 1992). Mesejo *et al.* (2008) found that foliar application of GA_3 at a rate of 10 mg L^{-1} over the days preceding blooming decreased fertilization and seed formation in a self-incompatible cultivar of mandarin called "Clemenules" by either boosting ovule abortion or reducing pollen tube growth during cross-pollination. GA_3 has also been used to induce seedlessness in grapes, as it inhibits pollen germination and pollen tube development (Kimura *et al.*, 1996). Fruit number per tree and yield were significantly increased when GA_3 (50 mg L^{-1}) was applied in combination with trunk girdling after 30 days of full bloom. (Gravina *et al.*, 2016). Sulfur treatments also have been used to reduce the number of seeds in citrus fruits. Mesejo *et al.* (2006) demonstrated that utilizing sulfur-based products reduced the in vitro pollen germination of the "Fortune" mandarin in vitro. In addition, they found that applying 25 mg L^{-1} of $CuSO_4.5H_2O$ at full bloom increased the percentage of seedless fruits in "Clemenules" mandarin and reduced the number of seeds by 55–81%. Kheder *et al.* (2019) reported that using 25 mg L^{-1} $CuSO_4$ combined with GA_3 at 25 or 50 mg L^{-1} was more effective than GA_3 alone in increasing aborted seeds in "Balady" mandarine and improving fruit weight. Three times of application with GA3 (50 mg L^{-1}) combined with $CuSO_4$ (25 mg L^{-1}) during the flowering period increased seedless fruit by 31% (Gambetta *et al.*, 2013). Mesejo *et al.* (2008) and Garmendia *et al.* (2019b) found that the combination of $CuSO_4$ + GA_3 treatment increased floral style elongation in mandarine more than the control. As a result, Pollen tubes have to travel longer to reach the ovules, which results in reduced fertilization. Also, Garmendia *et al.* (2022) reported that sulfur inhibited pollen tube growth in treated flowers of "Nadorcott" mandarinby by 94-100%.

As far as we know, no research has been published on how different practices affect the number of seeds and fruit quality of "Succarri" oranges. Therefore, it is necessary to evaluate the previously suggested treatments for reducing seeds with this cultivar separately. To achieve this goal, this study was conducted to evaluate the efficiency of three main treatments, including the net covering (Cover), insect repellent (InsRep), and SulfurGA treatment (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3) in addition to, their combination on the viability of pollen grains and style length of treated flowers as well as seed formation and fruit quality of "Succarri" orange fruits.

Materials and Methods

Experimental conditions and plant material

The present study was carried out on five years old of acidless orange trees "Succarri" (*Citrus sinensis* L.) budded on Sour Orange (*Citrus aurantium* L.) rootstock, spaced at 5x5 meters apart (168 trees/fed.). Trees are grown in a private orchard located at Desouk district, Kafr El-sheikh governorate, Egypt (31° 07 N and 30° 41 E) on clay soil with a pH of 8. The Nile water was used in irrigation under a flood irrigation system. All trees received the required agricultural treatments, as is customary in this region. Seventy-two trees of the same age, size, vegetative growth, load, and free from visible disorders were selected for this investigation.

Treatments

The six studied treatments are summarized in Table (1). All treatments were repeated during the two successive growing seasons of 2021 and 2022.

In Cover treatments, each treated tree was completely covered by a white, 50% transparent light anti-insect net before 25 % of opened flowers. Covering was put on for about 30 days to keep pollinators and bees away. The insect repellent treatment consisted of a water solution containing 0.2% v/v of a natural repellent produced from the essence of hot pepper (*Capsicum annuum* L). SulfurGA was conducted as a foliar application of copper sulfate (25 mg L^{-1} $CuSO_4.5H_2O$) and, subsequently, 50 mg L^{-1} GA_3. A surfactant Tween 80 at 0.05% (v/v) was added to all treatments to obtain better retention and penetration of different solutions. Control trees were sprayed with water only). Spraying treatments were applied at 4-5 liters per tree using a 20-liter knapsack sprayer with a constant pressure of 2.5 bar. Foliar spraying was applied 3 times throughout the period 25, 60, and 90% of flowering.

Table 1. Summarization of the six conducted treatments on "Succarri" orange trees during 2021 and 2022 seasons

	Abbreviations	Treatments
1	Control	Spraying with tap water only
2	Cover	Covering trees with an anti-insect net
3	InsRep	Foliar spraying with insect repellent product (natural insect repellent based on hot pepper (*Capsicum annuum* L).
4	SulfurGA	Foliar spraying with 25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3
5	Cover + SulfurGA	Covering with an anti-insect net followed by foliar spray with (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3)
6	InsRep + SulfurGA	Foliar spraying with insect repellent product + (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3)

Measurements

Viability of pollen grains %

To evaluate the viability percentage of pollen grains, 10 flowers/ tree were collected from each treatment before dehiscence occurred after three days of the last application. Anthers were placed carefully on a slide and thoroughly crushed using a needle in two drops of acetocarmin (0.5 gm carmin + 45 ml acetic acid and 55 ml distilled water). After that, the samples were examined using the light Microscope (40x). Round-stained pollen grains (dark red) are considered viable or living pollen, and sterile or dead pollen is stained yellow or light red. Three fields were counted in each slide to estimate the percentage of viable pollen grains, as described by Berding and Skinner (1980). Determining the percentage of viable pollen grains is achieved by dividing the number of viable pollen grains by the total count of pollen grains %.

Floral style length

The length of the floral style (without the stigma or ovary) was measured after three days of the third application in 10 flowers per tree in the first season. Flowers from covered trees were collected carefully without removing the net covering. A light microscope with an ocular micrometer attached was used to measure the length.

Anatomical studies of seed formation

Fruitlets were collected at a diameter of 10-15 mm and preserved in FAA fixative (10 ml formalin, 5 ml glacial acetic acid, and 85 ml ethyl alcohol 70%). The stored samples were prepared as follows: washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax, cross-sectioned to a thickness of 20 µm, and double stained as described by (Nassar and El-Sahhar, 1998). Three slides containing three or four cross-sections from the middle of fruitlets for each treatment were examined and photographed using light microscopy (20X).

Yield and fruit quality

At harvest time (in the middle of December in both seasons), the fruits of all the trees were collected separately, and the number of fruits/tree were recorded. The average fruit weight (g) and juice weight (gm) were measured in 10 randomly selected fruits per tree. Then, the percentage of juice was calculated by dividing by the initial fruit weight (Juice %). The total yield per tree was calculated by multiplying the average fruit weight by the fruit number/tree. The number of complete (developed) and incomplete (aborted) seeds per fruit was counted. Then, the percentage of incomplete seeds was determined by dividing the number of incomplete seeds by the total number of seeds.

Peel color was measured in five selected fruits randomly at two opposite places using a portable colorimeter (FRU WR18, Shenzhen, China), and then the mean was calculated for each fruit. The color was recorded as L*, a*, and b* parameters, according to (McGuire, 2019). The L* value denotes the brightness, which ranges from 0 (black) to 100 (white). a* value refers to green (negative) or red (positive), while the negative b* value refers to blue and positive for yellow.

For chemical analysis, Three fruits in each replicate were chosen that were free from disease, insect infestation, or any mechanical damage. Soluble solids content (SSC%) was determined by a digital refractometer (Milwaukee, model

MA871, Milano, Italy). Titratable acidity (TA) was determined as mg citric acid/100 ml juice according to AOAC (2003).

Statistical analysis

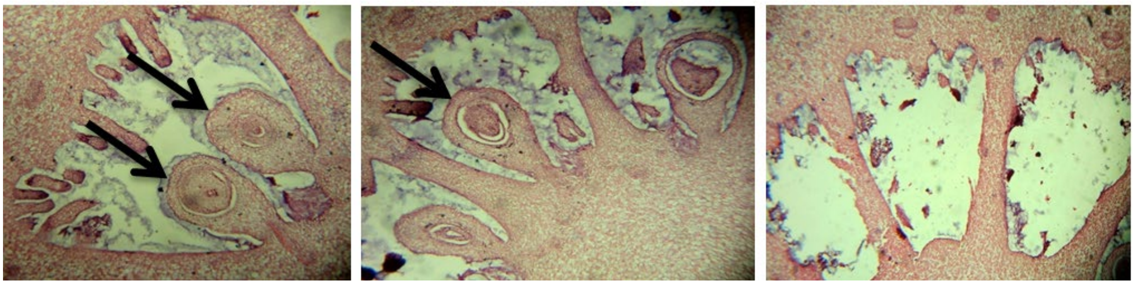
The Six examined treatments were distributed according to a randomized complete block design with four blocks, each block consisted of 18 trees and each replicate included 3 trees. Recorded data were analyzed using a one-way ANOVA test for each season separately. The Least Significant Difference (LSD) value were used to compare the significant difference between individual means of each treatment at $p \leq 0.05$. Statistical analysis was made using the software SPSS version 16 (SPSS, Inc., Chicago, IL, USA). The relationships between studied characteristics were collected as linear regression using Microsoft Excel program software.

Results

Effect of treatments on seed formation

The effect of different treatments on the number of fertilized ovules in the cross sections obtained from the middle of fruitlets was observed microscopically. In each segment, there were single or double-fertilized ovules; in some cases, no fertilized ovules were found (Fig. 1). Double-fertilized ovules were commonly seen with the control and InsRep treatments.

Developed and incomplete seeds of "Succarri" oranges are photographed (Fig. 2). Developed seeds were full and regular with large size compared to incomplete seeds, which looked deformed, wrinkled and small in size. The average weight of ten developed seeds was 3.2 g, whereas those of ten incomplete seeds were 1.1 g (unshown data). The data presented in Fig. 3 showed that Cover, InsRep, SulfurGA, and their combination have a statistically significant effect ($p \leq 0.05$) on the average number of total seeds formed per fruit and the percentage of incomplete seeds in "Succarri" fruits compared to Control, in both seasons. Data presented in Fig. 3 (A and B) exposed that in both seasons, the highest total number of seeds/fruit (20.4 and 23.4) and the lowest percentage of incomplete seeds (18.8 and 7.5 %) were recorded in Control, followed by InsRep treatment (19 and 22 total seeds) and (56.1 and 40.3% of incomplete seeds). The number of total seeds decreased significantly by using Cover alone (16.5and16.9) or combined with SulfurGA (17.7 and18.8) in (first and second) seasons. Also, the incomplete seed percentage increased significantly under Cover to reach (76.8 and 53.3%) in the first and second seasons, respectively. SulferGA and SulfurGA +InsRep treatments also decreased seed number/ fruit and incomplete seed% compared to Control and InsRep.



The arrows pointed to fertilized ovules in the segment.

Fig.1. Cross-section of "Succarri" oranges fruitlets.

Fig.2. Developed (A) and incomplete (B) seeds of treated "Succarri" oranges

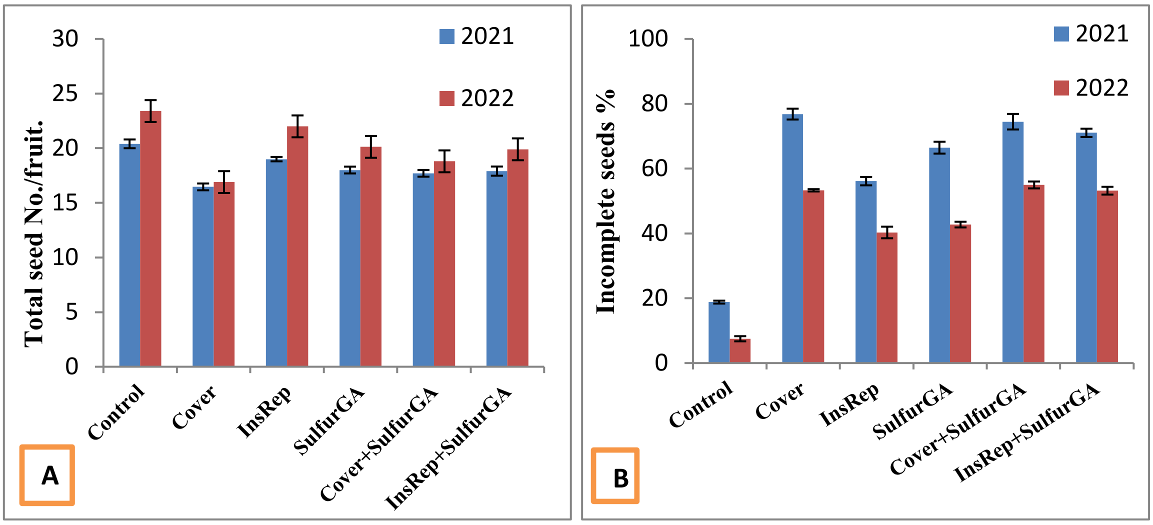


Fig.3. Effect of six studied treatments on A: total number of seeds/ fruit and B: incomplete seeds % of "Succarri" oranges during 2021 and 2022 seasons. Treatments are presented as C: control; Cover: net covering; InsRep: insect repellent; Sulfur: (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3). Error bars indicate the standard error

Effect of treatments on pollen grains viability %

Fig. 4 shows the differences between viable pollen grains (round-stained with dark red color) and dead pollen grains (stained yellow or light red with an irregular shape). The percentage of viable pollen grains is presented (Fig.5). SulfurGA+Cover significantly reduced the mean value of viable pollen grains

(58.1%), followed by SulfurGA only (65.0%) compared to Control (71.6%) and other treatments.

The relationships between seed numbers and the viability of pollen grains were calculated as linear regression. In general, there was a positive relationship between the pollen viability % and each of the total seed number/ fruit (R^2 = 0.37 and incomplete seed number/ fruit (R^2 = 0.47)(Table 2).

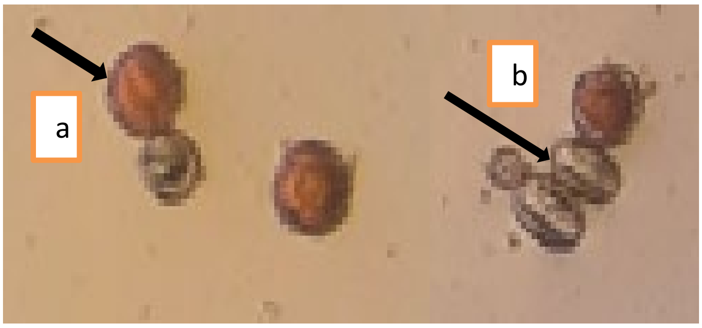


Fig. 4. Pollen grains of "Succarri" oranges, the arrows pointed to viable pollen grains (a) and dead pollen grains (b)

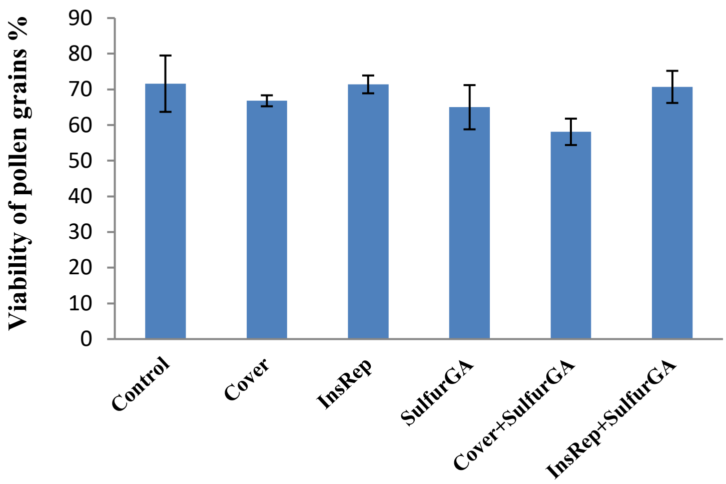


Fig. 5. Effect of six studied treatments on the viability percentage of pollen grains of "Succarri" oranges during 2022 and 2023 seasons. Treatments are presented as C: control; Cover: net covering; InsRep: insect repellent; SulfurGA: (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3). Error bars indicate the standard error.

Table 2. Linear regressions between total and incomplete seed number/ fruit, and pollen grains viability % and fruit weight.

	Total seed number/ fruit		**Incomplete seed number / fruit**	
	2021	2022	2021	2022
Pollen viability %	0.37	-	0.47	-
Fruit weight	0.11	0.17	0.08	0.01

Effect of treatments on floral style length

The effect of the treatments on the length and appearance of the floral style was observed in Fig. (6 and 7). SulfurGA treatments caused a significant increase in style length. The tallest average style lengths (8.5 and 8.3 mm) were recorded with Cover+SulfurGA and SulfurGA, respectively (Fig. 7). Cover+SulfurGA also caused a distinct bow in the style, as depicted in Fig. 6.

Control **SulfurGA** **InsRep** **Cover** **InsRep+ SulfurGA** **Cover+ SulfurGA**

Fig.6. Floral ovule, style and stigma of "Succarri" oranges under six studied treatments. Treatments are presented as C: control; Cover: net covering; InsRep: insect repellent; Sulfur GA: (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3).

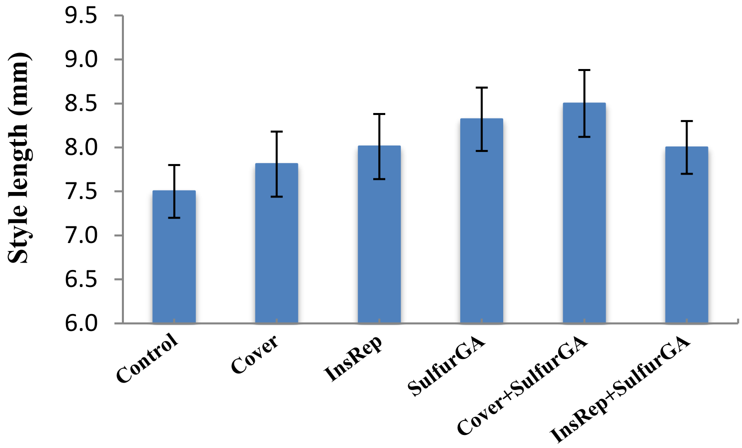


Fig.7. Effect of six studied treatments on the floral style length (mm) of "Succarri" oranges. Treatments are presented as C: control; Cover: net covering; InsRep: insect repellent; SulfurGA: (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3). Error bars indicate the standard error.

Effect of treatments on yield and fruit quality

The analysis of variance performed to evaluate the impact of treatments on fruit weight and yield showed statistically significant differences among treatments with a significance level of $p \geq 0.05$. Table 3. shows the mean values for fruit weight, fruit number per tree, and total yield (kg) per tree. The average fruit weight ranged from 154.3 to 175.0 in the first season and from 161.0 to 178.3 g in the second. Using SulfurGA alone or combined with InsRep resulted in the highest fruit weight, fruit number, and overall yield per tree throughout both seasons. Utilizing Cover treatments reduced fruit weight, fruit No/tree and the total yield of tree in both seasons, as compared to the control group.

Table 4 displays the impact of different treatments on SSC %, titratable acidity % and juice % of "Succarri" oranges during the seasons of 2021 and 2022. The results indicated significant differences in SSC in both seasons. The SSC % of the treated fruits ranged from 10.9 to 12 % and from 11.3 to 11.9 % in the first and second seasons, respectively. The highest SSC % were recorded with SulferGA and InsRep and their combination in 2021 and with SulferGA and SulferGA+ InsRep in 2022. Concerning titratable acidity, there were no statistically significant differences among each of the treatments. Regarding juice% within the fruit, The data reveals that the Cover treatment exhibited the lowest juice content percentage during its first season, while no significant differences in juice % were identified during the second one.

The effect of the studied treatments on fruit color was recorded as (L*, a*, and b*) parameters, Table 5. The three parameters showed that the effects of different treatments on fruit coloration are limited. The lowest a* values (representing green color) were obtained using the SulfurGA+ InsRep and SulfurGA+ Cover treatments.

Table 3. The effect of six studied treatments on the average fruit weight (g), fruit number/tree and yield/tree (Kg) of "Succarri" oranges during the 2021 and 2022 seasons

Treatments	Fruit weight (g)		Fruit No/tree		Yield/tree (kg)	
	2021	2022	2021	2022	2021	2022
Control	164.3^{c}	168.3^{c}	400.6^{c}	407.9^{c}	65.8^{d}	68.7^{c}
Cover	154.3^{e}	161.0^{e}	372.6^{e}	374.7^{e}	57.5^{f}	60.3^{e}
InsRep.	166.7^{b}	170.7^{b}	$405.0b^{c}$	414.1^{c}	67.5^{c}	70.7^{c}
SulfurGA	173.3^{a}	174.3^{a}	410.6^{b}	429.3^{b}	71.2^{b}	74.8^{b}
Cover+ SulfurGA	159.0^{d}	165.0^{d}	384.7^{d}	390.9^{d}	61.2^{e}	64.5^{d}
InsRep+ SulfurGA	175.0^{a}	178.3^{a}	419.1^{a}	437.4^{a}	73.3^{a}	78.0^{a}

Treatments are presented as C: control; Cover: net covering; InsRep: insect repellent; SulfurGA: (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3). Mean values followed by different letters are significantly different at $P \leq 0.05$.

Table 4. The effect of six studied treatments on SSC (%), titratable acidity% (mg citric acid/ 100 ml juice) and juice % of "Succarri" oranges during 2021 and 2022 seasons.

Treatments	SSC (%)		TA (%)		Juice % (ml)	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**
Control	11.3^{b}	11.5^{bc}	0.16^{a}	0.16^{a}	52.7^{b}	51.6^{a}
Cover	10.9^{c}	11.3^{c}	0.15^{a}	0.16^{a}	47.7^{c}	50.9^{a}
InsRep.	11.7^{a}	11.5^{bc}	0.16^{a}	0.17^{a}	53.6^{a}	51.2^{a}
SulfurGA	12.0^{a}	11.7^{ab}	0.17^{a}	0.19^{a}	52.6^{b}	50.9^{a}
Cover+ SulfurGA	11.3^{b}	11.4^{bc}	0.15^{a}	0.16^{a}	52.9^{b}	51.7^{a}
InsRep+ SulfurGA	12.0^{a}	11.9^{a}	0.17^{a}	0.19^{a}	52.8^{b}	54.3^{a}

Treatments are presented as C: control; Cover: net covering; InsRep: insect repellent; SulfurGA: (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3). Mean values followed by different letters are significantly different at $P \leq 0.05$

Table 5. The effect of six studied treatments on peel color (L*, a* and b*values) of "Succarri" oranges during 2021 and 2022 seasons

Treatments	L*		a*		b*	
	2021	2022	2021	2022	2021	2022
Control	51.2^{b}	52.9^{a}	28.7^{a}	24.7^{bc}	70.3^{b}	69.1^{a}
Cover	51.3^{b}	52.1^{a}	27.9^{a}	25.4^{b}	70.9^{ab}	64.5^{ab}
InsRep.	51.5^{b}	51.1^{a}	28.4^{a}	27.0^{a}	70.2^{b}	65.2^{ab}
SulfurGA	52.9^{a}	51.2^{a}	26.5^{b}	25.4^{b}	71.5^{a}	59.1^{ab}
Cover+ SulfurGA	51.9^{b}	52.5^{a}	24.3^{c}	23.2^{c}	69.3^{c}	57.2^{b}
InsRep+ SulfurGA	51.8^{b}	52.7^{a}	23.2^{c}	23.1^{c}	70.9^{ab}	66.6^{ab}

Treatments are presented as C: control; Cover: net covering; InsRep: insect repellent; SulfurGA: (25 mg L^{-1} $CuSO_4.5H_2O$ + 50 mg L^{-1} GA_3). Mean values followed by different letters are significantly different at $P \leq 0.05$

Discussion

In this study, we investigate the effect of three main treatments, including Cover (net covering), InsRep *(Capsicum annuum* L.) and SulfurGA (GA_3 at 50 mg L^{-1} + $CUSO_4.5H_2O$ at 25 mg L^{-1}), as well as their combinations on seed formation, yield and fruit quality of "Succarri" oranges. Mean values for all measured characters differed in the two studied seasons due to individual differences in treated trees and surrounding conditions, such as soil fertility, irrigation, water quality, and the environment. As such, these attributes may vary from one year to the next or from one place to another.

Seed formation and incomplete seeds

Net covering

"Succarri" oranges, under normal cross-pollination conditions in Egypt, contain more than 20 seeds per fruit (Galal and Fayed, 2014). Although Cover is an expensive strategy that negatively affects fruit set and is considered unsuitable for commercial use (Gravina *et al*., 2016; Garmendia *et al*., 2019a), we aimed to investigate the effects of Cover on the natural pollination processes and seed formation in "Succarri" oranges, as no existing literature are available on the impact of various techniques on the seed number in "Succarri" oranges.

Using net covering treatments reduced the total seed number and raised the percentage of incomplete seeds in both seasons. The lowest number of seeds (16.5 and 16.8 seeds/fruit) was recorded with Cover treatment compared to Control (20.4 and 23.4 seeds/ fruit) in the first and second seasons, respectively. These results are consistent with previous works indicating that net covering is the most effective method for reducing the number of seeds present in mandarin (Gravina *et al*., 2016; Garmendia *et al*., 2019a), But the decrease in seed number formed under Cover is less than expected since covering resulted in completely seedless fruits of "Afourer" mandarin (Garmendia *et al*., 2019a). Also, Gambetta *et al*. (2013) reported 98 to 99 % of seedless "Afourer" mandarin under net covering, compared to 2-4 seeds under cross-pollination conditions. This variation could be explained by the information that "Afourer" mandarin is a self-incompatible cultivar; it produces a high number of seeds under open pollination conditions while it produces seedless fruit in the absence of cross-pollination. Therefore, we suggested that "Succarri" oranges may be a self-compatible cultivar or at least partially self-incompatible, and it does not require foreign pollens or cross-pollination to complete fertilization.

SulfurGA

The application of SulfurGA alone or in combination with other treatments decreased seed number and increased aborted seeds %. These findings are in agreement with Kheder et al. (2019), who reported that foliar spray at 25 mg L^{-1} GA_3 in combination with 25 mg L^{-1} $CuSO_4$ reduced the number of developed seeds of "Balady" mandarine from 21.6 to 4.7 seeds/ fruit. Mesejo *et al*. (2006) found that using 25 mg L^{-1} $CuSO_4.5H_2O$ totally reduced in vitro pollen germination and prevented pollen tube extension when it was applied after 8 hours of germination. While using it before hand-pollinating on "Fortune" mandarin flowers decreased pollen germination, but it did not completely prevent it. According to Garmendia *et al*. (2022), sulfur is the most effective treatment for inhibiting pollen tube growth in treated "Nadorcott" mandarin flowers by 94-100%. Also, Beltrán *et al*. (2023) reported that sulfur-based products in different concentrations ranging from 0.2 to 20,000 mg L^{-1} inhibited the pollination of "Clemenules" mandarin and Nova tangelo via affecting pollen germination and pollen tube growth. When the concentration of sulfur increased, lower pollen germination was detected.

The formation of incomplete seeds by SulfurGA may also be attributed to the influence of GA_3. Talon *et al.* (1992) found that GA concentration is significantly higher in "Satsuma" mandarine (parthenocarpic var.) than in "Clementine" (non-parthenocarpic self-incompatible var). Also, The percentage of developed seeds/fruit of "Balady" mandarin was reduced to 52.0% when GA3 was sprayed at 200 ppm during full bloom and to 39.2% during the pre-anthesis stage compared to the control (Mohamed and Elgammal, 2018).

Under our experimental conditions, although SulfurGA treatments increased incomplete seed % in both seasons, it only reduced the average number of seeds/ fruit from 20 to 18 in the first season and from 23 to 20 in the second. Even though most of these seeds are incomplete, they are still noticeable and undesirable to consumers. Perhaps better results would be obtained by using higher GA concentrations. Since the initial study indicated a lower effect on seed formation, more studies are required to determine whether higher concentrations are beneficial for "Succarri" oranges or not.

Insect repellent

Many chemical repellents and pesticides are available on the market that can be used to keep pollinators away to reduce pollination. However, These compounds may damage bees or other beneficial insects (Sahebzadeh *et al*., 2009). Thus, we aimed to investigate how one of the safe components based on natural substances as hot pepper (*Capsicum annuum* L.), may decrease the formation of seeds without endangering beneficial insects like bees.

In our study, InsRep resulted in producing 19 and 22 seeds/fruit in the first and second seasons, respectively, indicating that it was unable to reduce the number of seeds to a satisfactory level. In contrast, Pons *et al*. (1995) suggested that Insect repellents could be useful for reducing the number of seeds in citrus fruit as they keep insects away, preventing pollination, which in turn reduces the number of seeds per fruit. On the other hand, our observation is consistent with that of (Garmendia *et al*., 2019a), who found no effect of zinc-based repellent and C*apsicum annuum* repellant on Afourer' mandarin, despite the farmers in Spain utilise these products to keep bees away from their crops and seeds.Garmendia *et al*. (2019a) also suggested that an insignificant effect of insect repellents may be attributed to the application on a "tree scale". While applying repellents on a "field scale" may cause a more significant impact. Results also may be explained by our suggested hypothesis that "Succarri" oranges exhibit a greater dependence on self-pollination than cross-pollination, so they do not need pollinators for successful fertilization; hence, the presence or absence of pollinators does not affect pollination and seed formation.

The observed positive correlations between the total number of seeds and the number of developed seeds per fruit, in relation to the percentage of viable pollen grains, lend support to our hypothesis that the fertilization process in "Succarri" oranges is primarily reliant on self-pollination rather than cross-pollination. Consequently, by reducing the viability of pollen grains, it is possible to decrease the number of seeds formed in "Succarri" oranges.

Yield and fruit quality

The impact of studied treatments on yield and fruit quality is complex to assess, as many endogenous and exogenous factors influence them, such as irrigation, fertilization, biotic and abiotic stresses, etc.

The total yield production can be affected by both the average fruit weight and fruit number per tree (Total yield = fruit weight × fruit number). Although alternate bearing is widely observed in citrus (Abobatta, 2019), we did not notice this phenomenon in "Succarri" oranges. Our findings showed that Cover produced the lowest yield, fruit weight and fruit number. Cover reduced fruit weight by 6 and 4.3% in 2021 and 2022, respectively. In terms of yield, the covered trees produced an average of 57.5 and 60.3 kg, whereas the control trees produced an average of 65.8 and 68.7 kg in 2021 and 2022, respectively, representing a yield drop in covered trees of 12.6 and 12.2%. Our findings are consistent with previous research (Gravina *et al*., 2016; Otero and Rivas, 2017; Garmendia *et al*., 2019a), which demonstrated a significant reduction in fruit weight, fruit number and overall yield production with covering. Also, Malerbo-Souza *et al*. (2003) observed a negative effect of covering on fruit set, compared to uncovered trees, which may also be the cause of the yield drop. Otero and Rivas (2017) documented a significant decrease in fruit numbers by 66% when trees were covered, Gambetta *et al*. (2013) noticed a reduction of up to 30% in fruit set, Garmendia, *et al*. (2019a) recorded a substantial decline of 56% in yield production.

The effect of Cover in reducing yield could be attributed to higher temperature and humidity under covering, which inhibits photosynthesis and causes higher fruit drop (Jifon and Syvertsen, 2003). In "Washington" Navel oranges, although it does not need cross-pollination to increase fruit set, there is evidence to show that pollination by bees may contribute to keeping fruit from dropping off, which increased fruit production (Sanford, 2011).

The horticultural practices utilized to reduce seeds, such as net covering, may potentially result in a reduction in fruit set and fruit weight and subsequently contribute to diminished crop yields. There is no agreement regarding the effect of seed number on fruit size. According to Otero and Rivas (2017), there is a highly positive relationship (R^2=0.89) between seed number and fruit size in "Afourer' mandarine". Chao (2005) showed a moderate correlation between these two parameters (R=0.59). In contrast, Garmendia *et al*. (2019a) found a weak relationship between them (0.15). In our study, we found a weak relation between total or incomplete seeds and fruit weight (R^2 less than 0.17). However, the slight reduction in fruit weight that we reported with "Succarri" oranges could be explained by the large number of seeds formed with all treatments.

Using SulfurGA alone or combined with InsRep resulted in the highest fruit weight, fruit number, and overall yield per tree throughout both seasons. According to Garmendia *et al*. (2019b), the application of gibberellic acid (GA) prior to flowering has been found to enhance crop output by promoting fruit set, particularly in cases when there is insufficient stimulation for fruit set.

In all treatments, there is no obvious trend in the differences in TSS, juice %, and peel color between fruits. Meanwhile, (El-Shereif *et al*., 2017) found that application of GA_3 at 25 mg L^{-1} on "Balady" mandarin maintained fruit characteristics such as firmness, vitamin C, TSS and TSS/acid ratio.Garmendia *et al*., (2019b) reported that the affect of GA3 applications on Citrus fruit ripening, rind coloration, softening, and juice volume is contingent upon the timing of application and the concentration utilized.

No significant impact of the treatments investigated on the total acidity percentage (TA%) was seen, perhaps due to the low acidity levels present in "Succarri" oranges. The acidity content in "Succarri" oranges is nearly negligible, about 0.1%, which is approximately one-tenth of the acidity levels observed in in other orange caltivars (Seminara *et al*., 2023).

Conclusion

Under our experimental conditions, bee repellents were not useful for reducing the seed number/fruit in "Succarri" oranges. SulfurGA treatment increased incomplete seed % in both seasons, but it only reduced the average number of total seeds/ fruit from 20 to 18 in the first season and from 23 to 20 in the second one. The lowest average seed number in both seasons (about 16 seeds/fruit) was recorded with Cover. But, it is still insufficient to satisfy consumers. Therefore, reducing the number of seeds/ fruit in "Succarri" oranges to meet consumers' preferences is still a goal that has not been achieved. So, more research is needed on this purpose, taking into consideration the hypothesis that

"Succarri" oranges depend in fertilization on self-pollination rather than cross-pollination.

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تأثير بعض المعاملات على عدد البذور والمحصول وجودة الثمار فى اشجار البرتقال الخالي من الحموضة "السكرى" (*Citrus sinensis* L.).

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الملخص

يفضل المستهلك الثمار قليلة البذور فى الاستهلاك الطازج. بينما يحتوي صنف البرتقال الخالي من الحموضة "السكرى" على الكثير من البذوروالتى يصل عددها إلى أكثر من 20 بذرة / ثمرة. وقد تم إجراء البحث الحالي لتقييم تأثير ثلاثة من المعاملات الموصى بها لتقليل اعداد بذور الحمضيات وهى استخدام التغطية بالشباك، استخدام طارد الحشرات الطبيعي (المعتمد على الفلفل الحار) ومعاملة الكبريت والجبرلين (25 ملجم/لتر كبريتات نحاس و50 ملجم/لتر جبرلين)، بالإضافة إلى توليفاتها لتقليل عدد البذور في ثمار البرتقال السكرى. تم إجراء الرش الورقي ثلاث مرات عند25 و60 و90% من تفتح الأزهار، خلال موسمي النمو لعامي 2021 و2022. اظهرت النتائج أن استخدام معاملة الكبريت والجبرلين بمفردها أو مع المعاملة بطارد الحشرات اعطت أكبر وزن للثمرة واعلى عدد للثمار والمحصول الكلى/ شجرة خلال كلا الموسمين. سجلت أعلى مستويات للسكريات الذائبة الكلية مع معاملة الكبريت والجبرلين ومعاملة طارد الحشرات سواء كل منهما على حدة او المعاملة بهما معًا في عام 2021, ومع معاملة الكبريت والجبرلين منفردة او مصحوبة بالمعاملة بطارد الحشرات في عام 2022. كما ادت معاملة الكبريت والجبرلين إلى زيادة النسبة المئوية للبذور غير المكتملة في كلا الموسمين وتقليل متوسط عدد البذور/الثمرة من 20 إلى 18 في الموسم الأول ومن 23 إلى 20 في الموسم الثاني. سجل أقل متوسط لعدد البذور في كلا الموسمين (حوالي 16 بذرة/ ثمرة) مع التغطية. كما وجد ان اجمالى عدد البذورو عدد البذور غير الكاملة/ ثمرة لها علاقة بحيوية حبوب اللقاح في الازهار المعاملة. ساهمت النتائج المتحصل عليها فى وضع نظرية فرضية بأن تكوين البذور في البرتقال السكرى يعتمد اساسا على التلقيح الذاتي أكثر من اعتماده على التلقيح الخلطي.

الكلمات المفتاحية: *ثمار بدون بذور، التغطية، طارد الحشرات، جبرلين، كبريتات النحاس*