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Gibberellin (GA_3) and Copper Sulfate Pentahydrate ($CuSO_4 \cdot 5H_2O$) Reduce Seeds per Fruit and Increase Fruit Quality in Bac Son Mandarin Fruit

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Abstract: The aim of this study was to find the optimal gibberellic acid (GA_3) and copper sulfate ($CuSO_4 \cdot 5H_2O$) concentrations to reduce the number of seeds and increase the quality of Bac Son mandarin fruit. In experiment 1, the control plants (without GA_3) were sprayed with water, and the remaining plants were sprayed with different concentrations of GA_3 (50, 75, 100, or 125 ppm). In experiment 2, the control plants (without $CuSO_4 \cdot 5H_2O$) were sprayed with water, and the remaining plants were sprayed with different concentrations of $CuSO_4 \cdot 5H_2O$ (50, 75, 100, or 125 ppm). Spraying GA_3 at 100 ppm in 2018 produced the lowest seed number and the highest theoretical yield. In 2019, spraying GA_3 led to a lower seed number and a higher actual yield compared with the control. Similarly, spraying $CuSO_4 \cdot 5H_2O$ significantly reduced the number of seeds/fruit and significantly increased the theoretical yield in 2018. In 2019, the total number of seeds/fruit was significantly lower in the $CuSO_4 \cdot 5H_2O$ treatments than in the control. Importantly, both GA_3 and $CuSO_4 \cdot 5H_2O$ treatments did not adversely affect the fruit's biochemical parameters or yield. These findings demonstrate that spraying GA_3 or $CuSO_4 \cdot 5H_2O$ at a certain concentration can effectively reduce the number of seeds per fruit in Bac Son mandarin without compromising fruit quality or yield.

Keywords: Bac Son mandarin; seed reduction treatment; gibberellin (GA_3); copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)

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1. Introduction

Gibberellic acid (GA_3) belongs to a group of plant hormones known as gibberellins, which strongly influence seed development and fruit growth [1]. The quality of citrus fruit is evaluated using several characteristics, including taste, fruit color, fruit flesh ratio, flesh softness, number of seeds per fruit, nutrient contents, etc. [2,3]. The main objective of breeders is to select varieties with seedless or low-seed fruit, as this characteristic is highly valued in citrus fruits. Bac Son yellow mandarin (*Citrus reticulata* Blanco) is a large, round, flattened, ripe fruit with a bright yellow color, and a shiny, attractive appearance. The sweet taste is also slightly sour and, especially when peeled, this mandarin has a special taste. Chemical treatments can be used to produce seedless citrus fruits [3]. Seedlessness is highly appreciated by consumers both in conserved or processed fruits (e.g., tomato sauce and frozen eggplant) as well as in fruit for fresh consumption (e.g., citrus, grape, apple, and banana) [4]. Moreover, seeds can promote fruit deterioration, as in eggplant and watermelon [3]. In this case, the absence of seeds can increase the shelf life of the fruits.

GA is a well-known plant hormone [5]. GA has been used to enhance the different yield-related and crop cycle processes of *Citrus* spp. and other crops [6,7]. Farmers aim to produce high-quality fruit yields. To obtain this goal with fruit trees, flowering, fruit, seed number, and yield (fruit size and number) must be controlled according to tree capacity [8].

The number of seeds per fruit was reduced from 3.7 to 2.3 (38%) via treatment with GA_3 (50 mg/L) and CuSO4 (25 mg/L) [9]. The seed number per citrus fruit was reduced 35% by copper sulfate and GA_3 treatment [10]. The application of GA_3 has been widely studied for its impact on fruit set and seed development in various plant species [2]. Gibberellic acid acts as a plant growth regulator, and its exogenous application can influence the hormonal balance within a plant, affecting fruit development and seed formation [1]. The application of GA_3 significantly reduced the seed number in fruit [11]. These findings suggest that GA_3 can be used as an effective tool to create seedless or low-seeded citrus varieties.

Copper sulfate, a mineral nutrient, has been investigated for its potential role in reproductive processes in plants. Copper is involved in various physiological and biochemical processes, including hormone metabolism and enzyme activity [12]. The underlying mechanisms by which copper sulfate affects seed development in citrus are not yet fully understood and require further study. Copper sulfate may influence the hormone signaling or enzyme activity involved in seed formation [13]. Copper sulphate pentahydrate is used instead of copper sulphate in certain applications due to its advantageous properties. The pentahydrate form of copper sulphate contains five water molecules (H_2O) associated with each copper sulphate molecule ($CuSO_4$). This hydrated form provides certain benefits such as increased stability, solubility, and ease of handling. The presence of water molecules in the copper sulphate pentahydrate crystals enhances its solubility in water, making it easier to dissolve and mix with other solutions. This improved solubility allows for better distribution and absorption of copper ions by plants when applied as a foliar spray or soil amendment. Furthermore, the hydrated form of copper sulphate exhibits better stability, as the water molecules act as a protective layer around the copper sulphate molecule, reducing the risk of oxidation and degradation. This stability ensures that the copper sulphate remains effective and maintains its desired properties for longer periods, providing more consistent results. As such, the objective of this study was to determine the optimal concentrations in GA_3 and $CuSO_4 \cdot 5H_2O$ applications to minimize seed count and increase the quality of Bac Son mandarin fruit.

2. Materials and Methods

2.1. Experimental Site

Two experiments were performed on 16-year-old trees of Bac Son rootstock mandarin. A total of 150 plants were used in 2 separate experiments, with 75 plants assigned to each experiment. Each experiment was replicated 3 times, with 5 plants per replicate. The irrigation and fertilization were identical for all sample trees in the experiments. Management was conducted under standard culture conditions without other treatments, except for the experimental factors. The GA_3 (China) ingredients included 75% purity gibberellic acid as a fine, white powder. For the copper sulfate ($CuSO_4 \cdot 5H_2O$), the raw materials were imported from Taiwan (ToBa Trading Production Co., Ltd.) and had a composition of $CuSO_4 \cdot 5H_2O$, which was a blue powder with 97% purity.

2.2. Experimental Design

Experiment 1 included five treatments. The control treatment (without GA_3) involved spraying the plants with water; the remaining plants were sprayed with GA_3 at four different concentrations (50, 75, 100, or 125 ppm). Experiment 2 included five treatments. The control treatment (without $CuSO_4 \cdot 5H_2O$) involved spraying the plants with water, and the remaining plants were sprayed with one of four $CuSO_4 \cdot 5H_2O$ concentrations (50, 75, 100, or 125 ppm).

The experiment was conducted with a randomized complete block design (RCBD) with three replications per treatment. Five plants were used per replication. GA_3 was sprayed on the plants 3 times: first, 5–7 days before flower bloom (BBCH 60); second, when the flowers were in full bloom (BBCH 65); and third, 5–7 days after flower bloom (BBCH 69). Flower status was determined in the experiment according to the classification defined by the BBCH scale [14]. The plants underwent two rounds of spraying with $CuSO_4 \cdot 5H_2O$. The initial application occurred when the plants reached 60% bloom, and the second spraying was performed after the inflorescence had fully bloomed. A three-liter solution was applied to each plant, ensuring even distribution to adequately moisten the flower cluster, especially in shaded areas.

2.3. Measurements

The number of flowers/trees is determined by using nylon to cover the ground around the base of the tree up to the projection of its canopy; simultaneously, nylon is used to enclose the canopy, isolating the experimental tree from other trees. The number of flowers/trees is equal to the total number of flowers and young fruits collected on the nylon sheet beneath the tree's base, plus the remaining fruits on the tree. The fruiting rate percentage was calculated by dividing the total number of fruits on a tree by the overall number of flowers on the plant. The fruit number per tree was also recorded. The number of seeds per fruit and fruit height and diameter were determined using 10 randomly selected fruits per tree. The fruit height and diameter were manually measured using a digital caliper (Digital, Mitutoyo Co., Ltd., Tokyo, Japan). The number of segments/fruit was counted. The number of seeds/fruit was also counted considering the numbers of firm and flat seeds. Firm seeds are seeds that have normal shapes and sizes, carrying the characteristic seed traits of the variety. Flat seeds are seeds that are smaller than 1/3 of the normal seed size of the variety or seeds that have a flat, thin shape, and when the seed coat is peeled off, the embryo is found to be incomplete. The average fruit weight was also determined. The theoretical yield was determined as the number of fruits/tree multiplied by the average fruit weight. The actual yield was determined by directly weighing the number of fruit on each tree when harvested.

$$\text{* Ratio of edible parts (\%)} = (\text{W fruit} - (\text{W seeds} + \text{W peel}))/\text{W fruit} \times 100, \text{ W: weight}$$

The biochemical indicators of fruit including Brix, dry matter content, total acid, reducing sugar content, and vitamin C were analyzed on 10 fruits, and the analyses were conducted at the Fruit and Vegetable Quality Control Department of the Fruit and Vegetable Research Institute. The dry matter content (%) was determined according to the method reported by Nasiruddin et al., 2019 [15]. The vitamin C content (mg) was determined following the method of Nasiruddin et al., 2019 [15]. The total acid content (%) was determined following the method of McDonald et al., 2013 [16]. Degrees Brix (%) was determined following the method of McDonald et al., 2013 [16].

2.4. Statistical Analysis

All parameters were recorded from 10 fruits (n = 10). The data were analyzed using SPSS software (version 20.0; IBM Corp., Armonk, NY, USA). The experimental results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The mean values of the treatment groups were compared using Tukey's test, at a significance level of $p \leq 0.05$.

3. Results and Discussion

3.1. Effects of Gibberellin (GA_3) on Reducing the Number of Seeds/Fruit in Bac Son Citrus

3.1.1. Effects of GA_3 on the Fruit Set Ratio of Bac Son Citrus

The fruit set ratio after 30 days of flower pruning and the fruit set ratio in the 100 and 125 ppm GA_3 treatments were significantly higher than in the control in 2018. The fruit set ratio in the 75, 100, and 125 ppm GA_3 treatments was significantly higher than that in the control in 2019. The fruit set ratio 30 days after flower pruning ranged from 4.97% to 5.31% in 2018 and 4.81% to 5.25% in 2019. The control treatment achieved a fruit set ratio of 4.83% in 2018 and 4.81% in 2019. The GA_3 treatments achieved a stable fruit set ratio ranging from 2.48% to 2.52% in 2018 and 2.46% to 2.49% in 2019. The control treatment achieved 2.38% and 2.39% in 2018 and 2019, respectively. The stable fruit set ratio in the 75, 100, and

125 ppm GA_3 treatments was significantly higher than that in the control in 2018. However, no significant differences were observed in 2019 (Table 1).

Table 1. Effect of GA_3 on total number of flowers monitored per tree, number of set fruit after 30 days of flower pruning, fruit set ratio after 30 days of flower pruning, stable fruit set ratio of Bac Son citrus.

Index / GA_3 Concentration	Total Number of Flowers Monitored per Tree (Flowers)	Number of Set Fruit after 30 Days of Flower Pruning (Fruits)	Fruit Set Ratio after 30 Days of Flower Pruning (%)	Stable Fruit Set Ratio (%)
50 ppm	13.7845	685.1 b	4.97 b	2.48 b
75 ppm	13.3245	679.3 b	5.10 b	2.50 a
100 ppm	13.6649	725.6 a	5.31 a	2.52 a
125 ppm	13.5473	713.9 a	5.27 a	2.51 a
Control	13.5642	655.2 b	4.83 b	2.38 c
Significance	NS	**	**	***
50 ppm	13.9677	671.4 b	4.81 b	2.47
75 ppm	14.3902	720.5 a	5.01 a	2.46
100 ppm	14.4578	758.6 a	5.25 a	2.49
125 ppm	14.2105	733.6 a	5.16 a	2.47
Control	13.7891	658.5 b	4.78 b	2.39
Significance	NS	**	**	NS
A × B	NS	*	NS	NS

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; *** significant differences at $p > 0.001$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among the treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of year (in all treatments); and AB: interaction between A (treatments) and B (years).

Based on the findings of this study, it was observed that the Bac Son citrus trees produced a large number of flowers but had a high fruit drop rate. This phenomenon is a common issue in citrus production, resulting in lower yields and economic losses for farmers. However, the application of GA_3 as a spray treatment at 75, 100, and 125 ppm reduced the fruit drop rate in Bac Son citrus trees in 2018. This suggests that GA_3 may be an effective treatment to improve fruit set and reduce fruit drop in Bac Son citrus trees. Among the GA_3 treatments, the 75, 100, and 125 ppm concentrations were effective in achieving a higher stable fruit set ratio (Table 1). The use of GA_3 as a plant growth regulator for blueberry and citrus has been extensively studied [1,2]. GA_3 application can improve fruit set and reduce fruit drop in various crops, including citrus. For example, Moon et al. [17] found that GA_3 application increased the fruit set ratio and reduced the fruit drop rate of Satsuma mandarins. In conclusion, the application of GA_3 spray treatments can be an effective method to improve fruit set and reduce fruit drop in Bac Son citrus trees.

3.1.2. Effect of GA_3 on the Yield Parameters of Bac Son Citrus

In 2018, the application of GA_3 at concentrations of 50, 75, 100, and 125 ppm did not result in significant differences in terms of harvested fruit per tree compared with the control group. However, the application of GA_3 at 100 and 125 ppm led to a higher average fruit weight per tree compared with the control. At 100 ppm, GA_3 significantly increased the theoretical yield compared with the control. No significant differences in actual yield were observed in 2018. All treatments improved the harvested fruit per tree in 2019. GA_3 application at 100 ppm positively affected harvested fruit per tree. GA_3 application at 100 and 125 ppm significantly increased the average fruit weight and yield compared with the control. A higher actual yield was found for all GA_3 treatments than in the control group (Table 2).

Table 2. Effects of GA_3 on harvested fruit per tree, average fruit weight, theoretical and actual yield of Bac Son citrus in Bac Son, Lang Son.

Index / GA_3 Concentration	Harvested Fruit per Tree (Number)	Average Fruit Weight (g)	Yield (kg/Tree)	
			Theoretical	Actual
2018				
50 ppm	342.4	102.7 b	35.2 ab	34.1
75 ppm	347.6	103.4 ab	35.9 ab	34.8
100 ppm	355.2	104.1 a	37.0 a	35.9
125 ppm	343.4	104.2 a	35.8 ab	34.7
Control	317.9	102.3 b	32.5 b	31.5
Significance	NS	**	**	NS
2019				
50 ppm	363.7 ab	103.1 ab	37.5 ab	36.3 a
75 ppm	367.3 ab	102.6 b	37.7 ab	36.5 a
100 ppm	372.8 a	104.3 a	38.9 a	37.8 a
125 ppm	357.1 b	104.2 a	37.2 ab	36.0 a
Control	338.5 c	102.7 b	34.8 c	33.7 b
Significance	***	*	**	*
A × B	NS	*	*	NS

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; *** significant differences at $p > 0.001$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among the treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of year (in all treatments); and AB: interaction between A (treatments) and B (years).

The study results indicate that the use of 100 and 125 ppm of GA_3 can significantly increase the fruit set ratio, yield, and number of fruits per tree harvested from Bac Son citrus trees. The high number of flowers produced by the trees in this study and the usually high fruit drop rate of this species suggest that these trees may benefit from the application of GA_3 to improve fruit retention and increase yield (Table 2). Previous studies have also demonstrated the effectiveness of GA_3 in increasing fruit yield in various fruit crops, such as apples, cherries, and peaches [18]. GA_3 can stimulate fruit growth and development by promoting cell division, elongation, and differentiation, as well as enhancing the synthesis and transport of carbohydrates to the fruit [19]. However, the use of GA_3 should be carefully managed to avoid negative impacts on the environment and human health. The optimal GA_3 concentration and application timing may vary depending on the crop and growing conditions; excessive use may lead to phytotoxicity and residues in the fruit (Bazrafshan-Jahromi et al., 2021).

3.1.3. Effect of GA_3 on the Physiological Parameters of Bac Son Citrus

For fruit trees in general and citrus fruit in particular, fruit quality is not only evaluated using the content of nutrients in the fruit but also the morphological characteristics such as the fruit size, shape, and color. High-quality products are preferred by consumers, so these products have higher economic value. The quality indicators of the Bac Son citrus fruit in this study are shown in Table 3.

In 2018, the application of different concentrations of GA_3 did not result in significant differences in fruit height, fruit diameter, or the number of segments per fruit compared with the control group. However, significant differences were observed in the total number of seeds per fruit, the seeds per fruit, and the edible portion of fruit. The plants in the GA_3 treatments showed a significant decrease in the total number of seeds per fruit compared with the control group. This indicates that GA_3 application of 100 and 125 ppm led to a reduction in the number of seeds and firm seeds in Bac Son mandarin fruit. Additionally, there was no significant difference in edible fruit between the GA_3 treatments and the control group. In 2019, the application of different concentrations of GA_3 did not result in significant differences in fruit height, fruit diameter, or the number of segments per fruit compared with the control group (Table 3). The fruits in the GA_3 treatments consistently showed significant reductions in the total number of seeds per fruit and firm seeds and a significant increase in the edible portion of the fruit compared with the control group. This indicates that GA_3 significantly reduced the number of seeds per fruit and increased the fruit quality.

Table 3. Effect of GA_3 treatment on fruit height, fruit diameter, number of segments/fruit, total number of seeds/fruit, seeds/fruit (seeds), and edible portion of Bac Son mandarin fruit.

Index / GA_3 Concentration	Fruit Height (cm)	Fruit Diameter (cm)	Number of Segments/Fruit (Segments)	Total Number of Seeds/Fruit (Seeds)	Seeds/Fruit (Seeds)		Edible Portion (%)
					Firm seed	Small seed	
				2018			
50 ppm	4.76	5.90	11.6	30.6 b	26.8 b	3.8 c	79.7 ab
75 ppm	4.86	6.46	11.2	29.8 b	25.3 b	4.5 b	80.8 ab
100 ppm	4.74	6.22	11.2	26.5 c	18.7 c	7.8 a	81.7 a
125 ppm	4.81	6.18	12.0	28.7 b	23.5 bc	5.2 b	81.1 a
Control	4.76	6.14	11.8	33.4 a	30.5 a	2.9 c	78.9 b
Significance	NS	NS	NS	**	***	***	*
				2019			
50 ppm	4.80	5.92	11.5	29.3 b	24.7 b	4.6 b	80.4 ab
75 ppm	4.49	5.95	11.6	28.2 b	23.3 b	4.9 b	80.2 ab
100 ppm	4.77	5.96	11.4	25.8 c	17.5 c	8.3 a	80.6 ab
125 ppm	4.81	6.12	11.7	26.4 c	21.9 bc	4.5 b	81.1 a
Control	4.79	6.05	11.7	32.8 a	30.1 a	2.7 c	79.8 b
Significance	NS	NS	NS	***	***	***	**
A × B	NS	NS	NS	**	***	**	**

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; *** significant differences at $p > 0.001$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among the treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of year (in all treatments); and AB: interaction between A (treatments) and B (years).

These findings are consistent with those of previous studies that have shown the efficacy of GA_3 in increasing fruit set and yield in various crops, including citrus trees [19]. Although the mechanical indices of physiological parameters such as fruit height, fruit diameter, and number of segments per fruit were not significantly different between the GA_3-treated and control plants, the application of GA_3 at 100 ppm and 125 ppm significantly affected the number and size of seeds per fruit. The GA_3-treated trees had a slightly lesser decrease in the total number of seeds/fruit and firm seeds than the control trees but had a larger number of small seeds. This may have occurred due to GA_3 affecting the development of ovules and the process of fertilization, leading to changes in seed number and size [20]. Overall, the results of this study suggest that GA_3 can be an effective tool for improving fruit set, yield, and seed characteristics in Bac Son citrus trees.

3.1.4. Effect of GA_3 on the Internal Quality Parameters of Bac Son Citrus

In 2018, the application of different concentrations of GA_3 did not result in significant differences in degrees Brix (sugar content), reducing sugar content, or dry matter content compared with the control group. However, significant differences were observed in the total acid and vitamin C contents. The total acid content was significantly reduced in the

50, 75, and 100 ppm GA_3 treatments compared with that of the control. The vitamin C content significantly increased under 75 and 125 ppm GA_3 treatments compared with that of the control (Table 4). In 2019, similar trends were observed. We found no significant difference in degrees Brix and dry matter, vitamin C, and reducing sugar contents among the treatments. The GA_3 treatments significantly reduced the total acid content compared with the control. Notably, in both years, the control group consistently showed higher total acid contents (Table 4). This suggested that the GA_3 treatments had a significant effect on reducing the acidity of the fruits compared with the control group.

Table 4. Effect of GA_3 on brix, dry matter, total acid, reducing sugar, and vitamin C of Bac Son mandarin fruit.

Index / GA_3 Concentration	Brix (%)	Dry Matter (%)	Total Acid (%)	Reducing Sugar (%)	Vitamin C (mg/100 g)
50 ppm	9.8	11.28 b	0.871 ab	5.14 ab	27.29 b
75 ppm	10.1	11.58 b	0.771 b	5.53 a	29.09 a
100 ppm	10.2	12.0 a	0.865 ab	5.00 b	27.80 b
125 ppm	10.2	11.6 b	0.92 a	5.20 ab	28.00 a
Control	10.0	11.8 ab	0.891 a	5.38 ab	27.27 b
Significance	NS	*	*	**	**
50 ppm	10.3	11.99 b	0.845 b	5.48	29.37 ab
75 ppm	10.6	12.97 a	0.862 b	5.74	28.96 b
100 ppm	10.5	12.78 a	0.872 b	5.62	29.09 ab
125 ppm	10.4	12.86 a	0.859 b	5.24	28.65 b
Control	9.6	11.75 b	0.927 a	5.63	30.85 a
Significance	NS	NS	**	NS	***
A × B	NS	NS	NS	NS	NS

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; *** significant differences at $p > 0.001$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among the treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of year (in all treatments); and AB: interaction between A (treatments) and B (years).

No consistent significant differences were observed in the internal quality parameters (i.e., degrees Brix and reducing sugar content) during both years studied. This suggested that the application of GA_3 did not have any adverse effects on the quality of Bac Son mandarin fruit. The reported findings are in line with those of previous studies investigating the effect of GA_3 application on citrus fruits. For example, Moon et al. [17] found that the application of GA_3 resulted in a significant reduction in seed number in Satsuma mandarin fruit without affecting the fruit-quality parameters such as soluble solid content, titratable acidity, and vitamin C content. Similarly, in a study on Washington navel orange trees [21], the application of GA_3 resulted in a significant reduction in seed number and had no adverse effects on the fruit quality parameters such as total soluble solid content, acidity, and vitamin C content. These findings suggested that the use of GA_3, as a plant growth regulator, is a useful tool for reducing seed number in citrus fruits without affecting their quality. Our findings suggest that the application of GA_3 can be an effective method for reducing the seed number of Bac Son mandarin fruit without changing the biochemical parameters or the internal quality parameters. This finding is consistent with those of previous studies on citrus fruits and highlights the potential of GA_3 as a plant growth regulator for increasing the quality of citrus fruits.

3.2. Effect of Copper Sulfate ($CuSO_4{\cdot}5H_2O$) on Reducing the Number of Seeds per Bac Son Mandarin Fruit

3.2.1. Effects of $CuSO_4{\cdot}5H_2O$ on the Fruit Set Ratio of Bac Son Mandarin

Regarding the total number of flowers monitored per tree, no significant differences were observed among the different copper sulfate concentrations in either year. This suggests that copper sulfate did not have a notable impact on the overall flower production of mandarin trees. It is important to note that the flower count remained relatively consistent across all treatments and the control group. However, when examining the number of fruit set after 30 days of flower pruning, significant differences were observed in both years. In 2018, the 100 ppm concentrations of copper sulfate resulted in a significantly higher fruit set than the control group (Table 5). Similarly, in 2019, the 75 ppm and 125 ppm concentrations led to a significantly higher fruit set. The stable fruit set ratio, which represents the long-term stability of the fruit set, showed no significant differences among the copper sulfate treatments in both years. This indicates that the different concentrations of copper sulfate did not have a lasting impact on the fruit set ratio of mandarin trees. The stable fruit set ratio remained relatively consistent across all treatments.

Table 5. Effect of $CuSO_4{\cdot}5H_2O$ on total number of flowers monitored per tree, number of set fruit after 30 days of flower pruning, fruit set ratio after 30 days of flower pruning, stable fruit set ratio of Bac Son mandarin in Bac Son, Lang Son.

Index / $CuSO_4{\cdot}5H_2O$	Total Number of Flowers per Tree	Number of Fruits Set after 30 Days of Flower Pruning	Fruit Set Ratio 30 Days after Flower Pruning (%)	Stable Fruit Set Ratio (%)
		2018		
50 ppm	13.6814	663.5 b	4.85 b	2.46
75 ppm	13.5437	673.1 ab	4.97 ab	2.41
100 ppm	13.8334	690.3 a	4.99 ab	2.45
125 ppm	13.4793	678.0 ab	5.03 a	2.49
Control	13.4956	658.3 b	4.88 b	2.43
Significance	NS	**	*	NS
		2019		
50 ppm	13.7467	670.2 b	4.88	2.39
75 ppm	14.2343	698.4 a	4.91	2.38
100 ppm	14.0261	654.8 b	4.67	2.41
125 ppm	14.2328	690.9 a	4.85	2.39
Control	14.1161	662.1 b	4.69	2.40
Significance	NS	**	NS	NS
A × B	NS	*	NS	NS

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among the treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of year (in all treatments); and AB: interaction between A (treatments) and B (years).

The results of this study suggest that copper sulfate, particularly at the concentration of 100 ppm in 2018 and 75 ppm and 125 ppm in 2019, can have a positive influence on the fruit set of mandarin trees (Table 5). This study was in line with a previous study that showed that $CuSO_4{\cdot}5H_2O$ was effective in having a positive influence on the fruit set of citrus [22].

3.2.2. Effect of $CuSO_4{\cdot}5H_2O$ on the Yield Parameters of Bac Son Citrus

In 2018, the application of $CuSO_4{\cdot}5H_2O$ at different concentrations did not result in significant differences in the number of harvested fruit per tree. However, significant differences were found in the average fruit weight and theoretical yield. Among the $CuSO_4 \cdot 5H_2O$ treatments, the 50 ppm treatment showed a significantly higher average fruit weight compared to the control group. This indicated that the application of $CuSO_4 \cdot 5H_2O$ at 50 ppm positively affected the average fruit weight of Bac Son mandarin trees. The actual yield of the trees in the $CuSO_4 \cdot 5H_2O$ treatments did not significantly differ from that of the trees in the control group. However, the theoretical yield of all $CuSO_4 \cdot 5H_2O$ treatments was slightly higher than that of the control. In 2019, significant differences were found in the number of harvested fruit per tree and the average fruit weight. The number of harvested fruit per tree in the 50 ppm $CuSO_4 \cdot 5H_2O$ treatment at (denoted by the letter "a") was significantly higher than in the control group (Table 6). This suggested that the application of $CuSO_4 \cdot 5H_2O$ at 50 ppm positively influenced the fruit production in the Bac Son mandarin trees. The average fruit weight in the 75 ppm $CuSO_4 \cdot 5H_2O$ treatment was significantly higher than that in the control group. The average fruit weight being higher at 75 ppm and not at 50 ppm could be due to the fact that this year, there was a higher number of fruit on the tree which contributed to a lower average weight compared to the previous year. In terms of theoretical and actual yields, those of the $CuSO_4 \cdot 5H_2O$ treatments did not significantly differ from those of the control group (Table 6).

Table 6. Effect of $CuSO_4 \cdot 5H_2O$ on harvested fruit per tree, average fruit weight, theoretical and actual yield and of Bac Son mandarin in Bac Son, Lang Son.

Index / $CuSO_4 \cdot 5H_2O$	Harvested Fruit per Tree (Number)	Average Fruit Weight (g)	Yield (kg/tree)	
			Theoretical	Actual
2018				
50 ppm	317.6	104.2 a	33.1 a	32.1
75 ppm	322.5	103.5 ab	33.4 a	32.4
100 ppm	323.4	102.6 b	33.2 a	32.2
125 ppm	322.9	102.1 b	33.0 a	32.0
Control	316.2	102.9 b	32.5 b	31.5
Significance	NS	**	*	NS
2019				
50 ppm	340.7 a	102.5 ab	34.9	33.8
75 ppm	335.2 b	103.1 a	34.6	33.5
100 ppm	337.5 b	101.1 b	34.1	33.0
125 ppm	338.2 b	101.3 b	34.3	33.2
Control	334.9 b	101.2 b	33.9	32.8
Significance	*	**	NS	NS
A × B	NS	*	NS	NS

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among the treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of year (in all treatments); and AB: interaction between A (treatments) and B (years).

The lack of significant effect of $CuSO_4 \cdot 5H_2O$ on fruit yield is consistent with the findings of previous studies that investigated the effect of copper-based fungicides on citrus fruit production. For example, in a study on Valencia orange trees, the researchers found that the application of copper-based fungicides did not significantly affect fruit yield: the average fruit weight, fruit diameter, and yield were not significantly different between the copper-treated and control trees. Similarly, a study by El-Gioushy et al., 2021 [23], on navel orange trees found that the application of copper-based fungicides did not significantly affect fruit yield. In summary, the application of different concentrations of $CuSO_4 \cdot 5H_2O$ did not significantly affect the number of harvested fruit per tree or yield compared with the control group in either year. However, significant differences were observed in the average fruit weight, with the trees in the 50 and 75 ppm treatments consistently producing a higher average fruit weight (Table 6).

3.2.3. Effect of $CuSO_4{\cdot}5H_2O$ on the Physiological Parameters of Bac Son Citrus

In terms of fruit growth, we recorded fruit height and diameter. The data showed that the fruit height did not significantly differ among the different concentration groups compared with the control group in both 2018 and 2019. The number of segments and the total number of seeds per fruit were also measured. In 2018, the number of segments per fruit was significantly higher in the 125 ppm treatment group than in the control group; in 2019, no significant differences were observed among the groups. However, the total number of seeds per fruit was significantly lower in the treated groups (50, 75, 100, and 125 ppm) than in the control group in both 2018 and 2019 (Table 7). The data showed the tendency that as the copper sulfate dose increased, the less seeds the fruit had.

Table 7. Effects of $CuSO_4{\cdot}5H_2O$ on fruit height, fruit diameter, number of segments/fruit, total number of seeds/fruit, seeds/fruit (seeds), and edible portion of Bac Son mandarin fruit (2018 and 2019).

Index / $CuSO_4{\cdot}5H_2O$	Fruit Height (cm)	Fruit Diameter (cm)	Number of Segments/Fruit (Segments)	Total Number of Seeds/Fruit (Seeds)	Seeds/Fruit (Seeds)		Edible Portion (%)
					Firm Seed	Small Seed	
2018							
50 ppm	4.62	6.58	11.8 ab	26.6 b	23.2 b	3.4 b	81.0
75 ppm	4.64	6.44	11.2 ab	25.5 b	19.8 b	5.7 a	81.1
100 ppm	4.52	6.26	10.2 b	26.5 b	22.3 b	4.2 a	80.6
125 ppm	4.52	6.28	12.6 a	18.8 c	17.4 c	1.4 c	81.2
Control	4.60	6.24	11.8 ab	33.6 a	30.8 a	2.8 b	80.7
Significance	NS	NS	**	***	***	*	NS
2019							
50 ppm	4.41	6.47	10.8	26.8 b	16.6 b	10.2 a	80.3
75 ppm	4.51	6.34	10.3	18.7 c	12.9 b	5.8 b	80.4
100 ppm	4.52	6.44	10.4	18.3 c	12.1 b	6.2 b	80.5
125 ppm	4.50	6.30	10.6	11.6 c	5.2 c	6.4 b	81.2
Control	4.56	5.95	10.5	31.2 a	25.7 a	5.5 b	80.4
Significance	NS	NS	NS	NS	***	**	NS
A × B	NS	NS	NS	NS	**	*	NS

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; *** significant differences at $p > 0.001$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among the treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of the year (in all treatments); and AB: interaction between A (treatments) and B (years).

The data also provided information about the type of seeds present in the fruits, specifically firm and small seeds. In both years, the trees in the 125 ppm treated groups had significantly fewer firm seeds than those in the control group. However, the number of small seeds did not show consistent patterns across the different concentrations and years. Lastly, the edible portion percentage, which represents the proportion of the fruit that is considered edible, was measured; no significant differences were found in the edible portion percentage among the different concentration groups compared with the control group in either year. In summary, the experimental results indicated that the tested concentrations $CuSO_4{\cdot}5H_2O$ had limited effects on the fruit growth parameters (fruit height and diameter) and the edible portion percentage. However, the treatments resulted in a decrease in the total number of seeds per fruit and the number of firm seeds. This finding is consistent with those of a previous study that demonstrated the effectiveness of copper-based fungicides in reducing the seed number in citrus fruits [22,23]. The application of 25 mg/L of $CuSO_4 \cdot 5H_2O$ at full bloom to entire tangor trees under cross-pollination conditions resulted in a significant reduction in the average number of seeds per fruit by 55–81% and a significant increase in the percentage of seedless fruits. Importantly, this treatment did not have a negative impact on fruit yield [22]. The reduction in seed number may have practical implications for the fruit-processing industry, as seedless fruits are often preferred due to their convenience and ease of consumption.

3.2.4. Effect of $CuSO_4 \cdot 5H_2O$ on the Internal Quality Parameters of Bac Son Citrus

Regarding the fruit quality indicators, the degrees Brix represents the sugar content in the fruit, where higher values generally indicate sweeter fruit. We found no significant differences in the degrees Brix among the different $CuSO_4 \cdot 5H_2O$ treatments in both 2018 and 2019. The dry matter percentage reflects the solid content of the fruit, which indicates fruit texture and density. In 2018, the 125 ppm treatment group had significantly lower total acid percentages than the other treated groups. In 2019, the 50 ppm treatment group had a significantly lower total acid percentage compared with the control group, whereas the other treatment groups showed no significant differences. Although differences due to treatments are seen in different years, these differences are not consistent since the variation is not the same each year due to the treatments. No significant differences were observed among the different treatment groups for either parameter in 2018. However, in 2019, the control group had significantly higher reducing sugar content than the 75, 100, and 125 ppm treatment groups (Table 8). Vitamin C content is an important nutritional indicator for fruit. In 2018, the control group had the highest vitamin C content, with it being significantly higher than that in the treatment groups. In 2019, the 75 and 100 ppm treatment groups had significantly higher vitamin C contents compared with the other groups (Table 8).

Table 8. Effect of $CuSO_4 \cdot 5H_2O$ on the brix, dry matter, total acid, reducing sugar, and vitamin C of Bac Son mandarin fruit.

Index / $CuSO_4 \cdot 5H_2O$	Brix (%)	Dry Matter (%)	Total Acid (%)	Reducing Sugar (%)	Vitamin C (mg/100 g)
50 ppm	10.3	12.65 a	0.900 a	5.52	30.00 b
75 ppm	9.9	11.30 b	0.932 a	5.00	27.30 b
100 ppm	10.0	11.52 b	0.830 ab	5.52	29.00 b
125 ppm	10.2	11.52 b	0.790 b	5.56	27.92 b
Control	10.1	12.62 a	0.911 a	5.46	32.73 a
Significance	NS	*	*	NS	**
50 ppm	10.4	12.04	0.713 c	6.02 a	31.86 b
75 ppm	10.1	11.26	0.962 a	5.75 b	34.86 a
100 ppm	10.3	12.72	0.854 ab	5.78 b	38.91 a
125 ppm	10.3	12.04	0.912 a	5.52 b	32.53 b
Control	10.0	11.86	0.869 ab	6.20 a	28.90 c
Significance	NS	NS	**	***	**
A × B	NS	NS	*	NS	**

NS: no significant differences; * significant differences at $p > 0.05$; ** significant differences at $p > 0.01$; *** significant differences at $p > 0.001$; values represent the means of three replicates with 10 fruits (n = 3). Different letters indicate significant differences among treatments, determined using Tukey's test. A: effect of treatment (in both years); B: effect of year (in all treatments); and AB: interaction between A (treatments) and B (years).

The application of $CuSO_4 \cdot 5H_2O$ at different concentrations did not affect the quality parameters of Bac Son mandarin, including degrees Brix and dry matter in 2019. These results are consistent with those of a previous study. For example, a study [24] on the effect of $CuSO_4$ on the quality of tomato fruit showed that the application of $CuSO_4$ did not significantly affect the degrees Brix or vitamin C content of the fruits. Similarly, researchers [25] found that the application of $CuSO_4$ did not significantly affect the Brix degree or vitamin C content of grapes.

4. Conclusions

In conclusion, the results of this study demonstrate that spraying 125 ppm GA_3 significantly reduces the number of seeds per fruit in Bac Son mandarin without changing the biochemical parameters or the quality. Similarly, spraying $CuSO_4{\cdot}5H_2O$ also significantly reduced the number of seeds per fruit and did not negatively impact the quality parameters or yield. Moreover, copper sulfate shows a tendency that the higher the dose, the lower the number of seeds, mainly due to the reduction in the number of firm seeds. These findings provide valuable insights for farmers and researchers in the citrus industry in selecting appropriate treatments to improve the marketability of Bac Son mandarin. Further research is needed to explore the mechanisms behind the observed effects and to optimize the treatment conditions for better results.

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