

Effect of Paclobutrazol and Tryptophan foliar application on floral and quantitative traits of fruits in three apple cultivars

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Abstract

An experiment was carried out in a private orchard at Diyala Governorate during 2023 season on three apple cultivars, namely 'Sharabi', White ' (Al-Kaghdhi) and 'Al-Ibrahimi', four years old to study the effect of spraying with Paclobutrazol at concentrations (0, 250 and 500 mg/L) and spraying with Tryptophan at concentrations (0, 25 and 50 mg/L) on growth and yield of the three cultivars. The experiment comprised 27 treatments, replicated three times, with an experimental unit consisting of a tree; so the number of trees was 81. The Nested Design was adopted and the data were analyzed according to GenStat statistical program. The results showed: the cultivar Al-Kaghdhi was significantly distinguished by giving the shortest flowering period, the number of flowers per branch, the number of set fruits, and the earliest full maturity, while the cultivars 'Sharabi' and 'Al-Ibrahimi' were significantly superior in marketable yield per tree. Spraying the apple trees with Paclobutrazol at the concentration 500 mg/L led to earliness in flowering date, the number of flowers per branch, the number of set fruits, the percentage of fruit set, and the marketable yield per tree. Also, spraying with Tryptophan at the concentration 50 mg/L gave a significant increase in the number of set fruits, the percentage of fruit set, and the marketable yield per tree.

Introduction

The apple (*Malus domestica* Borkh) is in the family Rosaceae, as one of the most popular deciduous fruit trees distributed throughout temperate regions worldwide. At the world level China is the leading, producing 40,500,000 million tons followed by United States of America and Poland [1]. The number of apple trees cultivated in Iraq is about 2,632,299 million trees, with an annual production of 79,413 tons and an average production of 30.17 kg per tree, with Baghdad ranking first, followed by Al-Anbar governorate, then Salah al-Din governorate [2]. Plant growth regulators are defined as non-nutritive organic compounds that can stimulate, inhibit, or modify the physiological activities of the plant using low concentrations of them [3.]

Paclobutrazol is a growth retardant used in controlling plant growth, as it works to reduce vegetative growth and increase the formation of flower initials in the buds, giving early and abundant flowering, thus increasing the productivity of fruit trees [4]. The efficacy of Paclobutrazol depends on its inhibition of the biosynthesis of gibberellins in the plant, as it inhibits the action of the enzyme Kaurene oxidase and Cyt P-450, and therefore prevents the oxidation of ent-Kaurene to ent-Kaurenoic acid in the biosynthetic pathway for the natural production of gibberellins and works to inhibit cell elongation without causing inhibition of their division, as the cells continue to divide without elongation [5.]

L-Tryptophan is one of the essential amino acids; it is an aliphatic amino acid, works on

the biosynthesis of proteins, and consists of an aliphatic amino group and a carboxyl group, and also contains an indole side chain, and therefore belongs to the group of mono-peptide aromatic amino acids [6]. It has an important role in accelerating growth as it helps in the formation of plant tissues and plays an important role in the decomposition of cells into proteins, as well as stimulating plant growth, and thus it has numerous effects on the flowering and growth of the plant [7, 8]. Tryptophan contains 13.72% nitrogen in its structure, which is released through the photosynthesis process inside the plant or in the root zone, which contributes to enhancing crop productivity [9]. It is an aromatic amino acid produced via the Shikimate pathway, which activates chromosomes [10]. Foliar spraying with tryptophan led to the stimulation of growth regulators and photosynthesis, which was positively reflected in improving plant growth [11]. It also plays a protective and defensive role in the plant [12]. Based on the above and considering the high medical and nutritional importance of apples, it is essential to focus on their cultivation by expanding the cultivated area, identifying major challenges and limitations that restrict their spread, and paying attention to the factors affecting plant growth and flowering.

Materials and Methods

This study was conducted in private orchard, Baqubah city on three apple's cultivars aged 4 years old at the space (4 x 4m) according to Nested design experiment. The experiment consisted of three factors the first factor was 4 years old cultivars including Al-Sharabi, Al-Kaghdhi and Al-Ibrahimi symbolized (V1, V2, V3). The second factor was Paclobutrazol spraying (0, 250 and 500 mg. L^{-1}), denoted by (P1, P2, P3). The third factor was spraying with Tryptophan at

concentrations (0, 25, and 50 $mg.L^{-1}$), symbolized as (T1, T2, T3). The experiment included 27 treatments with three replicates. Each tree was considered an experimental unit, resulting in a total of 81 trees. A Nested design was used, and the data was analyzed utilizing the GenStat statistical software, and the average values were compared through the LSD test at a significance level of 0.05.

Studied Traits:

Flowering Date:

Determined by calculating the number of days from the beginning of the year until the date of full flowering (the stage where about 70 – 80% of the flowers on the tree are fully open). Al-Sharabi 9/3/2023, Al-Kaghdhi 4/3/2023 and Al-Ibrahimi 8/3/2023

Number of Flowers per Branch (flower branch $^{-1}$):

After the plants reached the full flowering stage, the count of flowers on every labeled branch (four branches), and the result was divided by the number of branches tagged

Number of Fruit Sets (fruits•branch $^{-1}$):

The number of fruit sets was counted on the marked branches 2–3 weeks after full bloom,

including only the small fruits that persisted and did not drop.

Fruit set percentage(%)

The total number of flowers per tree was estimated by multiplying the average number of flowers per labeled branch by the total number of branches per tree. Subsequently, the total number of set fruits per tree was recorded, and the fruit set percentage was calculated using the following formula: Fruit set (%)=(Number of fruit sets)/(Total number of flowers) x 100 [13]

Maturity date for each cultivar

It was determined by counting the number of days from full bloom to the harvest date for each cultivar.

Marketable Yield per Tree (kg)

The yield for each experimental unit (tree) was harvested, and the weight of the yield of each experimental unit was calculated separately after excluding injured and malformed fruits.

Results and Discussion

Flowering Date (days)

As seen in Table 1, White (Al-Kaghghi) cultivar was the most distinct cultivar with the shortest flowering period of 79.41 days. Conversely, the highest values in terms of

flowering duration were reported Sharabi and Al-Ibrahimi cultivars with 83.41 and 83.52 days, respectively (Table 1). Spraying with Paclobutrazol at the concentration of 500 mg•L⁻¹ showed the minimum days to flowering (80.30 days), and no application showed the longest duration of 83.19 days. No significant differences were observed among tryptophan spray levels regarding flowering onset date .

The interaction between cultivars and Paclobutrazol application, as well as the interaction between cultivars and tryptophan application, did not significantly affect this trait. However, the interaction between Paclobutrazol at 500 mg•L⁻¹ and the absence of tryptophan spray (T1) contributed to the earliest flowering onset, recorded at 79.89 days. Conversely, the longest flowering duration (83.56 days) was observed in the interaction treatment combining no Paclobutrazol (P1) with tryptophan at 50 mg•L⁻¹ (T3) .

The three-way interaction (cultivar × Paclobutrazol × tryptophan) did not exert a significant effect on flowering onset date.

Table 1. Effect of cultivars, Paclobutrazol, tryptophan, and their interaction on flowering date (days)

V * P	T3	T2	T1	P	Cultivars
84.33	84.67	83.67	84.67	P1	V1
84.11	84.33	84.33	83.67	P2	
81.78	81.00	82.00	82.33	P3	
80.56	81.00	80.00	80.67	P1	V2
80.33	81.00	80.00	80.00	P2	
77.33	77.33	78.00	76.67	P3	
84.67	85.00	84.67	84.33	P1	V3
84.11	84.67	84.00	83.67	P2	
81.78	82.33	82.33	80.67	P3	
N.S 1.09 LSD	N.S 1.57			LSD 0.05	
V * T	T3	T2	T1	V	
Mean cultivars				V1	
83.41	83.33	83.33	83.56	V1	
79.41	79.78	79.33	79.11	V2	
83.52	84.00	83.67	82.89	V3	
0.95 LSD	N.S 1.09			LSD	
P * T					

Mean Paclobutrazol	T3	T2	T1	P
83.19	83.56	82.78	83.22	P1
82.85	83.33	82.78	82.44	P2
80.30	80.22	80.78	79.89	P3
0.48	LSD	N.S	0.83	LSD
	T3	T2	T1	T
	82.37	82.11	81.85	Mean tryptophan
	N.S	0.48		LSD

Number of flowers per branch (flower branch-1)

Referring to Table 2, White cultivar showed a significantly higher number of flowers/branch (1512.1 flowers/branch-1) as compared with other cultivars. The Ibrahimi had the fewest flowers with 1340.8 flowers per branch-1. Spray application of Paclobutrazol at 500 mg L-1 was the best treatment in terms of flowers per branch with

a value of 1473.4 flowers branch-1. Meanwhile, the treatment without Paclobutrazol spraying produced the lowest number of flowers per branch, 1343.6 flowers per branch-1. Tryptophan levels showed no significant difference in the number of flowers per branch. The findings revealed that both two-way and three-way interactions had no significant impact on the number of flowers per branch.

Table 2. Effect of cultivars, Paclobutrazol, tryptophan, and their interaction on number of flowers per branch (flower branch-1)

V * P	T	T2	T1	P	Cultivars
	T3				V
1298.5	1328.0	1292.7	1274.8	P1	V1
1335.3	1346.8	1335.3	1323.7	P2	
1428.2	1436.6	1425.3	1422.6	P3	
1454.5	1470.0	1444.2	1449.3	P1	V2
1511.7	1489.6	1519.4	1526.1	P2	
1570.1	1578.7	1565.5	1566.1	P3	
1277.7	1271.0	1272.1	1290.1	P1	V3
1322.8	1331.1	1314.3	1323.1	P2	
1421.8	1448.5	1416.3	1400.6	P3	
67.1^{N.S}	LSD	77.3^{N.S}		LSD 0.05	
V * T					
Mean cultivars	T3	T2	T1	V	
1354.0	1370.5	1351.1	1340.4	V1	
1512.1	1512.8	1509.7	1513.8	V2	
1340.8	1350.2	1334.2	1337.9	V3	
65.5	LSD	67.1^{N.S}		LSD	
P * T					
Mean Paclobutrazol	T3	T2	T1	P	
1343.6	1356.3	1336.3	1338.1	P1	
1389.9	1389.2	1389.7	1390.9	P2	
1473.4	1488.0	1469.0	1463.1	P3	
18.2	LSD	31.6^{N.S}		LSD	
	T3	T2	T1	T	
	1411.1	1398.3	1397.4	Mean tryptophan	
	N.S	18.2		LSD	

Number of fruit set (fruit tree-1(

The results of Table 3 show the significant superiority of the white variety in the number of fruit set, 799.6 fruits, while the Ibrahimi variety had the lowest fruit set number, reaching 637.8 fruits. Spraying with Paclobutrazol at a rate of 500 mg L-1 significantly surpassed the number of fruit set, yielding 794.1 fruits, while the lowest number of fruit set was for the no-spray treatment, reaching 636.7 fruits. Spraying with 50 mg L-1 of tryptophan significantly enhanced fruit set, reaching 731.4 fruits, while the number of fruit set decreased for the no-spray treatment,

to 686.1 fruits. The results show the significant superiority of the interaction between the white variety and Paclobutrazol spraying. At a rate of 500 mg L-1, the number of fruit set was 846.1 fruits, and the number of fruit set decreased in the interaction between the Ibrahimi variety and not spraying with Paclobutrazol to 557.1 fruits. Regarding the interaction between the varieties, spraying tryptophan and the interaction between spraying the two substances did not significantly affect this trait. There were no notable differences in the three-way interaction among the study factors regarding the fruit set.

Table 3. Effect of cultivars, Paclobutrazol, tryptophan, and their interaction on Number of fruit set (fruit tree-1.(

V * P	T	T	T	P	Cultivars
	T3	T2	T1		v
590.6	624.7	578.3	568.7	P1	V1
662.0	672.7	661.3	652.0	P2	
779.2	835.7	758.7	743.3	P3	
762.6	789.3	754.7	743.7	P1	V2
790.1	774.7	789.0	806.7	P2	
846.1	870.7	837.0	830.7	P3	
557.1	555.3	550.7	565.3	P1	V3
599.1	630.3	591.7	575.3	P2	
757.1	829.3	753.0	689.0	P3	
126.5^{N.S}	LSD	138.1^{N.S}		LSD 0.05	
V * T					
Mean cultivars	T3	T2	T1	V	
677.3	711.0	666.1	654.7	V1	
799.6	811.6	793.6	793.7	V2	
637.8	671.7	631.8	609.9	V3	
124.9	LSD	126.5^{N.S}		LSD	
P * T					
Mean Paclobutrazol	T3	T2	T1	P	
636.7	656.4	627.9	625.9	P1	
683.7	692.6	680.7	678.0	P2	
794.1	845.2	782.9	754.3	P3	
28.3	LSD	49.0^{N.S}		LSD	
	T3	T2	T1	T	
	1411.1	1398.3	1397.4	Mean tryptophan	
	28.3			LSD	

Fruit set percentage(%)

The results from Table 4 indicate that there are no significant variances among the cultivars in terms of fruit settling percentage. Paclobutrazol spraying significantly outperformed the cultivar in settling percentage, achieving 13.45%, while the lowest settling percentage was for the no-spray treatment, 11.78%. Setting percentage increased significantly when tryptophan was sprayed at a rate of 50 mg L-1, reaching 12.87%. The lowest settling % was for the no-spray treatment, reaching 12.20%.

The results show a significant superiority in settling percentage for the interaction between the Sharabi cultivar and the 500 mg L-1 Paclobutrazol spray (13.59%). The lowest settling percentage was 10.90% for the interaction between the Ibrahimy cultivar and the no-spray treatment. No significant differences were observed between the interaction between the varieties, spraying with tryptophan, and the interaction between spraying the two substances on the fruit set (%). However, the three-way interaction did not have a significant impact on the fruit set percentage

Table 4. Effect of cultivars, Paclobutrazol, tryptophan, and their interaction on fruit set percentage .(%)

V * P	T	T2	T1	P	Cultivars
	T3				V
11.34	11.70	11.17	11.16	P1	V1
12.35	12.39	12.40	12.26	P2	
13.59	14.50	13.29	12.99	P3	
13.10	13.42	13.06	12.82	P1	V2
13.06	13.00	12.98	13.21	P2	
13.47	13.80	13.36	13.26	P3	
10.90	10.92	10.82	10.97	P1	V3
11.31	11.83	11.25	10.86	P2	
13.30	14.31	13.28	12.31	P3	
1.78	LSD	1.98^{N.S}		LSD 0.05	
V * T					
Mean cultivars	T3	T2	T1	V	
677.3	711.0	666.1	654.7	V1	
799.6	811.6	793.6	793.7	V2	
637.8	671.7	631.8	609.9	V3	
1.76^{N.S}	LSD	1.78^{N.S}		LSD	
P * T					
Mean Paclobutrazol	T3	T2	T1	P	
636.7	656.4	627.9	625.9	P1	
683.7	692.6	680.7	678.0	P2	
794.1	845.2	782.9	754.3	P3	
0.42	LSD	0.73^{N.S}		LSD	
	T3	T2	T1	T	
	1411.1	1398.3	1397.4	Mean tryptophan	
	0.42			LSD	

Maturation date for each cultivar (days.(

Results of Table 5 revealed that White cultivar has the significant effect on maturity date for all the cultivars as having the lowest maturity days which is 83.4 days, whereas the Ibrahimim cultivar had highest value with 124.5 days. The control without Paclobutrazol spray was significantly better and took the shortest time to maturity (101.3 days) while for treatment having a Paclobutrazol spray concentration of 500 mg L-1 it required the most extended period to mature (104.4 days). Tryptophan spray levels had no significant effect on the maturity date for each cultivar. The results of the table indicate that all two- and three-way interactions did not significantly affect the maturity date for each cultivar

Marketable Yield per Tree (kg tree-1.)

Results in Table 6 reveal no significant differences among the three cultivars in

Table 5. Effect of cultivars, Paclobutrazol, tryptophan, and their interaction on maturation date for each cultivar (days)

V * P	T3	T2	T1	P	Cultivars v
98.7	98.3	99.3	98.3	P1	V1
98.9	98.7	98.7	99.3	P2	
101.2	102.0	101.0	100.7	P3	
82.0	81.3	82.3	82.3	P1	V2
82.4	82.0	83.0	82.3	P2	
85.7	85.7	85.0	86.3	P3	
123.3	123.0	123.3	123.7	P1	V3
123.9	123.3	124.0	124.3	P2	
126.2	125.7	125.7	127.3	P3	
1.4^{N.S}	1.8^{N.S}			LSD 0.05	
V * T					
Mean cultivars	T3	T2	T1	V	
99.6	99.7	99.7	99.4	V1	
83.4	83.0	83.4	83.7	V2	
124.5	124.0	124.3	125.1	V3	
1.3	1.4^{N.S}			LSD	
P * T					
Mean Paclobutrazol	T3	T2	T1	P	
101.3	100.9	101.7	101.4	P1	
101.7	101.3	101.9	102.0	P2	
104.4	104.4	103.9	104.8	P3	
1.3	0.9^{N.S}			LSD	
	T3	T2	T1	T	
	Mean tryptophan	
			LSD	

marketable yield per tree. Paclobutrazol application at 500 mg•L⁻¹ significantly increased marketable yield, achieving the highest value of 95.0 kg•tree⁻¹, whereas the lowest yield (87.0 kg•tree⁻¹) was recorded in the untreated control (0 mg•L⁻¹ Paclobutrazol). Tryptophan application levels did not significantly affect marketable yield per tree.

The interaction between the Al-Ibrahimi cultivar and Paclobutrazol at 500 mg•L⁻¹ was significantly superior in yield, reaching 95.2 kg•tree⁻¹. Conversely, the lowest yield (85.9 kg•tree⁻¹) was observed in the interaction between the White cultivar and the absence of Paclobutrazol application.

There are no significant differences for the effect of the interaction between cultivars and Tryptophan spraying or between the spraying of the two substances.

Table 6. Effect of cultivars, Paclobutrazol, tryptophan, and their interaction on marketable yield per tree (kg tree⁻¹)

V * P	T	T	T	P	Cultivars
	T3	T2	T1		V
26.14	27.85	25.81	24.76	P1	V1
28.46	28.91	27.76	28.71	P2	
33.12	35.83	32.03	31.50	P3	
25.99	27.24	25.38	25.35	P1	V2
27.31	27.68	27.13	27.13	P2	
30.43	32.04	29.86	29.40	P3	
24.54	25.03	24.27	24.31	P1	V3
27.13	28.59	26.99	25.80	P2	
35.14	38.74	34.82	31.87	P3	
6.86	7.6^{N.S}			LSD 0.05	
V * T					
Mean cultivars	T3	T2	T1	V	
29.24	30.86	28.53	28.32	V1	
27.91	28.99	27.46	27.29	V2	
28.94	30.79	28.69	27.33	V3	
6.84^{N.S}	6.86^{N.S}			LSD	
P * T					
Mean Paclobutrazol	T3	T2	T1	P	
25.55	26.71	25.15	24.81	P1	
27.63	28.39	27.29	27.21	P2	
32.90	35.54	32.24	30.92	P3	
1.03	1.78^{N.S}			LSD	
	T3	T2	T1	T	
	30.21	28.23	27.65	Mean tryptophan	
	1.03			LSD	

Discussion

The differences among cultivars in floral characteristics, such as flowering date, flower quantity branch-1, number of fruit sets, and fruit set percentage (Tables 1–4), may be attributed to the fact that each cultivar possesses a distinct genetic variation that controls its floral traits. In addition, cultivars differ in their response to environmental conditions such as temperature, light, and humidity, resulting in variations in floral characteristics. Each cultivar has specific physiological properties and varying concentrations of hormones responsible for floral growth and fruit set, such as gibberellins and auxins [13.]

It is also observed that the Sharabi and Ibrahim cultivars showed results close to each other compared with the White cultivar, which could be due to the genetic similarity between

these two cultivars. The differences among cultivars in maturity date (Table 5) may be related to variations in flowering date (Table 1) or to genetic differences, as the genetic factor represents the main determinant for the timing of flowering initiation and the thermal requirements needed to break winter dormancy, which vary among cultivars. Furthermore, cultivars differ in their response to temperature and day length, leading to variations in the initiation of flowering among cultivars grown in the same region [14, 15.]

The superiority of Paclobutrazol spraying at 500 mg L⁻¹ in certain floral traits, including flowering duration, flower quantity branch-1, number of fruit sets, and fruit set percentage (Tables 1–4), may be attributed to its influence on the hormonal balance within the plant, which promotes flowering and improves

flower quality, particularly specially in fruit trees like apple and mango[13.]

.The reduction in gibberellin levels resulting from Paclobutrazol spraying decreases excessive vegetative growth (height and branching), leading to carbohydrate accumulation. This, in turn, increases the ratio of flowering to vegetative growth and improves nutritional balance in favor of floral development [16.]

The increase in the number and percentage of fruit sets (Tables 3 and 4) resulting from spraying with tryptophan at 50 mg L⁻¹ may be due to enhanced auxin synthesis within floral tissues and ovaries, stimulating cell division and elongation within the ovary even before complete fertilization (early or parthenocarpic fruit set). Auxin also helps maintain flowers and prevent their abscission due to its inhibitory effect on the abscission layer, improves flower nutrition, and promotes pollen tube growth, thereby increasing the number and percentage of fruit sets [17.]

The reason for the increased yield per tree for the Sharabi and White cultivars (Table 6) is attributed to the increased fruit set rate and the increased number of fruit set (Tables 3 and 4), which leads to an increase in the yield per tree.

Conclusion

The findings of this study showed that in the White cultivar (Al-Kaghdhi), exhibited superior performance in several floral traits, including shorter flowering and maturity periods, higher numbers of flowers and fruit sets were observed while higher percentage of fruit set was also observed compared with the other two cultivars. The application of Paclobutrazol at 500 mg L⁻¹ sprayed

The reason for the improvement in yield indicators, such as the percentage of fruit set and the date of maturity for each variety when spraying trees with Paclobutrazol at a rate of 500 mg L⁻¹, may be due to its important physiological effects on the plant, as it works to regulate growth and stimulate a balance between vegetative growth, flowering, and yield through several mechanisms, including inhibiting the production of gibberellins, which reduces excessive vegetative growth and directs the food reserve towards the formation of flowers and fruits, thus increasing fruit set and improving the quality of the fruits. As a result of reducing vegetative growth, the accumulation of carbohydrates in the tissues increases, providing a critical energy source for flower and fruit development and improving overall fruit quality. Paclobutrazol also stimulates early and uniform flowering, which leads to improving the date of fruit set and increasing the percentage of fruit set. It also works to increase the concentration of some important hormones, such as cytokinins, which contribute to the floral organs [18]. The reason for the improvement in the quantitative and qualitative characteristics of the crop when spraying tryptophan at a rate of 50 mg L⁻¹ may be due to the improvement in the floral characteristics when spraying tryptophan at a rate of 50 mg L⁻¹, including the number and percentage of fruit set.

enhanced flower development, induced early and uniform flowering, improved marketable yield per tree by manipulating the vegetative and reproductive growth. Consequently, 50 mg L⁻¹ Tryptophan application also improved fruit set and yield as a result of auxin formation through the increased content of flower retaining. Generally, the application of Paclobutrazol with tryptophan was effective in

enhancing reproductive performance and productivity of apple trees at Diyala

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