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Impact of fertilizer doses on soil properties, vegetative growth, fruit quality and biochemical compounds of strawberry (*Fragaria* × *ananassa* Duch)

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Abstract

Fertilizers are important for plant growth, yield enhancement, and achieving food security, but overdose can have negative effects. This study sought to determine the impact of different fertilizer doses on soil properties, vegetative growth, fruit quality, and biochemical compounds of strawberry (*Fragaria* × *ananassa* Duch) cv. 'Camarosa'. During the years 2022-2023, strawberry seedlings were grown in pots in a greenhouse. Four increasing dosages of fertilizers were applied. The results showed that following treatment, electrical conductivity and soil organic matter rose to maximum values of 176.26 µS. cm^{-1} and 2.63%, respectively, with d2 treatment. However, the pH was initially high before dropping considerably to 7.34 with d4 dosage. Treatment d1 was highly suggested for enhanced fruit number (5.8) and total yield (729 g. $plant^{-1}$). The treatment d2 produced the highest fruit weight (24.3 g), volume (27.71 cm^3), length (5.55 cm), and width (5.15 cm). The medium dose d3 drastically increased leaf area (2652.76 cm^2), total soluble solids (7.4 °Brix), total phenols (1,332.54 mg GAE· L^{-1}) and antioxidant activity (89.53%), but not significantly. The maximum petiole length (12.66 cm), petiole number (29.4), chlorophyll content, runners number (6), roots length (22.33 cm), fresh root and shoot weight (73.11 and 96.41 g), root and shoot dry weight (10.84 and 30.64 g), were recorded at d4 treatment. In control plants, all of these measures were lower, despite having higher fruit pH (3.72), titratable acidity (0.87%), and flowers number (4.40). In conclusion, increasing fertilizer doses may affect soil and strawberry plant qualities, and farmers must use them appropriately.

Keywords: biochemical compounds; fertilizer; *Fragaria x ananassa* Duch; fruit quality; soil properties; vegetative growth

Introduction

The strawberry is the most widely known commercial berry because it has a notable nutritional content that includes proteins, fats, carbohydrates, fibers, vitamins (A and C), minerals (Na, K, Fe, Cu, and Zn) as well as antioxidant composition (Total phenolic, Total flavonoids and total tannin) that can be used to treat a

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variety of chronic diseases (Rahman *et al*., 2021). Strawberries have bioactive potential, including photoprotective and anti-proliferative properties, due to their total polyphenol content and higher antioxidant activity compared to other fruits (Nowicka *et al*., 2019; Salas-Arias *et al*., 2023). They are promising dietary source to prevent diabetes because they are high in pelargonidin, which has strong α-glucosidase inhibitory activity (Park *et al*., 2020). According to a recent study, strawberry stem hot water and ethanol extracts are valuable as natural compounds with anti-inflammatory and antioxidant properties (Yoo and Lee, 2023). A study additionally showed that two phytosteroids found in strawberries had anti-Alzheimer and anti-inflammatory properties (Mahnashi and Alshehri, 2022). Strawberries are also known to have anti-allergic and antimicrobial activity owing to their flavonoid contents (Chirumbolo, 2014; Cárdenas-Valdovinos *et al*., 2018). All of these benefits have made the strawberry consumed by a large number of people, farmers and others have hurried to grow it. In growing strawberry, excellent yields of high-quality fruit are dependent on correct cultural practices, notably the application of fertilizers appropriately. In order to apply fertilizers more efficiently, the farmer is required to determine the nutritional needs of the strawberry plant (Ulrich *et al*., 1992). There was a high correlation between growth parameters and addition of nutrients (Shirko *et al*., 2018). Fertilizers are used for high yields due to their elements that are essential for plant development, soil fertility, and the reduction of environmental damage (Shaji *et al*., 2021; Bafoev *et al*., 2022). With limited arable area, fertilizer application becomes essential to improving agricultural production and achieving food security (Randive *et al*., 2021). According to a study, the calculated budgets for N, P, and K show that commercial fertilizer accounts for the majority of nutrient inputs required maintaining present crop production levels (Stewart *et al*., 2005). The fertilizers used can change the properties of the soil, making it more or less suitable for plant culture, and thus indirectly affect the plant, as well as directly affect the morphological and physiological characteristics of the plant when they were sprayed. Bai et al. (2020) demonstrated that long-term application of chemical fertilizers altered the chemical and microbiological characteristics of the soil. According to Kilic *et al*. (2021), the type of fertilizer investigated (organic, chemical, and organic + chemical fertilizer) can also affect the fruit quality parameters of strawberries, specifically fruit color parameters, soluble solid content, total acidity, fruit firmness, and vitamin C. Khalil and Agah (2017) found that foliar application of mineral, organic, and biological fertilizers significantly improves vegetative growth, including leaf area, number of leaves, chlorophyll levels…etc, fruit quality (fresh weight, number of fruits, soluble solids), yield, and bioactive components such anthocyanin concentration. Furthermore, using biofertilizers, i.e., effective microorganisms and bio fertile coupled with 100% of the necessary dose of NPK as mineral fertilizers and compost boosted the majority of growth characteristics, chemical composition, total fruit production and its components, physical quality, and chemical constituents of strawberry fruits (Hassan, 2015). In most cases, a producer will stick with his fertilizer practice in so far as production and fruit quality are satisfactory. However, producers frequently continue to use the guessing strategy and overestimate their ability to improve fertilizer practices, which results in over fertilizing. It is crucial to note that while an insufficient amount of nutrients reduces potential crop yield, an overabundance can have a negative impact on plant physiology and crop quality. A sufficient dose of nutrients is required for a high yield of excellent quality and biological value (Ulrich *et al*., 1992; Hopkins et al., 2020).

The present study aims to evaluate the impact of different doses of a variety of commercials fertilizers on some properties of soil, vegetative plant growth, quality of fruits, and biochemical compounds of strawberry during the period of cultivation.

Materials and Methods

Soil sampling and analysis

The soil samples were collected from a farm on the same site located in the wilaya of Jijel, 30 km East of Algeria. The soil sample was taken from the top 30 cm of the soil in October, before any crops were planted. The soil was divided into two parts: 1.5 kg for seeding in pots and 0.5 kg for physicochemical analysis. All analysis was done both before and after cultivation and treatment. The international Robinson pipette method was used to determine soil texture (Rouiller *et al*., 1994). The pH H_2O of the soil was measured at a 1:2.5 soil/water ratio, using a pH meter (Mathieu and Pieltain, 2003), the electrical conductivity (EC) was evaluated using a conductivity meter (Mathieu and Pieltain, 2003). Organic matter (OM) was quantified using the modified Walkley and Black method, 1 g of soil was treated with 10 ml of 1N of potassium dichromate solution ($K_2Cr_2O_7$) followed by addition of 20 ml of concentrated sulfuric acid. Shake for one minute and leave to stand for 30 minutes at room temperature. 200 ml of distilled water, 10 ml phosphoric acid (H_3PO_4) and 1 ml of diphenylamine were added to the mixture. Titrate with the standard 0.2 N ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 6H_2O$) solution. Carry out a blank test using the same procedure with no soil). Organic carbon content was calculated as:

$$\text{Organic Carbon (OC) (\%)} = (n' - n) \times 0.9975 \times 0.1 \qquad (1)$$

Where: n': the volume of ferrous solution used in the titration of soil.

n: the volume of ferrous solution used in the blank titration.

The quantify organic carbon was then multiplied by 1.724 to determine soil organic matter, as follows: (Mathieu and Pieltain, 2003).

$$\text{Organic matter (OM) (\%)} = \text{OC} \times 1.724 \qquad (2)$$

Plant material and treatment

The strawberry frigo plant (*Fragaria* × *ananassa* Duch) cv. 'Camarosa' was used in the experiment. The planting date was November 1, 2022. Plants were grown in plastic pots having volume of 1.491 cm^3 in a plastic greenhouse. Five plants were used as replications for each dosage level, with one plant per pot. The plants were watered with tap water. The treatment considered the application foliar of only fertilizers, with no pesticides utilized in this research. The treatment was started from November 2022 and was continued until May 2023. The treatments were as follows:

Control plants or (d0) not fertilized, were sprayed only with water.

Plants treated with fertilizers at dose 1 (d1).

Plants treated with fertilizers at dose 1 × 2 (d2).

Plants treated with fertilizers at dose 1 × 3 (d3).

Plants treated with fertilizers at dose 1 × 4 (d4).

- The choice of fertilizer and the dose d1 used depends on the practices of farmers in the region. The treatment included:

1) NPK 18-18-18 (Total Nitrogen: 18%, (Nitric Nitrogen: 9.9% + Ammoniacal Nitrogen 8.1%), Phosphorus pentoxide soluble in water (P_2O_5): 18%, Potassium Oxide soluble in water (K_2O): 18%, Boron (B) - 0.01%, Copper (Cu) chelated by EDTA 0.02%, Iron (Fe) chelated by EDTA 0.05%, Manganese (Mn) chelated by EDTA 0.05%, Molybdenum (Mo): 0.001, Zinc (Zn) chelated by EDTA 0.02%).

2) Activeg or (NPK 20-20-20): (20% N + 20% $\mathbf{P_20_5}$ + 20% $\mathbf{K_20}$ + 0,4% MgO + 0,8% $\mathbf{SO_3}$ + oligo-element).

3) NPK 13-40-13 (N: 13%, P_2O_5: 40%, K_2O/ 13%)

4) Fertigoful (N: 8.8, P_2O_5: 2.7, K_2O: 7.1, MgO: 0.11, SO_3: 0.10, B: 0.04, Cu: 0.01, Fe: 0.02, Mn: 0.04, Mo: 0.004, Zn: 0.03).

5) NPK 12-61-00 (N: 12%- P_2O_5: 61%- K_2O: 0%).

The fertilizer 13-40-13 was applied every month during November and December, while Activeg, 18-18-18, Fertigoful and 12-61-00 were applied every 15 days in February, March, April, and May. The amount of dose 1 (d1) applied for all fertilizers was 400 mg. L^{-1}, except for fertigofol, where d1 equalled 1 ml. L^{-1}. All fertilisers were applied as foliar sprays.

The effect of fertilizing on strawberry plants was observed by investigating soil properties, vegetative growth parameters, yield and fruit quality and biochemical properties of Fruits. The date of the first open flower and the first mature fruit was also noted.

Vegetative growth parameters

The effect of treatment by fertilizers on vegetative growth parameters was assessed monthly from January to June by measuring per plant: the area of total trifoliate leaves, petiole length, petiole number, stolon numbers, flower numbers, fruits number, content of chlorophyll a and b. The leaf area calculated concerned all the leaves of the plant. The petiole length was measured from the base to the trifoliate attachment zone. The number of flowers and fruits per plant were counted throughout the flowering and harvesting periods, respectively. The number of runners was calculated, approximately every 5-6 days, as soon as new runners appeared they were removed after being recorded to prevent the formation of daughter plants and to ensure that the treatment only affects the parent plant. The leaf chlorophyll concentration was measured using acetone at 80% according to Holden (1975). The absorbance of the extracts was obtained with a spectrophotometer at 645 and 663 nm. The chlorophyll values were determined using equations (3) and (4) respectively (Arnon, 1949):

$$\text{Chlorophyll a} = 12.7 \times DO.\,663 - 2.69 \times DO.\,645 \quad (3)$$

$$\text{Chlorophyll b} = 22.9 \times DO.\,645 - 4.68 \times DO.\,663 \quad (4)$$

After harvest, plants were removed from their pots, shoots and roots were washed with distilled water, weighed for fresh root and shoots, and then dried in an oven at 70 °C until consistent weight was achieved; the length of the root was also measured prior to drying.

Yield and fruit quality

Ripe fruits were collected every (3-5) day during the harvesting period. The fruits were measured immediately for length, width, weight, volume, and number before being stored at -20 °C until analysis. For determination of titratable acidity, total soluble solids and pH, Fruits were thawed at room temperature before being processed in a juice extractor for obtaining natural fruit juice. Three replicates of juice (20 ml each) were centrifuged at 4,000 rpm for a one hour. All juices were analysed the same day when they were prepared, also were used for analysis of polyphenols content and antioxidant activity. The total soluble solids (TSS) were determined using a refractometer (WYA ABBE). The pH was measured directly on the supernatant clear juice obtained. The titratable acidity (TA) of diluted juice solution was evaluated by titrating to pH 8.3 with 0.1 M NaOH (Ornelas-Paz *et al*., 2013) and calculated according to Gunness *et al*. (2009). The total fruit yield was calculated in June; all the mature fruits collected per replication during the experiment were weighed and expressed as g $plant^{-1}$.

The biochemical characteristic

After harvesting, the biochemical characteristics of strawberry fruits were studied in terms of polyphenol concentration and antioxidant activity. Strawberry antioxidant activity was evaluated using the method described by Molyneaux (2004) with slight modifications. The free-radical l, l-diphenyl-2-picrylhydrazyl (DPPH) was used. Samples of clear juice were diluted at a ratio of 1: 6 which were mixed with 0.1 mM methanolic solution of DPPH and placed in a dark place at room temperature for 30 min. Reduction in the absorbance of DPPH at 517 nm was recorded. Fruit juice antioxidant activity was estimated using equation (5).

$$\text{Antioxidant activity (\%)} = (A0 - As / A0) \times 100 \quad (5)$$

Where: A0: the absorbance of the treatment under control conditions
As: the absorbance of the sample (Zahid *et al.*, 2021).

The Folin-Ciocalteau micro method of Waterhouse was used for the determination of total polyphenols. 20 µl of diluted fruit juice (1:1 (v/v)) was mixed with 1.580 µl of distilled water and 100 µl of Folin-Ciocalteau reagent. After that, 300 µl of sodium carbonate solution (200 g. L^{-1}) was added to the mixture and agitated. After 30 minutes of incubation in a water bath at 40 °C, the absorbance of the combination was measured against a prepared blank at 765 nm. Total polyphenolics were expressed as mg of gallic acid equivalents per L of fruit juice (mg GAE· L^{-1}) (Jakobek *et al.*, 2007).

Electrolyte leakage

Membrane permeability in leaves was assessed by electrolyte leakage (EL) according to Shi *et al.* (2006). 10 leaf discs (10 mm in diameter) from young fully grown leaves from two plants per replicate per each treatment were inserted in 50 mL glass vials and washed with distilled water to eliminate every electrolyte. Vials were filled with 30 mL of distilled water and left in the dark for 24 hours. After the incubation time, the electrical conductivity (EC1) of the solution was measured. Vials were heated in a 95 °C water bath for 20 minutes before being cooled to room temperature and the electrical conductivity (EC2) measured. Ion leakage was calculated using the following equation (6)

$$EL (\%) = C1 / C2 \times 100. \quad (6)$$

Statistical analysis

Results were provided as mean ± standard deviation, with five replicates for each assay. SPSS statistical package version 16.0 was used to perform all statistical analyses. One-way ANOVA was used for analyzing parameters that required a comparison between different treatments and controls. Also, Two-way ANOVA was used to test the effects of the two factors: months of cultivation and fertilizer treatment, as well as their interaction (months of cultivation × treatment) on the vegetative growth parameters studied. In the presence of significant differences ($P < 0.05$) between means, the Tukey post hoc test was conducted.

Results

Effect of treatment on soil characteristics

The data gathered in Table 1 showed that fertilizer treatment had significant effects on all soil chemical properties. Before cultivation, the organic matter value was 0.82 ± 0.18%, but it increased after cultivation and treatment. The application of the d2 treatment caused the highest organic matter value of 2.63 ± 0.11%, which then declined despite increasing the fertilizer dose. Significant changes of soil organic matter observed before and after planting, as well as differences between untreated and treated soils after planting. The same results were observed for electrical conductivity, which was low at 73.46 ± 3.07 µS. cm-1 before to cultivation, but increased until treatment with d2, reaching a maximum value of 176.26 ± 25.09 µS. cm-1 before decreasing. Statistically, the electrical conductivity values of the soil before and after planting differed significantly, notable variations between treated and untreated soils following planting as well. The pH, on the other hand, was high prior to cultivation, peaking at 7.9 ± 0.005, and then significantly reducing throughout cultivation and treatment, reaching a low of 7.34 ± 0.06 with d4 treatment. The texture of the soil was sandy (Table 1).

Table 1. Physicochemical characteristics of soil before and after cultivation and treatment

	Before cultivation	After cultivation and treatment				
		Control: d0	d1	d2	d3	d4
OM (%)	0.82 ± 0.18 a	1.6 ± 0.24 b	2.61 ± 0.04 c	2.63 ± 0.11 c	2.59 ± 0.16 c	2.33 ± 0.53 c
pH	7.9 ± 0.005 a	7.89 ± 0.01 ab	7.83 ± 0.04 ab	7.78 ± 0.06 b	7.6 ± 0.02 c	7.34 ± 0.06 e
EC ($\mu S.cm^{-1}$)	73.46 ± 3.07 a	103.16 ± 1.6 a	175.66 ± 5.67 b	176.26 ± 25.09 b	171.4 ± 20.94 b	151.66 ± 8.38 b
Texture soil						
Silt (%)	0±0	Sandy soil				
Clay (%)	4±0.001					
Sand (%)	96±0.005					

The data presented are mean ± standard deviation. Different letters between treatments denote significant differences (Tukey test, $p < 0.05$).

Electrolyte leakage

The electrolyte leakage in the control plants was 43.10 ± 5.48%; on the other hand, the treated plants revealed an insignificant decrease in electrolyte leakage. The plants that received d2 treatment had the lowest value (39.84 ± 5.0%) (Table 2).

Table 2. Effect of treatment on length of roots, fresh and dry weight of root, fresh and dry weight of shoot and electrolyte leakage

Treatments	Length of roots (cm)	Fresh weight of root (g)	Dry weight of root (g)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Electrolyte leakage (%)
Control	21.33 ± 1.52 a	17.08 ± 1.42 a	3.05 ± 0.72 a	17.81 ± 1.14 a	4.99 ± 0.55 a	43.10 ± 5.48 a
d1	22.66 ± 1.15 a	37.03 ± 6.40 ae	6.4 ± 0.98 ab	35.66 ± 5.03 a	10.7 ± 2.09 a	42.93 ± 2.19 a
d2	21 ± 1.00 a	44.24 ± 2.06 be	7.41 ± 0.71 bc	69.5 ± 8.5 b	21.08 ± 1.08 b	39.84 ± 5.0 a
d3	20.33 ± 1.52 a	60.68 ± 3.22 bc	9.26 ± 1.25 bc	77.46 ± 11.15 b	24.46 ± 3.27 bc	42.74 ± 6.08 a
d4	22.33 ± 2.51 a	73.11 ± 17.92 c	10.84 ± 3 c	96.41 ± 19.69 b	30.64 ± 6.5 c	41.77 ± 20.51 a

The data presented are mean ± standard deviation. Different letters between treatments denote significant differences (Tukey test, $p < 0.05$)

Effect of treatment on vegetative growth parameters

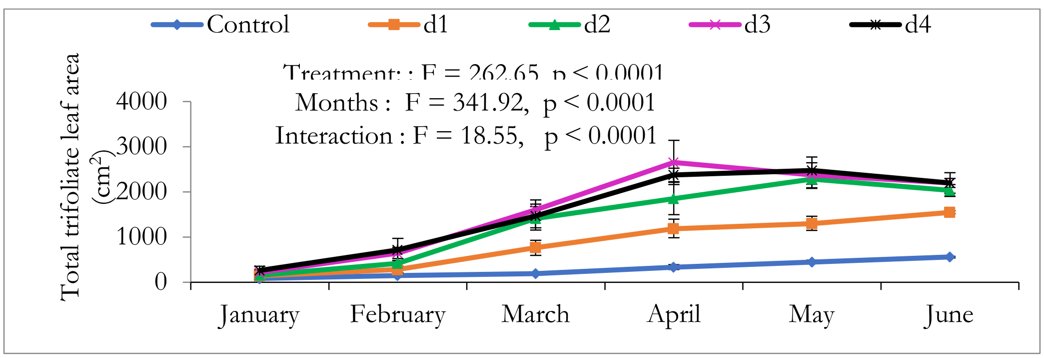
The current study reported that applying different fertilizers at various doses had significant impact on vegetative growth parameters.

(A)

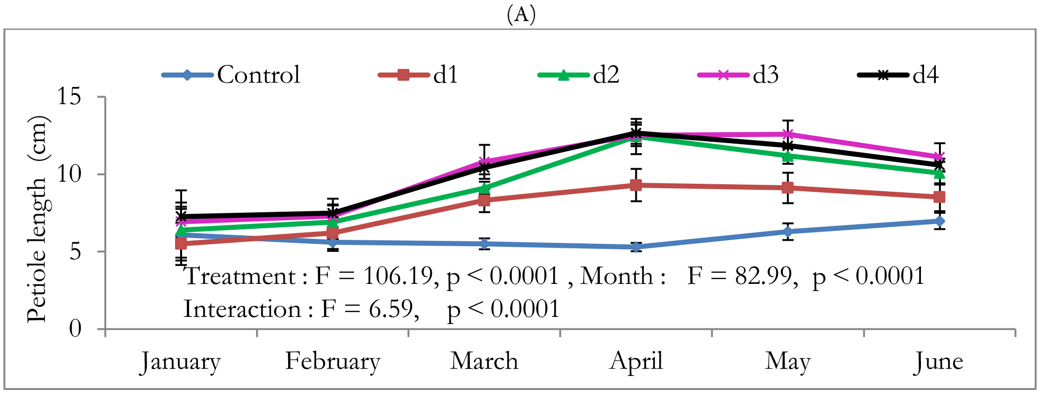
(B)

Figure 1. Control and treated strawberry plants A: in flowering, B: in fruiting

The ANOVA results revealed that fertilizer treatment had a significant effect on total leaf area, petiole length, petiole number, runner number, flowers number, fruits number, and chlorophyll a and b content ($p < 0.05$). It was additionally found that the period of cultivation in month had a significant effect on all of these parameters ($p < 0.05$). The interaction treatment × period of cultivation was significant for all measured parameters ($p < 0.05$) (Figures 2, 3, 4, 6 and Tables 3 and 4).



(A)



(B)

Figure 2. Effect of treatment and period of cultivation in months on: A: Total trifoliate leaf area, B: petiole length of strawberry control and treated plants

Two-way ANOVA test statistics (F and P-values for fertilization, months of cultivation, and their interaction).

The control plant had the lowest mean value of leaf area, which significantly rose with increasing fertilizer doses showing its greatest values after treatment with d3. The lowest values were observed in January, and they rose considerably over time to stabilize in April. Indeed, no significant difference was seen in the last three months of the experiment (Table 3). The highest leaf area value was obtained in April for the d3 concentration with 2,652.76 ± 492.1 cm^2 and the lowest in January for the control plants (81.75± 12.65 cm^2) (Table 4 and Figure 2A).

The lowest petiole length value was obtained at the control plant level, increasing significantly with increasing fertilizer dose until attaining its maximum values from d3 onwards. The lowest values of petiole length were found in January, increasing significantly over the cultivation period to reach a peak in April, then declining significantly until June (Table 3). The highest value of petiole length was obtained in April for dose d4 (12.66 ± 0.69 cm) on treated plants. The lowest was observed in April on control plants (5.3 ± 0.27 cm). (Table 4 and Figure 2B).

Table 3. Tukey post-hoc test results for the effect of treatment and cultivation period on the parameters investigated

	Total leaf area	Petioles length	Petioles number	Runners number	Flowers number	Fruits number	Chlorophyll a content	Chlorophyll b content
Treatment								
Control	A	A	A	A	A	AB	A	A
d1	B	B	B	B	A	A	A	B
d2	C	C	C	C	B	AB	BC	C
d3	D	D	D	D	B	AB	B	D
d4	D	D	E	E	B	B	C	E
Months								
January	a	a	a	/	/	/	a	a
February	b	a	b	a	/	/	b	a
March	c	b	c	a	a	/	b	a
April	d	c	d	b	b	a	ae	b
May	d	ce	e	b	c	b	ae	b
June	d	be	f	c	c	c	e	b

Different letters in the same column between treatment or month meant significantly different (Tukey test, $p < 0.05$).

The control plants had the lowest petiole number, which enhanced significantly with increasing treatment dose to reach the maximum value in plants treated with the d4 dose. The lowest value was reported in January, rising significantly during the growing season to reach the highest level in June (Table 3). The maximum petiole number was found in plants treated with dosage d4 in June (29.4 ± 0.89), and the lowest in plants treated with dose d2 in January (2±0) (Table 4 and Figure 3A).

There was no stolon or runner recorded in the control plants throughout the cultivation period. In contrast, we noted a low number of runners in the plants treated with dosage d1, and as the fertilizer dose was increased, the number of runners rose significantly until it reached its maximum in the plants treated with dose d4. (Table 3). There was no record of runner growth in January; Runners began to appear in February with the lowest value, and during the growing season, their number increased significantly, reaching its peak in June. The greatest number of runners was recorded in June in plants treated with d4 with a value of 6±0, while the lowest number of runners was reported in February in plants treated with d1 (1±0)(Table 4 and Figure 3B).

The treated plants started to flower on March 3; in contrast, the control plants did not begin to flower until April 10 (Figures 1A and 4A). The number of flowers increased in the treated plants until it reached a maximum value, then decreased and increased again; the same trend was observed in the control plants, but with a delay. The highest values were recorded in plants treated with d1, followed by d3, d4, and finally d2. It should be highlighted that plants that were not fertilized had the highest values, followed by plants that were fertilized with lower dosages d1. As demonstrated in Table 3, there was a significant difference between the results. Throughout the growing season, values increased from low in March to high levels in April and then decreased progressively until June. The maximum value of flowers number was obtained in May for the control plants, followed by the treated plants with d1 in April and the lowest in May for the treated plants with d4 (Table 4 and Figure 4A). Ripe strawberries were gathered for the earliest time from d2-treated plants on April 27, while control fruits were harvested late on May 19 (Figure 1B and 4B). The absence of ripe strawberries was noted in April, except in plants treated with modest doses of d2 and d3. All treated plants showed a considerable increase in fruit production in May, with the highest value observed in plants treated with dosage (d1) (5.8 ± 1.3), and then decreased significantly in June, reaching a minimum value of 0.4± 0.4 in plants treated with d2. And stopping production in plants treated with the highest doses of d3 and d4.

In comparison to control plants, ripe fruit production began in May and increased significantly in June, reaching an estimated maximum value of 4. This study ended in June; therefore, no decline has yet been shown. There was no statistically significant difference between control plants and plants treated with d2 or d3 (Figure 4B and Tables 3 and 4).

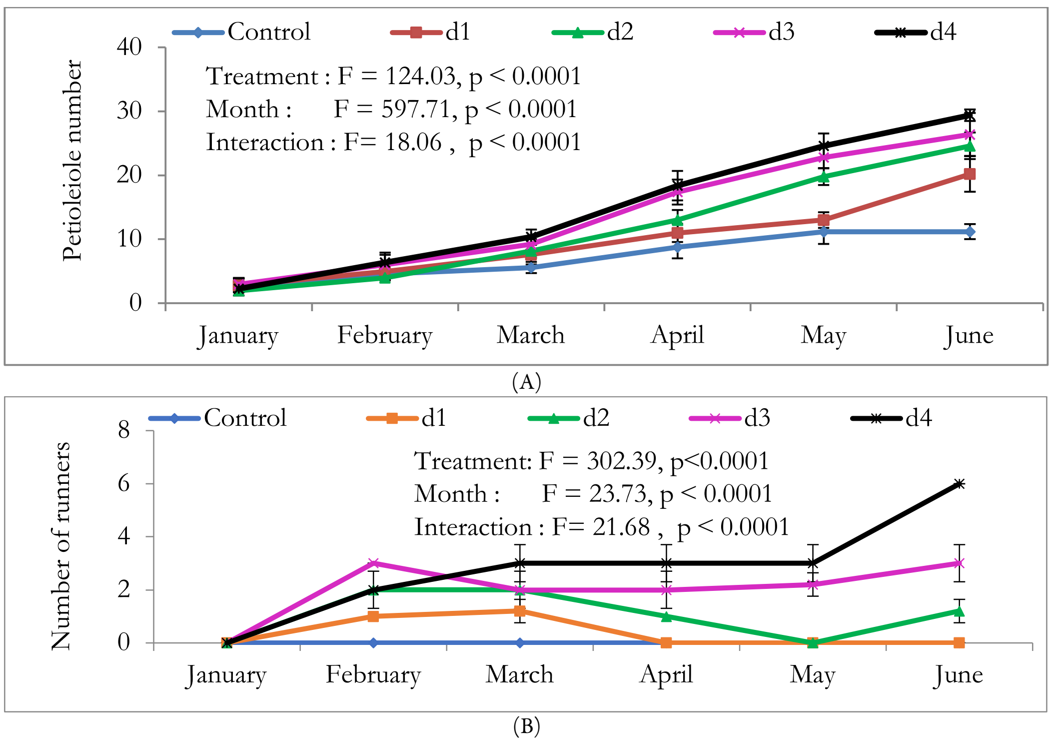
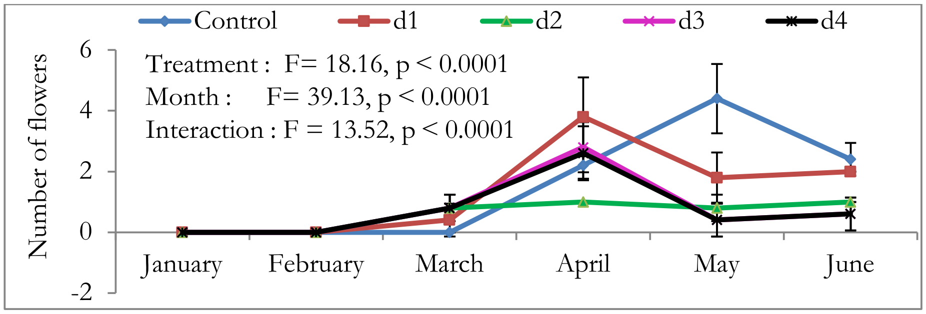


Figure 3. Effect of treatment and period of cultivation in months on: A: petiole number, B: number of runners of strawberry control and treated plants

Two-way ANOVA test statistics (F and P-values for fertilization, months of cultivation, and their interaction).



(A)

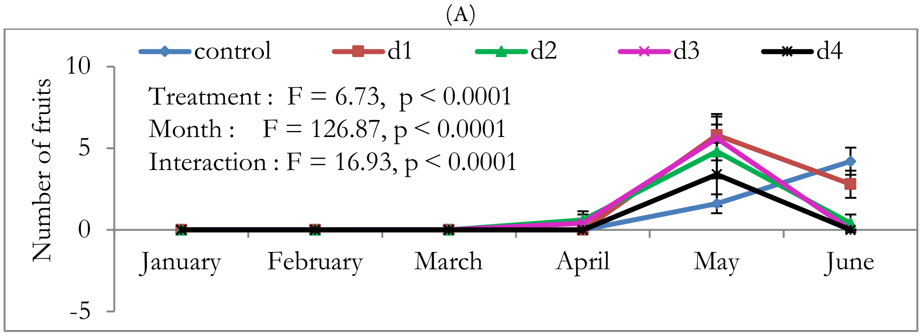


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Figure 4. Effect of treatment and period of cultivation in months on: A: number of flowers, B: fruits number of strawberry control and treated plants

Two-way ANOVA test statistics (F and P-values for fertilization, months of cultivation, and their interaction).

Table 4. Results of Tukey test of the interaction effect (treatment × month) on parameters studied

Interaction Treatment × Months		Total leaf area	petiole length	petiole number	runners number	flowers number	fruits number	Chlorophyll a content	Chlorophyll b content
Control	January	L	IJ	KL	/	/	/	CDE	FGHI
	February	KL	J	IJKL	F	/	/	BCDE	EF
	March	JKL	J	GHIJKL	F	H	/	CDE	EFG
	April	HIJKL	J	FGH	F	CDE	D	E	I
	May	HIJKL	HIJ	EF	F	A	CD	E	HI
	June	HIJK	GHIJ	EF	F	BCD	AB	E	GHI
d1	January	KL	J	KL	/	/	/	BCDE	CDE
	February	IJKL	IJ	HIJKL	E	/	/	BCDE	FGHI
	March	GH	EFGHI	FGHIJ	DE	GH	/	CDE	EFG
	April	FG	CDEF	EF	F	AB	D	E	FGHI
	May	F	CDEFG	E	F	CDEFG	A	E	FGHI
	June	EF	DEFGH	CD	F	CDEF	BC	E	FGH
d2	January	KL	HIJ	L	/	/	/	BCDE	DEF
	February	HIJKL	GHIJ	JKL	CD	/	/	A	AB
	March	EF	CDEFG	FGHI	CD	EFGH	/	BCDE	CDE
	April	CDE	A	E	E	DEFGH	D	DE	EFG
	May	ABC	ABC	CD	F	EFGH	AB	E	EFG
	June	BCD	BCDE	B	DE	DEFGH	D	E	CDEF
d3	January	JKL	GHIJ	KL	/	/	/	BCDE	AB
	February	HIJ	FGHIJ	GHIJK	B	/	/	B	AB
	March	DEF	ABCD	EFG	CD	EFGH	/	BCD	AB
	April	A	A	D	CD	BC	D	E	EFG
	May	AB	A	BC	BC	GH	A	BCDE	EFG
	June	ABC	ABC	AB	B	FGH	D	E	EFG
d4	January	IGKL	FGHIJ	KL	/	/	/	BCDE	AB
	February	HI	FGHIJ	GHIJK	CD	/	/	BC	BCD
	March	EF	ABCDE	EF	B	EFGH	/	A	A
	April	AB	A	D	B	BC	D	CDE	A
	May	AB	AB	B	B	GH	BC	BCDE	BC

	June	ABC	ABCDE	A	A	FGH	D	E	DEF

Different letters in the same column between categories meant significantly different (Tukey test, $p < 0.05$).

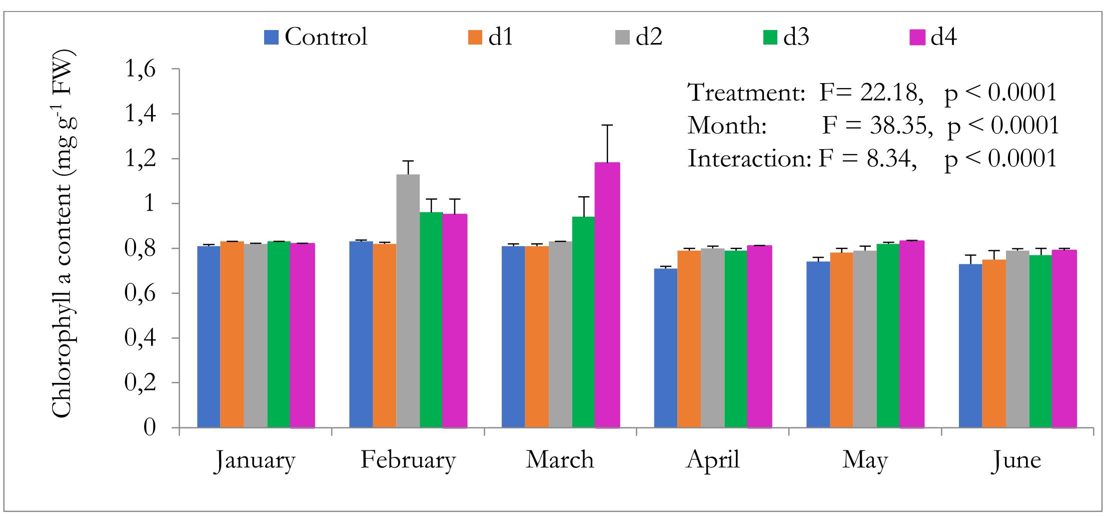
Chlorophyll a concentration was lowest in control plants and those treated with d1, and increased with increasing fertilizer dose, reaching its greatest levels in plants treated with d4 (Table 3 and Figure 6A). The greatest values were recorded in February, followed by March, and decreased gradually until reaching a low value in June, with significant variation between months (Table 3). The maximum chlorophyll a value was found in March for the d4 dose, with a value of 1.18±0.17 mg g^{-1} FW, and the lowest in April in the control plants, with a value of 0.71±0.01 mg g^{-1} FW (Table 4 and Figure 6A).

Chlorophyll b content was lowest in control plants and increased with increasing treatment dose to reach a maximum with the d4 dose. It should be highlighted that there was a substantial difference between the treatments (Table 3). The highest average chlorophyll b values were observed over the first three months, with no notable difference reported, and then declined dramatically to stabilize from April. Similarly, no significant change was found between the last 3 months of experimentation (Table 3). The greatest value was recorded in April in d4-treated plants (0.66 ± 0.005 mg g^{-1} FW) and the lowest in April in control plants (0.31 ± 0.01 mg g^{-1} FW) (Table 4 and Figure 6B).

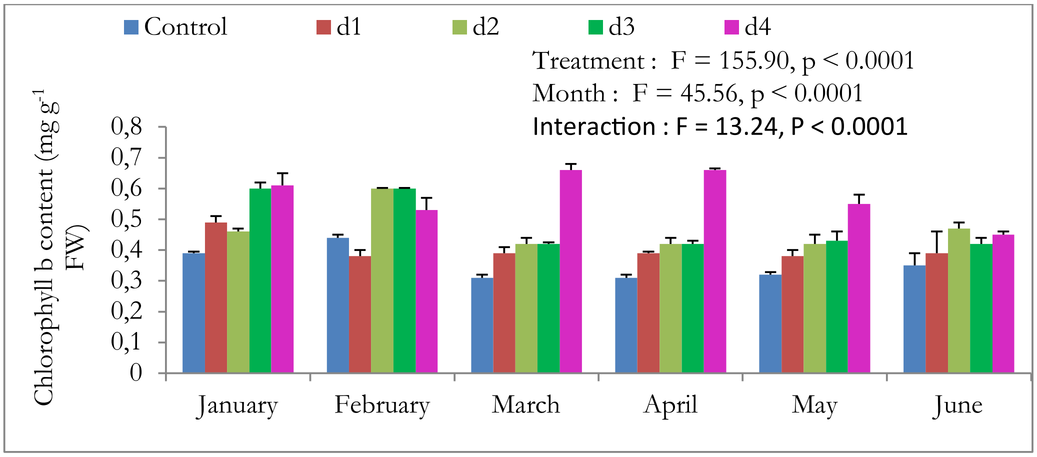
Despite the increased fertilizer dose, there was no significant difference in root length between control and treated plants. This could be due to the use of smaller pots reducing root penetration, but it would allow roots to develop horizontally, increasing their weight, as seen in Figure 5 and Table 2. Despite this, using d4 resulted in the greatest root length.

Figure 5. Fresh strawberry roots

A: Control, B: plants treated with d1, C: plants treated with d2, D: plants treated with d3, E: plants treated with d4.



(A)



(B)

Figure 6. The effect treatment and month on chlorophyll content (A): Chlorophyll a, (B): Chlorophyll b of strawberry control and treated plants

Two-way ANOVA test statistics (F and P-values for fertilization, months of cultivation, and their interaction).

Control plants had the lowest fresh and dry root weights, measuring 17.08 ± 1.42 g and 3.05 ± 0.72 g, respectively. These values increased significantly as fertilizer doses increased. The treatment dosage d4 provided the greatest mean values of 73.11 ± 17.92 g and 10.84 ± 3 g, respectively. Similarly for fresh and dry shoot weights, control plants exhibited the lowest values with 17.81 ± 1.14 g and 4.99 ± 0.55 g, respectively. As fertilizer doses increased, these values rose significantly. The treatment dosage d4 resulted in the highest mean values for fresh and dry shoot weights (96.41 ± 19.69 g and 30.64 ± 6.5 g, respectively).

Effect of treatment on yield and fruit quality

Although fertilizers have been proved to significantly elevate fruit quality and yield in comparison to controls with the lowest values, whether for fruit weight, volume, length, width, fruit number, total yield fruit, pH, titratable acidity, and total soluble solids; the application of fertilizers at high doses (d4) could negatively impact fruit quality and yield (Tables 5, 6 and Figure 1). The d2 treatment resulted in the greatest fruit weight of 24.3 ± 11.07 g, fruit volume of 27.71 ± 15.71 cm^3, fruit length of 5.55 ± 0.80 cm, and fruit width of 5.15 ± 1.10 cm (Table 5). The titratable acidity value was greater in control fruit (0.87 ± 0.05%), then decreased significantly in treated fruit, reaching a low of 0.58 ± 0.02% with d4 treatment. The pH values in control fruits were low (3.72 ± 0.02), but in treated fruits, increasing fertilizer dosages negatively influenced the values, which increased significantly from 3.72 ± 0.02 at d1 dose to a high of 3.91±0.05 at d4 (Table 6).

The total fruit yield value in the control fruits was 345.05 g $plant^{-1}$. Using fertilizers with the lowest dose d1 enhanced total yield of fruits, which obtained the greatest value (729 g $plant^{-1}$). Increasing the treatment dosages had a negative influence on the yield until it reached the lowest value 281.97 g $plant^{-1}$ with the maximal dose d4 (Table 5). The total solids soluble in control fruits were 6 °Brix, then increased dramatically in treated fruits with fertilizer used until treatment d3 when a higher value of 7.4 ± 0.54 °Brix was attained, then reduced significantly to 4.6±0.54 °Brix with the greatest dose d4 (Table 6).

Effect of treatment on the biochemical characteristic

The findings in Table 6 revealed that the lowest value of total phenolic content was found in control fruits (1.051.12 ± 149.64 mg GAE· L^{-1}) when compared to treated fruits with significant differences. As fertilizer doses rose, the value of phenolic compounds increased until moderate treatment (d3) resulted in the maximum total phenolic content with 1,332.54±98.49 mg GAE· L^{-1}. Which later lowered significantly with treatment d4 to 1,142.04 ± 73.05 mg GAE· L^{-1}. The antioxidant activity of the control and treated fruits did not differ significantly. Fertilizer treatment boosted antioxidant activity until dose d3 (89.53 ± 3.74%), then declined insignificantly to 84.88 ± 0.48% with treatment d4.

Table 5. Effect of treatment on fruit weight, fruit Volume, fruit length, fruit width and total yield fruit of strawberry

Treatments	Fruit weight (g)	Fruit Volume (cm^3)	Fruit length (cm)	Fruit width (cm)	Total yield fruit (g $plant^{-1}$)
Control	12.32 ± 6.44 a	13.25 ± 7.21 a	4.53 ± 1.00 b	3.98 ± 0.78 a	345.05
d1	17.4 ± 10.32 ac	17.85 ± 10.8 a	4.96 ± 1.15 ab	4.45 ± 0.99 ab	729
d2	24.3 ± 11.07 b	27.71 ± 15.71 b	5.55 ± 0.80 a	5.15 ± 1.10 b	680.43
d3	19.76 ± 11.43 bc	21.62 ± 13.40 ab	5.12 ± 1.03 ab	4.56 ± 1.22 ab	573.18
d4	17.62 ± 9.39 ab	18.75 ± 11.28 ab	4.78 ± 1.08 ab	4.58 ± 1.16 ab	281.97

The data presented are mean ± standard deviation. Different letters between treatments denote significant differences (Tukey test, $p < 0.05$).

Table 6. Effect of treatment on TSS, pH, TA, total phenolic content and antioxidant activity of strawberry

Treatment	TSS (°Brix)	pH	TA (%)	Total phenolic content (mg GAE· L^{-1})	Antioxidant activity (%)
Control	6 ± 0.0 a	3.72 ± 0.02 a	0.87 ± 0.05 a	1,051.12 ± 149.64 a	85.02 ± 2.97 a
d1	6.2 ± 0.44 a	3.72 ± 0.02 a	0.82 ± 0.03 a	1,139.75 ± 86.42 a	85.47 ± 4.28 a
d2	7 ± 0.0 b	3.73 ± 0.005 a	0.72 ± 0.01 b	1,165.20 ± 97.46 ab	86.78 ± 0.99 a
d3	7.4 ± 0.54 b	3.85 ± 0.01 b	0.65 ± 0.01 bc	1,332.54 ± 98.49 b	89.53 ± 3.74 a
d4	4.6 ± 0.54 c	3.91 ± 0.05 b	0.58 ± 0.02 c	1,142.04 ± 73.05 a	84.88 ± 0.48 a

The data presented are mean ± standard deviation. Different letters between treatments denote significant differences (Tukey test, $p < 0.05$).

Discussion

The fertilizers used in our study (13-40-13, Activeg, 18-18-18, Fertigoful and 12-61-00) supply the majority of the nutrients required for plant growth, with a much greater proportion of macronutrients (Nitrogen, Phosphorus, Potassium, Sulfur, and Magnesium) and a low percentage of micronutrients or trace elements (Iron, Zinc, Boron, Copper, Manganese, and Molybdenum); we cannot ignore any nutrient, as the resulting effect, will be the influence of all nutrients combined on the factors studied. Both macro and micronutrients have been proven to impact soil properties, plant growth, quality, yield, and biochemical compounds in fruit plants (Panigrahi *et al*., 2019; Bai *et al*., 2020). Before cultivation, the organic matter of soil was regarded poor, but it increased after cultivation and become normal (in the soil without treatment and d4-treated soil) to high (in the soil treated with d1 to d3) (Bertschinger *et al*., 2003). Soil electrical conductivity was low prior to planting and increased during planting and treatment. Though all values remained within the non-saline range. As a result, the effect of soil salinity on strawberry plants was negligible (Abrol *et al*., 1988). Before cultivation, the pH of the soil was moderately alkaline; after treatment, it progressively became slightly alkaline until treatment d4 reduced the pH level to neutral (Odutola Oshunsanya, 2019). Every plant requires a specific pH range in order to express its full growth potential. The optimum range of soil pH for strawberry cultivation is 5.5−6.5 (Pokhrel *et al*., 2015). The rise in EC could be attributed to the presence of nitrogen (Patriquin *et al*., 1993). The fertilizers applied in our study resulted in a reduction in pH, while increasing the electrical conductivity and soil organic matter in comparison to soil with no fertilizer, similar results were observed by several researchers using an inorganic fertilizer or in an increase in fertilizers doses (Abu-Zahra and Tahboub, 2008; Zhao *et al*., 2017; Bonanomi *et al*., 2020). The solubility and availability of nutrients for plants is heavily influenced by the pH. Iron, zinc, boron, copper, and manganese are soluble in acidic soil. Nitrogen, phosphorus, potassium, and sulfur, on the other hand, are readily available in a pH range near neutrality (Fernández and Hoeft, 2009). This could explain the decrease in electrical conductivity and organic matter in soil treated with d4, where soil pH was neutral and nutrients may have been absorbed by strawberry plants.

According to Arancon *et al*. (2004), 110 days after transplanting of strawberry, the total leaf area ranged between 500 and 700 cm^2 after using various fertilizers, and 220 days later, the total leaf area ranged between 3500 and 5500 cm^2. Our study found similar results for treated plants after three months of cultivation (in February). Additionally, continuous fertilizer application led to an increase in total leaf area throughout the final three months of cultivation (until 2,652.76 ±492.1 cm^2).

Şener and Türemiş (2016) revealed average root length (20.73 cm) for cultivar 'Camarosa', which are close to values found in our study. The mean number of runners obtained ranged from 1-3, with the exception of d4 in June, which reached 6. Yadav *et al*. (2020) obtained similar results for the cultivar 'Camarosa', with a range of 1-3 runners per treatment and the maximum number (3.68) observed when NPK fertilizers and vermicompost were combined. After fertilization, the dry weight of the roots grew to 10.84 ± 3 g and that of the shoots to 30.64 ± 6.5 g. Rafeii and Pakkish (2014) and Khan *et al*. (2021) discovered that when humic acid, $ZnSO_4$, and boric acid were applied to strawberry plants of cv. 'Camarosa', the dry weight of the root and shoot ranged between 12-13 g and 17-18 g, respectively.

The high average fruit length in our study was 5.55 cm, with a maximum width of 5.15 cm on treated plants. Senthilkumar *et al*. (2023) found a maximum value for fruit length (5.46 cm) and girth (3.01 cm) in organic inputs of strawberry cv. 'Camarosa' grown in a pot. While Yadav *et al*. (2020) recorded longest berries (3.78 cm) and highest width (2.517 cm) in strawberry cv. 'Camarosa' after using Vermicompost with NPK.

In this study, the control plants produced fruits weighing 12.32 ± 6.44 g and yielding 345.05 g. $plant^{-1}$, but the d1 application recorded the highest total yield value (729 g $plant^{-1}$). Rahman *et al*. (2014) reported that the fruit weight and total yield values for the 'Camarosa' variety were 13.81 g and 372.26 g $plant^{-1}$ respectively. Chowhan et al. (2016) found that the fruit weight for genotype 'Camarosa' was 16.38 g. In addition, the current findings are consistent with those of Rafeii and Pakkish (2014), who discovered the same value of fruit weight in control plants of strawberry cv. 'Camarosa' that rose with foliar application of fertilizer containing zinc or boron. According to Balci and Demirsoy (2008), using organic fertilizer including nutrients (N, P, K, Mn, Fe, Zn, and Mg) on strawberries resulted in a yield of 563.7 g $plant^{-1}$ in the 'Camarosa' variety.

pH, titratable acids, and total soluble solids are important parameters in determining strawberry fruit quality (Agulheiro-Santos *et al*., 2022). In the current investigation, the TA and pH in control fruits were 0.87% and 3.72, respectively. Amiri *et al*. (2022) also observed that the titratable acidity and pH values for the 'camarosa' cultivar were 0.85% and 3.51, respectively, at the overripe stage of the fruit. The TA values in treated plants ranged between 0.58 ± 0.02 and 0.82 ± 0.03%. Balci and Demirsoy (2008) reported values ranging from 0.61 to 0.65% for 'Camarosa' fruit treated with organic fertilizers. The use of a medium fertilizer dose d3 resulted in a total soluble solids value of 7.4 °brix, which was the same value found by Capocasa *et al*. (2008) for cultivar 'Camarosa'. Our results for fruit volume varied from 13.25 to 27.71 cm^3, as Khalil and Agah (2017) obtained comparable results after using organic + mineral fertilizers, with a strawberry fruit size of (25.83 cm^3). The total phenol levels ranged from 1,051.12 to 1,332.54 mg GAE· L^{-1}. These values are lower than those obtained by Piljac-egarac *et al*. (2009), who discovered 1,302.1 mg GAE· L^{-1}; and Avalos-Llano *et al*. (2020) who found 1,451 mg GAE· L^{-1}in strawberry. And Jakobek *et al*. (2007), who found 1,271.85 mg GAE· L^{-1}. The radical scavenging activity ranged between 84.88 to 89.53%; comparable results were achieved by Zahid *et al*. (2021), who revealed 84.50% in untreated plants.

The results obtained demonstrated that the usage of nutrient-containing fertilizers improved fruit quality and total yield over the control. Yadav *et al*. (2021) discovered equivalent results when micronutrients (Zn + Mo + B) were combined with macronutrients NPK via foliar spraying. Also, our findings agree with those of Kazemi (2014), who discovered that nutrients spray such as zinc and iron increased plant dry weight, number of runners, leaf area, root length, number of flowers, fruit weight, fruit pH, TA and TSS. Also, Arancon *et al*. (2004) found that food waste vermicomposts containing the combined nutrients (N, P, K, Mg, S, Fe, Mn, B, Cu) had a beneficial influence on strawberry shoot dry weights, leaf areas, number of runners, number of flowers, and yield when compared to other treatments.

The results appeared to be in general agreement with those reported by Mohamed *et al*. (2011) regarding the interaction effects of phosphate and zinc rates, which had beneficial impacts on number of runners, leaf area, and reflected earlier flowering and a greater number of flowers than the control, as well as fruit weight, length, fruit diameter, and TSS. In contrast, increased fertilizer doses led in a decrease in titratable acidity.

The d4 dose of fertilizers significantly increases chlorophyll, which may be attributed to the nutrients present in the fertilizers. This is supported by the findings of Anchal *et al*. (2023), who discovered that foliar application of the micronutrients combination (B+ Cu + Fe + Zn) at greater concentrations induced an enhancement of growth characteristics such as chlorophyll content and leaf area. Iron and copper are also known to play a function in increasing the chlorophyll content of leaves. In addition, plants treated with d4 produced fewer fruits and more runners. This could be owing to the excessive amounts of fertilizers applied. This is consistent with the findings of Fujishige and Yamane (1996), who concluded that neutral strawberries have poor runner number due to persistent flowering throughout the growth season. The administration of growth regulator and chemical fertilizer (NPK 15-15-15) per plant increased growth parameters, plant weight in several cultivars, and considerably increased runner production.

The use of fertilizers improved fruit quality characteristics in comparison to controls, but the concentration of nutrients in fertilizers also affects; Our findings agree with those of Sayğı (2022), who reported that fertilizer vermicompost, that contains a lower level of macronutrients (NPK) and micronutrients (Zn, Cu, Fe, Mg), produced a higher yield and fruit weight than chicken manure, which has a lower yield and fruit weight despite having a higher level of macronutrients and micronutrients. Also, the results of Rafeii and Pakkish (2014) indicated that humic acid, ZnSo4, and boric acid application improved reproductive and vegetative characteristics of strawberry cv. 'Camarosa' compared to control treatment.

Singh and Maurya (2004) investigated the effect of foliar sprays of $ZnSO_4$, $FeSO_4$, $MnSO_4$, and H_3BO_3 alone and in combination. The spray of these micronutrients was found to be responsive in boosting mango flowering, fruiting, and yield. Yadav et al. (2013) found that foliar applications of 0.1% H3BO3 + 0.5% ZnSO4 + 0.5% FeSO4 on low-chill peaches resulted in quality fruits. While, Trejo-Téllez and Gómez-Merino (2014) concluded that nitrogen has an enormous impact on strawberry quality. Furthermore, when plants have deficits in B and Zn, fruit set is reduced. Surprisingly, the nutrients P, Mg, Cu, Fe, and Mn have no noticeable direct impact on plant growth or fruit quality. Compost added to soil improved chlorophyll content, plant dry weight, TSS, fruit fresh weight, yield, and size (Wang and Lin, 2002).

Membrane damage was evaluated indirectly by measuring solute leakage (electrolyte leakage) from cells. The increased permeability for ions and electrolytes generated by salt stress is well manifested in the cellular membrane dysfunction, which may be easily assessed by the efflux and electrolytes (Lutts *et al*., 1996). The high dose of fertilizer utilized in our study caused no damage to the leaf membranes. In contrast, the use of fertilizers decreases the electrolyte leakage, this in line with results of Abdullah *et al*. (2022) who found that potassium and calcium decreased EL. Also, According to El-Tohamy *et al*. (2006), electrolyte leakage was 40.07% in control plants and reduced following foliar P and K application on pepper plants.

Conclusions

Fertilizers are important for plant growth, yield enhancement, and achieving food security, but overdose can have negative effects. In this study, the use of fertilizers in various doses significantly influenced soil parameters, plant growth characteristics, yield, fruit quality and biochemical compounds in strawberry. The increase in fertilizer treatment dose has a considerable positive influence on all soil chemical parameters, increasing moderately EC and MO and decreasing pH, all of which provide ideal conditions for growing strawberries. Low fertilizer dosages improve fruit quality and yield more than high doses, but high doses improve vegetative growth characteristics significantly more than low doses. Treatment d1 was highly suggested for enhanced number of fruits and total yield fruit. The use of d2 treatment was an ideal choice as it has obtained the greatest results for weight, volume, length, and width of fruits. The use of medium dose d3 drastically raised total phenolic and also increased antioxidant activity, but not significantly; it was additionally the dose recommended for improved leaf area and TSS. The dose d4 provided the greatest benefit for petiole length, petiole number, runner number, chlorophyll content, root length, fresh and dry weight of root, and fresh and dry weight of shoot. It was also concluded that using fertilizers accelerated flowering and increased the number of blooms. In addition, the appearance of fruit was delayed in the control plants compared to the treated plants. On the other hand, the values of fruit pH, titratable acidity and flowers number were better in control plants than in treated plants.

Authors' Contributions

NF; Laboratory analysis, Investigation and Writing. MB; Revision Methodology and Supervision and BF; Make the Statistical Study. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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