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The Effects of Shading and Nutrient Management on Yield Quality of Vegetable Fern

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Abstract: This study investigated the optimization of shading and organic fertilizer applications on vegetable fern growth and yield quality in order to develop guidelines for farmers interested in sustainable vegetable fern production. The experiment was conducted in a split-plot design in RCBD with four replications. There were three main plots; no shading, 75% shading, and 96% shading. The five sub-plots consisted of no fertilizer application (control), chemical fertilizer at a rate of 92.80 kg N ha^{-1}, and cow manure at rates of 92.80, 185.60, and 278.40 kg N ha^{-1}. A comparison between different shading and fertilizer treatments, combined, demonstrated that 75% shading with the application of cow manure at the rate of 185.60 kg N ha^{-1} was the most appropriate management for vegetable fern production, with the highest marketable yield recorded (1128.54 kg ha^{-1} month^{-1}) and a 10-fold yield increase compared to the control (no shading and no fertilizer). This treatment also resulted in good yield quality (crunchy and tender), high concentrations of chlorophyll and vitamin C, and a safe amount of nitrate accumulation for consumers.

Keywords: *Diplazium esculentum*; cow manure; sustainable production; marketable fresh weight; yield quality

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1. Introduction

Diplazium esculentum (Retz.) Sw., more often known as the vegetable fern, is a perennial vegetable crop native to the tropical climates of Asia and Oceania. In certain parts of Thailand, the tiny delicate fronds of this fern (also known as fiddleheads) are eaten as a vegetable, while in others, they are boiled, blanched, or cooked in curries [1]. Vegetable fern possesses various positive nutritional features, including beta-carotene, folic acid, calcium, iron, phosphorus minerals, and anti-nutritional compounds, such as phytic acids, tannins, and trypsin, which are present in non-toxic concentrations [2,3]. The vegetable fern is also rich in flavonoids and inhibits reactive oxygen species activity [4] and inflammation [5,6]. Moreover, it was also determined that vegetable fern was able to suppress the type 2 diabetes-causing enzyme, alpha-glucosidase. In addition, it has been reported that vegetable fern extract, at a concentration of 125 micrograms per milliliter or higher, has proven effective against K526 cells or leukemia malignancy [7].

Vegetable fern can be cultivated in all parts of Thailand, particularly in locations with consistently moist soil conditions and cool, sunny weather. It typically grows densely along the edge of shaded forests or in marshy places around waterways. However, excessive exposure to light can result in poor growth and production. It might also result in burns and yellow fronds. In excessive shade, the sprouts grow lengthy and fragile [8,9]. Therefore, shading is a factor that must be well-regulated for the cultivation of vegetable ferns. Wang et al. [10] discovered that the flavonoid and phenol content in the leaves of four fern species increased in proportion to the light intensity. In contrast, the overall amount of nutrients and amino acids decreased. Although a small number of studies have focused on the effects of shading on vegetable ferns in Thailand, it was observed that farmers typically covered the ferns with shading material. Alternatively, vegetable fern has been grown underneath large trees in areas with adequate water supply, where limited crops are often planted for local markets. In addition to a lack of effective nutrient management, farmers have continued to use manure or chemical fertilizer based on availability and local capability. Particularly in the province of Phatthalung in Thailand, it was found that cow manure fertilizer was preferred for growing vegetable fern, due to the abundance of beef cattle in the area. Utilizing locally accessible cow manure has been suggested as an alternate nutrient management technique for the sustainable production of vegetable fern and as a way to lower the cost of production. Consequently, this research sought to discover the optimal shade conditions and cow manure application rates, as compared to chemical fertilizer, and their effects on the development, production, and quality of vegetable fern yields, especially under acidic soil conditions such as those found in a large range of lowland areas in Southern Thailand [8]. The information gained could be shared with farmers or used as a guideline for additional research and development in sustainable commercial production of vegetable fern.

2. Materials and Methods

2.1. Experimental Site and Soil Characteristics

The field experiment was conducted from September 2020 to February 2021 in Phatthalung Province (Latitude: 7.814278, Longitude: 99.945861), Thailand. The trials were located on a Lamphu La soil boundary (Fine, mixed, semiactive, isohyperthermic Typic Palehumults). The soil of the experimental field had a clay loam texture [11]. In addition, the chemical properties of cow manure used in this experiment were as follows: pH 8.23, EC 7.75 dS m^{-1}, 51.67% organic matter, 2.12% total N, 0.88% total P, 3.54% total K, and a C/N ratio of 14.11. The climate conditions of the study site during the growing season were characterized by the rainy (September-October) and dry seasons (November–February), with temperatures typically ranging from 26 °C to 34 °C throughout the experimental period.

2.2. Plant Materials and Experimental Design

One-year-old rhizomes of vegetable fern (*Diplazium esculentum* (Retz.) Sw.) were collected from a fern farmer on 23 August 2020. The average rhizome size was 3–4 cm in diameter and 10–15 cm in length. The leaves were pruned and the roots of these rhizomes were kept moist in plastic baskets and watered twice per day for seven days, during which new roots would be evenly distributed.

The experiment was arranged in a split plot in a completely randomized block design (RCBD) with four replicates. The main plots consisted of three levels of shading: (1) full sunlight or without shading (0%), (2) 75% shading, and (3) 96% shading. The light transmittances of the two shading nets (50% and 80% black shading nets, respectively) were determined by comparing the light intensity under full sunlight with that under the shade nets. The different chemical fertilizer and cow manure dosages of the subplots had five different amounts: (1) no fertilizer application (control), (2) 92.80 kg N ha^{-1} of chemical fertilizer (15-15-15 + 46-0-0) (CF 92.80N), (3) 92.80 kg N ha^{-1} of cow manure (CM 92.80N), (4) 185.60 kg N ha^{-1} of cow manure (CM 185.60N), and (5) 278.40 kg N ha^{-1} of cow manure (CM 278.40N) (Table 1).

After plowing (15 cm depth), 60 plots were prepared, each having dimensions of 4 m long and 1 m wide. The vegetable fern plants were planted in three rows per bed with 30 cm spacing both along and between the rows, with a density of 11 plants per m^2. Core plots for fiddlehead harvest were middle rows, leaving the two rows on the left and right as a border. The black shading nets were clipped onto bamboo cane frames, which were supported by cement posts for shading. The height of the nets was 1.8 m, and a distance of 0.3 m between the net and the canopy surface was maintained. For fertilization, the organic fertilizer (cow manure) was applied in two equal splits, 50% as a basal application and the remaining 50% as a top dressing at 90 days after transplanting. The chemical fertilizers were applied with a 15-15-15 formula (N-P-K) and urea (46-0-0), each in two equal splits.

15-15-15 formula (N-P-K) was used once, as a basal application, and a second time at 90 days after transplanting, whereas urea (46-0-0) was applied at 30 and 120 days after transplanting. Plants were irrigated equally in all plots every day, as required to maintain vigorous growth. Natural extracts were sprayed to eliminate both diseases and insects when needed, and mechanical methods permanently eliminated weeds. Temperature (°C) and relative humidity of the shaded and unshaded atmospheres were measured two times daily (12 a.m. and 4 p.m.) until harvest.

Table 1. Background plant nutrient contents in various fertilizers applied for each different shading condition.

Fertilizer Treatment	Application Rates (t ha^{-1})	The Proportion of Primary Elements in Fertilizer Applied for Each Treatment		
		N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Control (no fertilizer)	-	-	-	-
Chemical fertilizer 92.80 kg N ha^{-1} (15-15-15$_{187.50\ kg\ ha^{-1}}$ + 46-0-0$_{140.60\ kg\ ha^{-1}}$)	0.33	92.80	175.78	175.78
Cow manure 92.80 kg N ha^{-1}	4.38	92.80	38.50	154.88
Cow manure 185.60 kg N ha^{-1}	8.75	185.60	77.00	309.75
Cow manure 278.40 kg N ha^{-1}	13.13	278.40	115.50	464.63

2.3. Data Collection and Analysis

2.3.1. Soil Chemical Properties

Soil samples were taken before and after three harvesting times and were air-dried, crushed, and sieved through a 2 mm sieve to analyze specific soil chemical properties. The soil's pH and electrical conductivity (EC) were measured in water at 1:1 and 1:5, respectively, and determined by using a pH-EC meter (SciberScanPC510, EUTEC, Singapore). The organic matter was measured using the Walkley and Black [12] method, and the total N content was determined by using the Kjeldahl method. The available P was extracted from the soil using the Bray II method and determined by using a spectrophotometer (UV-1280, Shimadzu, Japan) at 880 nm. The available K was extracted from the soil using a 1M NH_4OAc solution at pH 7.0 and measured using a flame photometer (410, Sherwood Scientific Ltd., Cambridge, UK) [13].

2.3.2. Plant Growth and Yield

This experiment collected random samples of vegetable fern (three plants per replication). To address the study question, three variables were considered. First, the vegetative growth of vegetable fern was determined as the plant height every 30 days from transplanting to the harvesting stage (90 days old). Plant height was measured from the soil level to the tip of the longest leaf. Secondly, the plant width (the average width measured at the 90° widest point of the plant) was measured every 30 days from transplanting to the harvesting stage (90 days old). Lastly, the crop's productivity was evaluated based on its fresh shoot weight; the vegetable fern case, the young fronds' weight, was also considered. The edible young fern frond and fresh shoot weight per plant were weighed separately. These parameters were measured immediately after harvesting.

2.3.3. Total Chlorophyll Content

For analysis of total chlorophyll content, the samples of vegetable fern frond were collected at first (90–120 days after transplanting), second (121–150 days after transplanting), and third (151–180 days after transplanting) harvesting months. To conduct the study, adapting a technique first described by Mackinney [14], the vegetable fern frond was pierced with a 6 mm cork borer into five pieces. These five pieces were placed in a test tube containing 10 mL of 80% acetone. Chlorophyll was extracted for 72 h at 4 °C in the dark. After that, the absorbance of the extract was measured using a spectrophotometer at

645 and 663 nm. Then, the chlorophyll concentration was calculated (μg cm^{-2}) using the following equation;

$$\text{Chlorophyll a} = [(12.7 \times \text{A663}) - (2.69 \times \text{A645})] \times (\text{V/A}) \quad (1)$$

$$\text{Chlorophyll b} = [(22.90 \times \text{A645}) - (4.68 \times \text{A663})] \times (\text{V/A}) \quad (2)$$

$$\text{Total chlorophyll (a + b)} = [(20.20 \times \text{A645}) + (8.02 \times \text{A663})] \times (\text{V/A}) \quad (3)$$

where V = Final volume of chlorophyll-extracting solution. A = Frond area of plant samples taken for chlorophyll.

2.3.4. Vitamin C Content

Vegetable fern shoots were collected at first (90–120 days after transplanting), second (121–150 days after transplanting), and third (151–180 days after transplanting) harvesting months and ground thoroughly to measure the vitamin C content. Next, a blender mixed 10 g of fresh ground sample powder with 50 mL of distilled water. The resulting mixture was filtered through a delicate fabric. Then, the volume was adjusted to 100 mL. The analysis process was modified from Babashahi–Kouhanestani et al. [15] by pipetting 2 mL of the vegetable fern extract into a flask, then adding 100 mL of distilled water and 5 mL of starch water. Subsequently, it was titrated with a stock solution of iodine until the solution became blue, indicating the equivalence point. The amount of iodine solution used in the titration was documented. Then, the resulting volume was used to calculate the ascorbic acid concentration, which was reported in milligrams per 100 g of fresh weight.

2.3.5. Nitrate Accumulation

Nitrate was measured with an ion-selective electrode (ISE) technique that was adapted from the procedure reported by Youngvises and Rithichai [16]. In brief, 50 mL of distilled water was added to 0.5 g of fresh samples (fiddleheads). The mixture was centrifuged at 5000× *g* for 15 min and then filtered through Whatman No.1 filter paper. Thereafter, the test solution was made in a test tube by mixing 9 mL of the sample and 1 mL of ammonium sulfate $2M(NH_4)_2SO_4$, and then electrode potential (mV) was measured with a nitrate combination electrode (Cole-Parmer, Vernon Hills, IL, USA). After data collection, concentration (mg kg^{-1} of fresh weight) was calculated by comparing results to a standard nitrate reference calibration curve.

2.4. Statistical Analysis

Experimental treatment effects were analyzed using randomized split plots with three replications. Data were analyzed through one-way analysis of variance (ANOVA) by using IBM SPSS Statistics, Version 26.0 software (IBM Crop., Armonk, NY, USA). The means were evaluated by Duncan's multiple range test ($p \leq 0.05$).

3. Results

3.1. Soil Chemical Properties

The experimental plots' soil had extremely acidic pH values (pH 4.80). The organic matter (1.67%) and total nitrogen (0.9 mg g^{-1}) in the soil were moderate, while there were high levels of available phosphorus (18.67 mg kg^{-1}) and low levels of available potassium (52.33 mg kg^{-1}). Different levels of shading and fertilizer rates resulted in significant differences in most chemical properties of treated soils. In terms of soil pH, 96% shading resulted in the lowest value for soil pH (4.73), whereas no differences in soil pH were observed as a function of fertilizer rate, which showed a range of 4.72–4.83. Regarding electrical conductivity (EC), the 96% shading treatments with 185.60 and 278.40 kg N ha^{-1} resulted in the highest EC values. These were not significantly different from the EC values from the no-shading treatments that received 185.60 and 278.40 kg N ha^{-1}. These EC values were in the range of 7.39–8.26 dS m^{-1} (Table 2).

Table 2. Selected soil properties before and after experiments.

Shading (S)	Fertilizer (F)	pH (1:1 H_2O)	EC (1:5 H_2O) (dS m^{-1})	Organic Matter (%)	Total N (mg g^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
Before experiment		4.63	0.04	1.67	0.90	19.00	52.00
After experiment (180 days after planting)							
0%	Control	4.73 c–f	1.46 fg	0.87 efg	0.43 de	11.00 d	77.00 k
	CF 92.80N	4.97 a	3.22 de	0.68 fg	0.33 e	11.67 d	94.67 f
	CM 92.80N	4.60 f	4.69 c	0.83 efg	0.43 de	14.00 bc	66.00 n
	CM 185.60N	4.87 a–d	7.58 ab	1.09 de	0.57 bcd	8.00 ef	91.33 g
	CM 278.40N	4.93 ab	7.39 ab	0.96 def	0.50 cd	14.67 b	119.33 a
75%	Control	4.90 abc	0.74 g	0.65 g	0.33 e	12.33 cd	75.00 l
	CF 92.80N	4.87 a–d	3.93 cd	1.06 de	0.57 bcd	6.00 f	70.33 m
	CM 92.80N	4.73 c–f	3.99 cd	1.21 bcd	0.60 bc	8.00 ef	77.33 j
	CM 185.60N	4.70 def	4.25 cd	1.41 abc	0.70 ab	10.67 d	95.67 e
	CM 278.40N	4.83 a–e	3.45 cd	1.01 de	0.53 cd	18.33 a	112.33 b
96%	Control	4.77 b–f	1.70 fg	1.51 ab	0.77 a	8.33 e	63.67 o
	CF 92.80N	4.67 ef	2.07 ef	1.54 a	0.77 a	11.33 d	78.33 i
	CM 92.80N	4.83 a–e	6.85 b	1.23 abc	0.60 bc	6.67 ef	87.33 h
	CM 185.60N	4.77 b–f	8.26 a	1.24 a–d	0.63 abc	11.33 d	97.33 d
	CM 278.40N	4.63 f	8.08 ab	1.14 cde	0.53 cd	11.00 d	106.00 c
Significance S × F		**	**	**	**	**	**
Mean for shading	0%	4.82 a	4.87 a	0.89 c	0.45 c	11.87 a	89.67
	75%	4.81 a	3.27 b	1.07 b	0.55 b	11.07 a	86.13
	96%	4.73 b	5.39 a	1.33 a	0.66 a	9.73 b	86.53
Significance S		*	**	*	**	**	ns
Mean for fertilizer	Control	4.80	1.30 d	1.01 b	0.51 b	10.56 b	71.89 d
	CF 92.80N	4.83	3.07 c	1.09 ab	0.56 b	9.67 b	81.11 c
	CM 92.80N	4.72	5.18 b	1.09 ab	0.54 b	9.56 b	76.89 cd
	CM 185.60N	4.78	6.70 a	1.25 a	0.63 a	10.00 b	97.67 b
	CM 278.40N	4.80	6.31 a	1.10 ab	0.52 b	14.67 a	109.67 a
Significance F		ns	**	**	*	**	**
Mean		4.79	4.51	1.10	0.55	10.89	87.44
C.V. (%)		1.94	16.28	14.92	13.74	10.60	6.45

Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant, *,** Significance at $p \leq 0.05$ and 0.01, respectively.

For the quantity of organic matter (OM), it was determined that all soil treatments resulted in decreased organic matter compared to the soil before planting. By evaluating each variable, it was determined that 96% shading led to the highest organic matter content in the soil after planting, 1.33%, while no shading resulted in 0.89% OM, and 75% shading resulted in 1.07% OM.

Nitrogen content was found to be proportional to organic matter content for the macronutrient composition in the soil after planting. Thus, the vegetable fern plots that were 96% shaded, and which had a greater OM content, had higher total nitrogen content in the soil than the non-shaded or 75% shaded plots. When the level of shade increased, the amount of available phosphorus in the soil after planting dropped. At 96% shade, the vegetable fern-planted soil contained the least amount of available phosphorus, 9.73 mg kg^{-1}. However, the light transmittance did not influence the available potassium concentration in the soil after planting, with soil potassium values ranging between 86.53 and 89.67 mg kg^{-1}. Nonetheless, it was shown that all fertilizer treatments at all rates improved the soil quantity of available potassium compared to no fertilizer. Thus, by applying 278.40 kg N ha^{-1} of cow manure, the soil after planting contained the most significant amount of potassium, 109.67 mg kg^{-1}. However, when analyzing the effects of both shading and fertilization on the available phosphorus and potassium content in the soil after planting, it was shown that 75% shading, in combination with the application of 278.40 kg N ha^{-1} of cow manure, resulted in the most significant available phosphorus concentration, namely, 18.33 mg kg^{-1}. Non-shading, combined with the application of cow manure at the rate of 278.40 kg N ha^{-1}, resulted in the highest amount of available potassium, which was 119.33 mg kg^{-1} (Table 2).

3.2. Plant Growth and Yield

3.2.1. Plant Length

The different shading levels influenced the height of vegetable ferns. At 30, 60, and 90 days after planting, the 96% shading exhibited the highest values of plant height as compared to other shading treatments, with average heights of 66.89, 72.60, and 75.56 cm, respectively. These were followed by vegetable fern grown under 75% shading, with average heights of 39.28 cm at 30 days, 52.07 cm at 60 days, and 65.24 cm at 90 days after planting. The different fertilizer applications significantly affected the height of vegetable ferns at 30 and 90 days after planting. The highest vegetable fern height at 30 days after planting was observed by applying 92.80 kg N ha^{-1} of chemical fertilizer, while cow manure, at a rate of 185.60 kg N ha^{-1}, resulted in the greatest height at 90 days after planting, which was 67.04 cm (Table 3).

Table 3. Growth at 30, 60 and 90 days after transplanting (DAT) and yield at first (90–120 DAT), second (121–150 DAT), and third (151–180 DAT) harvests of vegetable fern grown under different treatments of shading and fertilizer application.

Treatment	Plant Height (cm)			Plant Width (cm)			Yield (kg ha^{-1})		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	1st Harvest	2nd Harvest	3rd Harvest
Shading (S)									
0%	32.34 c	39.08 c	49.04 c	27.22 c	39.47 c	47.11 c	380.83 b	242.73 b	463.56 c
75%	39.28 b	52.07 b	65.24 b	34.81 b	53.60 b	65.52 b	694.09 a	815.79 a	818.12 a
96%	66.89 a	72.60 a	75.56 a	43.68 a	69.81 a	79.86 a	24.61 c	123.42 c	494.54 b
Fertilizer (F)									
Control	47.68 ab	52.46	61.25 b	34.22 b	54.79 b	63.27 ab	181.85 d	222.65 e	407.01 e
CF 92.80N	42.15 c	54.58	62.17 b	33.07 b	53.22 b	64.90 ab	427.54 b	400.77 c	520.98 c
CM 92.80N	46.25 ab	53.98	62.84 b	35.58 ab	52.15 b	61.17 b	315.67 c	320.13 d	431.21 d
CM 185.60N	49.77 a	57.58	67.04 a	39.03 a	59.38 a	67.73 a	461.98 a	560.99 a	787.18 b
CM 278.40N	45.00 bc	54.32	63.08 b	34.28 b	51.92 b	63.74 ab	445.50 ab	465.36 b	813.98 a
Significance									
S	**	**	**	**	**	**	**	**	**
F	**	ns	*	*	**	*	**	**	**
S × F	**	*	*	ns	**	**	**	**	**
CV (%)	9.54	10.05	10.28	13.36	8.12	7.81	6.69	9.70	4.56

Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant, *,** Significance at $p \leq 0.05$ and 0.01, respectively.

However, there was no difference ($p > 0.05$) in the height of vegetable ferns at 60 days after planting among the different fertilizer applications, which were in a range of 52.46–57.58 cm. Moreover, an interaction between the shade and fertilization variables was observed regarding the effect on the plant height at all days after planting. Results showed that 96% shading, regardless of the fertilization level, appeared to produce vegetable ferns with greater height than non-shading and 75% shading treatments. Notably, at 96% shading and cow manure application, at a rate of 185.60 kg N ha^{-1}, the plant height tended to be greatest at 30, 60, and 90 days after planting (Figure 1).

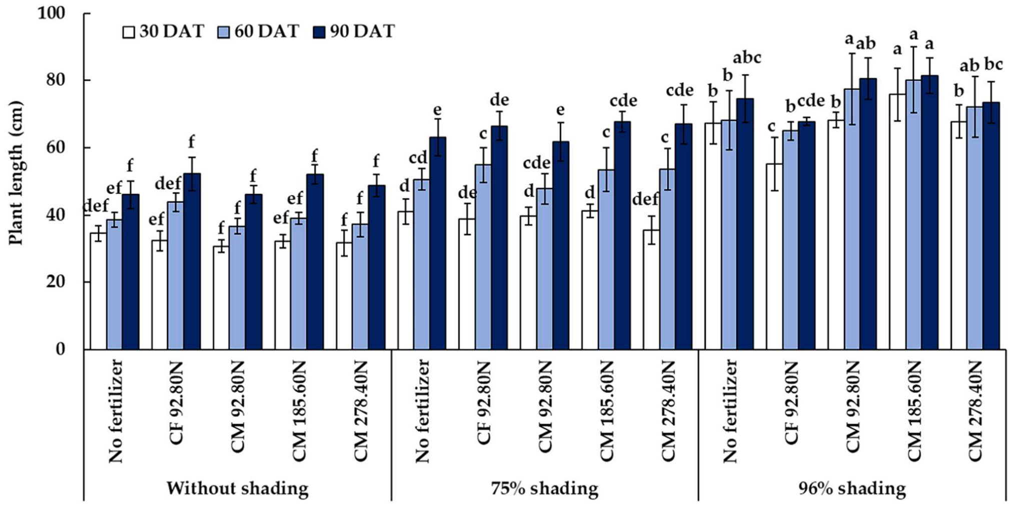

Figure 1. Plant length of vegetable fern grown under different shading and fertilizer application treatments at each DAT (days after transplanting). Values are means with standard deviations (n = 4). The different letters above the bars indicate statistically significant differences by Duncan's multiple range tests ($p < 0.05$) within each harvesting month.

3.2.2. Plant Width

Comparing the different levels of shading, it was noted that shading levels were positively correlated with plant width ($p \leq 0.05$). At 30, 60, and 90 days after transplanting, vegetable ferns under 96% shading developed the most expansive width, followed by the 75% shaded vegetable ferns, while the non-shaded vegetable fern had the narrowest average plant widths. However, when fertilizer application factors were observed, it was discovered that cow manure applied at a rate of 185.60 kg N ha^{-1} resulted in the greatest plant width throughout development (Table 3). In addition, at 60 and 90 days after transplanting, shading and fertilizer significantly affected the width of the vegetable fern ($p \leq 0.05$). The 96% shading treatment, paired with cow manure application at a rate of 185.60 kg N ha^{-1}, resulted in the greatest plant width at 60 and 90 days after transplanting. On the other hand, the absence of shading, in combination with all fertilization treatments, showed a trend towards lower average fern plant widths, which were not statistically different from one another (Figure 2).

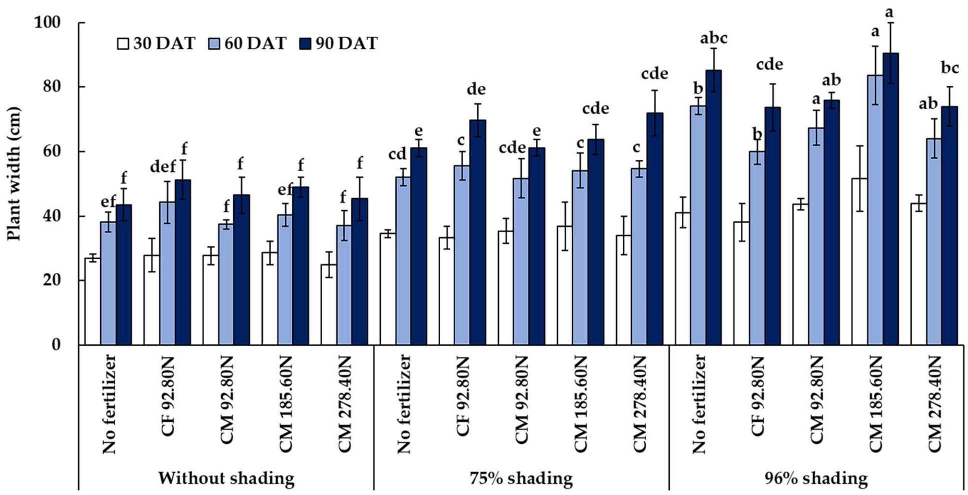

Figure 2. Plant width of vegetable fern grown under different shading and fertilizer application treatments at each DAT (days after transplanting). Values are means with standard deviations (n = 4). The different letters above the bars indicate statistically significant differences by Duncan's multiple range tests ($p < 0.05$) within each harvesting month.

3.2.3. Total Biomass Yield

Comparing different levels of shading, it was revealed that 75% shading had an influence ($p \leq 0.05$) on the total vegetable fern productivity in all three harvesting months, resulting in the maximum yields. In contrast, the 96% shading treatments produced the lowest overall yields of vegetable ferns. When fertilizer application was considered, it was discovered that it substantially impacted the total yield of vegetable fern during the third month of harvest ($p \leq 0.05$). The greatest vegetable fern yields of the first and second months were recorded in plots with cow manure fertilizer applied at a rate of 185.60 kg N ha^{-1}. This yielded 461.98 and 560.99 kg ha^{-1}, respectively. A rate of 278.40 kg N ha^{-1} of cow manure fertilizer did not significantly alter production in the first month (445.50 kg ha^{-1}). However, the greatest vegetable fern yield of the third harvesting month was recorded in plots with cow manure fertilizer applied at a rate of 278.40 kg N ha^{-1} (Table 3). Interestingly, an interaction ($p \leq 0.05$) was observed between shading and fertilization, affecting the total yield of vegetable fern throughout all three harvesting months. The vegetable fern produced the highest overall yield when 75% shading was supplemented with cow manure fertilizer at a rate of 185.60 kg N ha^{-1} throughout all three harvesting months. Vegetable fern grown without shading or fertilization resulted in the lowest corresponding yields (Figure 3).

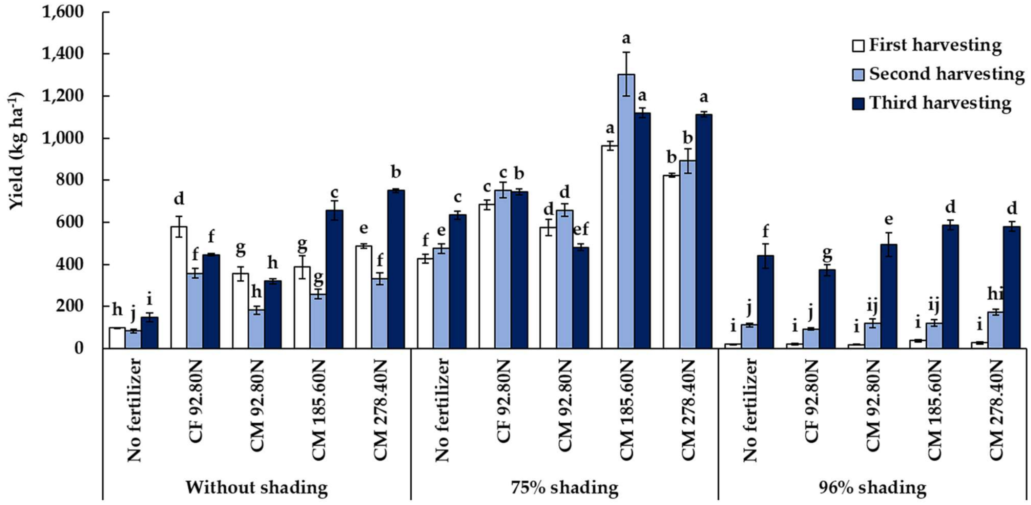

Figure 3. Vegetable fern yield under different shading and fertilizer application treatments at each harvesting time. Values are means with standard deviations ($n = 4$). The different letters above the bars indicate statistically significant differences by Duncan's multiple range tests ($p < 0.05$) within each harvesting month.

3.3. Yield of Biomass Quality

3.3.1. Total Chlorophyll Content

The level of shading had an influence ($p \leq 0.05$) on the total chlorophyll contents of vegetable fern for all three harvesting months. Increased shading levels were correlated with more chlorophyll production (Table 4). The total chlorophyll contents in the shoots of vegetable fern were significantly highest under the 96% shading treatment, followed by the 75% shading treatment. Conversely, the vegetable fern without shading exhibited the lowest total chlorophyll contents. The fertilizer treatments showed that there was a statistically significant difference ($p \leq 0.05$) in total chlorophyll contents during the second and third harvesting months. The vegetable fern treated with chemical fertilizer at a rate of 92.80 kg N ha^{-1} had the highest total chlorophyll yield throughout the three harvesting months: 7.26, 7.75, and 6.54 µg cm^{-2}, respectively. Likewise, total chlorophyll content yield in third-month vegetable fern was numerically highest (6.87 µg cm^{-2}) when treated with cow manure at a rate of 185.60 kg N ha^{-1}. However, the difference was not statistically significant (Table 4).

Table 4. Yield quality of vegetable fern at first (90–120 DAT), second (121–150 DAT), and third (151–180 DAT) harvests, grown under different treatments of shading and fertilizer application.

Treatment	Total Chlorophyll (µg cm^{-2})			Vitamin C (mg 100 g^{-1} Fresh Weight)			Nitrate (mg kg^{-1} Fresh Weight)		
	1st Harvest	2nd Harvest	3rd Harvest	1st Harvest	2nd Harvest	3rd Harvest	1st Harvest	2nd Harvest	3rd Harvest
Shading (S)									
0%	4.98 c	3.83 c	2.93 c	25.39 a	25.18 a	21.66 a	1975.0 b	1375.0 b	1990.0 b
75%	6.56 b	6.37 b	4.65 b	23.79 a	24.58 a	22.03 a	1965.0 b	1905.0 b	1925.0 b
96%	8.37 a	8.66 a	8.20 a	19.76 b	20.21 b	19.21 b	2975.0 a	3005.0 a	3030.0 a
Fertilizer (F)									
Control	6.70	4.58 c	3.06 d	21.27	22.96 ab	18.27 c	2000.0 d	2033.3 cd	2191.7 b
CF 92.80N	7.26	7.75 a	6.54 a	22.71	21.07 b	20.21 bc	2775.0 a	2725.0 a	2641.7 a
CM 92.80N	6.46	4.63 c	4.39 c	21.78	23.19 ab	21.05 abc	1975.0 d	2000.0 d	2025.0 c
CM 185.60N	6.34	7.18 b	6.87 a	23.99	23.72 ab	23.80 a	2166.7 c	2108.3 c	2133.3 b
CM 278.40N	6.41	7.27 b	5.43 b	25.14	25.66 a	21.50 ab	2608.3 b	2558.3 b	2583.3 a
Significance									
S	**	**	**	**	**	**	**	**	**
F	ns	**	**	ns	*	**	**	**	**
S × F	**	**	**	ns	**	*	**	**	**
CV (%)	13.45	8.69	12.83	15.60	14.85	15.52	5.79	4.75	4.47

Means with different letters within a column are significantly different at $p \leq 0.05$ by Duncan's multiple range test. ns = non-significant, *,** Significance at $p \leq 0.05$ and 0.01, respectively.

In addition, total chlorophyll content was revealed to be influenced significantly by shading level and fertilization ($p \leq 0.05$). In the first month, the highest total chlorophyll content appeared, with 96% shading in combination with no fertilizer treatment and the application of 92.80 kg N ha^{-1} of chemical fertilizer. For the second month, the total chlorophyll content was higher ($p \leq 0.05$) in 96% shading treatment, combined with no fertilizer treatment, chemical fertilizer at a rate of 92.80 kg N ha^{-1}, and cow manure at a rate of 278.40 kg N ha^{-1}. For the third month, the highest total chlorophyll content was found in 96% shading, combined with chemical fertilizer at a rate of 92.80 kg N ha^{-1}, and cow manure at rates of 92.80 and 185.60 kg N ha^{-1}. For all three harvesting months in this study, however, the results showed that 96%-shaded vegetable ferns, combined with chemical fertilizer at a rate of 92.80 kg N ha^{-1}, resulted in the most significant total chlorophyll content (Figure 4).

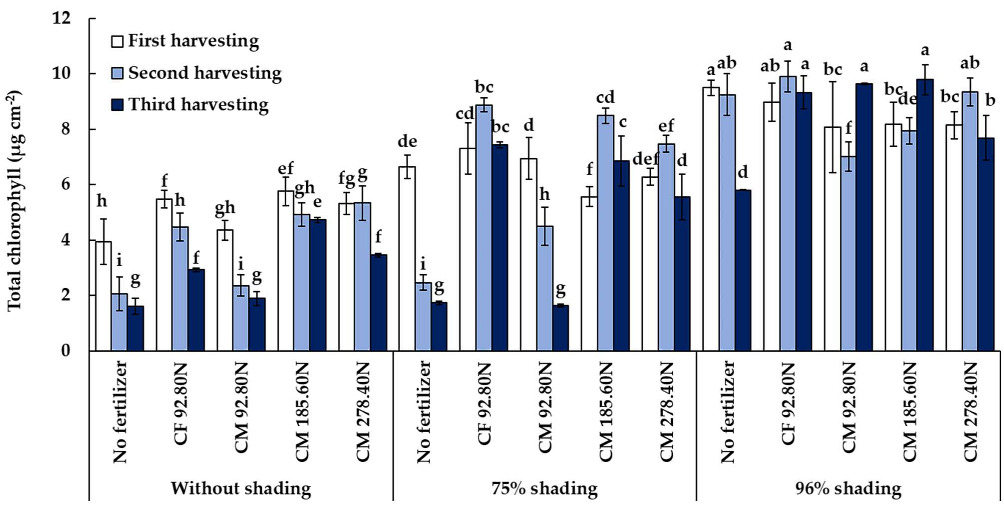

Figure 4. Total chlorophyll content in fiddleheads of vegetable fern grown under different shading and fertilizer application treatments at each harvesting time. Values are means with standard deviations (n = 4). The different letters above the bars indicate statistically significant differences by Duncan's multiple range tests ($p < 0.05$) within each harvesting month.

3.3.2. Vitamin C Content

Experimental results showed that shading significantly affected ($p \leq 0.05$) the vitamin C content of vegetable fern shoots harvested for all three months. The non-shaded vegetable fern yielded the highest vitamin C values for the first and second months, 25.39 and 25.18 mg 100 g^{-1} fresh weight, respectively. These values were not statistically different from those of the 75% shaded vegetable ferns, which had vitamin C contents of 23.79 and 24.58 mg 100 g^{-1} fresh weight. However, for the third-month yield, vegetable fern under 75% shading had the highest vitamin C content, at 22.03 mg 100 g^{-1} fresh weight. This value was nearly identical to a vegetable fern that was not shaded (21.66 mg 100 g^{-1} fresh weight). The 96% shaded vegetable fern had the lowest vitamin C content during the three months of harvesting (Table 4). Regarding the effect of fertilization, there was a statistically significant difference ($p \leq 0.05$) in vitamin C content during the second and third harvesting months. In the second harvesting month, it was found that the greatest vitamin C content, 25.66 mg 100 g^{-1} fresh weight, was found in vegetable fern treated with cow manure at a rate of 185.60 kg N ha^{-1}, which was significantly higher than that of the vegetable fern treated with chemical fertilizer at a rate of 92.80 kg N ha^{-1}. The highest vitamin C content, at 23.80 mg 100 g^{-1} fresh weight, was found in vegetable fern treated with cow manure at a rate of 185.60 kg N ha^{-1} during the third harvesting month. This value was significantly higher than that of the vegetable fern treated with no fertilizer and chemical fertilizer at a rate of 92.80 kg N ha^{-1}. In addition, the non-fertilized vegetable fern had the poorest vitamin C content during the third harvesting month (18.27 mg 100 g^{-1} fresh weight) (Table 4). The combined effects of shading level and fertilization rate on vitamin C content varied among different harvesting months. For the second harvesting month, the lower vitamin C content appeared in 75% shading in combination with the application of chemical fertilizer (92.80 kg N ha^{-1}) and 96% shading in combination with no fertilizer and the lower application rates of both cow manure and chemical fertilizer (92.80 kg N ha^{-1}). In the third harvesting month, the lower vitamin C content was found in vegetable fern treated with no shading combined with no fertilizer and the highest rate (278.40 kg N ha^{-1}) of cow manure, and with 96% shading combined with no fertilizer and the lower application rates of both cow manure and chemical fertilizer (92.80 kg N ha^{-1}) (Figure 5).

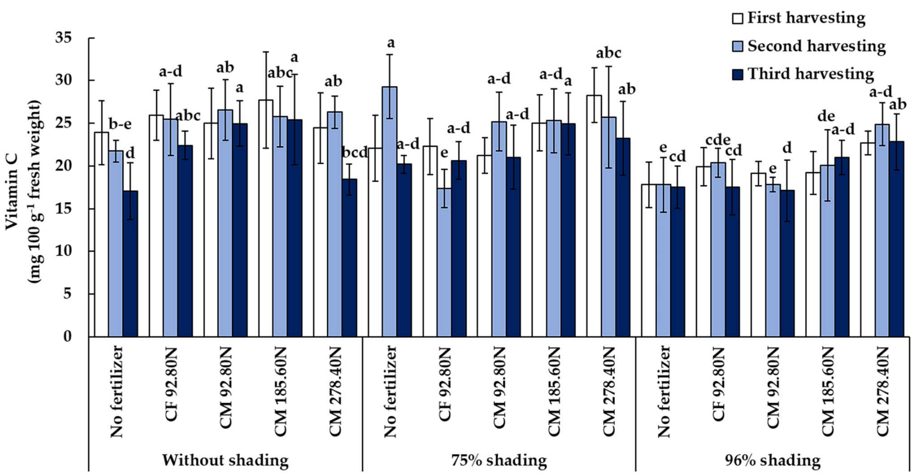

Figure 5. Vitamin C in fiddleheads of vegetable fern grown under different shading and fertilizer application treatments at each harvesting time. Values are means with standard deviations ($n = 4$). The different letters above the bars indicate statistically significant differences by Duncan's multiple range tests ($p < 0.05$) within each harvesting month.

3.3.3. Nitrate Accumulation

The shading level had an influence ($p \leq 0.05$) on nitrate accumulation throughout the three harvesting months of the vegetable fern. Analysis revealed that 96% shading resulted in the highest buildup of nitrates. Non-shaded and 75% shaded vegetable ferns accumulated less nitrate (Table 4). When fertilizer was considered, it was discovered that it had a statistically significant influence on nitrate buildup in all three months of vegetable fern production ($p \leq 0.05$). The use of chemical fertilizer (92.80 kg N ha^{-1}) resulted in the highest nitrate buildup in vegetable fern throughout the three harvesting months. The nitrate accumulation rates from chemical fertilizer were followed by those from cow manure fertilizer at a rate of 278.40 kg N ha^{-1}. Nonetheless, the vegetable fern had the lowest nitrate buildup when cow manure was applied at a rate of 92.8 kg N ha^{-1} (Table 4).

There was an interaction between shading and fertilization in terms of nitrate accumulation during the three harvesting months. The plots with 96% shading and chemical fertilizer at a rate of 92.80 kg N ha^{-1} exhibited the highest nitrate accumulation. In contrast, the non-shaded vegetable fern with cow manure application at a rate of 92.8 kg N ha^{-1} had the lowest nitrate buildup in all three-month yields. However, the nitrate buildup was not significantly different in the first and second-month yields between vegetable ferns that were not shaded and under 75% shading with no fertilizer (Figure 6).

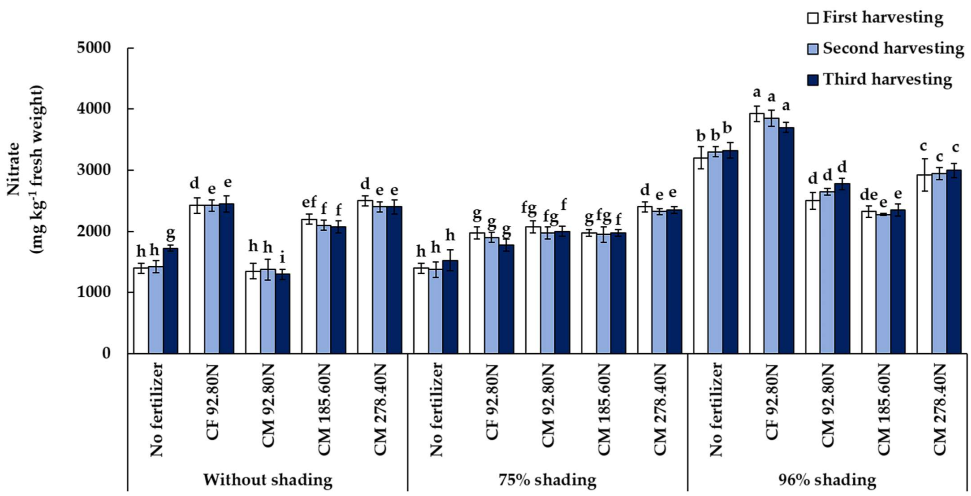

Figure 6. Nitrate content in fiddleheads of vegetable fern grown under different shading and fertilizer application treatments at each harvesting time. Values are means with standard deviations ($n = 4$). The different letters above the bars indicate statistically significant differences by Duncan's multiple range tests ($p < 0.05$) within each harvesting month.

4. Discussion

4.1. Soil Chemical Properties

Several direct and indirect effects of shading and fertilizer management on the soil chemical, physical, and biological properties of production systems have been reported [17–19]. For instance, Cruz et al. [17] noted that shade enhanced soil humidity and nutrient availability in the soil. In another study, Citak and Sonmez [20] highlighted that the chemical properties of soil varied among the application of three different organic soil amendments, including farmyard manure, chicken manure, and blood meal. During this experiment, the average soil pH at the experimental plots was strongly acidic (pH 4.79). Soil pH tended to rise significantly with higher shading treatments, although there was no observed difference in soil pH between different fertilizer applications (Table 2). Some literature also observed that soils under plant canopies tended to be more acidic than soils in open areas or under full sunlight. The slight acidity of soil within canopy zones (referring to shading) could be due to the accumulation of organic matter underneath tree canopy by litter fall; the litter falling on the soil undergoes decomposition and produces various organic acids. In addition, the increase in root respiration via lower carbon supply from the photosynthesis of aboveground litter under shading leads to the rise of carbon dioxide concentration in the soil atmosphere, which, on dissolution in water, produces carbonic acid. These acids lead to lower soil pH under shaded areas [21,22].

Soil pH is referred to as the main factor that influences plant growth and yield production. Several reports on fern growth being limited under low pH soil conditions [23–25]. However, plants, including fern species, vary immensely in their degree of tolerance to low-pH soil. This aligns with the fact that several ferns can grow in acidic soils, including *Nephrolepis exaltata*, which thrives in the pH range of 5.0 to 5.5, and *Polystichum polyblepharum*, which grows in soil between pH 5.1 and 6.5 [26]. Vegetative ferns, among others, are reported to thrive in the pH range of 5.5 to 7.5 [27]. In the current study, the range of pH values recorded in vegetable fern plots was 4.60 to 4.97 (very strongly acidic), which was relatively lower than those reported in the previous studies.

Although the change in the EC value under different shading was a complicated finding, fertilizer application dramatically increased the electrical conductivity of the soil after planting (Table 2). This was probably due to the EC value of cow manure (7.75 dS m^{-1}). This was supported by the findings of experiments with green oak lettuce, which showed that the soil's electrical conductivity rose from applying chicken manure [28]. According to the current organic matter result, the higher organic matter in the treated soil was observed under the higher transmittance. Previous studies showed that increased transmittance caused lower soil temperatures and maintained the soil moisture, benefitting soil microbial activity in decomposing organic matter [29]. In addition, the transmittance also reduced the loss of organic matter in the soil from hot or dry conditions when the soil was exposed to direct sunlight [30].

Furthermore, the total nitrogen content in the soil after planting appeared to follow the same trend as organic matter. Treated soils under low light intensity exhibited a higher total nitrogen content, while the highest total nitrogen content occurred at intermediate fertilization of cow manure (185.60 kg N ha^{-1}). Instead of correlating to the amount and type of fertilizer added, soil nitrogen content was observed to be higher under the shade, which could be linked to various factors, including properties of soil (physical, chemical, and biological properties) [31] and environmental conditions (runoff and erosion) [32]. For instance, reduction of soil temperatures and evaporation, along with increases in soil aggregation due to shade, should physically protect soil organic matter. This contributes to higher nitrogen availability, as it may reduce nitrogen leaching to underground soil compared to unshaded soil [33,34]. Moreover, Dodd et al. [29] noted that nitrogen mineralization rates were reduced by light intensity, which could result in lower availability of organic nitrogen sources for decomposition.

The higher application of cow manure resulted in increased phosphorus and potassium availability in the soil after planting, similar to the research findings of Zhang et al. [35] and Li et al. [36]. The finding could suggest that increasing cow manure increases plant-available nutrients by enhancing beneficial microbe activity, leading to the decomposition and mineralization of organic matter in the manure [37]. Although the effects of shading on soil phosphorus availability were less clear, as frequently noted in previous reports [38,39], the amount of available phosphorus tended to decrease when light intensity decreased. The highest shading treatments employed in this research significantly reduced the available phosphorus in the soil (Table 2), in contrast to the finding of Whitehead and Isaac [40], who demonstrated that higher levels of extractable phosphorus in rhizobox soils were found under shading treatment, compared with no-shading treatments. However, soil phosphorus availability is generally affected by environmental factors (light intensity, temperature, humidity), soil reaction, and chemical reactions among nutrients. The current study recorded a very strongly acidic (pH 4.79) average soil pH, with the lowest pH value (4.73) recorded in the most shaded plots (96% shading). Low-pH soils or acidic soils (soil pH < 5.5) are known for being problematic, with low nutrient availability, ion toxicities, and nutrient content imbalances. Based on our results, after comparing different shading conditions, the lowest level of phosphorus availability in the soil was observed under the 96% shading, where the lowest soil pH was also recorded. This could be explained by a reduction of phosphorus availability via chemical reactions with toxic ions, such as hydrogen (H) or aluminum (Al), making it less obtainable by plants [31].

4.2. Plant Growth and Yield

Shading is an essential manipulation for many plants, especially plants that need a shot photoperiod or some plant species, like vegetable fern and other edible fern species, for which several studies demonstrated the beneficial effects of partial shading on growth and yield (when compared with plants grown in full sunlight or fully shaded conditions) [8,9,41]. Indeed, the vegetable fern is categorized as a natural inhabitant of shady areas and can tolerate full shade. However, it can produce a higher yield when it grows under optimum light intensity levels, which differ among fern species [10]. Our study pointed out that the more-shaded (96% shading) plants had significantly higher differences in plant length and plant width from 30 to 90 days after transplanting (Table 3), which agreed with reports on other plant species, such as muskmelon [42] and alfalfa [43]. This was probably because the plants exhibited the adaptive response of shade avoidance triggered by a light intensity reduction. These responses include increased plant height and larger leaf size, via increasing cell division and elongation, to gain more surface to access more light intensity [43,44]. In addition, some literature revealed that increases in photosynthetic rates and leaf areas of shaded plants could be attributed to lower temperatures in the microenvironment, which are more favorable to metabolic and enzymatic activity [45,46]. Furthermore, in the current study, we observed higher shading, resulting in lower temperature of the microenvironment (Figure S1). However, to our knowledge, the significantly highest fresh yield of vegetable fern resulted from 75% shading, as compared to those from full sunlight and 96% shading conditions. As reported by Gardner et al. [47], optimal shade not only keeps plants from overheating but also stimulates the production and activity of additional growth-promoting auxin and gibberellin. The current study results from optimal shading for fresh yield production were higher than those reported by Duncan et al. [1], who observed that the optimal shading for vegetable fern production was 50% shading, or in other fern species reported by Shim et al. [48]. According to Shim et al. [48], three distinct species of evergreen ferns, *Dryopteris nipponensis* Koids., *Cyrtomium falcatum* (L.F.) Presl., and *Onychium japonicum* (Thunb.) Kunze, grew better in partial shade (30–60%) than in direct sunlight or at higher shading levels.

To find the most suitable conditions for fresh biomass production in vegetable fern, attention must be paid, not only to shading levels, but also to optimum nutrient provision, as a lack of macronutrients can inhibit plant growth and physiological responses [49,50]. In our study, the observed plant width increased when fertilization with cow manure was raised from 92.80 to 185.60 kg N ha^{-1}. Furthermore, the highest yield of vegetable fern was recorded under cow manure application at 185.60 kg N ha^{-1} in the first and second months of harvest. In comparison, the highest fresh yield at the third harvest was observed under the cow manure application at a rate of 278.40 kg N ha^{-1} (Table 3). Calculation of total yield from the three harvesting months showed that the vegetable fern yield under different fertilization rates ranged from 811.51 to 1810.16 kg ha^{-1}, with the lowest fresh yield recorded from non-fertilized plots and the highest recorded from plots that received cow manure application at a rate of 185.60 kg N ha^{-1}. Results of the research presented here showed that plots treated with optimal fertilization with cow manure at a rate of 185.60 kg N ha^{-1} produced a 113% higher yield than non-fertilized plots. Furthermore, the total yield of vegetable fern under this optimal fertilization rate was 5% greater than that under the highest rate of cow manure application (278.40 kg N ha^{-1}), which had a total yield from all three harvesting months of 1724.83 kg ha^{-1}. According to current results, the application of higher-than-optimum levels of cow dung fertilizer will not increase yield but may result in higher costs and a more extended payback period, which was observed in our previous study [51]. Therefore, proper fertilization rates are important and should be included in future guidelines that farmers can apply.

In combination with fertilization, shading significantly affected the yield of vegetable fern in all harvesting months (Figure 3). Significantly higher yields were observed in vegetable fern grown under 75% shading and supplemented with cow manure fertilizer at a rate of 185.60 kg N ha^{-1} throughout all three harvesting months. In addition, the average yield per month from three harvesting months of vegetable fern grown under the optimal conditions of both shading and fertilization was calculated as 1128.54 kg ha^{-1}. The average monthly yield in the current study was within the average range reported by Duangsa et al. [8] and Trail et al. [41], who studied ferns under acidic and fertile soil conditions, respectively. According to the report of Yumkham et al. [52] and Trail et al. [41], vegetable ferns can survive more than 8–10 years, and fiddleheads (marketable fern fronds) can increase for several years via increasing clumps and fern founds. Based on these reports, we assumed that the vegetable fern yield tended to increase with more harvest periods as plants grow, and that this could result in higher average monthly fresh yields than what was reported in the present study.

4.3. Yield Quality

Total chlorophyll mainly includes chlorophyll a and b, the primary pigments of plant photosynthesis, which can absorb light energy and transfer electrons through the reaction centers to convert light energy into chemical energy of organic molecules in the form of sugars. The chlorophyll biosynthesis pathway is usually stimulated by light, which involves the expression of genes encoding critical enzymes for this synthesis [53]. However, photoinhibition may occur when light intensity exceeds the plant's required levels to maximize photosynthetic output. Optimal light intensity, under moderate shading, can reduce chlorophyll degradation from photoinhibition, which can obstruct chlorophyll synthesis [54]. Nevertheless, chlorophyll content in plants frequently enhances under long-term low light intensity, as evidenced in Boston fern [55] and also in other species, such as maize [56], sage [57], alfalfa [43], and tomato [58]. Our study also observed a similar trend, which showed that increased shading levels were correlated with higher chlorophyll production in vegetable fern shoots (Table 4). Under limited light conditions, plants can adapt to maintain photosynthetic activity by increasing photosynthetic light-harvesting antennae (e.g., chlorophyll) to capture more of the required light energy under light-deficit stress [53,57].

Nitrogen is an essential component in chlorophyll structures. Increases in nitrogen fertilizers usually cause increased chlorophyll content in leaves. This trend has been found in studies of apple [59] and sugar beet [60]. Our study observed a similar tendency, albeit only in the second (121–150 DAT) and third (151–180 DAT) harvesting periods, under the different nitrogen rates of cow manure application. It was also reported that the chlorophyll content of Boston fern was not influenced by the application of cow dung and urine, namely jeevamrit [61]. The current study also compared plots treated with chemical fertilizer and cow manure of the same nitrogen rate. Vegetable fern grown under the chemical fertilizer treatment produced higher chlorophyll content than those grown under the cow manure treatment (Table 4). This may be due to the immediate availability of macronutrients in chemical fertilizers, compared to cow manure, which can take several months to become available. Furthermore, when focusing on the combined effects of shading and fertilizer application, our study results revealed that chlorophyll production in vegetable fern shoots tended to be higher in higher shading conditions, with or without fertilizer application. It seemed, therefore, that chlorophyll production was influenced by light intensity more than fertilizer application, in line with a report by Putri et al. [62].

Low light intensity is thought to cause low concentrations of vitamin C in plant tissues, in agreement with the results of vitamin C concentrations in vegetable fern in this study, as well as other reports on tomato [63] and crisp lettuce [64]. This may be because vitamin C comprises L-ascorbic acid (AA), synthesized from sugar supplied by photosynthesis. Consequently, it seems logical that plants growing under intense light conditions would have a high vitamin C concentration. However, the influence of fertilizer on vitamin C concentrations in vegetable fern was not evident in the current study. It varied among the different harvesting periods, leading to unclear results when considering the factors of shading and fertilization (Figure 5). Although many studies have found *a* negative correlation between vitamin C concentration and nitrogen fertilizer supply [65,66], other reports have found the opposite, as was the case in a butterhead lettuce study [67]. Thus, the relationship between nitrogen supply and vitamin C concentration in plant tissues depends on crop type; causes of these effects may also be due to different species of plants studied [68].

Nitrate accumulation is strongly affected by light intensity because plants usually accumulate more nitrates when they are grown in low light-intensity conditions. In our study, nitrate contents observed in fresh-weight vegetable fern shoots were observed over 2500 mg NO^3 kg^{-1} (European Union limit for lettuce) under 96% shading in all the harvesting months. In addition, the use of chemical fertilizer resulted in the highest nitrate buildup in vegetable fern throughout the three harvesting months (Table 4). This was in line with the findings of a review by Nuñez de González et al. [69], which stated that nitrate buildup in yield was greatest when chemical fertilizers were used, as well as the results of Hassan et al. [70], which found that *Cosmos caudatus* grown in organic soil had more vitamin C than that grown in soil fertilized with chemical fertilizer. In contrast, chemical fertilizers resulted in more accumulated nitrates. One explanation could be that chemical fertilizers dissolve in water and release nitrogen more rapidly than cow manure. It was interesting to note, in this study, that nitrate accumulation was highest under the highest shading, combined with the application of chemical fertilizer, with the vegetable fern grown under 96% shading along with chemical fertilizer at a rate of 92.80 kg N ha^{-1} showing (significant) highest nitrate accumulation rates throughout the three harvesting months: 2925, 2950, and 3000 mg kg^{-1} fresh weight, respectively (Figure 6). This amount of nitrate largely exceeded the limits set by the European Union (EU) commission. However, the 75% shading, supplemented with cow manure fertilizer application at a rate of 185.60 kg N ha^{-1}, not only gave the highest total yield of vegetable fern from the three harvesting months, but also exhibited the lowest nitrate accumulation in plant tissues, which did not exceed the limits set by the EU.

5. Conclusions

Based on the impact of light shading and nutrition management on vegetable fern development and quantity, and quality of yield observed in this study, it was concluded that vegetable fern growth, production, and quality were influenced by the presence or absence of shade and by fertilization type and amount. Optimal growth required a shading level of 75% and fertilization with cow manure at a rate of 185.60 kg N ha^{-1}, all of which contributed to healthy vegetable fern development and the greatest monthly average production. The vegetable fern was crunchy and tender, maintaining compatibility with even the highest demands. They were also rich in vitamin C and contained nitrates in proportions acceptable for human consumption.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9020259/s1, Figure S1: The temperature of the microenvironment in the study area for vegetable production under different shading treatments.

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