

Evaluation of Different High Tunnel Protection Methods for Quality Banana Production in Bangladesh

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Citation: Shormin Choudhury, Nazrul Islam, Atiqur Rahman Shaon and Jamal Hossain (2023) Evaluation of different high tunnel protection methods for quality banana production in Bangladesh. *J Plant Sci Crop Protec* 6(1): 102.

Abstract

High tunnels can provide several benefits to horticultural crops, including environmental stress protection such as hail, frost, excessive rainfall, and high wind. In hot and sunny areas, high tunnel is one of the cooling ways for modifying the microclimate and maximizing crop development. Present study was carried out to assess the effect of different type of high tunnels on banana growth, yield, and fruit quality characteristics. Net houses, poly net houses, UV poly shed houses, and open field (control) conditions are among the experimental treatments. The results revealed that the plants produced in the poly net house condition had maximum pseudo stem height (171.00cm), stem girth (68.66 cm), chlorophyll content (57.63), number of fruits (140), number of hands (9.66), individual fruit weight (125.00) and pulp: peel ratio (3.35) of bananas as compared to the other treatments. Quality parameters like total soluble solid (21.78°Brix), ascorbic acid (10.24 mg/100g), total sugar (25.44%), and reducing sugar (15.75%) were higher in fruits grown in poly net house. The study revealed that the poly net house is the best growing environment for bananas in terms of growth, yield, and quality attributes.

Keywords: Shed houses; Banana; chlorophyll content; fruit yield; quality

Introduction

Food production through agricultural activities is crucial for our existence, but productivity might be increased with a better understanding of how changes in temperature, rainfall, and relative humidity (RH) affect agricultural output. It is one of the most popular fruit crops in Bangladesh and is consumed more frequently than any other fruit due to its year-round availability. Because they are readily available throughout the year, bananas contribute significantly to the reduction of poverty because they offer farmers and rural residents as well as dealers and retailers a steady source of income. The fruit has low-fat content and is a good source of vitamins, edible fiber, potassium, phosphorus, and calcium. Geographical location, productivity, and production period of various crops are determined by growth development and crop quality under a certain set of climate characteristics.

According to survey data of banana growers, one of the most significant elements determining the growth and productivity of bananas is the environment [1]. Because of its high unpredictability, the climate is the most essential component in agricultural production, as the atmosphere in which crops are grown can be changed by a protected environment. The conditions under which plants are grown must be measured, and environmental elements that influence fruit quality must be considered. Temperature and relative humidity are two environmental factors that have a significant impact. Temperature has a significant impact on banana development and growth. Banana growth and development are adversely affected by extreme, higher, and lower temperatures. A study German [2] found that the ideal temperature for banana growth and development is around 27 °C. High temperature stress alters plant physiological and biochemical responses, lowering crop quality and yield. Additionally, the growth of bunches is halted at temperatures higher than 38 °C or lower than 9 °C [3].

Fruits grown under shed house had substantially more total soluble solids, ascorbic acid, sugar and total phenol content [4]. By regulating the environment with the use of a greenhouse and employing various protected structures, such as plastic houses, lath houses, cloth houses, net houses, shed houses, etc., nearly ideal climatic conditions can be produced. The temperature behavior inside the high tunnel is critical because it affects metabolic activity, water and nutrition absorption, gas exchange, carbohydrate generation and expenditure, and growth regulators [5]. Temperature is a factor that has significant effect on chemical composition of the fruits [6]. It interferes in the formation of sugars, due to cell division and multiplication in the fruits, the alteration in the biosynthetic enzymatic activity of carbohydrates and the increase in the transpiration rate [7]. By encouraging the crops to grow before and after their natural growing season and extending their overall lifespan, higher than normal temperatures, controlled humidity, or increased artificially induced light levels under protection can increase yields and improve quality [8]. Protected cultivation practices can be defined as a cropping technique where in the micro-climate surrounding the plant body is controlled partially or fully as per the requirement of the crop species grown during their period of growth. The most practical way to fulfill the aims of protected agriculture is to adapt the natural environment using strong engineering concepts to enhance optimum plant growth and production with increased input usage efficiency.

Protected agriculture is now being expanded to boost agricultural productivity. There has been minimal research into banana cultivation under protected condition. To satisfy the growing demand for fruit crops, there is an urgent need to examine the cultivation and suitability of banana production, as well as quality measurement inside different shed houses. The experiment attempted to determine the efficacy of various shed house cultivation methods against open field methods on banana yield and quality.

Material and Methods

Plant Materials and Growing Conditions

This experiment was performed at the Horticulture farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh from February to October 2021. Dhaka is located at 23°42' 37" N (Latitude), 90°24' 26" E (Longitude), and has an average elevation of 4 meters (13.12 ft.) according to the National Mapping Organization of Bangladesh. The banana cv. BARI khola 1 was planted at 1.2 m

× 1.2 m spacing under four protected cultivation system, those are open field condition (control), net house (60 mesh), poly shed house (naturally ventilated polyhouse; entire roof and half the portion of four sides covered with poly sheet, the remaining half covered with 25% shed net) and UV poly shed house (Fan pad UV polyhouse; fully covered with UV film sheet) (Figure 1). The experiment was laid out with 4 numbers of treatments with 4 replications. During the experiment, all essential cultural practices and plant protection measures were followed uniformly for all the plots. In each replication, five plants were randomly selected for observations on fruit production, yield, and physio-chemical parameters. Three fruits from each plant and each treatment were harvested for various biochemical analyses. Temperature and relative humidity were recorded during the growing period in all environments, to monitor the actual environmental conditions in which the plants were grown.

Measurement of Growth Parameters

Five plants in each treatment and each replication were used for pseudo stem length, stem diameter, and leaf chlorophyll content at flowering stages. Plant height was measured from the base of the plant to the top of the plant. The stem diameter of each plant was measured from a height of 10 cm above the ground.

SPAD Chlorophyll Meter Reading

Leaf chlorophyll content was measured using a SPAD-502 chlorophyll meter on the first fully expanded leaves (Minolta, Tokyo, Japan). In each shed house, the measurements were obtained from the center of the lamina of five randomly selected plants.

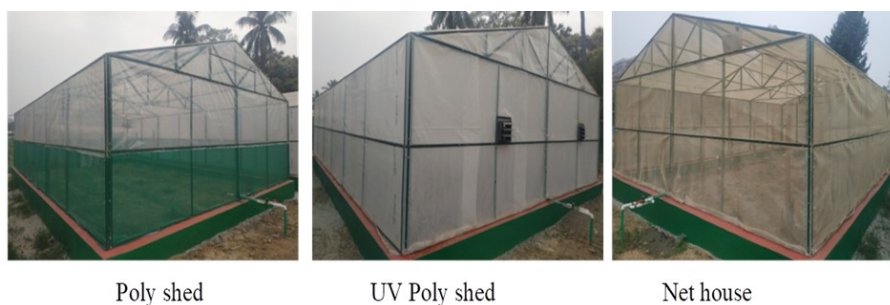


Figure 1: Banana grown in different high tunnel shed houses

Measurement of Yield Contributing Parameters

Days to flower initiation were recorded for all the selected five plants in each treatment. The number of hands/bunches, number of fingers/hands, number of fingers/bunch was recorded by counting the fruits that reached harvestable ripeness. The weight of individual fruits (g), fruit length, breadth, diameter and pulp: peel from each selected hand was taken with the help of an electronic top pan balance and slide calipers.

Measurement of Biochemical Parameters

Total Soluble Solids (TSS) Content

The TSS content of banana was measured by a digital refractometer (MA871; Romania). A drop of banana juice was obtained with a dropper and placed on the refractometer prism. The refractometer showed a reading of total soluble solids.

pH Determination

The fruit juices of individual treated strawberries were filtered separately, and pH was measured using a digital pH meter (HI 2211; Romania).

Titrateable Acidity (TA %)

The fruit samples (5g) were macerated by the mortar and pestle for the determination of the TA. After macerated, samples were filtered and added water to make 100 ml of volume. Then 10 ml of stock solution was taken in a conical flask and 2 drops of phenolphthelin were added. The solution was titrated three times with 1N NaOH. The titration was stopped until the pink color appeared.

Vitamin C Determination

The Vitamin C content of banana was calculated using the technique previously described by [9]. A 5gm banana fruit sample was blended, and the juice was sieved with filter paper (Whatman No. 1). The volume was made up to 100 ml by adding 5% oxalic acid solution. The titration was done with dye solution 2, 6-dichlorophenol indophenol. The mean observations provided the amount of dye required to oxidize an unknown concentration of a definite amount of L-ascorbic acid solution, using L-ascorbic acid standard. A 5ml solution was taken for titration each time, and the pink color determined the last point of titration, which remained for 10 seconds.

Total Sugar

Two banana pulps were divided into little bits, dropped into ethyl alcohol that was just beginning to boil, and left for 10 minutes (10 ml of alcohol was used per g of pulp). Using 2 to 3 ml of alcohol per g of tissue, the extract was re-extracted for 3 minutes in hot 80% alcohols after being filtered through two layers of cloth. After cooling, the extract was run through two layers of cotton. Through Whatman No. 41 filter paper, both extracts were purified. Over a steam bath, the volume of the extract was evaporated to 25% of the volume, and then cooled. This concentrated extract was then transferred to a 100 ml volumetric flask and filled to the appropriate level with distilled water.

Total sugar (%) = Amount of sugar obtained/ Weight of sample×100

Reducing Sugar

A 3ml aliquot of the extract was pipette into each test tube, along with 3ml of DNS reagent, which was thoroughly mixed together. The test tube was heated in a bath of boiling water for five minutes. When the contents of the tubes were still warm after the color had developed, 1ml of 40% Rochelle salt was added. Next, the test tubes were cooled under running tap water. 3 ml of distilled water and 3 ml of DNS reagent were combined in a tube to create a reagent blank, which was then handled similarly. The absorbance of the solution was measured at 575 nm in a colorimeter.

Reducing sugar (%) = Amount of reducing sugar obtained/ Weight of sample×100

Non-Reducing Sugar

Non-reducing sugar content of banana pulp was calculated by using the following formula:

% non-reducing sugar = % total sugar - % reducing sugar

Color Measurement

Using a Minolta Chroma meter (Model CR400) that was set up with a D65 illuminant and 10o observer angle, the colors of the banana skin were measured nondestructively. L*, a*, b*, and C* were used to represent the color values. The reading was programmed to require six random points on average for each fruit. To prevent light leakage from the colorimeter's light, the device

must be entirely in contact with the fruits.

Statistical Analyses

The experiments used a randomized complete block design (RCBD) with four replications for each treatment and five plants in each replicate. Statistical analyses were conducted with version 9.4 of the Statistical Analysis System (SAS) (SAS Institute, Cary NC, USA). The mean value among the treatments was statistically significant when $P < 0.05$. All results were presented with the mean standard error (SE) from the replicates.

Results and Discussion

Temperature and Relative Humidity (RH) Conditions

Under protected conditions, temperatures can be monitored and managed, and better plant growth could be expected. Different shed houses and open field conditions influenced the temperature. Data on the temperatures for each treatment was measured at 12 pm daily during the experimental period. The average monthly temperature varied approximately 27.28 to 36.80 °C, as shown in Table 1. In our experiment, it was found that air temperature in UV poly shed was always more than net poly shed, net house and open field condition. According to Varma and Bebbler [10], low or very high temperatures have negative impact on banana growth and development. A mean temperature of about 26 °C will lead to good annual banana production [11].

During the experimental period, the relative humidity for each treatment was measured at 12 pm daily. From February to October, the average monthly relative humidity ranges between 64.52 and 80% during the day (Table 1). Compared to other growing conditions the relative humidity was comparatively lower in UV poly shed condition. The lower relative humidity inside the UV poly house may be due to fully covered with UV film sheet. Relative humidity reduces evaporation loss from plants, resulting in optimal nutrient utilization and cell turgidity, which is useful in enzyme activity and leads to a higher yield [12]. A relative humidity level of approximately 77% was considered to be optimal for good yield of banana [11].

Month	12 hrs							
	Open field		UV Poly shed		Poly net house		Net house	
	Temp (°c)	RH (%)	Temp (°c)	RH (%)	Temp. (°c)	RH (%)	Temp. (°c)	RH (%)
February	27.28	68.15	28.24	68.03	26.80	69.65	26.09	70.01
March	32.31	70.52	33.14	68.11	31.25	74.15	30.55	77.19
April	34.5	70.10	36.05	67.25	33.08	72.32	30.61	73.87
May	32.08	80.25	34.19	75.63	31.02	79.42	29.62	75.53
June	32.03	75.00	34.00	70.21	31.56	79.12	30.78	80.53
July	32.84	74.20	36.80	70.41	31.21	75.65	32.29	70.82
August	31.53	72.03	35.06	71.32	30.22	74.78	31.61	72.97
September	32.53	72.09	34.08	66.25	32.27	74.13	31.34	75.93
October	31.70	70.04	33.25	68.42	31.04	70.25	30.27	70.13
November	29.43	70.95	30.93	68.07	28.19	72.53	27.77	73.19

Table 1: Monthly average air temperature (°C) and relative humidity (%) at 12 hrs in different shed houses and open field during February to October 2021.

Growth Parameters

The pseudo stem height and girth were influenced significantly with different growing environments (Table 2). The highest pseudo stem height (175.67 cm) was recorded in net house condition whereas the girth size (68.66 cm) were recorded in poly net house condition at harvesting stage and the lowest pseudo stem height (151.67 cm) and the pseudo stem girth (51.00 cm) were recorded in open field condition [13], observed that the girth of a banana pseudostem was significantly reduced in the open field compared to the shade house. [14] also conducted an experiment with green house and open field condition on capsicum annum cv. California Wonder. The growth characters, like plant height, number of primary branches, number of leaves, number of fruits per plant, length of fruits and girth of fruits found significantly better under green house as compared to open field condition.

Banana grown in different protected condition had shown significant effect on leaf chlorophyll content at harvesting stage (Table 2). The highest leaf chlorophyll content (57.63 %) was recorded in poly net house condition. Whereas the lowest leaf chlorophyll content (40.46 %) was recorded in open field condition. Shed net house modify the light concentration which affect the chlorophyll concentration. The leaves grown under poly net house reduced levels of light and contain more chlorophyll than leaves grown under the open conditions. The reason for the more chlorophyll in the leaves grown under poly net house could be attributed to the purpose of capture of scattered radiation which ultimately is needed for the plant growth by the production of the carbohydrates. Shaded leaves typically contain more total chlorophyll than control leaves (open field). Although shade-grown leaves do not receive direct sunlight, they produce more chlorophyll to capture diffuse radiation and produce the carbohydrates required for plant growth [15]. [16] reported that the chlorophyll content of shaded strawberry plants was 1.3 times higher than that of unshaded plants.

Different sheds	Pseudo stem length (cm)	Stem diameter (cm)	Chlorophyll content
Open space	151.67 b	51.00 c	40.46 c
UV poly shed	164.00 ab	65.66 ab	45.73 bc
Poly net house	171.00 a	68.66 a	57.63 a
Net house	175.67 a	60.66 b	51.23 ab
LSD _{0.05}	16.05	6.24	6.90
CV%	5.15	5.39	7.52

Table 2: Growth parameters of banana grown under different sheds and open field condition. Mean \pm S.E. (n = 15). Means with the same letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Yield Contributing Parameters

The early emergence of inflorescence (5.85 months) was recorded when fruits were grown in UV poly shed condition, followed by poly net house growing condition (6.26 months), while banana grown in open field condition took 7.16 months to take inflorescence emergence (Table 3). However, it was discovered that plants cultivated in a greenhouse began to bloom 6.9–7.0 months after planting, whereas plants grown outdoors began to bloom 9.2–10.10 months after planting. Likewise, [17] showed that fruit maturation length in greenhouse-grown bananas was shorter compared open-field cultivation.

Days required to harvesting after fruit setting was significantly influenced by different growing environments (Table 3). The minimum harvesting time (3.55 months) was recorded for the poly net house condition, and the maximum harvesting time (4.30 months) was recorded for the control (open space condition). It was observed that open space condition delayed the harvesting time over the poly net house condition. The highest bunch length (1st hand to last hand) (85.33 cm) was recorded when the fruits were grown in poly net house condition and the lowest (64.00 cm) was recorded when fruits grown in open field condition. It was noted that spikes were observed later in open field condition but it required less periods in greenhouse, and harvesting time after

spikes presence was much less than cultivation in open field condition [18].

The maximum number of hands per bunch (9.66) was observed in poly net house condition and the minimum number of hands per bunch (7.33) was found in open space condition. Maximum number of fingers/bunch (140) was recorded in poly net house protected condition and minimum number (93) was recorded in open field condition (Figure 2 & Table 3). The number of fruits was significantly higher in the poly net house, which could be attributed to better environmental conditions, particularly higher relative humidity and moderate temperature, which may have aided in maintaining pollen viability and preventing desiccation of pollen on the stigmatic surface.

The maximum number of fingers hand⁻¹ (14.50) was observed in poly net house condition and the minimum No. of fingers hand⁻¹ (12.67) was found in open space condition (Table 3). Plants planted under poly house conditions produced more fruits (38) with greater length (4.4 cm), diameter (5.4 cm), and fruit weight (68 g) than those grown in the open field. Kaur and Kaur [19] investigated the performance of Red Lady papaya grown under protected and open field conditions, discovering that protected cultivation improved flowering, fruiting, and yield. Altinkaya and Gubbuk. [20]. Found that Yield components of bananas were found to be better that grown under protected cultivation compared to those grown in open-field cultivation.



Figure 2: Banana fruits grown in different high tunnel shed houses (a: open field, b: UV poly shed, c: poly net house, d: net house).

Different sheds	Inflorescence initiation (month)	Inflorescence initiation to harvesting (month)	1st hand to last hand length (cm)	No. of hands/bunch	Total no. of fingers/bunch	No. of fingers/hand
Open space	7.66 a	4.30 a	64.00 c	7.33 b	93.00 c	12.67 b
UV poly shed	5.85 c	3.95 b	78.00 b	9.33 a	125.67 b	13.48 ab
Poly net house	6.26 b	3.55 c	85.33 a	9.66 a	140.00 a	14.50 a
Net house	6.56 b	3.57 c	68.00 c	8.66 a	122.67 b	14.20 a
LSD _{0.05}	0.374	0.232	4.92	1.087	12.37	1.532
CV%	3.08	3.21	3.54	6.60	5.46	5.94

Table 3: Yield contributing parameters of banana grown under different sheds and open field condition. Mean \pm S.E. (n = 15). Means with the same letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Yield Parameters

Fruit length varied significantly between treatments. The fruits produced in poly net house conditions had the longest fruit length (16.17 cm), whereas net house conditions had the shortest (14.41 cm). Maximum breadth of the banana fruit (3.46 cm) was recorded in poly net house growing condition and the minimum fruit breadth (2.89 cm) was recorded in open field condition.

Different growing environments also positively influenced the diameter and fruit breadth of banana finger and the effect different growing condition was significant. Among the different growing conditions, UV shed condition produced the highest diameter of finger (12.13 cm) followed by the poly net house (12.08 cm) and the lowest diameter (11.52 cm) was recorded for open field condition. The maximum individual fruit weight (125.00 g) was observed in poly net house condition and the minimum average fruit weight (102.18g) was found in open space condition. According to Darini [21] indicated that compared to open field conditions, banana yield and spike weight increased dramatically in the greenhouse.

The poly net house condition produced the highest pulp/peel ratio (3.35), whereas the open field condition produced the lowest (2.21). Because the pulp/peel ratio in banana fruit has previously been demonstrated to rise with ripening, the higher pulp/peel ratio at higher temperature and low humidity shows that these bananas were at a more advanced stage of ripening [22]. The greater weight loss could be the second cause for the higher pulp/peel ratio at higher temperatures and lower humidity. Because the pulp mass of banana fruit grows during ripening due to an increase in water content, this could be related to water migration from peel to pulp and into the surrounding air [23].

Different shed	Fruit length (cm)	Fruit breadth (cm)	Fruit diameter (cm)	Individual fruit weight (g)	Pulp: peel
Open space	15.66 b	2.89 d	11.52 b	102.18 d	2.21 c
UV shed	15.50 b	3.24 b	12.13 a	117.50 b	3.18 a
Poly net house	16.17 a	3.46 a	12.08 a	125.00 a	3.35 a
Net house	14.41 c	3.13 c	11.68 b	109.80 c	2.97 b
LSD _{0.05}	0.404	0.079	0.236	6.22	0.189
CV%	1.39	1.33	1.06	2.91	3.44

Table 4: Yield contributing parameters of banana grown under different sheds and open field condition. Mean \pm S.E. (n = 15). Means with the same letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Measurement of Biochemical Parameters

The data presented in table 4 showed the quality character viz., TSS, TA, pH, reducing sugar, non-reducing sugars, total sugars in banana which were affected due to various growing environments. All these parameters had significant difference through various treatments.

The maximum total soluble solids (21.78%) was found in poly net house growing condition and minimum total soluble solids (TSS) (16.77%) was found in UV poly shed condition. The decrease in TSS during the season has been related to an increase in temperature, which causes an increase in respiration rate and, as a result, a decrease in the concentration of sugars, organic acids, and soluble solids in the fruits [24, 25].

The maximum titratable acidity (TA) (0.57%) was found in net house growing condition followed by UV poly shed condition (0.55%) and TA (0.40%) was found in poly net shed condition. The highest pH (5.64) was recorded in poly net house condition and the lowest p^H (4.92) was recorded in UV poly shed condition. Lower pH and higher titratable acidity in uv poly shed due to higher temperature. Similar result was found by [26] who reported that the lower TSS (total soluble solids) and pH of Caber-

net-Sauvignon grapes were in high temperature than in the low temperature. Higher total acidity and berry weight was recorded under low temperatures than in the high temperature. [27] concluded that the increase in TSS content during proper maturation under net poly shed house could be attributed to starch degradation and quick metabolic transformation in soluble compounds, primarily sugars, as a result of the favorable micro-climatic conditions [27]. The decreased acidity of fruits cultivated in sheltered environments may be due to the plant's lower photosynthetic activity (shading in protected environments).

The ascorbic acid content of banana was significantly affected by different protected condition. The highest ascorbic acid content of banana (10.24 mg/100 g) was recorded in poly net house condition. Whereas the lowest titratable ascorbic acid content (8.96 mg/100 g) of banana was recorded in net house protected condition. [28] reported that the cultivation systems can have a greater influence on the chemical composition of the strawberry fruits. [29] also reported that compared to open-field fruits, protected--condition capsicum fruits showed greater ascorbic acid and total soluble solids (TSS).

The maximum total sugar (25.44%), reducing sugar (15.5%) and non-reducing sugar were recorded in poly net house growing environment, whereas the minimum total sugar (21.17%) and non-reducing sugar (7.87%) were recorded in open space condition. Higher sugar content in poly net house fruits may be attributable to increased light intensity and photosynthetic plant activity in this crop environment. The yield and quality of bananas grown under greenhouses are both desirable [30].

Different sheds	TSS	TA%	pH	Vitamin Cmg/100g	Total sugar	Reducing sugar	Non-reducing sugar
Open space	20.89 a	0.43 b	5.18 b	9.38 ab	21.17 c	13.30 c	7.87 b
UV shed	16.77 c	0.55 a	4.92 c	9.17 b	21.76 c	12.17 d	9.59 a
Poly net house	21.78 a	0.40 b	5.64 a	10.24 a	25.44 a	15.75 a	9.68 a
Net house	18.33 b	0.57 a	5.27 b	8.96 b	23.01 b	14.21 b	8.79 ab
LSD _{0.05}	0.992	0.059	0.181	0.983	0.812	0.853	1.154
CV%	2.71	6.45	1.83	5.54	1.89	3.27	6.82

Table 5: Quality parameters of banana grown under different sheds and open field condition. Mean \pm S.E. (n = 15). Means with the same letter are not significantly different at P = 0.05 by Duncan's multiple range test.

Color Measurement

Significant changes in color values (L*, a*, b*) during ripening for the period of were presented in table 5 & figure 3. It can be observed from the table, that the lightness value (L* value) and yellowness (b* value) increased during the ripening. This might be caused by the breakdown of chlorophyll, which then makes the yellow carotenoid pigments visible [31]. The highest L* and a* value was recorded in fruits of poly net house condition and the lowest value of L and b value was recorded in fruits in UV poly shed conditions. The lower value of L and b might be due to the development of brown flecks over the peel, spots were observed by [32] on ripening the banana. The maximum a value (greenness) was recorded in fruits of UV poly shed conditions. Banana fruit's greenness value increased from negative to positive. This confirms that banana peel has been de-greened. Similar results were reported by [33].



Figure 3: Skin color of banana fruits grown under different high tunnel shed houses (a: open field, b: UV poly shed, c: poly net house, d: net house).

Different sheds	Color measurement		
	L*	a*	b*
Open space	68.37 a	7.14 b	47.56 ab
UV shed	62.0 b	9.79 a	43.36 c
Poly net house	68.87 a	3.98 c	49.20 a
Net house	62.16 b	3.41 c	45.33 bc
LSD _{0.05}	4.74	0.77	3.351
CV%	3.86	6.74	3.84

Table 6: Average color values of banana grown under different sheds and open field condition.

Conclusion

Our results revealed that high tunnel protective cultural environment is best suited and beneficial for the growth, yield and quality of banana. Fruits grown under poly net house had substantially more total soluble solids, vitamin C and sugar content. Furthermore, in the poly net house, the fruit peel color, yield, and yield contributing parameters of the banana were higher. From the present investigation it is concluded that banana can be grown successfully in a poly net house with improved plant growth, fruit weight, color, yield, and quality.

Author Contributions

Conceptualization N.I., and S.C.; investigation, S.C., and A.R.S.; writing—original draft preparation, S.C.; writing—final submission, N.I., S.C. and M.J.H. revisions and responses to reviewers. All authors have read and agreed to the published version of the manuscript.

Funding

This project was supported by National Agricultural Technology Program Phase-II Project (NATP-2), Bangladesh.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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