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# The Effect of Two Concentrations and Three Applications of Nitrobenzene on Growth and Yield of Bottle Gourd (*Lagenaria siceraria* L.)

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#### Authors' contributions

This work was carried out in collaboration among all authors. Authors TM and KK planned the experiment and lead the research. Authors MAS, TM and KK designed and carried out the research. Authors MAS and MEH performed the statistical analysis. Authors MAS and MA carried out the research on the field. Authors SSR, KF and JIJ collected the data. Authors MAS and MEH wrote the manuscript. Authors MA, SSR, KF and JIJ managed the literature searches. All authors provided critical feedback, helped shape the research, analysis, read and approved the final manuscript.

#### Article Information

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## **ABSTRACT**

The experiment was conducted in the Horticultural Farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh with aim to assess the influence of two concentrations and three applications of nitrobenzene in relation to control ( $C_0$ ) without nitrobenzene on the growth and yield of bottle gourd. Two concentrations of nitrobenzene applied were: @ 2 ml/L referred as  $C_1$  and 3 ml/L referred as  $C_2$  in relation to a control ( $C_0$ ) with water instead of nitrobenzene. Further, the above treatments were applied number of times, e.g. One spray ( $N_1$ ), Two spray ( $N_2$ ) and Three spray ( $N_3$ ). In case of different number of concentrations of nitrobenzene the maximum number of male flower (51.06), the maximum number of female flower (40.18), the highest number of fruit harvested per plant (10.95), the highest yield (55.69 t/ha) was found from  $C_1$  treatment, whereas the lowest from  $C_0$ 

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treatment. For the number of sprays the highest number of flowers (male 49.02 and female 38.05), the maximum number of fruit per plant (10.32), the highest yield (52.94 t/ha) was recorded from  $N_3$  treatment, while the minimum from  $N_1$  treatment. Due to combined effect, the maximum number of flower (male 57.78 and female 47.12), the maximum number of fruit harvested per plant (12.55), the highest yield (63.38 t/ha) with net income (Tk. 587384.7) and BCR (2.23) was observed from  $C_1N_3$  treatment combination, while the lowest from  $C_0N_1$  treatment combination. So, the economic analysis revealed that the  $C_1N_3$  treatment combination appeared to be the best for achieving the maximum growth, yield and economic benefit of bottle gourd.

Keywords: Application; benefit-cost ratio; bottle gourd; nitrobenzene; yield.

#### 1. INTRODUCTION

Bottle gourd (Lagenaria siceraria L.) is one of the most important vegetables grown worldwide. It belongs to family Cucurbitaceae originated from wild populations in southern Africa [1]. The bottle gourd can be easily distinguished from other pumpkin varieties by its white flowers and characteristic fruit, seed and leaf shapes [2]. Tropics and subtropics areas are the best for its cultivation and it is one of the most favorite and crucial vegetable crops grown in Bangladesh both in the Rabi and Kharif season. Bottle gourd is one of the most highly praised vegetables consumed widely and it is a major source of vitamins and minerals [3]. It is reported as an easily digestible vegetable, which keeps the body cool and prevents constipation [4]. It is good for people suffering from indigestion and biliousness [5]. The total production of bottle gourd 232000 ton during the year 2017-2018 [6]. The yield of vegetables in our country is not satisfactory in comparison to our requirement. Bottle gourd is an annual, tendril bearing and cross-pollinated crop due to its monoecious nature. Cross pollination occurs from 60 to 80%. Sex expression and sex ratio in cucurbits are important factors for governing the yield. All the cultivars of cucurbits differ in production of pistillate flowers. It is a tendency of all the cucurbits to produce more number of male flowers and less number of pistillate and hermaphrodite flowers. The exogenous application of plant growth regulators has proved to be quite useful in the regulation of flowering and fruiting. Plant growth regulators play a key role in controlling internal mechanisms of plant growth by interacting with key metabolic processes such as nucleic acid metabolism and synthesis. Different plant regulators are auxins, gibberellins, nitrobenzene, cytokinins, abscisic acid (ABA) and ethylene, etc. Among them, nitrobenzene (20% w/w) plays an important role for higher growth and yield of vegetables. 'Flora' is a commercially available plant growth substance containing 20% (w/w)

nitrobenzene. Nitrobenzene 20% used to repair the hormonal function of a plant thus promotes the flowering activity and growth of roots [7,8]. The application of nitrobenzene produces the highest number of fruits and flowers per plant [9,10]. Nitrobenzene is quickly absorbed into the plants. It influences the bio-chemical pathway of the plants to uptake more nutrients from the soil. It also increases the nutrient use efficiency thus improves the vegetative growth. Induces profuse flowering and helps in the retention of the flowers and fruits [9]. Nitrobenzene spray resulted in a high number of inflorescences in Jatropha [11].

Besides the concentration of nitrobenzene, number of the spray of growth regulators have effect on the production. Four sprays of nitrobenzene during 40, 55, 80 and 105 days after sowing (DAS) improve the yield up to 40% [12]. Number of spray of plant growth regulator play an important role for producing maximum yield [12]. Unfortunately, very limited researches have been carried out regarding the use of growth the effect of various concentrations and the number of the spray of nitrobenzene on bottle gourd for higher growth and yield. A detailed and systematic study is needed to find out the suitable concentration, optimum number of spray and suitable combination concentration of nitrobenzene and the number of spray for maximum growth, yield and economic benefit of bottle gourd in Bangladesh.

## 2. MATERIALS AND METHODS

# 2.1 Experimental Site and Experimental Framework

The research was conducted at the Horticultural Farm of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 and Bangladesh during the period of October 2018 to March 2019. During the rabi season the experiment was carried out. The location of the experimental site is situated at 90°22'E longitude and 23°41'N latitude. The altitude of 8.6 meters

above sea level. The soil was having a texture of sandy loam with pH and organic matter 5.47 -5.63 and 0.83%, respectively two concentrations of nitrobenzene applied were: @ 2ml/L referred as C1 and 3 ml/L referred as C2 in relation to a control (C<sub>0</sub>) with water instead of nitrobenzene. Further, the above treatments were applied number of times, e.g. One spray (N<sub>1</sub>), Two spray  $(N_2)$  and Three spray  $(N_3)$ . The two factors experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications where the experimental area was divided into three equal blocks representing the replications to minimize the soil heterogeneous effects. The length of the experimental area 24 m and width 15 m and the total area of the experimental plot was 360 m<sup>2</sup>. The total area is divided into three equal blocks. Each block was divided into 9 plots where 9 treatments combination were allotted at random. There was a total of 27 unit plots in the experiment. The size of each plot was 4 m × 2 m. The distance maintained between two blocks and two plots were 1 m and 0.5 m, respectively. Both the row to row and plant to plant distances were 2 m and 2 m, respectively.

# 2.2 Planting Materials and Nitrobenzene (20% W/W)

The seeds of variety BARI Lau-3 were collected from the Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Flora was used as the source of nitrobenzene (20% w/w), Flora is a product of ACI Formulations Ltd. which contain nitrobenzene (Nitrobenzene 20% w/w). It was collected from ACI Formulations Ltd.

# 2.3 Preparation and Application of Nitrobenzene

To prepare the solutions, 2 ml and 3 ml flora were mixed with 1 L of water in a separate container. No nitrobenzene was applied on  $C_0N_1$ ,  $C_0N_2$ ,  $C_0N_3$  plots. Nitrobenzene at 2ml/L concentration was applied on  $C_1N_1$  plot at 20 DAT with the help of sprayer. Nitrobenzene at 2 ml/L concentration was applied on  $C_1N_2$  plot at 20 DAT and 40 DAT with the help of sprayer. Nitrobenzene at 2 ml/L concentration was applied on  $C_1N_2$  plot at 20 DAT, 40 DAT and 60 DAT with the help of sprayer. Nitrobenzene at 3 ml/L concentration was applied on  $C_2N_1$  plot at 20 DAT with the help of sprayer. Nitrobenzene at 3 ml/L concentration was applied on  $C_2N_1$  plot at 20 DAT with the help of sprayer. Nitrobenzene at 3 ml/L concentration was applied on  $C_2N_2$  plot at 20 DAT and 40 DAT with the help of sprayer.

Nitrobenzene at 3 ml/L concentration was applied on  $C_2N_3$  plot at 20 DAT, 40DAT and 60 DAT with the help of sprayer.

# 2.4 Economic Analysis

The cost of production was calculated to find out the most economic combination of the concentration of nitrobenzene and the number of sprays. All input cost like the cost for land lease and interest in running capital was computing in the calculation. The interests were calculated @ 13% at a simple rate. The market price of bottle gourd was considered for estimating the return. Analyses were done according to the procedure of Gomez and Gomez [13]. The benefit-cost ratio (BCR) was calculated as follows:

BCR = 
$$\frac{\text{Gross return per hectare (Tk)}}{\text{Total cost of production per hectare (Tk)}} \times 100$$

# 2.5 Statistical Analysis

The recorded data on different parameters were statistically analyzed using Statistic 10 software. The significance of the difference among the treatments means was estimated by the least significant difference test (LSD) at 5% level of probability.

## 3. RESULTS AND DISCUSSION

#### 3.1 Vein Length (M)

Significant influence was not found in terms of vine length of bottle gourd at 20 days after transplanting (DAT) affected by different concentrations of nitrobenzene but later at 40, 60 and 140 DAT there were significant differences in vine length (Table 1). At 140 DAT the highest vine length (12.23 m) was observed in C<sub>2</sub> (Nitrobenzene @ 3ml/L) treatment while the lowest vine length (8.45 m) was found in C<sub>0</sub> (control) treatment. At 20 DAT there were no significant differences because the first dose of nitrobenzene was applied at 20 DAT. It revealed that nitrobenzene increased plant height, which might be due to the regulating effect of exogenous application of nitrobenzene. Several authors [8-10,14-16] observed a similar trend of results. They opined that the application of nitrobenzene increases plant height. Vine length of bottle gourd was not varied significantly for the different numbers of spray at 20 and 40 DAT but vine length varied significantly at 60 and 140 DAT (Table 2). At harvest (140DAT), the highest vine length (11.30 m) was performed by  $N_3$ (three spray) treatment while N<sub>1</sub> (one spray) treatment gave the lowest vine length (9.51 m). There were no significant differences at 20 and 40DAT because the first dose of nitrobenzene was applied at 20 DAT and at 40 DAT all treatment plots get only one spray. Significant variation was not observed due to the combined effect of the plant on different concentrations and the number of sprays in terms of vine length of bottle gourd at 20 DAT (days after transplanting) but vine length varied significantly at 40, 60 and 140 DAT (Table 3). At harvest (140 DAT), the highest vine length (13.28 m) was observed in treatment combination  $C_2N_3$  and lowest vine length (8.1 m) was observed in  $C_0N_1$  treatment combination which is statistically similar to  $C_0N_3$  treatment combination.

# 3.2 Number of Leaves per Plant

Significant influence was not found in terms number of leaves per plant of bottle gourd at 20 days after transplanting (DAT) affected by different concentrations but later at 40, 60 and 140 DAT there were significant differences in the number of leaves per plant (Table 1). At harvest (140 DAT), the highest number of leaves per plant (333.56) was observed in  $C_2$  (Nitrobenzene @ 3 ml/L) treatment while the lowest number of leaves per plant (269.44) was found in  $C_0$  (control) treatment [14,15] recorded that application of nitrobenzene increases leaf number. The number of leaves per plant of bottle

gourd was not varied significantly for different numbers of spray at 20 and 40 DAT but the number of leaves per plant varied significantly at 60 and 140 DAT (Table 2). At harvest (140DAT) the highest number of leaves per plant (322.50) was performed by N<sub>3</sub> (three spray) treatment while N<sub>1</sub> (one spray) treatment gave the lowest number of leaves per plant (283.22). There were no significant differences at 20 and 40 DAT because the first dose of nitrobenzene was applied at 20 DAT and at 40 DAT all treatment plots got only one spray. Significant variation was not observed due to the combined effect of different concentrations and the number of spray on numbers of leaves per plant of bottle gourd at 20 DAT (days after transplanting) but the number of leaves per plant varied significantly at 40, 60 and 140 DAT and (Table 3). At harvest (140 DAT), the highest number of leaves per plant (365.33) was observed in treatment combination C<sub>2</sub>N<sub>3</sub> treatment combination and the lowest number of leaves per plant (264.17) in C<sub>0</sub>N<sub>1</sub> treatment combination. The treatment effect was statistically identical to C<sub>0</sub>N<sub>2</sub> and C<sub>0</sub>N<sub>3</sub> treatment combination. The results of the present study indicated that combined effect of different concentrations and number of combinations induced better growing condition which ultimately led to the production of more leaves per plant.

Table 1. Effect of different concentrations of nitrobenzene on vine length and number of leaves per plant at different days after transplanting of bottle gourd

Treatment	Vine length (m)				Number of leaves per plant			
	20	40	60	140	20	40	60	140
	DAT	DAT	DAT	DAT	DAT	DAT	DAT	DAT
C <sub>0</sub>	0.28	0.93 c	2.14 c	8.45 c	9.50	14.83 b	40.61 c	269.44 c
$C_1$	0.27	1.18 b	3.03 b	11.10 b	9.44	18.22 a	49.68 b	310.00 b
$C_2$	0.28	1.30 a	3.41 a	12.23 a	9.11	19.61 a	54.88 a	333.56 a
LSD(0.05)	0.0390 <sup>NS</sup>	0.0594	0.1123	0.3247	1.5426 <sup>NS</sup>	2.0328	0.9746	8.6419
CV%	13.84	5.24	3.95	3.09	16.65	11.69	2.03	2.86

Table 2. Effect of different number sprays of nitrobenzene on vine length and number of leaves per plant at different days after transplanting of bottle gourd

Treatment	Vine length (m)				Number of leaves per plant			
	20	40	60	140	20	40 DAT	60 DAT	140
	DAT	DAT	DAT	DAT	DAT			DAT
N <sub>1</sub>	0.28	1.14	2.57 b	9.51 c	9.38	17.33	43.29 b	283.22 c
$N_2$	0.29	1.15	3.04 a	10.96 b	9.05	18.50	51.27 a	309.28 b
$N_3$	0.27	1.12	2.97 a	11.30 a	9.61	16.83	50.61 a	322.50 a
LSD(0.05)	0.0390 <sup>NS</sup>	0.0594 <sup>NS</sup>	0.1123	0.3247	1.5426 <sup>NS</sup>	2.0328 <sup>NS</sup>	0.9746	8.6419
CV%	13.84	5.24	3.95	3.09	16.65	11.69	2.03	2.86

Table 3. Combined effect of different concentrations and number of sprays of nitrobenzene on vine length and number of leaves per plant at different days after transplanting of bottle gourd

Treatment		Vine len	gth (m)		N	umber of lea	ives per p	lant
combina-	20	40	60	140	20	40	60	140
tion	DAT	DAT	DAT	DAT	DAT	DAT	DAT	DAT
$C_0N_1$	0.30	0.93 d	2.06 e	8.10 h	10.83	15.50 cd	38.33 h	264.17 f
$C_0N_2$	0.27	0.97 d	2.15 e	8.72 g	9.16	15.16 cd	42.33 g	270.83 f
$C_0N_3$	0.26	0.91 d	2.23 e	8.54 gh	8.5	13.83 d	41.16 g	273.33 f
$C_1N_1$	0.23	1.15 c	2.75 d	9.71 f	8.17	17.16 bcd	44.37 f	288.83 e
$C_1N_2$	0.31	1.21 bc	3.22 c	11.51 d	9.16	19.16 ab	51.33 d	312.33 d
$C_1N_3$	0.28	1.19 bc	3.13 c	12.09 c	11.00	18.33 abc	53.33 c	328.83 c
$C_2N_1$	0.30	1.35 a	2.91 d	10.74 e	9.16	19.33 ab	47.16 e	296.67 e
$C_2N_2$	0.29	1.28 ab	3.77 a	12.65 b	8.83	21.16 a	60.16 a	344.67 b
$C_2N_3$	0.25	1.27 ab	3.54 b	13.28 a	9.33	18.33 abc	57.33 b	365.33 a
LSD(0.05)	0.0768 <sup>NS</sup>	0.1028	0.1944	0.562	2.8518 <sup>NS</sup>	3.5209	1.688	14.968
CV%	13.84	5.24	3.95	3.09	16.65	11.69	2.03	2.86

In column, means with a same small letter (s) are not significantly different and those with different small letters are significantly different (LSD at 5% level of significance). Where,  $C_0$  = Control (Water only),  $C_1$  = Nitrobenzene @ 2 ml/L,  $C_2$  = Nitrobenzene @ 3 ml/L. Where,  $N_1$  = One spray,  $N_2$  = Two spray,  $N_3$  = Three spray

# 3.3 Number of Primary Branches per Plant at Harvest

The number of branches per plant showed significant differences due to the application of different concentrations of nitrobenzene (Table 4). At harvest (140 DAT) the maximum number of branches (19.99) was found from C2 (Nitrobenzene @ 3ml/L) treatment while the minimum number of branches (14.82) was counted from C<sub>0</sub> (Control) treatment. Chowdhury et al. [16] found that application of nitrobenzene in rice increases tiller number. This result is in agreement with the findings of Pandita et al. [17]. The application of the different number of sprays of nitrobenzene showed significant variations on the number of branches per plant (Table 5). At harvest (140 DAT) the maximum number of branches (19.03) was found in N<sub>3</sub> (Three spray) treatment while the minimum number of branches (15.44) was recorded in N₁ (One spray) treatment. The result also indicated that the increasing rate of the number of sprays significantly increased the number of branches. The combined effect of different concentrations and the number of sprays performed a wide range of variations on the number of branches per plant at harvest (Table 6). At harvest (140 DAT) the highest number of the branch (22.64) was counted from C<sub>2</sub>N<sub>3</sub> treatment combination while the minimum number of branches per plant (14.02) was found from C<sub>0</sub>N<sub>1</sub> treatment combination.

#### 3.4 Number of Female Flowers per Plant

The recorded data on number of female flowers per plant was significant with different

concentrations of nitrobenzene (Table 4). The result showed that the maximum number of female flowers (40.18) was found from treatment C<sub>1</sub> (Nitrobenzene @ 2 ml/L). On the other hand minimum number of female flowers (27.15) was found from C<sub>0</sub> (Control) treatment. The number of female flowers per plant showed statistically significant impact due to different number of spray of nitrobenzene application in bottle gourd cultivation (Table 5). Due to influence of different number of spray of nitrobenzene the maximum number of female flowers per plant (38.05) was recorded in N<sub>3</sub> (three spray) treatment while the minimum number of female flowers per plant (29.50) was observed in  $N_1$  (one spray) treatment. This might be due to that nitrobenzene helped in proper reproductive development in bottle gourd. Remarkable variation was found on number of female flowers per plant influenced by combination of different concentrations and number of spray of nitrobenzene (Table 6). It was verified that maximum number of female flowers per plant (47.12) was recorded from the treatment combination of C<sub>1</sub>N<sub>3</sub>. On the other hand, the minimum (25.16) number of female flowers per plant was recorded from combination treatment of C<sub>0</sub>N<sub>1</sub>, which is statistically similar to C<sub>0</sub>N<sub>2</sub> treatment combination. Nitrobenzene can be used as spray or in granular form, which increases flowers forming substances by altering auxin, cytokinin, gibbrellic acid and ethylene ratio favourably tilting to a higher level of flowers forming substances, thereby increasing flowers by more than 40 to 45% [18]. Nuruzzaman et al. [15] also found that plants treated with nitrobenzene more flowers produce strawberry. They found that application of nitrobenzene produce number of flowers in strawberry over control.

# 3.5 Days Required to First Male Flowering

Different concentrations of nitrobenzene had a significant influence on days required to first male flowering of bottle gourd (Table 7). It was observed that maximum days (69.95 days) to first male flowering was required in C2 (Nitrobenzene @ 3 ml/L)) treatment whereas minimum days to first male flowering (58.03 days) was required in C1 (Nitrobenzene @ 2 ml/L) treatment. This is might be due to the regulating effect of exogenous application of nitrobenzene that influences early floral initiation. [19-21] they stated that, PGRs promotes vegetative growth, increases the photosynthetic and metabolic activities causing more transport and utilization of photosynthetic products resulting early flowering in different plants. vegetative Nitrobenzene promote growth increases the photosynthetic and metabolic activities causing more transport and utilization of photosynthetic products resulting in early flowering in different plants [10,22]. Ahmad et al. [23] also found that those JP-27 cherry tomato plants required less time to flowering which are treated with nitrobenzene over control. The data on days required to first male flowering was found to be significant in terms of the different number of sprays of nitrobenzene on bottle gourd (Table 8). Results exposed that maximum days (65.75 days) to first male flowering were required in N₁ (one spray) treatment whereas minimum days (61.14 days) to first male flowering was required in N<sub>3</sub> (three spray) treatment which is statistically identical to N<sub>2</sub> (two spray) treatment. Significant variation was remarked on days to first male flowering influenced by the combination of different concentrations and the number of spray of nitrobenzene on bottle gourd (Table 9). It was observed that maximum (71.88 days) to first male flowering were required C<sub>2</sub>N<sub>3</sub> treatment combination whereas minimum days (53.16 days) to first male flowering were required from C<sub>1</sub>N<sub>3</sub> treatment combination.

# 3.6 Days Required to First Female Flowering

The days required to first female flowering showed a significant difference for different concentrations of nitrobenzene application (Table 7). Due to application of different concentrations of nitrobenzene, the maximum days required to

first female flowering (70.83 days) was recorded in C<sub>2</sub> (Nitrobenzene @ 3 ml/L) treatment while the minimum day to first female flowering (61.88 days) was recorded in C1 (nitrobenzene @ 2ml/L) treatment. Application of the different number of spray on bottle gourd showed a significant effect on days to first female flowering (Table 8). The maximum days required to first female flowering (67.73 days) was found in N<sub>1</sub> (One spray) treatment while the minimum days to first female flowering (65.05 days) was recorded in N<sub>3</sub> (three spray) treatment which is statistically identical to N<sub>2</sub> (two spray) treatment. This might be due to the application of nitrobenzene helped to early flowering in bottle gourd plants. Days to female flowering were significantly influenced by the combination of different concentrations and the number of spray of nitrobenzene (Table 9). It was verified that the maximum days required to first female (73.83 days) flowering was found from C<sub>2</sub>N<sub>3</sub> treatment combination whereas minimum days to first female (58.16 days) were obtained from treatment combination C<sub>1</sub>N<sub>3</sub>.

#### 3.7 Number of Male Flowers per Plant

The data on number of male flower per plant was found to be significant in terms of for different concentrations of nitrobenzene application on bottle gourd (Table 7). The maximum (51.06) number of male flower per plant was recorded from C<sub>1</sub> (Nitrobenzene @ 2 ml/L) treatment which is statistically significant from other treatments while the minimum number of male flower per plant (38.73) was recorded from C<sub>0</sub> (Control) treatment. This might be due to that nitrobenzene helped in proper reproductive development in bottle gourd. Application of different number of spray on bottle gourd showed significant effect on number of male flower per plant (Table 8). The maximum number of male flower (49.01) was recorded from N<sub>3</sub> (Three spray) treatment which is statistically significant from other treatments while the minimum (41.78) number of male flower per plant was recorded from N<sub>1</sub> (One spray) treatment. Combined effect between plant different concentrations and number of spray of nitrobenzene showed a statistically significant variation in consideration to number of male flower per plant. The maximum number of male flower per plant (57.78) was recorded from the treatment combination of C<sub>1</sub>N<sub>3</sub> while the minimum number of male flower per plant (37.36) was recorded from combination treatment of C<sub>0</sub>N<sub>2</sub> which is statistically similar to C<sub>0</sub>N<sub>1</sub> treatment combination (Table 9). Babu et al. [11]

observed nitrobenzene spray resulted in high number of inflorescences per plant followed by brassinolide and gibberellic acid in jatropha. Kohombange et al. [24] also observed that application of nitrobenzene increases the number of flowers in bell pepper.

Table 4. Effect of different concentrations of nitrobenzene on number of primary branches per plant at harvest, number of female flowers per plant of bottle gourd

Treatment	Number of primary branches per plant at harvest	Number of female flowers per plant
C <sub>0</sub>	14.82 c	27.15 c
C <sub>0</sub> C <sub>1</sub>	17.66 b	40.18 a
$C_2$	19.99 a	35.02 b
LSD(0.05)	0.576	1.4582
CV%	3.32	4.32

Table 5. Effect of different number of sprays of nitrobenzene on number of primary branches per plant at harvest, number of female flowers per plant of bottle gourd

Treatment	Number of primary branches per plantat harvest	Number of female flowers per plant
$N_1$	15.44 c	29.50 c
$N_2$	17.99 b	34.80 b
$N_3$	19.03 a	38.05 a
LSD(0.05)	0.576	1.4582
CV%	3.32	4.32

Table 6. Combined effect of different concentrations and number of sprays of nitrobenzene on number of primary branches per plant at harvest and number of female flowers of bottle gourd

Treatment combination	Number of primary branches per	Number of female
	plant at harvest	flowers
$C_0N_1$	14.02 g	25.16 h
$C_0N_2$	15.24 f	27.39 gh
$C_0N_3$	15.20 f	28.90 fg
$C_1N_1$	16.05 ef	31.24 ef
$C_1N_2$	17.67 d	42.19 b
$C_1N_3$	19.27 c	47.12 a
$C_2N_1$	16.27 e	32.10 e
$C_2N_2$	21.08 b	34.83 d
$C_2N_3$	22.64 a	38.14 c
LSD(0.05)	0.9977	2.5257
CV%	3.32	4.32

In column, means with a same small letter (s) are not significantly different and those with different small letters are significantly different (LSD at 5% level of significance). Where,  $C_0$  = Control (Water only),  $C_1$  = Nitrobenzene @ 2 ml/L,  $C_2$  = Nitrobenzene @ 3 ml/L. Where,  $N_1$  = One spray,  $N_2$  = Two spray,  $N_3$  = Three spray

Table 7. Effect of different concentrations of nitrobenzene on number of days required to firstmale flower, days required to first female flower, number of male flower of bottle gourd

Treatment	Days required to first male flower	Days required to first female flower	Number of male flower
C <sub>0</sub>	61.11 b	65.50 b	38.73 c
$C_1$	58.03 c	61.88 c	51.06 a
$C_2$	69.95 a	70.83 a	46.29 b
LSD(0.05)	1.1138	1.4378	1.0283
CV%	1.78	2.2	2.29

Table 8. Effect of different number of sprays of nitrobenzene on number of days to first male flower, days to first female flower, number of male flower of bottle gourd

Treatment	Days required to first male flower	Days required to first female flower	Number of male flower
$N_1$	65.75 a	67.73 a	41.78 c
$N_2$	62.14 b	65.44 b	45.28 b
$N_3$	61.14 b	65.05 b	49.01 a
LSD(0.05)	1.1138	1.4378	1.0283
CV%	1.78	2.2	2.29

Table 9. Combined effect of different concentrations and number of sprays of nitrobenzene on number of days required to first male flower, days required to first female flower, number of male flower, number of female flower of bottle gourd

Treatment combination	Days required to first male flower	Days required to first female flower	Number of male flower
$C_0N_1$	64.01 d	69.02 bc	38.82 fg
$C_0N_2$	60.94 e	64.33 d	37.36 g
$C_0N_3$	58.39 f	63.16 d	40.02 f
$C_1N_1$	65.24 d	66.83 c	42.90 e
$C_1N_2$	55.69 g	60.66 e	52.50 b
$C_1N_3$	53.16 h	58.16 f	57.78 a
$C_2N_1$	68.01 c	67.33 c	43.64 e
$C_2N_2$	69.95 b	71.33 b	45.98 d
$C_2N_3$	71.88 a	73.83 a	49.26 c
LSD(0.05)	1.9291	2.4904	1.7811
CV%	1.78	2.2	2.29

In a column, means with similar letter (s) are not significantly different and those having dissimilar letter (s) are significantly different by LSD at 5% level of significance. Where,  $C_0$  = Control (Water only),  $C_1$  = Nitrobenzene @ 2 ml/L,  $C_2$  = Nitrobenzene @ 3 ml/L, Where,  $N_1$  = One spray,  $N_2$  = Two spray,  $N_3$  = Three spray

#### 3.8 Days Required to First Fruit Harvest

Significant variation was observed on days required to first fruit harvest of bottle gourd influenced by different concentrations nitrobenzene (Table 10). Results indicated that maximum days required to first fruit harvest (85.96) days) was recorded from  $C_2$ 3ml/L) treatment. (Nitrobenzene @ minimum days to first fruit harvest was (77.42 days) recorded from C<sub>1</sub> (Nitrobenzene @ 2 ml/L) treatment. Nuruzzaman et al. [15] found that plants treated with nitrobenzene required minimum day to first fruit harvest in strawberry. Days to first fruit harvest of bottle gourd was significantly varied due to different number of spray of nitrobenzene (Table 11). It was examined that maximum days required to first fruit harvest (83.23 days) was recorded from N<sub>1</sub> (one spray) treatment while minimum days required to first fruit harvest (79.65 days) was recorded from treatment N<sub>3</sub> (three spray). Combined effect between different concentrations and number of spray nitrobenzene was found to be significant on days

required to first fruit harvest. Among the combination treatments, the days required to first fruit harvest was maximum (89.06 days) in the treatment combination  $C_2N_3$ , while the days required to first fruit harvest was minimum (73.01 days) in the treatment combination of  $C_1N_3$ . The treatment combination  $C_1N_2$ ,  $C_0N_2$  and  $C_0N_3$  also showed that it require minimum days required to first fruit harvest but significantly different from treatment combination of  $C_1N_3$  (Table 12).

#### 3.9 Fruit Length (Cm)

Variation on fruit length differed significantly due to different concentrations of nitrobenzene at growth stage (Table 10). Results revealed that maximum fruit length (36.29 cm) was observed from  $C_2$  (Nitrobenzene @3 ml/L) treatment and minimum fruit length (31.88 cm) was found from  $C_0$  (Control) treatment. This result is similar with Kohombange et al. [24], they found that application of nitrobenzene incesases fruit length of bell pepper. Significant influence was observed in terms of fruit length of bottle gourd influenced by different number of spray (Table

11). Result signified that maximum fruit length (35.42 cm) was observed from N<sub>3</sub> (three spray) treatment which is statistically identical to N<sub>2</sub> (two spray) treatment and minimum fruit length (32.80 cm) was found from N<sub>1</sub> (one spray) treatment. Combined effect of different concentrations and number of spray showed statistically significant variation on fruit length of bottle gourd (Table 12). The result showed that maximum fruit length (38.08 cm) was recorded from treatment combination C<sub>2</sub>N<sub>3</sub> which is statistically similar to C<sub>2</sub>N<sub>2</sub> treatment combination and the minimum fruit length (31.04 cm) was found from the treatment combination C<sub>0</sub>N<sub>1</sub> which is statistically similar to C<sub>0</sub>N<sub>3</sub> treatment combination. From the above result it revealed that application of nitrobenzene increases the fruit length.

# 3.10 Fruit Diameter (Cm)

Different concentrations of nitrobenzene significantly influence fruit diameter of bottle gourd (Table 10). It was found that the highest fruit diameter (13.26 cm) was recorded from C<sub>2</sub> (Nitrobenzene @3 ml/L) treatment which was significantly different from others whereas the lowest fruit diameter (11.21 cm) was recorded inC<sub>0</sub> (Control). It showed that fruit diameter of bottle gourd increased with the increase of concentration of nitrobenzene. Nuruzzaman et al. [15] reaveled that application of nitrobenzene increases the fruit diameter of strawberry. Fruit diameter of bottle gourd was varied significantly due to different number of spray (Table 11). The result revealed that highest fruit diameter (12.82 cm) was recorded from N<sub>3</sub> (three spray) treatment which was significantly identical to N<sub>2</sub> (two spray) treatment whereas the lowest fruit diameter (11.77 cm) was recorded from N<sub>1</sub> (one spray) treatment. Significant variation was remarked on fruit diameter influenced by combined effect of different concentrations and number of spray (Table 12). Results exposed that the highest fruit diameter (14.27 cm) was obtained from the treatment combination of C<sub>2</sub>N<sub>3</sub> which is statistically similar to the treatment combination of C2N2. The lowest fruit diameter (11.02 cm) was obtained from treatment combination of C<sub>0</sub>N<sub>1</sub> which is statistically similar with the treatment combination of C<sub>0</sub>N<sub>2</sub> and C<sub>0</sub>N<sub>3</sub>. From the above result it revealed that application of nitrobenzene increases the fruit diameter.

# 3.11 Individual Fruit Weight (Kg)

There was significant variation on individual fruit weight of bottle gourd influenced by different concentrations of nitrobenzene. It was observed that the highest individual fruit weight (2.11 kg) was achieved from C2 (Nitrobenzene @ 3 ml/L) treatment whereas the lowest individual fruit weight (1.96 kg) was achieved from C<sub>0</sub> (Control) treatment (Table 13). Individual fruit weight of bottle gourd was significantly influenced by different number of spray of nitrobenzene. It was found that the highest individual fruit weight (2.08 kg) was achieved from N<sub>3</sub> (two spray) treatment which is statistically identical with N<sub>2</sub> (two spray) treatment whereas the lowest individual fruit weight (1.98 kg) was achieved from  $N_1$  (one spray) treatment (Table 14). Combined effect between different concentrations and number of spray of nitrobenzene was found to be significant of individual fruit weight (Table 15). Among the combination treatments, the individual fruit weight was maximum (2.17 kg) in the treatment combination C<sub>2</sub>N<sub>2</sub> which is statistically similar to C<sub>2</sub>N<sub>3</sub> and the individual fruit weight was minimum (1.89 kg) in the treatment combination of C<sub>0</sub>N<sub>1</sub> which is statistically similar to C<sub>2</sub>N<sub>3</sub> treatment combination (Table 15). Several authors [8,24,25] also found that plants treated with nitrobenzene produce maximum fruit weight.

# 3.12 Number of Fruit Harvested per Plant

Significant variation was observed on number of fruit harvested per plant of bottle gourd influenced by different concentrations of nitrobenzene (Table 13). Results indicated that the maximum number of fruit harvested per plant (10.95) was recorded from C<sub>2</sub> (Nitrobenzene @ 3ml/L) treatment while the minimum number of fruit harvested per plant was (8.01) recorded from C<sub>0</sub> (Control) treatment. The number of fruit harvested per plant of bottle gourd showed statistically significant impact due to different number of spray of nitrobenzene application (Table 14). Due to influence of different number of spray of nitrobenzene the maximum number of fruit harvested per plant (10.32) was recorded from N<sub>3</sub> (three spray) treatment while the minimum number of fruit harvested per plant (8.61) was from  $N_1$  (one spray) treatment. This might be due to that nitrobenzene helped in proper reproductive development in bottle gourd. Combined effect of different concentrations and number of spray showed statistically significant variation on number of fruit harvested per plant of bottle gourd (Table 15). The result showed that maximum number of fruit harvested per plant recorded from (12.55)was combination C<sub>1</sub>N<sub>3</sub> which is significantly different from other treatment combinations. The minimum

number of fruit harvested per plant (7.88) was found from the treatment combination  $C_0N_1$  which is statistically identical to  $C_0N_2$  and statistically similar to  $C_0N_3$  treatment combination. From the above result it revealed that application of nitrobenzene increases number of fruit harvested per plant. This might be due to that nitrobenzene helped in proper reproductive development in bottle gourd. This result is also in agreement with the findings of several authors [10,15,22,24].

# 3.13 Yield per Plant (Kg)

Significant variation was noted on yield per plant gourd affected different bottle bγ concentrations of nitrobenzene (Table 13). The result revealed that highest yield per plant (22.28 kg) was observed from C<sub>1</sub> (Nitrobenzene @2 ml/L) treatment while lowest yield per plant (15.68 kg) was found from  $C_0$  (control) treatment. Yield per plant of bottle gourd was significantly varied due to different number of spray of nitrobenzene (Table 14). It was examined that the highest yield per plant (21.18 kg) was observed from N<sub>3</sub> (three spray) treatment while the lowest yield per plant (17.08 kg) was found from N<sub>1</sub> (one spray) treatment. It indicated that with the increase of number of spray of nitrobenzene plant increased. vield per Combined effect between different concentrations and number of spray showed a statistically significant variation for yield per plant (Table 15). The result indicated that the highest yield per plant (25.35 kg) was observed from C<sub>1</sub>N<sub>3</sub> treatment combination while the lowest yield per plant (14.90 kg) was found from C<sub>0</sub>N<sub>1</sub> (One spray) treatment which is significantly similar with treatment combination C<sub>0</sub>N<sub>2</sub>. It indicated that with the increase of number of spray of nitrobenzene yield per plant increased. This might be due to that nitrobenzene helped in proper reproductive development in bottle gourd.

#### 3.14 Yield per Plot (Kg)

Significant variation was noted on yield per plot of bottle gourd affected by different concentrations of nitrobenzene (Table 13). The result revealed that the highest yield per plot (44.55 kg) was observed from C<sub>1</sub> (Nitrobenzene @ 2 ml/L) treatment while the lowest yield per plot (31.37 kg) was found from C<sub>0</sub> (Control) treatment. Yield per plot of bottle gourd was significantly varied due to different number of spray of nitrobenzene (Table 14). It was examined that the highest yield per plot (42.35 kg) was observed from  $N_3$  (Three spray) treatment while the lowest yield per plot (34.17 kg) was found from N<sub>1</sub> (One spray) treatment. It indicated that with the increase of number of per sprav of nitrobenzene vield increased. Combined effect between different concentrations and number of spray showed a statistically significant variation for yield per plot (Table 15). The result indicated that the highest yield per plot (50.70 kg) was observed from C<sub>1</sub>N<sub>3</sub> treatment combination while the lowest yield per plot (29.81 kg) was found from C<sub>0</sub>N<sub>1</sub> treatment combination which is significantly similar with treatment combination C<sub>0</sub>N<sub>2</sub>. It indicated that with the increase of number of spray of nitrobenzene yield per plot increased. This might be due to that nitrobenzene helped in proper reproductive development in bottle gourd.

# 3.15 Fruit Yield per Hectare (Ton)

Significant variation was noted on fruit yield per hectare of bottle gourd affected by different concentrations of nitrobenzene (Fig. 1). The result revealed that the highest fruit yield per hectare (55.70 t) was observed from C<sub>1</sub> (Nitrobenzene @2 ml/L) treatment while the lowest fruit vield per hectare (39.21 t) was found from C<sub>0</sub> (Control) treatment. This results is similar with [8,16,23,24,25], they also found that application of nitrobenzene increase yield of different crops. Fruit yield per hectare of bottle gourd was significantly varied due to different number of spray of nitrobenzene (Fig. 2). It was examined that the highest fruit yield per hectare (52.94 t) was observed from N<sub>3</sub> (Three spray) treatment while the lowest fruit yield per hectare (42.71 t) was found from  $N_1$  (One spray) treatment. It indicated that with the increase of number of spray of nitrobenzene fruit yield per hectare increased.

Table 10. Effect of different concentrations of nitrobenzene on days to first fruit harvest, fruit length, fruit diameter of bottle gourd

Treatment	Days to first fruit harvest	Fruit length (cm)	Fruit diameter (cm)
C <sub>0</sub>	80.57 b	31.88 c	11.21 c
$C_1$	77.42 c	34.89 b	12.67 b
$C_2$	85.96 a	36.29 a	13.26 a
LSD(0.05)	0.9325	0.6889	0.4753
CV%	1.16	2.01	3.84

Table 11. Effect of different number of sprays of nitrobenzene on days required to first fruit harvest, fruit length, fruit diameter of bottle gourd

Treatment	Days to first fruit harvest	Fruit length (cm)	Fruit diameter (cm)
N <sub>1</sub>	83.23 a	32.80b	11.77 b
$N_2$	80.66 b	34.83 a	12.55 a
$N_3$	79.65 c	35.42 a	12.82 a
LSD(0.05)	0.9325	0.6889	0.4753
CV%	1.16	2.01	3.84

Table 12. Combined effect of different concentrations and number of sprays of nitrobenzene on days required to first fruit harvest, fruit diameter, fruit length of bottle gourd

Treatment combination	Day to first fruit harvest	Fruit length (cm)	Fruit diameter (cm)
$C_0N_1$	84.40 c	31.04 g	11.02 f
$C_0N_2$	79.25 e	32.42 ef	11.44 ef
$C_0N_3$	78.06 e	32.18 fg	11.17 ef
$C_1N_1$	83.23 cd	33.48 de	12.42 cd
$C_1N_2$	76.01 f	35.17 c	12.57 cd
$C_1N_3$	73.01 g	36.02 bc	13.01 bc
$C_2N_1$	82.08 d	33.88 d	11.87 de
$C_2N_2$	86.74 b	36.91 ab	13.63 ab
$C_2N_3$	89.06 a	38.08 a	14.27 a
LSD(0.05)	1.6151	1.1933	0.8232
CV%	1.16	2.01	3.84

In column, means with a same small letter (s) are not significantly different and those with different small letters are significantly different (LSD at 5% level of significance). Where,  $C_0$  = Control (Water only),  $C_1$  = Nitrobenzene @ 2 ml/L,  $C_2$  = Nitrobenzene @ 3 ml/L. Where,  $N_1$  = One spray,  $N_2$  = Two spray,  $N_3$  = Three spray

Table 13. Effect of different concentrations of nitrobenzene on number of fruits harvested, individual fruit weight, yield per plant, yield per plot, yield per hectare of bottle gourd

Treatment	Number of fruits harvested	Individual fruit weight(kg)	Yield per plant(kg)	Yield per plot(kg)
C <sub>0</sub>	8.01 c	1.96 c	15.68 c	31.37 c
$C_1$	10.95 a	2.04 b	22.28 a	44.55 a
$C_2$	9.55 b	2.11a	20.21 b	40.43 b
LSD(0.05)	0.5012	0.0555	0.7415	1.483
CV%	5.32	2.73	3.86	3.86

Table 14. Effect of different number of sprays of nitrobenzene on number of fruits harvested, individual fruit weight, yield per plant, yield per plot, yield per hectare of bottle gourd

Treatment	Number of fruits harvested	Individual fruit weight(kg)	Yield per plant(kg)	Yield per plot(kg)
N <sub>1</sub>	8.61 c	1.98 b	17.08 c	34.17 c
$N_2$	9.59 b	2.06a	19.91 b	39.83 b
$N_3$	10.32 a	2.08 a	21.18 a	42.35 a
LSD(0.05)	0.5012	0.0555	0.7415	1.483
CV%	5.32	2.73	3.86	3.86

Combined effect between different concentrations and number of spray showed a statistically significant variation for fruit yield per hectare (Table 15). The result indicated that the highest fruit yield per hectare (63.38 t) was observed from  $C_1N_3$  treatment combination while the lowest fruit yield per hectare (37.26 t) was

found from  $C_0N_1$  (one spray) treatment which is significantly similar with treatment combination  $C_0N_2$ . It indicated that with the increase of number of spray of nitrobenzene fruit yield per hectare increased. This might be due to that nitrobenzene helped in proper reproductive development in bottle gourd.

Table 15. Combined effect of different concentrations and number of sprays of nitrobenzene on number of fruits harvested, individual fruit weight, yield per plant, yield per plot and yield per hectare of bottle gourd

Treatment combination	Number of fruits harvested	Individual fruit weight(kg)	Yield per plant(kg)	Yield per plot(kg)	Yield per hectare (t)
$C_0N_1$	7.88 f	1.89 d	14.90 g	29.81 g	37.26 g
$C_0N_2$	8.02 f	1.98 cd	15.88 fg	31.76 fg	39.70 fg
$C_0N_3$	8.12 ef	2.00 c	16.26 f	32.53 f	40.66 f
$C_1N_1$	8.93 de	2.01 c	17.94 e	35.89 e	44.86 e
$C_1N_2$	11.37 b	2.07 bc	23.54 b	47.07 b	58.84 b
$C_1N_3$	12.55 a	2.02c	25.35 a	50.70 a	63.38 a
$C_2N_1$	9.02 d	2.04 c	18.40 e	36.81 e	46.01 e
$C_2N_2$	9.37 d	2.17 a	20.33 d	40.65 d	50.81 d
$C_2N_3$	10.27 c	2.14 ab	21.92 c	43.83 c	54.79 c
LSD(0.05)	0.868	0.0961	1.2843	2.5687	3.21
CV%	5.32	2.73	3.86	3.86	3.86

In a column, means with similar letter (s) are not significantly different and those having dissimilar letter (s) are significantly different by LSD at 5% level of significance. Where,  $C_0$  = Control (Water only),  $C_1$  = Nitrobenzene @2 ml/L,  $C_2$  = Nitrobenzene @3 ml/L,  $N_1$  = One spray,  $N_2$  = Two spray,  $N_3$  = Three spray

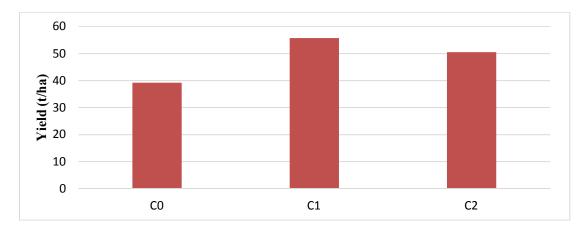


Fig. 1. Effect of different concentrations of nitrobenzene on yield per hectare of bottle gourd Where,  $C_0$  = Control (Water only),  $C_1$  = Nitrobenzene @ 2 ml/L,  $C_2$  = Nitrobenzene @ 3 ml/L

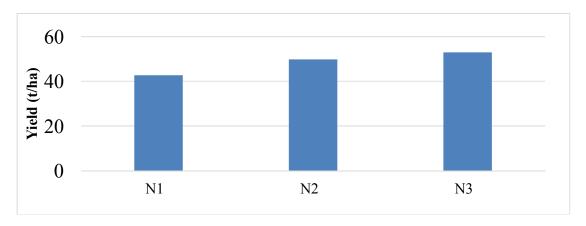


Fig. 2. Effect of different number of sprays of nitrobenzene on yield per hectare of bottle gourd Where,  $N_1$  = One spray,  $N_2$  = Two spray,  $N_3$  = Three spray

Table 16. Cost and return of bottle gourd cultivation as influenced by different concentrations and number of spray of nitrobenzene

Treatment	Cost of production (Tk / ha)	Yield (branch number)	Return (branch) (Tk)	Yield (t/ha)	Return (Fruit) (Tk)	Gross return (Tk/ha)	Net return (Tk /ha)	BCR
$C_0N_1$	473118	6255	62550	37.26	558900	621450	148332	1.31
$C_0N_2$	473118	6495	64950	39.7	595500	660450	187332	1.40
$C_0N_3$	473118	7755	77550	40.66	609900	687450	214332	1.45
$C_1N_1$	474017.1	9255	92550	44.86	672900	765450	291432.9	1.61
$C_1N_2$	474916.2	10005	100050	58.84	882600	982650	507733.8	2.07
$C_1N_3$	475815.3	11250	112500	63.38	950700	1063200	587384.7	2.23
$C_2N_1$	474466.7	9750	97500	46.01	690150	787650	313183.3	1.66
$C_2N_2$	475815.3	12255	122550	50.81	762150	884700	408884.7	1.86
$C_2N_3$	477164	15255	152550	54.79	821850	974400	497236	2.04

Note:  $C_0$  = Control (Water only),  $C_1$  = Nitrobenzene @ 2 ml/L,  $C_2$  = Nitrobenzene @ 3 ml/L and  $N_1$  = One spray,  $N_2$  = Two spray,  $N_3$  = Three spray Price of branch = 10 Tk./four branch, Price of bottle gourd = 15 Tk./Kg

# 3.16 Economic Analysis

Input costs for land preparation, fertilizer, irrigation, equipment for making trellis and manpower required for all the operations from seed sowing to harvesting, interest on fixed capital of land (Leased land by loan basis) and miscellaneous cost were calculated for unit plot and converted into cost per hectare (Table 16). Price of bottle gourd branch and fruit were considered as per market rate. The economic analysis presented under the following headings:

#### 3.16.1 Gross return

The combination of different concentrations and number of spray of nitrobenzene showed different values in terms of gross return under the trial (Table 16). The highest gross return (Tk. 1,06,3200) was found from the treatment combination  $C_1N_3$ and the second highest gross return (Tk. 9,82,650) was obtained in  $C_1N_2$ . The lowest gross return (Tk. 621450) was obtained from  $C_0N_1$ .

#### 3.16.2 Net return

In case of net return, different treatment combination showed different levels of net return under the present trial (Table 16). The highest net return (Tk. 5, 87, 384.7) was obtained from the treatment combination  $C_1N_3$  and the second highest net return (Tk. 5, 07, 733.8) was found from the combination  $C_1N_2$ . The lowest (Tk. 148332) net return was found from  $C_0N_1$  treatment combination.

#### 3.16.3 Benefit cost ratio

The combination of different plant growth regulators and number of spray of nitrobenzene for benefit cost ratio was different in all treatment

combination (Table 16). The highest benefit cost ratio (2.23) was found from the treatment combination  $C_1N_3$  and the second highest benefit cost ratio (2.07) was found from  $C_1N_2$  treatment combination. The lowest benefit cost ratio (1.31) was found from the  $C_0N_1$  (control) treatment combination. From the economic point of view, it was apparent from the above results that the treatment combination of  $C_1N_3$  was more profitable than rest of treatment combinations [26.17].

# 4. CONCLUSION

experiment concludes that the nitrobenzene concentrations of and its application significantly affected the production and the yield of bottle gourd. Among the treatment, C1with nitrobenzene 2 ml/L was one the most suitable for maximizing the growth and yield of bottle gourd. However, among the number of applications, it is observed that the three applications provide maximum growth and yield of bottle gourd. The combinations of C<sub>1</sub> and N<sub>3</sub>treatment provide maximum growth and the yield of the bottle gourd, and therefore, we recommend three applications (spray) of 2 ml/L nitrobenzene concentrations for maximizing the bottle gourd growth and yield.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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