ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt
International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Effect of Boron on Growth and Yield Parameters of Cauliflower (*Brassica oleracea* var *botrytis* cv Snow Mystique) in Terhathum, Nepal

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Article Information

Received: 26 January 2022
Revised version received: 20 March 2022
Accepted: 23 March 2022
Published: 29 March 2022

Cite this article as:
N. Poudel et al. (2022) Int. J. Appl. Sci. Biotechnol. Vol 10(1): 41-49. DOI: 10.3126/ijasbt.v10i1.44158

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Keywords: Boron; cauliflower; growth; hollowing

Abstract

A study was conducted to determine the effect of different levels of boron on growth and yield parameters of Snow Mystique variety of cauliflower at farmer's field in Myanglung, Terhathum, Nepal during the winter season of 2019 AD. Five levels of boron *viz,* control (0 kg/ha), 0.5 kg/ha, 1.0 kg/ha, 1.5 kg/ha, and 2.1 kg/ha were applied in the field that was laid out in Randomized Complete Block Design (RCBD) with four replications. Data were recorded regarding plant height, canopy cover, and leaf number at 30, 45, 60, and 75 days after transplanting while yield parameters like curd diameter, curd yield, and biological yield after harvest. Days to curd initiation, days to harvest, and incidence of hollowing were also recorded in this study. Results revealed that boron had significant effect on growth as well as yield parameters of cauliflower, where the value of these parameters were higher at higher boron dose. Boron at the rate of 2.1 kg/ha produced significantly higher curd yield (67.59 t/ha), curd diameter (25.21 cm) and biological yield (72.27 t/ha) whereas the value of these parameters were lowest with control. Similarly, boron had significant effect on incidence of hollowing where the application of boron at the rate of 2.1 kg/ha recorded the minimum incidence of hollowing (15.27 %). Results from this experiment concludes that application of boron at the rate of 2.1 kg/ha could be suggested for cauliflower growing farmers of Terhathum district for increased growth and yield of cauliflower.

Introduction

Cauliflower *Brassica oleracea* var. *botrytis* (2n=2x=18) is one of the important vegetable crops belonging to the family Brassicaceae. It is a nutritionally rich vegetable that can be eaten in raw, cooked, and pickled forms (Noor et al., 2014). It contains nutritive value of vitamins like vitamin A, thiamine, riboflavin, niacin, vitamin C, and minerals like calcium and iron (Ahmed & Ali, 2013). Besides, cauliflower contains a higher amount of dietary fiber, folic acid, water, and ascorbic acid possessing affluent nutrient density (Eimon et al., 2019). Pre-floral white fleshy apical meristem also known as curd is the edible part of cauliflower.

In Nepal, cauliflower is cultivated throughout all the geographical regions due to its wider adaptability. The total production of cauliflower across the country is 574,795 tons under the area of 35,764 hectares with a productivity of 16.07 tons per hectares (MOALD, 2018/19). In Terhathum, cauliflower is grown in an area of around 185 hectares with an average annual production of 2868 tons and productivity of 15.50 tons per hectares (MOALD, 2018/19).

Cauliflower production and productivity are seriously affected by the level of micronutrients in the soil. Boron, manganese, and molybdenum are important micronutrients that enhance the growth and yield of cauliflower (Chaudhari et al., 2017). The narrow range of toxicity and deficiency makes the boron unique to other micronutrients, thus a slight variation in available boron can cause toxicity or deficiency in a single growing season of plants (Batabyal et al., 2015). Various soil factors such as pH, texture, moisture, temperature, organic matter content, and clay mineralogy affect the availability of boron in the soil to plants (Goldberg et al., 2000). Common deficiency symptoms of boron include the death of the terminal growing point of the plant leaving rosette in the plant, curled and brittle leaf, and deformed flowers and fruits (Gupta & Solanki, 2013). Contrastingly, yellowing followed by browning and death of leaf tip indicates boron toxicity in most of the plants (Wilcox, 1960). Boron plays a major role in the maintenance of structural integrity of the plant membranes, cell division and elongation, metabolism of biomolecules like carbohydrates and nucleic acids, protein synthesis, sugar translocation, as well as uptake of essential plant nutrients from the soil. Therefore, the deficiency of boron affects all these processes (Ahmad et al., 2009; Pilbeam & Kirkby, 1983; Shaaban, 2010; Shireen et al., 2018).

The growth and yield components of cauliflower are seriously affected by the level of boron applied (Batabyal et al.; Dhakal et al., 2009; Subedi et al., 2020. The deficiency of boron in cauliflower causes browning of curds and the development of hollow stems which directly affects the marketing of cauliflower (Farooq et al., 2018).

A study by Silanpaa (1982) revealed the deficiency of boron in Nepalese soil. The author has also mentioned that the great majority of Nepalese soil samples are texturally on the coarse side. So, the widespread boron deficiency, acute or hidden is more likely to exist in Nepal, which limits the yield of crops that requires a higher amount of boron. Furthermore, farmers of Nepal being unaware of the optimum dose of boron to be applied, haphazard boron application might lead to deficiency or toxicity symptoms in cauliflower reducing the yield of the crop.

Several studies have been conducted on boron requirements of cauliflower in various parts of the world but there is very limited information under Nepalese soil conditions on this aspect. Moreover, no research work has been reported on the effects of boron in cauliflower production in the Terhathum district. The present research, therefore, was conducted to determine the effects of different doses of boron on the quality and yield attributes of cauliflower in the Terhathum district of Nepal.

Materials and Method

Location of the Experimental Site

The experiment was carried in the farmer field of Myanglung municipality, Terathum, Nepal from January 2019 to April 2019 with geographical location of the site, $27^{o}7^{'}38^{"}$N and $87^{o}32^{'}10^{"}$E and the elevation of 1500 meters above sea level. The area is characterized by a temperate climate with a unimodal rainfall pattern. Soil type is sandy loam and acidic with reddish-black color.

Experimental Design and Treatment Details

The field experiment was laid out on Randomized Complete Block Design (RCBD) with four replication and five different level of boron (B) namely B0: Control (0 kg B/ha), B1: 0.5 kg B/ha, B2: 1.0 kg B/ha, B3: 1.5 kg B/ha, and B4: 2.1 kg B/ha. Commercially available borax (10.5 % B) was used as a source of boron. Individual plot size was 5.13 m^2 area having 18 plants maintaining row to row distance of 60 cm and plant to plant distance of 45 cm. Cauliflower variety Snow Mystique was used as planting material (Table 1).

Table 1: Treatments details

Treatment	Borax (kg/ha)	Boron (kg/ha)
B0	0	0
B1	5	0.5
B2	10	1
B3	15	1.5
B4	20	2.1

Data Observation and Statistical Analysis

Four randomly selected plants considering border effects were selected for the measurement of required parameters. Growth parameters like plant height, canopy cover and leaf number and yield parameters like curd yield, curd diameter and biological yield were recorded following the standard methods. Further, the days to curd initiation and days to curd maturity were recorded by observing the field regularly. The incidence of hollowing was calculated based on the formula given by Mehotra and Aggarwal (2015). The formula used for calculating the incidence of hollowing is given below.

$$\text{Incidence of hollowing} = \frac{\text{Number of plants showing hollow stem}}{\text{Total number of plants under observation}} \times 100$$

The data thus obtained above were subjected to statistical analysis using MS Excel and R studio version 3.6.2. Duncan Multiple Range Test (DMRT) was applied to compare the treatment superiority at a 5% level of significance. Correlation analysis was used to show the relationship between the level of boron applied, growth parameters at harvest, and yield parameters.

Results and Discussion

Effect of Boron on Growth Parameters of Cauliflower

Plant Height:

Data regarding plant height (Table 2) revealed that the different levels of boron had a significant effect on cauliflower plant height at 45, 60, and 75 days after transplanting (DAT).

At 45 DAT, the highest plant height (43.78 cm) was recorded from the plots treated with 1.5 kg B/ha which was statistically at par with that obtained from 2.1 kg B/ha i.e. 40.26 cm. The lowest plant height (36.16 cm) was observed in plots treated with 0.5 kg B/ha which was statistically similar to that obtained from control plots (36.34 cm). Similar results were obtained at 60 and 75 DAT, where the plots treated with 1.5 kg B/ha and 2.1 kg B/ha recorded statistically similar plant height and were significantly superior to other treatments. The lowest plant height was observed in plots with control treatment (Table 2).

Plant Canopy Cover:

Data regarding the canopy cover of cauliflower is represented in Table 3. Statistical analysis showed that different levels of boron had a significant effect on the canopy cover of cauliflower at various DAT. Maximum canopy cover observed at 45, 60, and 75 DAT (42.72 cm, 51.64 cm, and 64.57 cm respectively) were recorded from the plots treated with 2.1 kg B/ha. While plots treated with 0.5 kg B/ha recorded minimum canopy cover at 45 and 60 DAT, the control plot showed the minimum canopy cover of 75 DAT.

Table 2. Effect of different doses of boron on plant height of cauliflower-

Treatment	Plant height (cm)			
	30 DAT	**45 DAT**	**60 DAT**	**75 DAT**
B0 (Control)	8.78^{a}	36.34^{c}	39.06^{c}	66.29^{c}
B1 (Boron 0.5 kg/ha)	9.28^{a}	36.16^{c}	44.69^{bc}	70.29^{bc}
B2 (Boron 1.0 kg/ha)	9.53^{a}	36.63^{bc}	44.99^{bc}	66.95^{c}
B3 (Boron 1.5 kg/ha)	9.69^{a}	43.78^{a}	53.23^{a}	75.21^{a}
B4 (Boron 2.1 kg/ha)	10.28^{a}	40.26^{ab}	48.39^{ab}	73.13^{ab}
CD (0.05) :	NS	3.66**	5.99**	5.95*
SEM(±) :	0.20	0.53	0.85	0.86
CV (%) :	9.41	6.15	8.32	5.49

NS = non-significant, *, and ** represent significant at 5 % and 1% level of significance respectively. Treatment means followed by a common letter(s) in the superscript within a column are not significantly different among each other and different letter(s) represents the statistical difference based on DMRT at 0.05 level of probability. SEM=standard error of the mean, CD= critical difference, and CV=coefficient of variance

Table 3: Effect of different doses of boron on canopy cover of cauliflower-

Treatment	Canopy Cover (cm)			
	30 DAT	**45 DAT**	**60 DAT**	**75 DAT**
B0 (Control)	8.00^{a}	36.06^{b}	44.12^{bc}	53.91^{b}
B1 (Boron 0.5 kg/ha)	8.46^{a}	35.91^{b}	41.02^{c}	59.04^{b}
B2 (Boron 1.0 kg/ha)	9.71^{a}	35.86^{b}	46.64^{abc}	57.37^{b}
B3 (Boron 1.5 kg/ha)	10.00^{a}	37.97^{b}	49.14^{ab}	57.85^{ab}
B4 (Boron 2.1 kg/ha)	9.31^{a}	42.72^{a}	51.64^{a}	64.57^{a}
CD (0.05) :	NS	4.35*	6.24*	5.55*
SEM (±) :	0.24	0.63	0.90	0.80
CV (%) :	11.91	7.49	8.71	6.15

NS = non-significant, and * represent significant at 5 % level of significance. Treatment means followed by a common letter(s) in the superscript within a column are not significantly different among each other and different letter(s) represents the statistical difference based on DMRT at 0.05 level of probability. SEM=standard error of the mean, CD= critical difference, and CV=coefficient of variance

Table 4: Effect of different doses of boron on leaf number of cauliflower

Treatments	Leaf number /plant			
	30 DAT	**45 DAT**	**60 DAT**	**75 DAT**
B0 (Control)	7.81[c]	9.75[d]	10.87[d]	11.68[d]
B1 (Boron 0.5kg/ha)	8.00[bc]	9.68[d]	11.25[cd]	12.12[c]
B2 (Boron 1.0kg/ha)	8.18[bc]	10.25[c]	11.62[c]	12.37[bc]
B3 (Boron 1.5kg/ha)	8.25[b]	10.75[b]	12.12[b]	12.56[b]
B4 (Boron 2.1kg/ha)	8.87[a]	11.31[a]	12.62[a]	13.25[a]
CD :	0.40**	0.37***	0.47***	0.41***
SEM (±) :	0.05	0.05	0.06	0.05
CV (%) :	3.17	2.32	2.61	2.10

** and *** represent significant at 1 % and 0.1 % level of significance respectively. Treatment means followed by a common letter(s) in the superscript within a column are not significantly different among each other and different letter(s) represents the statistical difference based on DMRT at 0.05 level of probability. SEM=standard error of the mean, CD= critical difference, and CV=coefficient of variance

Leaf Number:

Statistical analysis of data revealed that boron had a significant effect on leaf number. At 30 DAT, plots treated with boron 2.1 kg B/ha recorded maximum leaf number (8.87) whereas control plots recorded a minimum number of leaves (7.81). Similar results were obtained at 45, 60, and 75 DAT where plots treated with 2.1 kg B/ha recorded maximum leaf number (11.31, 12.62, and 13.25 respectively). An increase in leaf number with increasing boron dose was seen (Table 4).

Effect of Boron on Yield Parameters of Cauliflower

Curd Diameter

Statistical analysis of data showed significant variation in curd diameter at different doses of boron applied (Table 5). Curd diameter ranged from 17.17 cm to 25.21 cm. The maximum curd diameter was recorded from the plots treated with 2.1 kg B/ha (25.21cm) while the minimum (17.17 cm) was recorded from the control plots. An increasing trend of curd diameter with increasing boron dose was seen.

Curd Yield:

The curd yield of cauliflower was significantly affected by boron application (Table 5). Mean values of data showed that plots treated with 2.1 kg B/ha produced higher curd yield i.e. 67.59 tons/hectares (t/ha) followed by 1.5 kg B/ha (61.21 t/ha), and 1.0 kg B/ha (58.05 t/ha) respectively. While lowest curd yield was recorded from the control plots (44.08 t/ha) which was statistically at par with that obtained from 0.5 kg B/ha (46.65 t/ha).

The yield response of cauliflower to different levels of boron is shown in figure 1. The coefficient of determination (R^2) was 0.81 which implies that 81 % of the variation in curd yield of cauliflower was due to different levels of boron applied in the field.

Biological Yield

Data regarding the biological yield of cauliflower as affected by different boron doses are shown in Table 5. Statistical analysis showed significant variations in the biological yield of cauliflower at different levels of boron. The biological yield of cauliflower ranged from 47.68 t/ha to 72.27 t/ha. Among the different treatments, plots treated with boron 2.1 kg B/ha recorded maximum biological yield (72.27 t/ha) followed by 1.5 kg B/ha (64.99 t/ha), 1.0 kg B/ha (61.98 t/ha), 0.5 kg/ha (50.90 t/ha), and control (47.68 t/ha) respectively.

Effect Of Boron on Days to Curd Initiation, Days to Harvest, And Days from Curd Initiation to Harvest of Cauliflower

Data regarding the effect of boron on days to curd initiation and days to harvest is presented in Table 6. Statistical analysis of data revealed the significant effect of boron on days to curd initiation. Maximum days required for curd initiation (67.50 days) were recorded from control plots which were statistically at par with that of 0.5 kg B/ha (67.43 days). While plots treated with 2.1 kg B/ha (65 days) recorded minimum days required for curd initiation followed by 1.5 kg B/ha (65.68 days) and 1.0 kg B /ha (66.68 days) respectively. Similarly, plots treated with 2.1 kg B/ha required significantly lower days to harvest i.e. 77.43 days followed by 1.5 kg B/ha (78.37 days), 1 kg B/ha (79.68 days), control plots (81.18 days), and 0.5 kg B/ha (80.87 days) respectively. The minimum number of days from curd initiation to harvest was required by treatment of 2.1 kg B/ha i.e.12.43 days. While control plots required the maximum number of days (13.68 days) to harvest from curd initiation

Table 5: Effect of different doses of boron on yield parameters of cauliflower

Treatments	Yield parameters			
	Curd diameter (cm)	**Curd yield (kg/plot)**	**Curd yield (t/ha)**	**Biological yield (t/ha)**
B0 (control)	17.17[d]	22.61[c]	44.08[c]	47.68[d]
B1 (Boron 0.5 kg/ha)	18.48[cd]	23.93[c]	46.65[c]	50.90[c]
B2 (Boron 1.0 kg/ha)	21.21[bc]	29.77[b]	58.05[b]	61.98[c]
B3 (Boron 1.5 kg/ha)	22.88[ab]	31.40[ab]	61.21[ab]	64.99[b]
B4 (Boron 2.1 kg/ha)	25.21[a]	34.66[a]	67.59[a]	72.27[a]
CD (0.05) :	2.92***	3.71***	7.24***	6.79***
SEM (±) :	0.42	0.53	1.05	0.98
CV (%) :	9.05	8.47	8.47	7.40

*** represents significance at 0.1 % level of significance. Treatment means followed by a common letter(s) in the superscript within a column are not significantly different among each other and different letter(s) represents the statistical difference based on DMRT at 0.05 level of probability. SEM=standard error of the mean, CD= critical difference, and CV=coefficient of variance

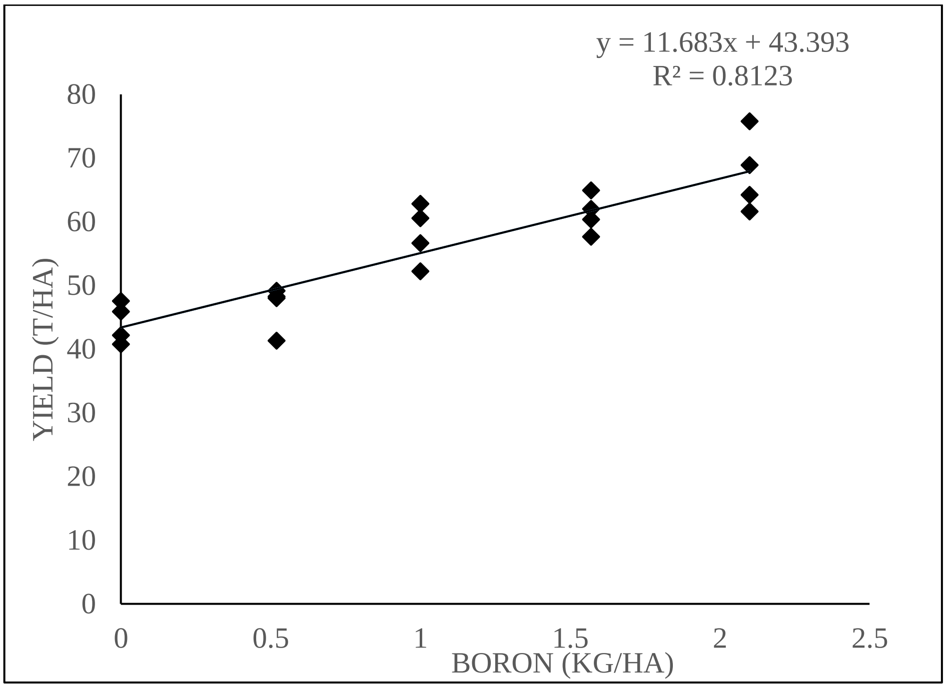

Fig. 1: Response of curd yield of cauliflower to different levels of boron applied

Table 6: Effect of different doses of boron on days to curd initiation, days to harvest, and days from curd initiation to curd harvest of cauliflower

Treatments	Days to curd initiation	Days to harvest	Days from curd initiation to harvest
B0 (Control)	67.50[a]	81.18[a]	13.68[a]
B1 (Boron 0.5kg/ha)	67.43[a]	80.87[a]	13.43[ab]
B2 (Boron 1.0kg/ha)	66.68[b]	79.68[b]	13.00[bc]
B3 (Boron 1.5kg/ha)	65.68[c]	78.37[c]	12.68[cd]
B4 (Boron 2.1kg/ha)	65.00[d]	77.43[d]	12.43[d]
CD (0.05) :	0.58***	0.72***	0.48***
SEM (±) :	0.08	0.10	0.07
CV (%)	0.57	0.59	2.43

*** represents significance at 0.1 % level of significance. Treatment means followed by a common letter(s) in the superscript within a column are not significantly different among each other and different letter(s) represents the statistical difference based on DMRT at 0.05 level of probability. SEM=standard error of the mean, CD= critical difference, and CV=coefficient of variance

Table 7: Effect of different doses of boron on the incidence of hollowing in cauliflower

Treatment	Incidence of hollowing
B0 (Control)	91.67^{a}
B1 (Boron 0.5 kg/ha)	63.88^{b}
B2 (Boron 1.0 kg/ha)	44.44^{c}
B3 (Boron 1.5 kg/ha)	29.16^{d}
B4 (Boron 2.1 kg/ha)	15.27^{e}
CD (0.05) :	9.85***
SEM (±) :	1.43
CV (%) :	13.08

*** represents significance at 0.1 % level of significance. Treatment means followed by a common letter(s) in the superscript within a column are not significantly different among each other and different letter(s) represents the statistical difference based on DMRT at 0.05 level of probability. SEM=standard error of the mean, CD= critical difference, and CV=coefficient of variance

Table 8: Correlation matrix showing correlation among different doses of boron, growth parameters, and yield parameters of cauliflower

	BD	PH	LN	CC	CD	CY	BY
BD	1						
PH	0.52*	1					
LN	0.81***	0.39	1				
CC	0.53*	0.31	0.58**	1			
CD	0.86***	0.45*	0.71***	0.29	1		
CY	0.90***	0.38	0.77**	0.49*	0.72***	1	
BY	0.91***	0.39	0.78**	0.50*	0.74***	0.97***	1

*, ** and *** represent significant at 5 %,1 % and 0.1 % level of significance respectively. BD = Boron doses, PH = Plant height at harvest, LN = Leaf number at harvest, CC= Canopy cover at harvest, CD= Curd diameter, CY= Curd yield, BY= Biological yield

Effect of Boron on The Incidence of Hollowing

The effect of different doses of boron on the incidence of hollowing in cauliflower is presented in Table 7. Mean values of data thus obtained after statistical analysis showed that plants in control treatment showed the highest percentage of hollow splitting (91.67%) which was gradually reduced with increasing doses of boron applied. Plants treated with 2.1 kg B/ha showed the minimum incidence of hollowing (15.27%).

Correlation Among Different Doses of Boron, Growth Parameters, and Yield Parameters of Cauliflower

Correlation analysis of boron with growth parameters and yield parameters showed that yield parameters like curd diameter, curd yield, and biological yield were positively correlated with boron dose at p-value < 0.001 (Table 8). Similarly, vegetative parameters like plant height ($r=0.50^{*}$), leaf number ($r=0.81^{***}$), and canopy cover (0.53^{*}) at harvest were also positively correlated with levels of boron applied.

Discussion

Boron significanlty increased the plant height in our experiment. This result is in agreement with previous experiments conducted across different parts of the globe. Adhikary et al. (2004) reported similar results where they observed taller plant height at higher borax dose i.e 25 kg/ha. Similarly, Subedi et al. (2020) observed significantly taller plant at higher boron dose i.e 1.7 kg/ha, and the lowest plant height was recorded in control plots. The increase in plant height with increasing levels of boron can be attributed to the fact that boron promotes cell division and elongation and is especially required for actively growing regions of plant-like apical meristems (Ahmad et al., 2009), thus aiding in plant height.

Singh et al. (2013) revealed from their research findings that maximum foliage cover was obtained from the application of boron at the rate of 1.5-2 kg/ha i.e Borax 15-20 kg/ha, the findings of which are similar to the findings of our research work. This result is also in agreement with the research result of Moniruzzaman et al. (2007) where the authors observed larger plant spread at 1.5 kg B/ha and 2 kg B/ha and minimum plant spread at control plots.

Our result showed an increase in leaf number with increasing boron dose. It is supported by the research result of Kant et al. (2013) which showed that boron significantly increases the number of leaf in cauliflower. Enhancement of nutrient uptake, photosynthesis, and higher metabolic activity in plants due to boron might have resulted in more leaf number.

An increase in curd diameter with increasing boron dose was observed in our experiment. This finding is in close harmony with the research results of Hassan et al. (2018) where the authors reported higher curd diameter in higher boron dose and vice versa. Similarly, Farooq et al. (2018) reported higher curd diameter in broccoli with increasing boron dose. This result is also in agreement with the research result of Alam and Jahan (2007) where the authors reported an increase in head diameter in cabbage up to the boron dose of 3 kg/ha.

Our data revealed that boron had a significant effect on increasing the curd yield. The result thus obtained is in close conformity with the research results of earlier experiments of a similar pattern. Kotur (1991) reported significantly higher curd yield at plots treated with 1.5 kg B/ha and 2 kg B/ha compared to control plots. Similar to our result Hassan et al. (2018) obtained a linear increase in curd yield with an increasing dose of boron. The finding of the present study is also supported by research results of Islam et al. (2015) which showed the significant effect of boron dose on increasing the curd yield of broccoli. This increase in curd yield with increasing boron dose might be due to the role of boron on uptake of essential plant nutrients and metabolism and transport of essential biomolecules in plants (Ahmad et al., 2009). So, the low concentration of boron in plants retards the growth of the whole plant ultimately reducing the yield of the plant. Likewise, the low concentration of boron in plants activates the enzymatic and non-enzymatic oxidation using phenol as the substrate that results in higher quinine and oxidase concentration which renders the growth and development of the plant (Shireen et al., 2018).

An increase in biological yield with increasing boron dose was obtained in this experiment. This result is supported by the research result of Farooq et al. (2018), where the authors reported a significant and linear increase in the biological yield of broccoli with an increasing dose of boron applied. Further, boron dose is significantly and positively correlated with leaf number, canopy cover, curd diameter, and curd yield in our experiment, thus recording higher biological yield at a higher boron dose.

Boron significantly reduced the incidence of hollowing in our experiment. The result thus obtained is in close conformity with results obtained by Islam et al. (2015) which showed a decrease in hollow stem disorder with an increase in boron level up to the 2 kg/ha in broccoli. Similarly, this finding is in agreement with the research findings of Batal et al. (1997) which showed a decrease in hollow stem incidence in cauliflower with an increase of boron levels up to 8.8 kg/ha. Our result is further supported by the research result of Sartori de Camargo and da Costa Mello (2009) where the authors reported higher hollow stem disorder in control plots compared to plots treated with 3 kg B/ha. However, results obtained by Hussain et al. (2012) showed an increase in hollow stem incidence of broccoli after 1 kg B/ha, which is found contradictory to our research result. Thus, the different results obtained above by various authors indicate that boron has a significant role in reducing the hollow stem in cruciferous crops, though their requirement varies from species to species.

It has been revealed that boron plays a significant role in the days required for curd initiation and harvest. Days required for curd initiation, days to harvest and days from curd initiation to harvest decreased with increasing boron dose. This result is supported by the research result of Kant et al. (2013). It might be due to the positive effect of boron on the growth rate of the plant.

Conclusion and Recommendation for Future Research

The highest yield of cauliflower was obtained when boron was applied at the rate of 2.1 kg/ha. So, it would be reasonable to suggest cauliflower growing farmers of Terhathum apply boron at the rate of 2.1 kg/ha for maximum production. However, our experiment consists of only four different boron dose and yet the highest dose has produced significantly higher yield. So, it will be important that future research investigate on effect of boron dose higher than 2.1 kg B/ha on cauliflower production.

Authors' Contribution

Nabin Poudel, Purnima Baral, Muna Neupane, Sundar Man Shrestha, Arjun Kumar Shrestha, and Sandesh Bhatta: conceived and designed the experiments; perform the experiments; analysed and interpreted the data; wrote and reviewed the manuscript. All contributed equally in all part.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

Acknowledgment

The author thanks Agriculture and Forestry University, Nepal and Prime Minister Agriculture Modernization Project/Government of Nepal for providing this platform. The supervising committee of the author is acknowledged for providing the author with appropriate guidelines and the farmers of Myanglung, Terhathum is acknowledged for providing field and other necessary resources to conduct research.

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