



Research Article

Effect of Ethephon Doses on Vegetative Characters, Sex Expression and Yield of Cucumber (*Cucumis sativus* cv. Bhaktapur Local) in Resunga Municipality, Gulmi, Nepal

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Abstract

A study on the effect of ethephon doses on vegetative characters, sex expression and yield of cucumber (*Cucumis sativus* cv. Bhaktapur Local) was conducted in Gulmi, Nepal during 21st April to 24th August 2017. The experiment was laid in single factor Randomized Complete Block Design (RCBD) with 4 replications and 5 treatments. Four different doses of ethephon 100, 200, 300 & 400 ppm with control were applied. Two sprays of ethephon was made one at two true leaf stage and other at four true leaf stage. Ethephon was found to reduce the plant height compared to control, minimum (238.8 cm) with 400 ppm and maximum (310.4 cm) with the control. The number of nodes and branches per plant were found to be highest with 300 ppm. Ethephon was found to shift first male flowers and female flowers towards upper and lower nodes respectively. The plot treated with 300 ppm ethephon, bearing 20.31 female flowers per plant was found superior to other doses for increasing total female flowers. Maximum and minimum number of male flowers per plant was recorded with control (107 per plant) and 400 ppm (46.90 per plant) respectively. Similarly, 300 ppm of ethephon was found superior for reducing (male: female) sex ratio. Maximum yield 27.51 t/ha was recorded with 300 ppm and minimum yield of 17.48 t/ha with the control. 57% increment in the yield was observed with 300 ppm ethephon as compared to the control. Thus, proper use of ethephon is found to be beneficial to farmers.

Introduction

Cucumber (*Cucumis sativus* L.) (2n=14), belonging to the family Cucurbitaceae, because of its varied usefulness, excellent flavour, texture and medicinal value, is among the most prized vegetable (Sebastian et al., 2010). Cucumber is a summer vegetable generally grown under open field conditions, optimum temperature for growth and development being 20°C to 30°C. Cucumber has a diverse array of unisexual or bisexual flowering sex phenotypes.

However, the majority of cucumber hybrids are gynodioecious (Wang et al., 2011). Sex expression is an important factor that has a positive effect on yield and that constitutes a major component of cucumber improvement programs. The sex appearance of cucumber is closely connected with its genetics as well as its chemical and environmental conditions (Arpan, 1974). Female and male flower ratio

may vary from 1:15 to 1:13. It can be minimized by some mechanical techniques and chemical practice.

Growth regulators have tremendous effects on vegetative characteristics, sex expression and flowering. Sex expression in cucumber is affected by the endogenous level of growth hormones, but can be triggered with the exogenous application of growth regulators (Singh and Singh, 1988). The two- or four-leaf stage are the critical stage at which the suppression or promotion of either sex is possible and therefore the exogenous application of plant growth regulators can alter the sex ratio and their sequence (Hossain *et al.*, 2006). Ethylene is one of the important growth regulator commercially available in form of ethephon in 39% S.L. in water aqua. *Chemically, ethephon is 2-Chloroethylphosphonic acid, which upon metabolism by plant, releases ethylene (Szyjewicz et al., 1984). Ethylene is an important natural plant hormone, used in agriculture to force the ripening of fruits (Wang et al., 2002). Thus, ethephon can be a cheap and easy way to enhance productivity of farmer.*

Cucumber is one of the most important vegetables in Nepal with its uses ranging from eating it raw as salad to using it for beauty purposes. Total area under cucumber cultivation in Nepal is 9691ha producing 148,702 t of fresh cucumber with productivity 15 t/ha while the productivity of Gulmi district is only 6t/ha (MoAD, 2017). The maximum yield potential of cucumber does not seem to be utilized due to lack of irrigation water, inappropriate selection of variety generally monoecious variety of cucumber is being cultivated which have high male flower compared to female resulting low yield, climate change has brought a shift in cultivation season and prolonged growth season of crop. Bhaktapur local variety of cucumber generally cultivated in our context is monoecious and bears higher male flowers 32/m² (Subedi & Sharma, 2005). Its varietal character is deteriorating due to factors such as, high number of misshapen fruits, high male: female ratio and short productive life of the crop. The number of female flowers in cucumber plant is one of the determinant of the ultimate yield so, it is very important to increase the number of female flowers to increase the number of fruits and ultimately the yield.

Among the cucurbits, cucumber as being popular among low-mid hills farmers who are residing near markets and/or road heads. Application of ethephon for inducing female flowers in cucurbits is very common practice abroad but still not much practiced in Nepal. Since cucumber is a crop having high nutritive value, have high demand in both international and national market thus have great potential for high value crop. Similarly, Gulmi district has been selected as vegetable block under Prime Minister Agriculture Modernization project (PMAMP) under Government of Nepal. District Agriculture Development Office(DADO), Gulmi has identified cucumber as one of

major product under the block program. Considering above scenario, present study was thus undertaken to study the effect of exogenous application of ethephon on vegetative characteristics, sex expression the yield of cucumber.

Materials and Methods

Experimental Site

The experiment was conducted at Resunga Municipality - 10 Simichaur, Gulmi located about 2 km south east of Tamghas (headquarter of Gulmi district). It lies at 28°02'N to 83°15'E / 28°03'N 83°25'E. The research site lies in the subtropical zone of Nepal, characterized by three distinct seasons namely, rainy monsoon (June-October), cool winter (November-February), and mild spring (March-May). Research was conducted during the month of April to August.

Experimental Material

Experiment was conducted under open field condition using Bhaktapur local variety of cucumber. Different concentration of ethephon (2- chloroethyl phosphonic acid) was applied to study its effect on vegetative character, flowering, yield and quality traits of cucumber.

Experimental Design

The experiment was conducted in Completely Randomised Block Design (RCBD). 4 replications of each treatment was done. Total field size for research was 68 m × 14 m consisting of 20 plots each of size 16 m × 12 m. Each plot consisted of single rows of 8 plants each, with spacing 200cm × 200 cm. 5 treatments with 4 replications was applied in field. Yield data and flower counts were taken on the centre row of each treatment.

Treatment Details

A total of 5 treatments was done on different doses of ethephon

T₁ = Control

T₂ = 100 ppm dose of ethephon

T₃ = 200 ppm dose of ethephon

T₄ = 300 ppm dose of ethephon

T₅ = 400 ppm dose of ethephon

Seedlings Preparation for Transplantation

The seeds of Bhaktapur local cultivar was sown under protected conditions in poly-bags of size 15 × 8 cm were used. Two seeds per polybag were sown and kept inside the poly-house with regular watering. After complete germination of the seed and seed being ready for transplantation. the seedlings were transplanted with spacing of 200 cm × 200 cm P-P × R-R., keeping 8 plants per plot and 5 plants were sample to document various observations on 21st April 2017.

Manure, Fertilizer and Irrigation

FYM was applied as basal dose and recommended dose of N:P:K @ 7:2:5 / ropani (equivalent to 140:40:100/ha) was applied 30 days after transplanting and nitrogen was applied at split dose. First irrigation was given on day of transplanting after transplanting of seedling then depending upon soil moisture condition irrigation was done and on days of application of fertilizer slight irrigation was done. The water requirement was almost met by natural rainfall. Trellis method of staking was done with bamboo stakes arranged in grid at distance 2m between two cucumber plants and plastic ropes were provided for training cucumber vine.

Preparation of Ethephon for Spray

The commercial form of ethephon, Thrill (ethephon 39% S.L) manufactured by Meerut Agrochemical Industries limited, India was used. It was initially diluted to 400 ppm by mixing 4.1 ml of ethephon in 4000 ml of water then 1000 ml of solution was taken out then the remaining 3000 ml was diluted with 1000 ml water to prepare 4L of 300 ppm solution again 2000 ml of 300 ppm solution was taken out and mixed with 1000 ml water to prepare 3L of 200 ppm solution similarly 1000 ml of 200 ppm was mixed with 1000ml water to prepare 2L of 100 ppm solution. For measuring the volume of ethephon a syringe of least count 0.1 ml was used.

Data Collection Technique

Vegetative characteristics and flowering behaviors were recorded. 5 plants were selected from each plot to record for different observations.

Morphological Measurements

Length of main stem(cm), number of primary branches per vine and number of nodes on main stem were observed and average was calculated.

Floral Measurements

Days to first female flower emergence after transplanting, node number for emergence of first female, days to first male flower emergence after transplanting, node number for emergence of first male, number of female flowers per plant, number of male flowers per plant, sex ratio were observed and average was calculated.

Metrical Measurements

Fruit length (cm), fruit circumference (cm), number of fruit per plant, fruit weight per vine (cm), fruit yield per hectare (mt/ha) were observed and average was calculated.

Data Analysis

All the recorded data were arranged systematically. A simple correlation and regression was established among the selected parameters with reference to Gomez and Gomez (1984). Different statistical tools as R and MS-EXCEL were used for the analysis of variance and other data analysis.

Results and Discussion

Plant Height

The plant height was affected by application of ethephon as with increase in level of ethephon there was decrease in height (Table 1). At 90 DAT, final height of plants showed considerable difference among treatments in order of 400 ppm (238.8 cm) < 300 ppm (267.5 cm) < 200 ppm (270.1 cm) < 100 ppm (298.9 cm) < control (310.4 cm). DMRT analysis at $p < 0.05$ showed similarities among control and 100 ppm, 300 & 400 ppm and among 100, 200 and 300 ppm.

According to Hayashi, Cameron and Carlson (2001), anti-gibberellic property of ethylene causes cessation of mitotic processes in meristem of root and shoot, affecting the length of plant which might be a possible reason behind the reduced height of plants. Ethylene also inhibits IAA transport in plant systems, inhibiting the elongation (Morgan & Gausman, 1966; Malloch & Osborne, 1976). Similar results have been observed in *Cucumis melo* L (Ouzounidou, Papadopoulou, Giannakoula, & Ilias, 2008). Decrease in plant height with increased ethephon concentration and death of all plants was seen at 5000 ppm (Bhandary *et al.*, 1974). Thus during the initial phase of vine growth effect of ethephon was anticipated since it release ethylene and the inhibitory effect of ethylene on shoot growth have been attributed to the reduction of auxin levels (Lieberman & Knegi, 1977) possibly via ethylene induced acceleration of auxin metabolism. Based on above facts a conclusion can be made that with increasing dose of ethephon the height of plants decreases proportionately.

Number of Nodes

It is evident from the Table 2 that number of nodes per plant increased with the increase in level of ethephon. Comparing number of nodes/plant at end, 300 ppm (67.38) and 200 ppm (67.15) ethephon produced significantly higher number of nodes/plant compared to control (53.90) and 100 ppm and 200 and 300 ppm are in par statistically. Similar trend was seen on all 15 DAT, 30 DAT, 45 DAT, 60 DAT and 75 DAT. Maximum number of nodes were seen in ethephon concentration 200 and 300 ppm and with increasing or decreasing concentration number of nodes/ plant is seen in decreasing.

Ethephon at 200-300 ppm proved superior to its higher levels and control for increasing number of nodes. This may be related to fact that ethephon at 200 and 300 ppm reduced plant height to a greater extent as compared to higher concentrations. The increased number of nodes due to ethephon treatment may be attributed to reduction in internodal distance by suppressing cell division. Rafeekhar *et al.* (2001), David and Loy (1982) and Arora *et al.* (1994) also reported reduction in internodal distance by application of ethephon in cucumber, in watermelon and long melon respectively and Singh (1985) also observed reduction in

internodal length and plant height but not on number of nodes per plant in cucumber.

Branching

At 90 DAT, the maximum number of branches were seen in case of 300 ppm (6.075) followed by 400 ppm (5.57), 200 ppm (5.45), 100 ppm(4.62) and least number of branches were found in control (4.32). With increasing dose of ethephon number of branches were also increasing but at higher dose of ethephon again the number of branches were

decreasing. Ethephon at 300 ppm followed, proved superior to its higher and lower concentrations respectively for increasing number of branches (Table 3). Marked change in plant phenotype giving high branched plants that resulted from changes in the nuclear genomes were observed by Selga and Selga (1993) and Rafeekhar *et al.*, 2001. The increase in number of branches due to ethephon spray is in agreement with earlier work of Miller *et al.* (1969), Bhandhary *et al.* (1974) and Singh (1985).

Table 1: Effect of different concentrations of ethephon on plant height of cucumber in Gulmi district, 2017

Concentration of Ethephon	Plant height at various days after transplanting(cm)					
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
Control	14.35 ^a	27.40 ^a	116.9 ^a	210.1 ^a	254.4 ^a	310.4 ^a
100 ppm	13.73 ^a	25.63 ^{ab}	116.3 ^a	199.1 ^a	253.5 ^a	298.9 ^{ab}
200 ppm	12.60 ^{ab}	24.20 ^b	107.7 ^{ab}	193.7 ^{ab}	231.6 ^b	270.1 ^{abc}
300 ppm	12.65 ^{ab}	24.10 ^b	105.9 ^{ab}	193.2 ^{ab}	233.7 ^b	267.5 ^{bc}
400 ppm	11.48 ^b	23.08 ^b	99.25 ^b	173.5 ^b	223.6 ^b	238.8 ^c
SEM	0.57	0.87	3.28	6.39	6.08	12.49
LSD	1.77 [*]	2.69 [*]	10.11 [*]	19.69 [*]	18.73 [*]	38.48 [*]
CV%	8.88%	7.01%	6.09%	6.59%	5.08%	9.01%

Means followed by the same letter (s) in a column are not significantly different at 5% level of significance as determined by DMRT

Table 2: Effect of different concentrations of ethephon on number of nodes per plant in cucumber in Gulmi district, 2017

Concentration of Ethephon	Number of nodes per plant at various days after transplanting					
	15 DAT	30 DAT	45DAT	60 DAT	75 DAT	90 DAT
Control	3.80 ^b	9.09 ^b	20.75 ^b	36.35 ^b	49.50 ^b	53.90 ^b
100 ppm	4.32 ^a	9.24 ^b	22.80 ^{ab}	36.10 ^b	49.90 ^b	54.10 ^b
200 ppm	4.57 ^a	11.64 ^a	26.25 ^a	43.22 ^a	62.53 ^a	67.15 ^a
300 ppm	4.58 ^a	12.03 ^a	26.60 ^a	44.26 ^a	61.10 ^a	67.38 ^a
400 ppm	4.42 ^a	11.31 ^{ab}	25.38 ^a	43.38 ^a	57.28 ^{ab}	62.38 ^{ab}
SEM	0.14	0.74	1.35	1.81	2.72	2.95
LSD	0.441 [*]	2.23 [*]	4.10 [*]	5.57 [*]	8.38 [*]	9.08 [*]
CV%	6.59%	13.86%	11.08%	8.90%	9.70%	9.64%

Means followed by the same letter (s) in a column are not significantly different at 5% level of significance as determined by DMRT

Table 3: Effect of different concentrations of ethephon on branching of cucumber in Gulmi district, 2017

Concentration of Ethephon	Branching per plant at various days after transplanting				
	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT
Control	0.82 ^b	1.90 ^b	3.70 ^b	4.15 ^b	4.32 ^c
100 ppm	0.83 ^b	1.90 ^b	3.80 ^b	4.07 ^b	4.62 ^{bc}
200 ppm	1.18 ^a	2.30 ^{ab}	4.40 ^{ab}	4.82 ^{ab}	5.45 ^{abc}
300 ppm	1.05 ^{ab}	2.40 ^a	5.00 ^a	5.50 ^a	6.07 ^a
400 ppm	1.32 ^a	2.45 ^a	4.60 ^a	5.12 ^a	5.57 ^{ab}
SEM	0.09	0.14	0.25	0.27	0.30
LSD	0.30*	0.42*	0.75*	0.84*	0.92*
CV%	19.07%	12.59%	6.59%	11.57%	11.43%

Means followed by the same letter (s) in a column are not significantly different at 5% level of significance as determined by DMRT

Table 4: Effect of different concentration of ethephon on flowering node and flowering date of cucumber in Gulmi district, 2017

Concentration of Ethephon	First flowering node		First flowering date (DAT)	
	Male	Female	Male	Female
Control	3.33 ^b	8.35 ^a	9.75 ^b	25.30 ^a
100 ppm	3.72 ^{ab}	8.15 ^a	9.90 ^b	24.09 ^a
200 ppm	4.55 ^a	6.70 ^{ab}	12.80 ^{ab}	21.25 ^{ab}
300 ppm	4.82 ^a	5.90 ^b	13.05 ^{ab}	18.23 ^b
400 ppm	4.95 ^a	5.05 ^b	15.05 ^a	17.84 ^b
SEM	0.37	0.60	1.12	1.79
LSD	1.14*	1.86**	3.48*	4.75*
CV%	17.32%	17.69%	18.63%	14.46%

Means followed by the same letter (s) in a column are not significantly different at 5% level of significance as determined by DMRT

First flowering node and first flowering date

Table 4 shows the data achieved by application of ethephon 400 ppm produced male flower at upper nodes (4.95) compared to other treatments being at par with 300 ppm (4.82) and 200 ppm (4.55). Control produced male flower at lower nodes (3.33) being similar with 100 ppm (3.72). It showed a trend of increasing node number for first male flower appearance with increasing dose of ethephon. In case of female flowers 400 ppm produced female flowers in lowest node (5.05) being at par with 300 ppm (5.90) and control produced female flowers in uppermost nodes (8.35) being at par with 100 ppm (8.15).

Similarly, earliest male flowering (9.75 DAT) was seen in control being par with 100 ppm (9.90 DAT) followed by 200 ppm (12.80 DAT), 300 ppm (13.05 DAT) and latter flowering in 400 ppm (15.05 DAT). Thus, with increase in concentration of ethephon delays the flowering of male

flower. Likewise, earliest female flowering was found in 400 ppm (17.85 DAT) being at par with 300 ppm (18.23 DAT) and latter flowering was found in control (25.30 DAT) being at par with 100 ppm (24.09 DAT). The result signifies that with increase of ethephon earliness in female flowering date occurs.

Anatomical study showed that in primordial stage all flowers carry both set of sex organs and transformation of male flower to female can be induced by application of certain chemicals (Ito *et al.*, 1954). The reason for earliest production of female may be attributed to the maximum increase in starch and carbohydrate with ethephon treatments (Singh & Singh, 1984).

Total male and female flowers and sex ratio

With regard to ethephon concentration highest number of male flowers were obtained in control (107.0) followed by 100 ppm (73.57), 200 ppm (56.23), and least number

obtained in 400 ppm (46.90) being at par with 300 ppm (52.94). The data were found statistically significant (Table 5).

Total number of female flowers were also found significant statistically. Plant treated with 300 ppm produced highest number of female flowers (29.97) per plant compared with higher and lower concentrations being in par with 200 ppm (29.27). Similarly, lowest number of female flowers were found in control (18.25) which signifies that increasing the dose of ethephon increases female flower but concentration higher than 300 ppm is detrimental.

Fruit length and circumference

Table 6 shows that highest fruit length was obtained in control (23.0 cm) being at par with 100 ppm (22.77 cm)

followed by 200 ppm (21.75 cm) being at par and similar 300 ppm (21.80 cm) also being similar with 100 and 400 ppm and lowest length was recorded in case of 400 ppm (20.70 cm).

With increasing ethephon concentration fruit circumference increased significantly. Among different ethephon concentration 300 ppm (23.38 cm) significantly increased fruit diameter than its upper and lower concentrations being at par with 200 ppm (22.45 cm) and 400 ppm (22.05 cm) . Also least circumference was recorded in control (18.17 cm) being at par with 100 ppm (18.85 cm).

Table 5: Effect of ethephon on total male and female flower and sex ratio of cucumber in Gulmi district, 2017

Concentrations of Ethephon	Total male and female flowers per plant and sex ratio		
	Male flower	Female flower	Sex ratio(m:f)
Control	107.0 ^a	18.25 ^b	5.87 ^a
100 ppm	73.57 ^b	21.28 ^b	3.47 ^b
200 ppm	56.23 ^{bc}	29.27 ^a	1.98 ^c
300 ppm	52.94 ^c	29.97 ^a	1.83 ^c
400 ppm	46.90 ^c	20.31 ^b	2.50 ^c
SEM	6.15	2.46	0.27
LSD	18.95 ^{***}	7.58 [*]	0.84 ^{***}
CV%	18.20%	20.67%	21.12%

Means followed by the same letter (s) in a column are not significantly different at 5% level of significance as determined by DMRT

Table 6: Effect of various concentration of ethephon on fruit length and circumference of cucumber in Gulmi district, 2017

Concentration of Ethephon	Average fruit length and circumference in cm	
	Length(cm)	Circumference(cm)
Control	23.0 ^a	18.17 ^b
100 ppm	22.77 ^a	18.85 ^b
200 ppm	21.75 ^{ab}	22.45 ^a
300 ppm	21.80 ^{ab}	23.38 ^a
400 ppm	20.70 ^b	22.05 ^a
SEM	0.44	1.02
LSD	1.36 [*]	3.16 [*]
CV%	4.02%	9.78%

Means followed by the same letter (s) in a column are not significantly different at 5% level of significance as determined by DMRT

Table 7: Effect of different concentrations of ethephon on yield characters of cucumber in Gulmi district, 2017

Concentrations of Ethephon	Individual fruit weight and number of fruits per plant		
	Weight (gm)	Number	Yield(mt/ha)
Control	442.7.0 ^b	15.75 ^{bc}	17.48 ^b
100 ppm	461.3 ^b	15.62 ^{bc}	18.04 ^b
200 ppm	511.3 ^{ab}	18.94 ^{ab}	23.79 ^a
300 ppm	562.3 ^a	19.83 ^a	27.51 ^a
400 ppm	505.5 ^{ab}	14.18 ^c	17.95 ^b
SEM	24.35	1.07	1.71
LSD	75.04*	3.32*	5.28**
CV%	9.81%	12.79%	16.35%

Means followed by the same letter (s) in a column are not significantly different at 5% level of significance as determined by DMRT

Fruit weight and number of fruits per plant

Fruit weight in 300 ppm (562.3 gm) was found superior to other treatments followed by 200 ppm (511.3 gm) being at par with 400 ppm (505.5 gm). Control had least fruit weight (442.7 gm) being at par with 100 ppm (461.3). Similarly, Highest fruit number was seen in 300 ppm (19.83) followed by 200 ppm (18.94) and higher or lower concentrations had decreasing fruit number and least number of fruits was seen on 400 ppm (14.18). Control (15.75) and 100 (15.62) ppm were found at par and bear average number of fruits. Increase in number of fruit per plant may be attributed to increase in pistillate flower per plant by application of ethephon (El kholy and Hafez, 1982; Shindhu *et al.*, 1982). The change in fruit quality by application of ethephon in this investigation is in line with findings of (Verma *et al.*, 1985). Highest yield was obtained in 300 ppm (27.51 mt/ha) being at par with 200 ppm (23.79 mt/ha) followed by 100 ppm (18.04 mt/ha), 400 ppm (17.95 mt/ha) and lowest yield in control (17.48 mt/ha), latter 3 treatments being at par (Table 7).

Practically commercial benefit of increasing the number of pistillate flowers will be achieved only if there is corresponding increase in weight and number of fruits. Ethephon at 300 was found superior in increasing the number of fruits and ultimate fruit yield per plant. The increase in yield was predominantly due to increased number of pistillate flowers, fruit number and was also linked to increase in fruit diameter and average fruit weight. Patrick (1982) and Wyse *et al.* (1980) suggested plant growth regulators are involved in all aspects of growth and differentiation and might have played role in sink strength which may be attributed to increase in yield. The increase in number of fruits twice and fruit yield thrice than control by application of cucumber seedlings with ethephon at 100 150 ppm was reported by Singh (1985), twice fruit yield of cucumber than control at 50-100 ppm.

Conclusion

The experiment brought some important information about effect of ethephon on morphological, floral and yield attributing character of cucumber. Among various concentrations of ethephon used application ethephon at 300 ppm was found superior to its other levels including control for increasing number of female flowers and inhibiting male flowers and reducing male: female sex ratio. Also 300 ppm was found superior for increasing yield of cucumber. Yield in 300 ppm was found almost twice compared to control. Plant height was found negatively correlated with concentrations of ethephon. Highest number of nodes and maximum branching was also found in 300 ppm. Thus 300 ppm ethephon can be used as best alternative to induce female flower and increase yield. The research widens the possibility of recommending the spray of ethephon as a regular practice for better yield. Further studies with other plant growth regulators can be conducted to see their effect on plant. Since PGR's are chemical substances, temperature parameters should be considered for further research on ethephon doses on cucumber.

Author's Contribution

Shiva Dhakal designed the research plan; performed experimental works, collected the required data & prepared the manuscript. Shiva Dhakal, Mahesh Karki, Pritee Subedi, & Aarati GC analysed the data, critically revised and finalization of manuscript.

Conflict of Interest

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

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