



Efficacy of Ethephon Doses on Vegetative and Reproductive Attributes of Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]

Ashish Pangeni^{1,a}, Sandip Kathayat^{1,b}, Pankaj Karki Dholi^{2,c,*}, Sujan Khanal^{1,d},
Garima Parajuli^{1,e}, Bharat Bimarsa^{1,f}

¹Tribhuvan University, Kathmandu, Nepal

²Agriculture and Forestry University, Bharatpur, Nepal

*Corresponding author

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ABSTRACT

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Watermelon is a widely cultivated cucurbitaceous crop with economic and dietary significance. The plant's ability to reproduce effectively relies on the harmonious growth and maturation of both its male and female flowers. At the farm level, determining the exact amount of ethylene required to regulate the development and sexual expression of individual floral buds remains uncertain, despite the known influence of exogenously applied ethylene on these processes. The goal of the research was to explore how the ethephon doses influence vegetative characteristics, sex expression, and watermelon yield. The experiment, employing a one-factorial Randomized Complete Block Design (RCBD) comprising five treatments and four replications, was conducted in a farmer's field in Buttabari, Jhapa. Using R-stat software, the data were analyzed at a 5% level of significance. Treatment consisted of Ethephon with four doses namely; 100ppm, 200ppm, 300ppm, 400ppm, and a control. Ethephon application was done twice, once at the two true-leaf stages and another at four true-leaf stages. The study revealed that plant height decreased with ethephon compared to the control, reaching a minimum of 280.1 cm at 400 ppm and a maximum of 307.1 cm in the control. Additionally, the highest branch numbers were observed with ethephon at 200 ppm. The plot treated with 200ppm ethephon was found to be more effective than alternative doses at increasing the total number of female flowers, bearing 15.25 female flowers per plant. Control (166.50 per plant) and 400 ppm (150.00 per plant) had the highest and lowest male flower counts per plant, respectively. A maximum yield of 25.12 t/ha and a minimum yield of 19.1 t/ha were determined at 200 ppm and control, respectively. Hence, farmers are found to benefit from ethephon doses of 200 ppm.

^a pangeniashish7@gmail.com

^b <https://orcid.org/0009-0009-0863-1425>

^b sandipkathayat5566@gmail.com

^b <https://orcid.org/0009-0004-9023-873X>

^c karkidholipankaj@gmail.com

^c <https://orcid.org/0000-0002-9453-6193>

^d sujan.751403@mrmc.tu.edu.np

^d <https://orcid.org/0009-0000-8167-3474>

^e garimaparajuli123@gmail.com

^e <https://orcid.org/0009-0008-5323-8043>

^f bbc37142@gmail.com

^f <https://orcid.org/0009-0001-4643-7916>



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Introduction

Fruit crops play a vital role in agriculture, contributing significantly to both the nutritional needs and economic prosperity of many regions worldwide. Among these, watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] stands out as a popular and economically important member of the cucurbit family (Cucurbitaceae). Cultivated primarily in tropical and subtropical regions, watermelon thrives in environments characterized by long periods without frost and consistently warm temperatures (Paris, 2015). Rich in immune-supportive vitamins such as Vitamin C and Vitamin A, along with essential minerals like potassium and magnesium, watermelon serves as a nutritious and refreshing addition to diets (Zia et al., 2021).

Despite its nutritional value and economic potential, watermelon cultivation faces challenges, particularly in regions like Nepal. Limited access to irrigation facilities, fertilizers, and other inputs, coupled with the seeding of monoecious varieties with imbalanced sex ratios, impedes farmers from realizing the maximum yield potential of watermelon (MoALD, 2021). The Icebox variety of watermelon, characterized by deep red flesh and a dark green rind, matures earlier than larger varieties due to its smaller size. However, like many monoecious varieties, it tends to produce a higher abundance of male flowers, hindering optimal production (Vegetable Research & Extension Center, n.d.).

To address these challenges and optimize watermelon production, the use of plant growth regulators such as ethephon presents a promising avenue. Ethephon, a chemical compound that releases ethylene upon application to plants, has been extensively studied for its effects on various plant processes, including fruit ripening, abscission, and growth inhibition (Lürssen, 2018; Hussain et al., 2020). Studies have shown that ethephon treatments can influence watermelon flower development and sex expression by altering hormone levels and gene transcription associated with ethylene synthesis and signaling, as well as other hormone-responsive genes (B. Yang, 2007; Rosli et al., 2012). Manipulating these hormonal pathways offers a potential solution to balancing the male-to-female flower ratio in monoecious watermelon varieties, thereby enhancing yield potential.

In light of these considerations, the objectives of our research are to evaluate the effects of various ethephon doses on vegetative parameters, reproductive expression, growth, and yield performance of watermelons. We hypothesize that ethephon doses will have a significant impact on these parameters, potentially addressing the challenges faced by watermelon growers in Nepal.

Materials and Method

Experimental Setup and Procedures

The research was conducted in a farmer's field located in Birtamod-6, Buttabari, Jhapa, Nepal, in 2022, at an elevation of 137 meters above mean sea level (26° 39' 20" N, 88° 0' 41" E). The tropical climate of the region, experiencing an annual rainfall of 250 to 300 cm and maximum and minimum temperatures of 42°C and 10°C in summer and winter respectively, provided the environmental backdrop for the study. This study was carried out from April to July. Watermelon Icebox KALYAN variety seeds were procured from an agrovet in the Birtamod market, while ethephon (2-chloroethyl phosphonic acid) was sourced from the laboratory of Mahendra Ratna Multiple Campus in Ilam, Nepal. The experiment followed a one-factorial Randomized Complete Block Design (RCBD) with five treatments and four replications. A total of 200 plants were utilized, arranged in 20 plots within the 44 m × 12 m research field, each containing 10 rows of plants spaced 150 cm × 100 cm apart. Throughout the experiment, data on vegetative growth, flowering patterns, and yield were collected at regular intervals to assess the effects of ethephon treatments on watermelon plants.

General Cultural Practices

Seedlings were prepared in poly bags of size 15*8cm under protected conditions and transferred on the field on February 15 with a 150cm * 100cm spacing as shown in Figure 1. 528 kilograms of well-rotted farmyard manure (FYM) with an NPK ratio of 55:55:55 kg/ha was used as fertilizer in the experiment. The basal dose consisted of a full dose of phosphorus and potassium, along with half the dose of nitrogen. The remaining half dose of nitrogen was applied 30 days after transplanting. Harvesting was conducted when the underside or belly of the fruit transitioned from a greenish-white color to a buttery yellow or cream hue. The yield and the factors influencing the yield were noted at each harvest.

Layout of Experiment

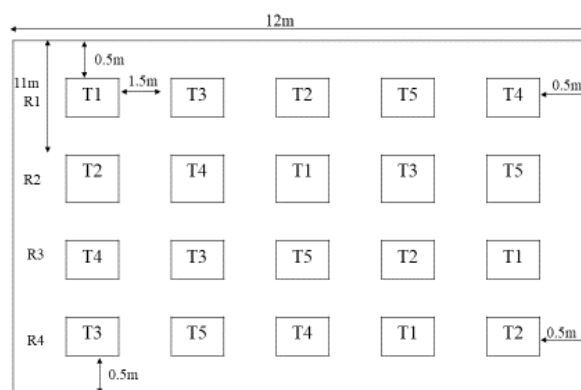


Figure 1. Layout of Experiment

Table 1. Treatment Detail

Treatment	Ethephon
T1	Control
T2	100ppm
T3	200ppm
T4	300ppm
T5	400ppm

Ethephon Solution Preparation and Data Collection

The commercial variant of ethephon, M-Phone (ethephon 40% SL), was utilized, prepared by diluting a 1000 ppm stock solution to create concentrations of 400 ppm, 300 ppm, 200 ppm, and 100 ppm. Ethephon application was done using a syringe with a least count of 0.1 ml at the 2-4 true leaf stages of the plants. Data collection included measuring plant height with a measuring tape, counting the number of fruits per plant and male/female flowers manually, and counting nodes and branches. The first flowering date was observed through field monitoring. Fruit diameter was measured using a tape measure, while fruit weight was recorded using a digital weight scale, allowing subsequent calculation of yield and ratio.

Statistical Analysis

Recorded data was systematically arranged based on various parameters. The data were entered into MS Excel Version 2019 following a standard format. Subsequently, the data underwent analysis of variance (ANOVA) suitable for the one-factorial Randomized Complete Block Design (RCBD) technique using R-stat version 4.2.1. To compare means, Duncan's Multiple Range Test (DMRT) was employed at a 5% significance level in cases where significant differences existed between the treatment means.

Results and Discussion

Vegetative Attribute

Plant Height

The data analysis (Table 2) showed that the use of ethephon at 15DAT, 30DAT, 45DAT, and 60DAT had a significant impact on the watermelon plant height. The highest plant height was recorded at (307.1 cm) at 60DAT on control and the lowest was recorded at 400ppm at 60DAT.

Ethylene's ability to stop the action of gibberellins in plants affects the growth of roots and shoots by slowing down cell division, which in turn leads to shorter plants (Hayashi et al., 2001). This alteration in gene expression resulted in a reduction in the amount of ethylene precursor, ultimately contributing to the decrease in plant height. Likewise, ethylene stimulates auxin biosynthesis and basipetal auxin transport, leading to the suppression of cell elongation, thereby inhibiting plant height, as noted by Negi et al. (2008). Ethepon also acts as an anti-gibberellin, inhibiting the action of gibberellic acid, which is a plant hormone responsible for stem elongation, resulting in reduced plant height (Hayashi et al., 2001). Due to ethylene's above-mentioned anti-auxin and anti-gibberellic acid properties, its application significantly reduces plant height.

Number of nodes.

The analyzed data from Table 3 revealed a highly significant impact of ethephon on the node number of watermelons at 15DAT, 30DAT, and 60DAT. The control group exhibited the highest number of nodes, while the

lowest number was observed in the 400 ppm treatment group up to 30 DAT. Similarly, 300 ppm shows the highest number of nodes (39.4) and control shows the lowest number of nodes (31.3) at 60 DAT.

It shows that the 300ppm ethephon application gave good results and an increase in node number. Ramezani et al. (2017) also reported that the use of ethylene increases the number of internodes in monoecious cucumber plants. This may be due to ethylene's effect in decreasing internodal distance in plants by increasing the responsiveness of cells to endogenous gibberellins and reducing the levels of abscisic acid (Azuma et al., 2001). A reduction in internodal distance caused by ethephon treatment attributed to the suppression of cell division (Dhakal et al., 2019).

Branching

From the observations presented in Table 4, it was noted that at 60DAT, the maximum number of branches was recorded in the case of the 200ppm treatment (11.00), followed by the 300ppm treatment (10.8), while the lowest number of branches was observed in the control group (8.7).

Table 2. Effect of ethephon doses on the height of watermelon plants treated with different doses of ethephon in 2022

Treatments	Plant height (cm)			
	15DAT	30DAT	45DAT	60DAT
400ppm	16.9±0.3 ^e	192.3±0.5 ^e	202.4±0.6 ^e	280.1±0.3 ^d
300ppm	20.9±0.5 ^d	210.8±0.8 ^d	230.0±0.5 ^d	286.6±0.9 ^c
200ppm	30.3±0.7 ^c	217.8±1.0 ^c	254.8±0.5 ^c	288.8±0.7 ^c
100ppm	36.6±0.4 ^b	232.8±0.8 ^b	272.8±0.5 ^b	302.1±0.9 ^b
control	38.6±0.4 ^a	237.1±1.3 ^a	289.5±1.6 ^a	307.1±0.8 ^a
LSD	1.6***	3.0***	2.5***	2.4***
CV (%)	3.6	0.9	0.7	0.5
Mean	28.7	218.1	249.9	293.0

The absence of a significant difference is denoted by identical letter(s) within the respective column. The asterisks (*, **, ***) represent the levels of statistical significance, with * indicating p < 0.05, ** indicating p < 0.01, and *** indicating p < 0.001.

Table 3. Number of nodes per plant of watermelon treated with different doses of ethephon in 2022

Treatment	Number of nodes			
	15DAT	30DAT	45DAT	60DAT
400ppm	2.4±0.2 ^c	13.3±1.6 ^b	32.9±1.5 ^a	38.8±0.3 ^a
300ppm	2.9±0.1 ^c	16.2±1.4 ^a	29.8±3.1 ^a	39.4±0.3 ^a
200ppm	3.1±0.1 ^{bc}	17.7±0.5 ^a	30.8±1.3 ^a	34.7±0.8 ^b
100ppm	3.7±0.1 ^{ab}	18.6±0.6 ^a	29.7±0.5 ^a	32.4±0.3 ^c
Control	3.9±0.4 ^a	18.9±0.2 ^a	29.7±0.8 ^a	31.3±0.6 ^c
LSD	0.7**	2.8**	4.2 ^{ns}	1.5***
CV (%)	13.5	10.8	8.9	2.7
Mean	3.2	16.9	30.6	35.3

The absence of a significant difference is denoted by identical letter(s) within the respective column. The asterisks (*, **, ***) represent the levels of statistical significance, with * indicating p < 0.05, ** indicating p < 0.01, and *** indicating p < 0.001.

Table 4. Branches number per plant in watermelon treated with different doses of ethephon in 2022

Treatment	Number of Branches			
	15DAT	30DAT	45DAT	60DAT
400ppm	1.7±0.4	5.7±0.4	8.3±0.3	9.6±0.1 ^{ab}
300ppm	1.6±0.2	4.8±0.5	8.7±0.6	10.8±0.1 ^a
200ppm	1.6±0.2	5.1±0.2	8.3±0.4	11.0±0.7 ^a
100ppm	2.2±0.2	5.3±0.3	8.0±0.2	10.0±0.3 ^{ab}
Control	2.2±0.1	5.0±0.3	7.4±0.2	8.7±0.5 ^b
LSD	0.7 ^{ns}	1.1 ^{ns}	1.2 ^{ns}	1.4*
CV (%)	23.7	13.4	9.6	9.1
Mean	1.9	5.2	8.1	10.0

The absence of a significant difference is denoted by identical letter(s) within the respective column. The asterisks (*, **, ***) represent the levels of statistical significance, with * indicating p < 0.05, ** indicating p < 0.01, and *** indicating p < 0.001.

More branches were seen as the ethephon dose increased, but fewer branches were seen as the ethephon dose increased further. The 200 ppm and 300 ppm concentrations of ethephon outperformed higher and lower concentrations, respectively. A raise in the number of productive branches was observed with the application of ethylene, as reported by Bahar et al. (2018). The increase in the number of productive branches with the application of ethylene might be due to its role in promoting cell division and expansion, as well as its involvement in processes such as endoreduplication and inhibition of cytokinesis, which contribute to increased branching (Dan et al., 2003). While ethylene is also involved in the activation of enzymes such as cellulase and polygalacturonase associated with the abscission process (Caser et al., 2015), its primary influence on branching likely stems from its regulation of hormonal pathways that promote growth and development, rather than abscission.

Number of staminate flowers

The data shown (Table 5) demonstrated that ethephon application had a significant impact on the staminate flower per plant. Control has the highest staminate flower recorded (166.5) and the lowest (150.0) was recorded at 400ppm treatment. Staminate flowers from 200 ppm and 300 ppm were recorded as statistically similar (156.3) and (154.3). By analyzing, it can be said that higher ethephon application reduces the staminate flowers.

Evidence suggests that the exogenous application of ethylene can decrease male flowers in plants by inducing earlier flower senescence and reducing the transcript abundance of ethylene receptor genes (Thongkum et al., 2015). The exogenous application of ethylene induces the expression of a repressor protein which inhibits the biosynthesis of viable pollen resulting in decrease in number of pistillate flowers (Bridge et al., 1994; Wang et al., 2010).

Number of pistillate flowers

The analyzed data (Table 5) showed that ethephon application had a significant impact on the female flower

per plant. On 200 ppm the highest (15.2) number of female flowers was recorded and the lowest (12) was in control. 100ppm and 300ppm ethephon applications gave statistically similar results.

Exogenous application of ethylene promotes female flower development in plants by inhibiting stamen development and potentially regulating ovary development through miRNA and TCP gene expression (Sun et al., 2010). Ethylene halts stamen development in female flowers, thereby promoting the formation of pistillate flowers in watermelons (Wang et al., 2010; Manzano et al., 2014).

Sex ratio

Analyzing the data from (Table 5) revealed that the use of ethephon had a significant impact on the sex ratio. The lowest (10.3:1) sex ratio (male: female) was recorded at 200ppm statistically similar to treatment (10.7:1) with 300ppm. At a higher dose of 400ppm, it starts to rise (11.6:1) and was observed highest in Control (13.7). In cucurbits, ethylene encourages femaleness rather than maleness, which ultimately lowers the male-to-female ratio. (Rudich et al., 1969).

First flowering date

Data from Table 6 indicated a significant influence of ethephon application on flowering days. The earlier male flowering (20.9 DAT) was observed in the control, and statistically similar results were obtained in 100 ppm (25.5 DAT), 200 ppm (28.2 DAT), 300 ppm (30.7 DAT), and 400 ppm (32.1 DAT) for later flowering. Consequently, an increase in ethephon concentration leads to a delay in the flowering date of male flowers. Similarly, the earliest female flowering was observed in control (25.6 DAT) and late female flowering was recorded at 200 ppm (32.8 DAT). Hence it was found that the application of ethephon delays the male flowering. The study also revealed that one spraying of ethephon at 500 ppm delays male flowering by 16 days (Edelstein et al., 1985). The application of ethylene delays flowering in male cucurbit flowers (Wang et al., 2010).

Table 5. Effects of ethephon doses on reproductive parameters of watermelon in 2022

Treatments	No. of staminate flowers	No. of pistillate flower	Sex ratio (M: F)
400ppm	150.0±2.160 ^d	13.0±0.7 ^{bc}	11.6±0.6 ^b
300ppm	154.3±1.750 ^{cd}	14.5±0.6 ^{ab}	10.7±0.6 ^b
200ppm	156.3±1.030 ^c	15.3±0.8 ^a	10.3±0.5 ^b
100ppm	161.0±0.707 ^b	14.0±0.6 ^{abc}	11.6±0.5 ^b
control	166.5±1.554 ^a	12.3±0.6 ^c	13.7±0.8 ^a
LSD	4.4***	2.0*	1.7**
CV(%)	1.8	9.5	9.6
Mean	157.6	13.8	11.6

The absence of a significant difference is denoted by identical letter(s) within the respective column. The asterisks (*, **, ***) represent the levels of statistical significance, with * indicating $p < 0.05$, ** indicating $p < 0.01$, and *** indicating $p < 0.001$.

Table 6. Effect of ethephon doses on the first days of male and female flowering of watermelon in 2022

Treatment	Days to first male flowers (DAT)	Days to first female flowers (DAT)
400ppm	32.2±0.6 ^a	35.4±0.8 ^a
300ppm	30.8±0.4 ^a	35.3±1.0 ^a
200ppm	28.3±0.3 ^b	32.9±0.5 ^b
100ppm	25.6±0.7 ^c	31.6±0.2 ^b
control	20.9±0.4 ^d	25.6±0.6 ^c
LSD	1.5***	2.1***
CV(%)	3.4	4.3
Mean	27.5	32.2

The absence of a significant difference is denoted by identical letter(s) within the respective column. The asterisks (*, **, ***) represent the levels of statistical significance, with * indicating $p < 0.05$, ** indicating $p < 0.01$, and *** indicating $p < 0.001$.

Table 7. Effect of ethephon on length of fruit and diameter of watermelon at Jhapa, Nepal in 2022

Treatment	Fruit length (cm)	Fruit diameter (cm)
400ppm	24.4±0.9 ^c	18.6±0.2 ^b
300ppm	27.6±0.9 ^{ab}	19.1±0.7 ^{ab}
200ppm	28.1±1.4 ^a	19.9±0.8 ^a
100ppm	27.7±0.8 ^{ab}	19.1±0.7 ^{ab}
control	25.8±0.2 ^{bc}	18.7±0.6 ^b
LSD	2.0**	1.0***
CV (%)	4.9	3.1
Mean	26.7	19.1

The absence of a significant difference is denoted by identical letter(s) within the respective column. The asterisks (*, **, ***) represent the levels of statistical significance, with * indicating $p < 0.05$, ** indicating $p < 0.01$, and *** indicating $p < 0.001$.

Table 8. Effect of ethephon doses on fruit per plant, average fruit weight, and total yield of watermelon in 2022

Treatment	No. of fruit per plant	Average weight of fruit (kg)	Total yield (MT/ha)
400ppm	2.1±0.2 ^b	2.5±0.1 ^c	19.6±0.8 ^{cd}
300ppm	2.6±0.1 ^a	2.9±0.1 ^b	21.9±0.7 ^{bc}
200ppm	2.6±0.1 ^a	3.3±0.1 ^a	25.1±0.1 ^a
100ppm	2.4±0.2 ^{ab}	3.3±0.1 ^a	23.1±1.3 ^{ab}
control	2.1±0.1 ^b	2.6±0.1 ^c	19.1±0.8 ^d
LSD	0.3*	0.3***	2.5**
CV (%)	9.4	6.5	7.5
Mean	2.3	2.9	21.8

The absence of a significant difference is denoted by identical letter(s) within the respective column. The asterisks (*, **, ***) represent the levels of statistical significance, with * indicating $p < 0.05$, ** indicating $p < 0.01$, and *** indicating $p < 0.001$.

Mean Comparison Value of Sex Ratio (M/F) and Total Yield (mt/ha)

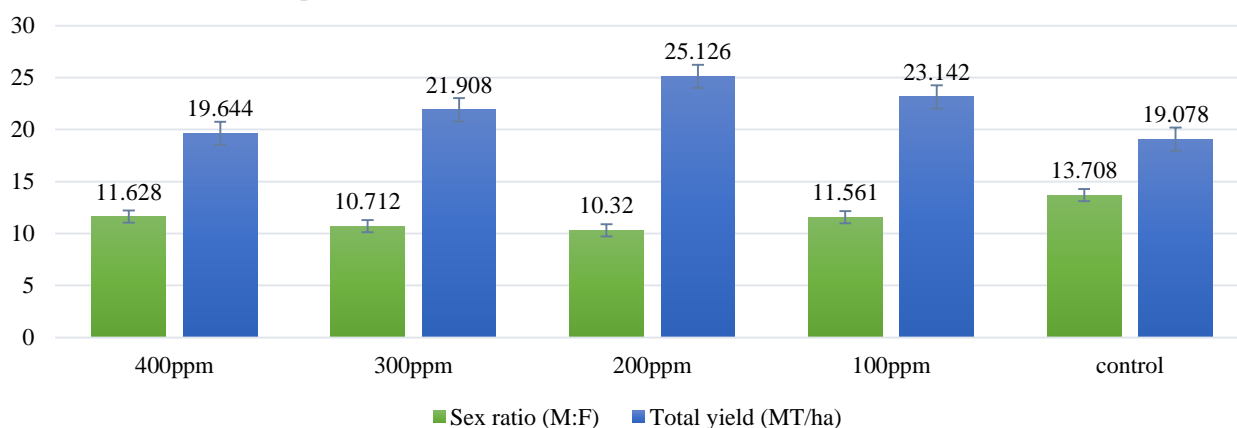


Figure 3. Effect of ethephon doses on Sex ratio and total yield of watermelon

Fruit diameter and length

The length of the watermelon fruit was found to differ significantly due to ethephon, as shown in (Table 7), The highest length of fruit was recorded at 200ppm (28.1 cm) while the lowest was at 400ppm (24.4 cm), showing that higher ethephon application decreases the fruit length.

It demonstrates that ethephon had a significant impact on fruit diameter. In 200 ppm, 100 ppm, and 300 ppm maximum fruit diameters of 19.9 cm, 19.1 cm, and 19.1 cm were recorded respectively. The smallest fruit diameter was noted in the control (18.7 cm) and 400 ppm (18.7 cm).

As the concentration of ethephon rose, there was a notable increase in fruit circumference and length (Dhakal et al., 2019). The possible cause may be ethylene's ability to affect hormonal and genetic mechanisms that control the fruit cell growth and division ultimately leading to an increase in fruit diameter (Xue et al., 1996).

Yield and Yield Attributing Parameters

Number of fruit per plant, Average weight of fruit

Fruit weight in 200 ppm (3.3 kg) was found to be better than other treatments, then 100 ppm (3.2 kg). 400ppm had the lowest fruit weight (2.5 kg) being statistically with the control (2.6 kg). Similarly, In 200 ppm and 300 ppm highest fruit numbers (2.6) and (2.5) were observed respectively whereas 400 ppm (2.062) had the lowest number of fruits.

Ethylene has been identified as a master regulator of sex determination in cucurbits, and the control of sex determination is closely linked to the control of fruit development and weight (Srividhya & Sujatha, 2017; An et al., 2020). The capacity of ethylene to stimulate respiration and raise levels of fatty acids and volatiles may be the cause of the plant's increased fruit production. (Hajam et al., 2018).

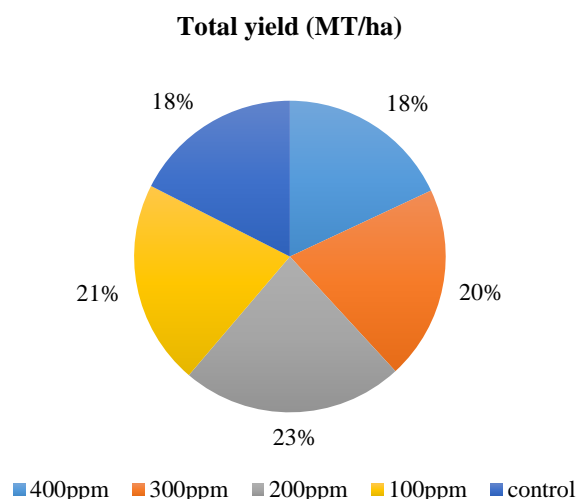


Figure 4. Impact of Different Ethephon Doses on the Total Yield of Watermelon (MT/ha)

Also, ethylene is involved in the fruit setting process and can cause fruit set without fertilization, resulting in the development of parthenocarpic fruit, and increasing the fruit yield (Martínez & Jamilena, 2021).

Productivity

Ethephon treatment significantly influenced the observed variable, as indicated in Table 8. In 200 ppm the highest yield (25.1 mt/ha) was recorded whereas in control lowest productivity was recorded (19.1 mt/ha).

The application of ethephon led to a rise in cucurbits yield, attributed to an increase in pistillate flowers, fruit weight, circumference, diameter, and the number of fruits per plant (Baral et al., 2022).

Conclusion

The findings of the present study suggest that ethephon exerts a beneficial impact on the sex expression, growth, and yield of watermelon. Among various concentrations of ethephon used, the level of 200 ppm was found to be better than all other levels of doses, including controls for boosting female flower production while reducing male floral production and the sex ratio. The yield of watermelon was also observed to be enhanced with the application of 200 ppm. There was a negative relationship between ethephon concentration and plant height. The maximum number of nodes was seen at 300 ppm concentration while the highest number of branches were discovered at 200 ppm concentration. So, the most effective substitute for promoting female flowering and increasing yield is 200 ppm. The research raises the possibility that ethephon spraying should be advised as a regular practice for increased yield. It is suggested to do similar research with expanded coverage on the effect of ethephon uses with multi-location and years to verify these early findings before drawing any firm conclusions.

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