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ENHANCEMENT OF FLOWER INDUCTION IN HARUMANIS MANGO (*Mangifera indica* L.) WITH ETHEPHON APPLICATION DURING OFF-SEASON

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Abstract:

In cultural practice of Harumanis tree, plant growth regulator such as paclobutrazol is applied at late vegetative stage to induce flowering. However, starting from 2020 to 2023, there is a sharp yield reduction due to the unexpected weather condition. Unexpected heavy rain had caused Harumanis flower to drop from the panicle before the pollination occur. Due to this problem, ethephon was identified as an alternative plant growth regulator for flower induction. Ethephon is an ethylene releasing agent that help mediate many aspects in plant growth and development such as shoot development and flower induction. Thus, the objective of this study is to observe the influence of ethephon application during off-season on vegetative growth stage and flower induction of harumanis tree. Total of 32 Harumanis trees (3 years old) in Research Plot, Unit Ladang Universiti Teknologi MARA Perlis were treated with ethephon in September 2023 by foliar spray until run-off on the plant canopy. Treatments were arranged according to Randomized Completely Block Design (RCBD) with three different concentrations of ethephon and being diluted 2 hours before application which are 400 ppm, 600 ppm, and 800 ppm and control (without application). The parameters were recorded 2 weeks interval: number of leaves, number of buds, length of bud, relative chlorophyll content, stomatal density, day to flowering, and number of flower panicles. The vegetative growth stage shows significant difference among concentrations applied where number of leaves is higher with 400 ppm. Meanwhile, the flower formation occurs after 2 months and 4 months of ethephon application at treatment of 800 ppm and 400 ppm respectively. However, there is no

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significant difference presented on the Harumanis physiological characteristics. As from this study, it can be concluded that the application of ethephon influences the growth of performance and flower formation of Harumanis when applied with specific concentration.

Keywords:

Harumanis Mango, Ethephon, Plant Growth Regulator, Vegetative Growth, Physiological Characteristics, Flower Induction

Introduction

Mangifera indica or its common name, mango is a perennial fruit plant that originated from India for thousands of years before being introduced to other tropical and subtropical regions around the world (Warschefsky & Wettberg, 2019). As with 41.6% of total global production, India is still the largest producer of mango in the world until today (Jameel, Naik, Madhumathi, Reddy, & Venkataramanaraz, 2018). There are about 850 species of mango that had been grouped into 73 genera under the order of Sapindales in the Anacardiaceae family. In tropical Asia, there are two sub-genera that divided from 70 species of Mangifera genus which are Limus and Mangifera where Harumanis is in the sub-genera Mangifera (Razak et al., 2019). In Malaysia, the most popular variety of mango is known as Harumanis (MA 128). It is mostly planted in the northern region of Malaysia, Perlis due to the temperature condition that suits well with growth pattern of Harumanis. Harumanis being the most awaited mango due to its perfect sweetness and pleasant aroma that provide a great eating quality (Sani, Abbas, Jaafar, & Ghaffar, 2018).

Harumanis grown at Unit Ladang UiTM Arau, Perlis has been successful in reaching a wide market of Harumanis in Perlis and other states over 10 years through its total production per season of more than 10,000 kilograms. However, in 2022, the total production decreases extremely as shown in Table 1, causing the yield harvested only 1,000 kilograms due to the microclimate factor which is heavy rain at the plantation area. The unexpected heavy rain causes the Harumanis flower to drop by turning it into dark colour before falling from the panicle. This situation is worst when the pollination did not occur yet before the flower begins to drop. Thus, plant growth regulator is the only way that can be applied to induce the flowering since weather condition cannot be controlled. Plant growth regulator is a chemical substance that influence the growth of a plant vegetative and reproductive growth. Ethephon is one of the plant growth regulators that are usually applied on mango crop to induce the flowering and influencing the vegetative growth in several country such as India and Philippine. It regulates plant growth and development by influencing various biological and non-biological processes such as stimulating leaf senescence and fruit ripening to improve earliness (Wang et al., 2023). This study aims to determine the effect of different ethephon concentration application on growth performance and physiological characteristics of Harumanis mango, also its ability to induce the flowering of Harumanis.

Table 1: Yield of Harumanis (2021-2023) in Unit Ladang UiTM Perlis

Year	Quantity (Box/2kg)	RM
2021	10,783	194,090
2022	8,308	166,154
2023	1,020	20,406
2024	140	8,285

Source: Unit Ladang UiTM Perlis

Harumanis Mango

Background

The origin of Harumanis was believed to be coming from the neighbour country, Indonesia in the region of Probolinggo. Harumanis or known as MA 128 at first was actually coming from Sungai Nibong, Kuala Selangor before being introduced by MARDI. After it had been registered with Department of Agriculture (DOA) on 28 May 1971, it is being domesticated widely in Malaysia. This variety of mango is well accepted by the Malaysian and it is also being a commercial crop chosen by farmers to be grown in their farm. In Perlis, 1,037 hectares of land had been used to be cultivated with Harumanis (Uda et al., 2020).

Figure 1: Harumanis Mango

Cultivation

Harumanis seed is polyembryonic which means the seed have nucellar embryos that is genetically identical to the mother plant which make Harumanis able to be propagated by its seed. However, for several practices in Perlis, farmers rather choose to propagate Harumanis by grafting instead of propagate it by seed. The propagation of Harumanis was done with mango var. Telur as this variety has a strong root system. Mango var. Telur being used as the rootstock in grafting while harumanis shoot will be used as the scion. Grafting technique applied was wedge grafting where the scion base was cut into a wedge shape, then the upper rootstock part was removed before vertical cut was made suitable with the length of scion cut.

Morphological Characteristics

Harumanis fruit shape will vary upon its variety such as round, oval, heart shape and kidney shape. The length of Harumanis range from 15 centimetres to more than 30 centimetres depending on the cultivar and shape. the seed of this mango variety has a length of 13 to 14 centimetres. The average weight of Harumanis begin from 300-650 grams which make

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the total income of yield is high even in low cultivation area. It has a thick skin with large and light-yellow dots, covered with a wax layer. The flesh texture of ripe Harumanis is smooth, firm, no fibre but less water content (Uda et al., 2020).

Growth Cycle

Cycle of Harumanis growth begin with its vegetative growth from July until December. At this stage, the stem and leaves are growing and collecting carbohydrate as the energy source that is required during the flowering period. The productive growth starts with the blooming of the flower until February. Productive growth is where the conversion of a vegetative bud at the terminal stem into a reproductive meristem. Fruit will first initiate during March with three stage of development which are pea stage, marble stage and pre-harvest stage. Pea stage will occur when the flower petal had dropped and also at this stage, fruit drop phenomena will happen. Fruit drop at pea stage happen due to the inability of the flower to pollinate or the sperm cell of the pollen did not reach ovary. The last stage of fruit drop will be at pre-harvest stage which considered as a serious problem since it will cause major losses on yield (Kumari & Kumar, 2022). Fruit can be harvested from April until June before the tree undergo pruning process in order to start the cycle of Harumanis growth again. However, for the first two years of harvesting Harumanis, the yields' quality is not as good as the next three years yields. In other words, Harumanis will provide the best quality of yield after three cycles of production.

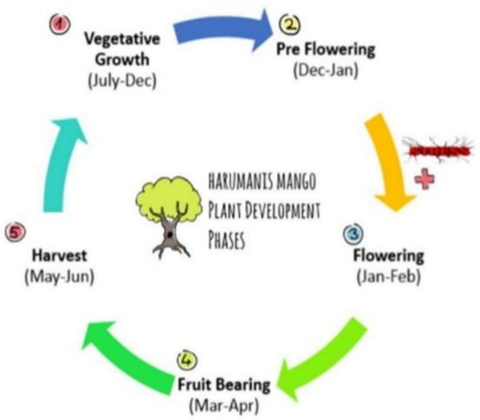

Figure 2: Harumanis Plant Development Cycle

Source: Uda et al., 2020

Flowering Cycle

Reproductive growth of Harumanis will only occur once a year for each given stem. The flowering involves the conversion of a vegetative bud at the terminal stem into a reproductive meristem. Then, it will develop a panicle that bears several thousand individual flowers that open acropetally where the flower will begin to bloom from the base to the apex in succession. The vegetative growth starts on July and ends in December as the pre-flowering phase is coming. At the vegetative stage, plant will focus on carrying photosynthesis and accumulating resources that will be needed for flowering and reproduction. Entering the pre-flowering phase, the flower induction process is stimulated where low temperature and low rainfall is required for at least two weeks or more. This flower induction process is also known as environment stress.

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Ethephon: Ethylene Releasing Compound

Ethephon As a Plant Growth Regulator

Plant growth regulator helps in increasing the yield and quality of mango tree (Gattass, Essa, Marzouk, & El-Nawam, 2018) beside playing an important role in inducing the flower. In plant biology, ethylene is considered as a major hormone when it mediated many complex aspects of plant growth, development and survival throughout the plant life cycle including seed germination, root and shoot development, leaves, and fruits abscission, inducing flower, sex determination and senescence of flowers and leaves. Besides the changes ethylene had given to the physiological characteristic of a plant, it also mediates adaptive responses to several stresses caused by the environment such as drought, flooding, pathogen attack and high salinity. For example, during flooding, ethylene will induce aerenchyma tissue that form an air channel in the leaves, stem, and root for the exchange gases process between root and shoot (Behera & Ramachandran, 2021). Aerenchyma will promote the oxygen diffusion to the submerge organ as the respond to the waterlogging stress (Yang et al., 2023)

The Effect of Ethephon During Vegetative Stage

The effect of ethephon application differs on the plant species, time of application, and the concentration of ethephon as it regulates the phases of plant growth and development by application to various growth sites (Bhadoria et al., 2018). A previous study on cucumber had proven that application of ethephon at 300 ppm will increase the number of primary and secondary branch rather than improving the stem elongation (Mir, Sadat, Amin, & Islam, 2019) as the ethylene is able to inhibit IAA transport in plant system (Dhakal, 2019). Meanwhile, the application of ethephon at 250 ppm on cucumber had supressed the elongation of internode which result in stunted growth. (Mir et al., 2019). In addition, a study on wheat crop had shown that ethephon affect on the plant height where it inhibits the transfer of auxin in the stem tissues before reducing the abilities to stimulate stem elongation (Hussein & Hashem, 2021). Moreover, a study of plant height in melon crop stated that the plant height will decrease as the concentration of ethephon increase and die at 5,000 ppm of ethephon concentration. This may cause by the anti-gibberellic property of ethylene that stop the mitotic process in root and shoot meristem which then reducing the plant height. However, the number of nodes in every plant does increase as the concentration increase which may be attributed to the internode distance reduction by suppressing the cell division (Dhakal, 2019).

The Effect of Ethephon on Mango Flowering Stage

In a previous study on mango flowering, 600 ppm and 1,000 ppm of ethephon was sprayed to increase the flowering percentage and shortened the flowering time. The result show that 1,000 ppm of ethephon concentration has higher percentage of flowering and faster flower production than 600 ppm. Other study had also shown 200 ppm of ethephon does shorten the flowering day and increase the percentage of flowering as to be compared with control but in small value (Patoliya, Tandel, Patil, & Patel, 2017). Thus, it may be concluded that the flowering percentage of mango and number of days to flowering will increase as the concentration increase (Maloba, Ambuko, Hutchinson, & Owino, 2017).

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Methodology

Study Site

The research was done at research plot in UiTM Arau, Perlis of a coordinate of 6°26’49.1” N 100°17’08.0” E. The total of 280 Harumanis trees were grown in the 6 acres area with planting distance of 9 m x 9 m. It had been planted on 2021 which mean the Harumanis tree now is around 3 years old. The average rainfall in the area is 1,952 mm every year with wind speed at 0.3 ms^{-1}. Annual temperature range between 24°C to 36°C with percentage humidity of 52.6–95.6%.

Research design

In this study, treatments were arranged according to Randomized Completely Block Design (RCBD). There are four treatments with 8 replications of each treatment. However, there are several trees being made as a border tree in between of the treatment tree for replicate 1, 7, and 8 because the number of the secondary branches is less than four. The total of Harumanis tree used for treatment application are 32 trees. Each of the trees being tagged with number treatment and replicate while there are four branches for each tree being tagged randomly for the data collection.

Field Maintenance

Prior to the application of ethephon, there are several field maintenances being done at the plot. One day before the application, pruning on shoot tip of the branch was done by using secateur as to stop the auxin production and influence the growth of lateral bud. Meanwhile for the moving activity, it was done once every 2 weeks by using mowing tractor. Other field maintenance such as fertilizer and pesticide application were done according to common practices of Unit Ladang UiTM Arau.

Application of Ethephon

Total of 32 trees of three years old Harumanis had been treated with different concentration of ethephon as shown in Table 1 by foliar spray. Ethephon was diluted in water 2 hours before being spray until run-off on the plant canopy around 500 ml for each plant by using knapsack sprayer in the morning. The application of the treatments was done on the first week of September 2023.

Table 1: Treatment of Ethephon Concentration (ppm) Per Plant

Treatment	Dosage/tree	Concentration (ml/L)
T1	-	Without ethephon (control)
T2	0.2ml + 500ml water	400
T3	0.3ml + 500ml water	600
T4	0.4ml + 500ml water	800

Data Collection

Data collection begins two weeks after ethephon application which is in September 2023 until December 2023. Growth performance of Harumanis tree was observed by collecting data on number of leaves, number of buds, and length of bud on four randomly tagged branches of each tree. meanwhile, physiological characteristics also being observed by measuring the relative chlorophyll content by using SPAD meter and also measure the stomatal density by using leaf

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impression method. Data collection on growth performance and physiological characteristics were done every two weeks interval after treatment application. As for flower production, days to flowering was recorded when the inflorescence at bud break stage after treatment application while number of flower panicles were recorded every two weeks interval after treatment application.

Figure 3: Data Collection on Relative Chlorophyll Content

Data Analysis

All of the data collected from the beginning until end of observation were recorded and arranged accordingly based on the parameters. The average value of each treatment was calculated and analysed in Statistical Package for the Social Science (SPSS) by using Analysis of Variance (ANOVA). The treatment means was separated by using Least Significance Difference (LSD) at the 5% level of significance.

Result and Discussion

Growth Performance

Number of Leaves

The application of ethephon significantly affect the number of leaves in Harumanis mango. Ethephon concentration at 400 ppm had shown the best result in number of leaves. However, there is a non-monotonic response for the different level of ethephon concentration which may be influenced by several factors such as environmental condition, tree's age, and specific physiological response of mango to different concentration of ethephon (Bhadoria, Nagar, Bharihoke & Bhadoria, 2018). Based on Figure 4, it can be seen that ethephon concentration at 600 ppm shows the lowest value on number of leaves as to be compared to other treatments. It was believed to be caused by the phytotoxicity symptom of ethephon such as leaf curling and discoloration (Henry, McCall & Whipker, 2021) which will lead to leaf wilting and falling. Meanwhile, ethephon concentration at 800 ppm is able to influence the number of leaves in Harumanis without causing phytotoxicity to the plant which then create the non-monotonic response.

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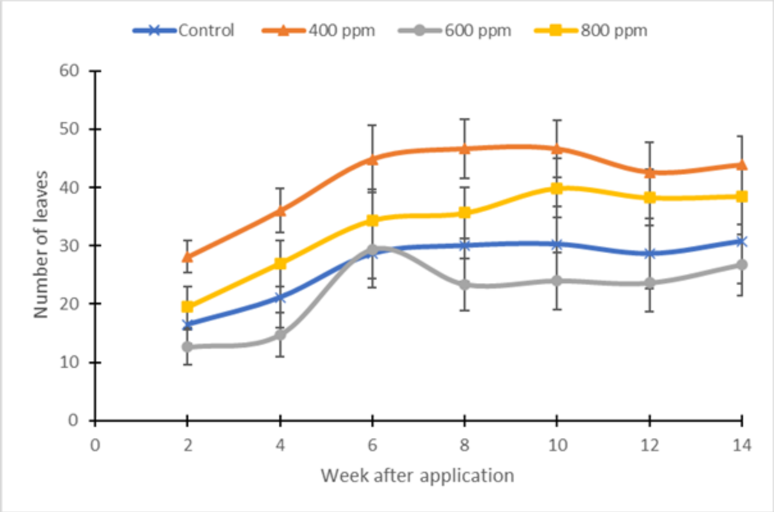

Figure 4: Effect of Ethephon Application on Number of Leaves at Week 2 Until Week 14

Number of Buds

Ethephon application on Harumanis mango had significantly affect the number of buds. Referring to the Figure 5, it can be seen that all of the treatments had influence the bud growth beyond the control treatment. However, the best concentration performed was ethephon at 600 ppm as it presents the highest value in number of buds among the other treatments along the observation period. The result from this analysis was related to previous study where the increase of ethephon concentration will lead to the reduction of internode distance by promoting the cell elongation which attribute to the increase of bud number on a branch (Dhakal, 2019). A study on sugarcane had proved that ethephon has the ability to perform early and higher rate of bud sprouting by showing stimulatory effects on bud sprouting and early growth characteristics by modulating sugar hydrolysis and its transport to growing shoot (Jain, Singh, Singh, & Chandra, 2018).

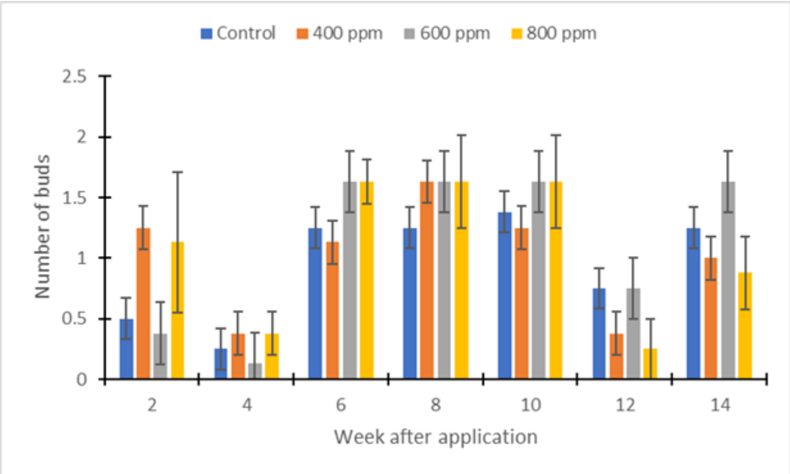

Figure 5: Effect of Ethephon Application on Number of Buds at Week 2 Until Week 14

Length of Buds

The effect of ethephon application in influencing bud length has no significance different between control treatment and ethephon application. Nevertheless, application at 400 ppm (T2)

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shows the best result in influencing the bud length when compared with the other treatments. It is because, since the first observation, the value presented was always higher than the control treatment. Only at week 4 and week 14, the length of bud is lower than the control treatment which may due to bud transformation to shoot since the observation of bud length stopped when the bud had turns into shoot. Based on the result from T2, it may be concluded that ethephon has the ability to influence the length of bud as referred to past study on sugarcane that stated that ethephon increase the enzyme activity of acid invertase and ATPase in sprouted buds by providing the cell with carbon and energy for underground bud growth (Jain, Solomon, Shrivastava, & Chandra, 2011).

Table 2: Effect of Ethephon Application on Length of Bud (Cm) at Week 2 Until Week 14

Treatment	Week						
	2	4	6	8	10	12	14
Control (T1)	0.33ab	0.40a	0.24a	0.22a	0.21a	0.47ab	0.42a
Ethephon 400 ppm (T2)	0.61b	0.23a	0.30a	0.26a	0.29a	0.53b	0.22a
Ethephon 600 ppm (T3)	0.11a	0.08a	0.24a	0.31a	0.24a	0.27ab	0.32a
Ethephon 800 ppm (T4)	0.31ab	0.30a	0.48a	0.38a	0.44a	0.09a	0.30a

Note: The same lowercase letter showed the mean value is in the same group which is not significantly different at $p \leq 0.05$ by LSD

Physiological Characteristics

Relative Chlorophyll Content

Relative chlorophyll content is related to the growth and health of Harumanis plant. Based on the result, it shows that plant treated with ethephon has no significant effect on relative chlorophyll content when compared to control treatment. However, concentration at 600 ppm had present a slight lower value among the other treatment which may indicate that the concentration may affect the growth and health of Harumanis by stressing the plant and decreasing the nutritional status (Kasim et al., 2018). Nevertheless, analysis based on the result says that ethephon application does not influence the relative chlorophyll content of Harumanis leaves even with different concentration applied.

Table 3: Effect of Ethephon Application on Relative Chlorophyll Content (µg cm^{-2}) by SPAD Meter at Week 2 Until Week 14

Treatment	Week						
	2	4	6	8	10	12	14
Control (T1)	49ab	55b	53a	53a	59a	52b	52b
Ethephon 400 ppm (T2)	49ab	50ab	50a	49a	58a	55b	53b
Ethephon 600 ppm (T3)	44a	47a	46a	50a	53a	45a	47a
Ethephon 800 ppm (T4)	53b	54ab	52a	56a	55a	50ab	52ab

Note: The same lowercase letter showed the mean value is in the same group which is not significantly different at $p \leq 0.05$ by LSD

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Stomatal Density

Based on the result obtain from stomatal density observation on Harumanis leaves for 14 weeks, generally can be said that ethephon application does not have significant impact on stomatal density in Harumanis leaves. The trend shown from week 2 until week 4 for all of the treatments are the same where the highest value of stomatal density between the treatment is variable. For example, the highest value at week 4 presented by T2 while highest value at week 6 was presented by T1 before switch back to T2. However, the change in stomatal density may cause by several factors such as the plant species or the environmental condition (Sakoda et al., 2020). Nevertheless, the data does not represent which concentration is the best to be applied to Harumanis tree as to influence the stomatal density of the leaves. Thus, says that ethephon application does not affect the photosynthetic in Harumanis.

Table 4: Effect of Ethephon Application on Stomatal Density (cm^{-1}) at Week 2 Until Week 14

Treatment	Week						
	2	4	6	8	10	12	14
Control (T1)	730a	827a	833b	770a	737a	687a	729a
Ethephon 400 ppm (T2)	720a	852a	760ab	806a	768a	722a	716a
Ethephon 600 ppm (T3)	730a	791a	718a	751a	726a	682a	681a
Ethephon 800 ppm (T4)	720a	785a	786ab	760a	741a	717a	721a

Note: The same lowercase letter showed the mean value is in the same group which is not significantly different at $p \leq 0.05$ by LSD

Flower Production

Days to Flowering

The result of ethephon application at off-season on days to flowering for Harumanis mango had indicate that suitable concentration of ethephon to be applied on Harumanis in order to induce flowering is at 800 ppm (T4). It is because the days to flowering of T4 is earlier than T2. From the day after ethephon application, flowering of Harumanis should be happening 112 days later. The difference presented by the treatment as to be compared with the actual stage of Harumanis flowering is 14 days and 56 days earlier for T2 and T4 respectively. This result had indicated that ethephon application is able to induce the flowering of Harumanis at off-season with higher concentration of ethephon which is related to the previous study by Maloba et al. (2017) that stated that higher ethephon concentration affected on higher production of mango flowering. However, the intermediate concentration which is T3 did not bear any flower panicle for the duration of 14 weeks of observation. This situation can be related to past study by Pratap et al. (2019) when the value of first flowering date of cucumber at ethephon 400 ppm is lower than application at 300 ppm and 600 ppm. The flowering of cucumber at 400 ppm is 19.85 while 300 ppm is 20.93 and 600 ppm is 22.13. In this case, it may indicate that the plant will be triggered only by the desired concentration which is 800 ppm and 400 ppm but not 600 ppm which then create a U-shaped curve (Agathokleous, 2017).

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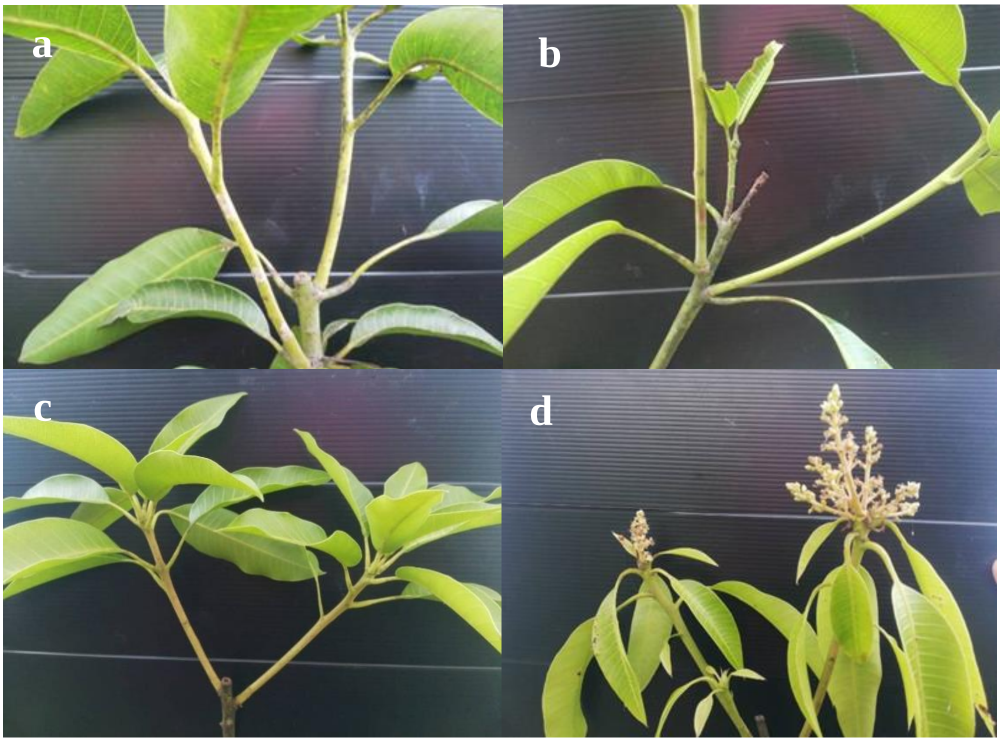

Figure 6: Tagged Branch of Harumanis Mango at Week 8 (a) Control Treatment (T1) (b) Ethephon 400 ppm (T2) (c) Ethephon 600 ppm (T3) (d) Ethephon 800 ppm (T4)

Table 5: Effect of Ethephon Application on Days to Flowering and Number of Panicles in Harumanis Mango

Treatment	Days to flowering	Number of panicles
Control (T1)	0	0
Ethephon 400 ppm (T2)	98	3
Ethephon 600 ppm (T3)	0	0
Ethephon 800 ppm (T4)	56	5

Number of Panicles

Application of ethephon does have significant effect on the number of panicles. It can be seen on the result presented by T2 and T4. The best result in inducing the flowering of Harumanis is T4 where the number of panicles is higher than T2. T2 present a lower total number of flower panicle because it begins to bear panicle at the last week of observation while T4 had bear flower since week 8 and remain its panicle until week 10. Flower panicles of T4 cannot remain longer due to the drying and detachment of the flower from the branches that caused by heavy rain during the period. Heavy rain had caused flower to detach from the panicles because Harumanis needs less rainfall amount during the flowering stage as to maintain the number of flowers on panicles (Uda et al., 2020). Meanwhile, number of flower panicles on T2 was lower than T4 because it bears on the last week of observation and with good weather condition where there is less amount of

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rain. If further observation was made, the actual number of flower panicle can be induced by T2 may be identified and compared to T4.

Conclusion

The aim of this study is to determine the effect of ethephon application towards Harumanis vegetative growth, physiological characteristics, and the ability in inducing flower during off-season. Based on the observation being made, the ethephon had influence the vegetative growth and induce the flowering of Harumanis. This statement can be supported by the result obtained that shows plant treated with ethephon 400 ppm had perform the most positive outcome on vegetative growth where it influences the number of leaves and length of bud of Harumanis mango. As for the flower induction, ethephon at 800 ppm, which is the highest concentration had performed the best result in shorten the flowering days of Harumanis and influence the production of flower panicles. However, the observation of physiological characteristics did not perform the desired result when there is no significant effect presented by all of the treatment applied, thus indicate that ethephon did not influence the physiological characteristics of Harumanis mango. In other words, application of ethephon can result best in vegetative growth and flower induction but not in physiological characteristics of Harumanis mango. Considering that the effect of ethephon application may vary depends on plant species, time of application and chemical concentration (Bhadoria et al., 2018), thus, it can be concluded that the ethephon is able to influence the vegetative growth of harumanis and induce the flowering but with suitable concentration and time of application. Building of these findings, this research may contribute to the understanding on how ethephon affect the growth of Harumanis while act as a guidance for the farmers to perform a better crop management on their field. Apart from that, the environmental impact of chemical use in agriculture can also be minimized when the optimal concentration for a specific plant being determined.

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