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Article

Ethephon-Induced Abscission of Oil Palm Fruits at Optimal Bunch Ripeness and Retting Period to Improve Commercial Seed Production

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Abstract: Oil palm seed producers typically require 10 months of various processes from pollination to seed germination to produce commercial *dura* × *pisifera* hybrid seeds. Conventional forced fruit shedding from underripe fresh fruit bunches (FFB) usually causes seed damage and an extended retting period (incubation for natural fruit abscission from spikelets), eventually leading to bunch rot and disease infection. As a fruit ripening agent, ethephon has been explored to hasten fruit abscission in many fruit crops and oil palm. Nevertheless, the previous studies in oil palm only focused on fruit shedding from FFB to improve oil extraction rate in oil mills without considering the actual FFB ripeness and retting period, which are critical for oil palm seed production. In this study, the application of ethephon containing buffer (adjusted to pH 9.0) to underripe FFB at 145 days after pollination (DAP), 135 DAP and 125 DAP resulted in 50% more fruit abscission after a 72-h incubation. Considering the minimal seed loss upon FFB harvest (<1%) and 50% reduction in retting period, underripe FFB at around 145 DAP was found to be optimum for seed production using ethephon treatment. The treatment, however, made negligible improvement in fruit detachment for ripe FFB at 150 DAP and older. Importantly, seed germination and culling rate at nursery stages were not significantly affected by the ethephon treatment. Hence, ethephon application can improve commercial seed production practices for oil palm.

Keywords: fresh fruit bunch; postharvest technology; ethephon; retting period; germination; seed abnormality

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1. Introduction

Oil palm (*Elaeis guineensis* Jacq.) is an important oil crop which supplies 35% of the global vegetable oils and fats [1]. Currently, oil palms have an economic life exceeding 20 years and can produce 10 times more oil per hectare than other oil crops [2]. One of the contributing factors to current yields is the switch in the 1960s of commercial *dura* planting to *dura* x *pisifera* hybrids (DxP), also termed *tenera*. This resulted in an increment of 30% oil yield in Southeast Asia [3,4]. Since then, the demand for palm oil has been increased with a rapid growth of world population especially in Malaysia has become the second largest producer and exporter of crude palm oil (CPO) [5,6]. The CPO production in Malaysia increased significantly from 92,000 tons in 1960 to 16.99 million tons in 2010, 19.4 million tons in 2020 and expected to increase to 19.6 million tons in 2021 [7,8]. This explains why commercial DxP seed production for planting in Malaysia has almost doubled from 1995 to 130 million in 2012 [9,10]. A cycle of seed production typically requires 10 months to complete various processes including controlled pollination, bunch harvesting, retting (incubation for natural fruit abscission), fruit shedding, dormancy breaking and germination (Figure 1). Therefore, improvement in production efficiency and seed recovery is important to ensure a continuous supply of high quality seeds to growing markets and improve the profitability of seed producers.

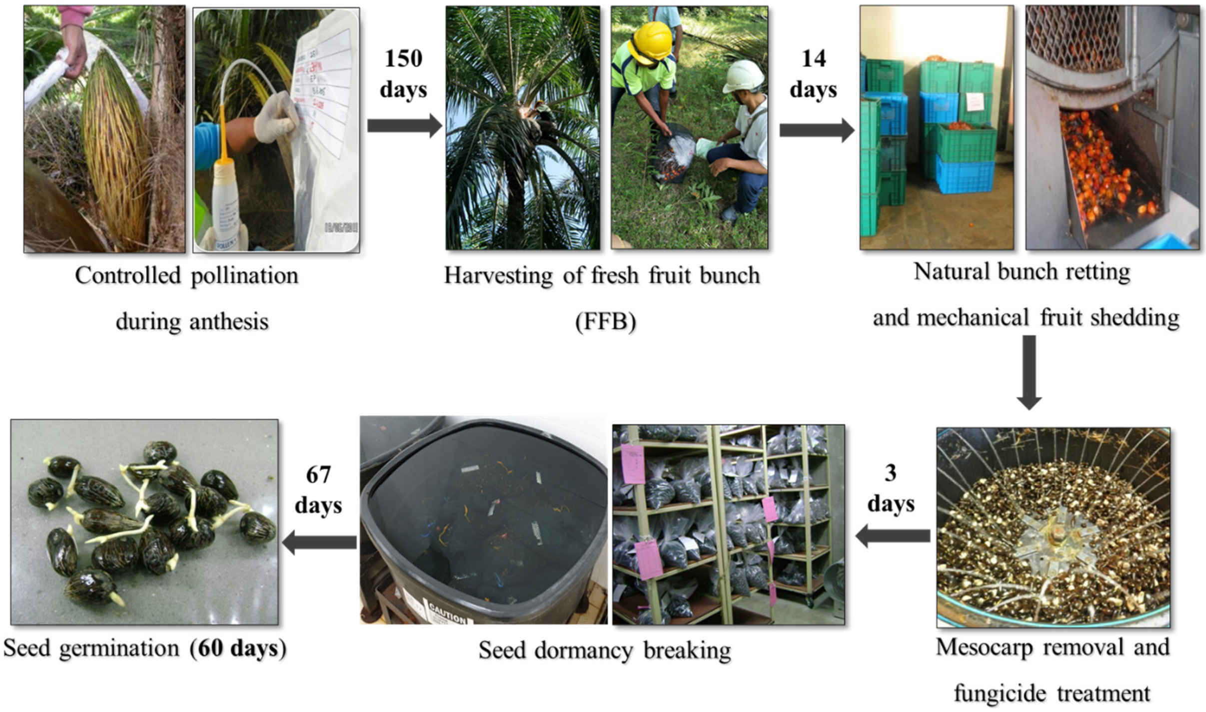

Figure 1. A process flow that involves various processes in a 10-month period for oil palm seed production. Conventional bunch retting allows natural abscission of fruits from spikelets, in which the process may take up to 14 days before fruit shedding by mechanical means.

A fresh fruit bunch (FFB) normally bears 1000 to 4000 fruits tightly wedged in the leaf axil of a palm. Similar to fruit crops such as red raspberry [11] and apple [12], abscission of oil palm fruits directly correlates to overall bunch ripeness [13]. The ripeness of commercial FFB for typical harvest standard is based on a minimum five naturally abscised fruits—known as loose fruits on the ground, which usually happens around 150–160 days after pollination (DAP), although it may not achieve the optimal oil extraction rates. This, however, does not apply to commercial seed production where harvesting is typically on the underripe FFB not more than 150 DAP for minimal loss of loose fruits during transport. Recovery of the loose fruit is totally restricted because some fruits can potentially originate from unintended parentage, causing genetic contamination. In a seed production facility, the fruits are mechanically shed from the spikelets of underripe FFB that have been retted for more than 10 days (Figure 1). Long retting period can increase the risk of fungal infection on seeds that may later reduce seed germination [14]. As a solution, ethylene can be used to accelerate uniform FFB ripeness and fruit abscission in oil palm.

Ethylene currently can be commercially obtained in the form of ethephon (2-chloroethylphosphonic acid) and it is widely used as an artificial ripening agent in many fruit crops. One example, ethylene has been applied in date fruits (*Phoenix dactylifera* L.) to increase ripening rate and facilitate early harvesting [15,16]. Indeed, the application of ethephon is not new to oil palm FFB, but it is only limited to accelerate fruit abscission to increase oil extraction rate in oil mills [17–20]. However, the applicability of ethephon/ethylene to increase the efficiency of oil palm seed production has yet to be reported. The current bunch ripeness standard with five or more loose fruits does not actually reflect the optimal time for underripe FFB harvest for seed production. Hence, our aim is (1) to evaluate the optimal FFB ripeness at the actual DAP for ethephon application to accelerate fruit abscission, (2) to allow the retting period to be shortened, and (3) to evaluate the impact of ethephon on seed germination and culling rates at the nursery stages were also studied.

2. Materials and Methods

2.1. Plant Materials

A total of 66 FFB derived from commercial Deli *dura* x AVROS *pisifera* origin were harvested from four seed gardens, namely PT100, PT101, PT105 and PT116 located at Dusun Durian Estate, Sungai Sedu Estate, Klanang Bharu Estate and East Estate in Selangor State, Malaysia which are maintained by Sime Darby Plantation Seeds and Agricultural Services Sdn. Bhd. (SDPSAS). The FFB were harvested at five different bunch ripeness stages, including 125, 135, 145, 150 and 160 DAP. At each DAP, the FFB was divided into two groups i.e., ethephon-treated and nontreated control as summarized in Table 1. The sample size for 160 DAP was small (6 FFB) because most of the bunches harvested were rotten due to being overripe. For recording purposes, each FFB was sealed with a netting bag to prevent loss of loose fruits during the harvesting process. Bunch weight and the number of loose fruits upon harvesting were recorded. The percentage of loose fruits of each FFB was then calculated based on the total number of fruits per FFB harvested.

Table 1. Number of fresh fruit bunch (FFB) harvested across different bunch ripeness.

Days after Pollination (DAP)	Number of FFB	
	Control	Ethephon-Treated
125	9	10
135	6	8
145	6	6
150	7	8
160	3	3
Total	31	35

2.2. Ethephon Treatment, Quantification of Loose Fruits and Retting Period

Approximately 200 mL of 0.5% (*v/v*) ethephon buffer adjusted to pH 9.0 with 5M sodium hydroxide was sprayed on each FFB [21]. Both ethephon-treated and control FFB (without buffer treatment) were placed into different covered boxes to prevent mixing of loose fruits during the retting process. The number of loose fruits were first counted after 72 h of incubation. Subsequently, the retting period of each FFB was observed daily and determined when all fruits were fully shed from a bunch using the mechanical method. The percentage of loose fruits detached after 72 h was calculated based on the total number of fruits per FFB.

2.3. Ethephon Effect on Seed Germination and Culling Rate for 145 DAP

The ethephon-treated and the nontreated fruits from 145 DAP were processed according to Malaysian Standard, namely MS 157:2005 [22] to produce germinated seeds. After dormancy breaking, the seed germination took place for 60 days with routine inspection and recording for every 10-day interval. The final percentage of seed germination of each FFB was calculated based on the total germinated seeds after 60 days. The seed germination of ethephon-treated FFBs and controls were then compared. A total of 100 seeds per FFB which were randomly selected from the ethephon-treated and the control FFB from 145 DAP was sown in a nursery. The first and second rounds of culling were carried out after 3 months and 9 months, respectively. The final percentage of culling per bunch was calculated based on the total seeds sowed.

2.4. Statistical Analysis

All comparisons were analyzed using the Kruskal–Wallis test in the Minitab 20 program [23]. Comparisons were conducted to address (a) the effect of FFB ripeness on fruit abscission upon harvesting, (b) the effect of ethephon treatment on fruit abscission after 72-h incubation and retting period across FFB ripeness, and (c) the effect of postethephon treatment on seed germination and culling at nursery. A significant difference was defined at the $p = 0.05$ threshold. All boxplots were constructed using *R 3.5.3* with package *ggplot2*.

3. Results

3.1. Effect of FFB Ripeness on Fruit Abscission upon Harvesting

Minimal loose fruits were observed upon FFB harvesting at 125 DAP and 135 DAP (Figure 2). Fruit abscission only started with loose fruit median = 0.27% at 145 DAP and significantly increased to 9.9% and 16.8% loose fruits at 150 DAP and 160 DAP, respectively with $p < 0.0005$.

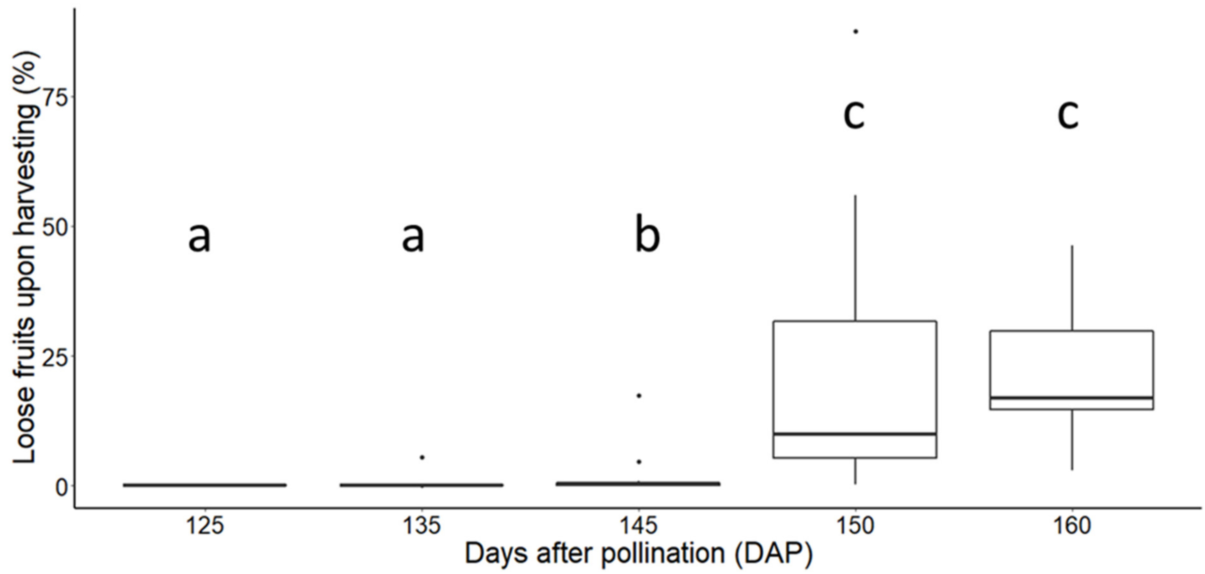

Figure 2. The boxplots represent median values, 25th–75th percentile of loose fruit percentage upon harvesting across FFB ripeness. Significant differences in loose fruit among the five DAP are denoted by a, b, and c based on Kruskal-Wallis test at the $p = 0.05$ threshold. Nineteen FFB harvested at 125 DAP have 0 loose fruit. Thirteen FFB harvested at 135 DAP have zero loose fruit with 1 outlier has 5.53% loose fruits. Ten FFB harvested at 145 DAP have less than 1.0% loose fruits, with 2 outliers having loose fruits 4.66% and 17.42%.

3.2. Effect of Ethephon Treatment on Fruit Abscission after 72-h Incubation and Retting Period across FFB Ripeness

The loose fruit percentage of 72-h postethephon treatment and the control across FFB ripeness is shown in Figure 3. Ethephon-induced loose fruits (median = 28.4%) started as early as 125 DAP, which was significantly 315 folds higher than that of the control FFB (median = 0.09%) with $p < 0.0005$. Subsequently, loose fruits of ethephon-treated FFB increased rapidly at 135 DAP (median = 51.4%) and peaked at 145 DAP (median = 53.4%), whereas the control FFB only peaked at median = 31.9% at 160 DAP which was delayed by 15 days and was 50% lower in loose fruits. In addition, the ethephon-induced loose fruit percentage at 150 DAP (median = 36.1%) and 160 DAP (median = 40.1%) dropped to a comparable level of control FFB.

Overall, the retting period reduced as FFB ripeness increased regardless of ethephon-treated and control FFB (Figure 4). Retting of the ethephon-treated FFB at 125 DAP was successfully reduced to a median of 9 days, while 3 more days were required by the control FFB. The trend continued and reached the bottom at a median of 4.5 days of retting for the ethephon-treated FFB at 145 DAP, which was significantly 50% shorter than that of the control FFB with $p = 0.023$. However, the gap of retting period between ethephon-treated and control FFB at 150 DAP and 160 DAP become comparable with p value of 0.109 and 0.197, respectively.

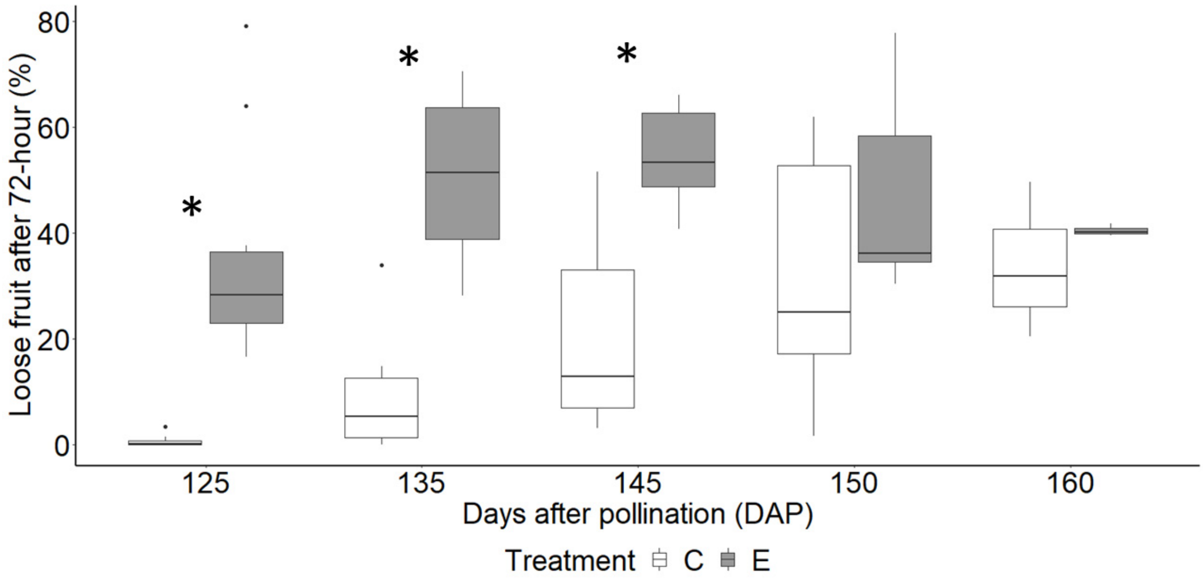

Figure 3. The boxplots represent median values, 25th–75th percentile of loose fruit percentage of ethephon-treated (E) and control (C) FFB after 72-h incubation across FFB ripeness. Significant difference in loose fruits between E and C are denoted by asterisk (*) based on the Kruskal–Wallis test at the $p = 0.05$ threshold.

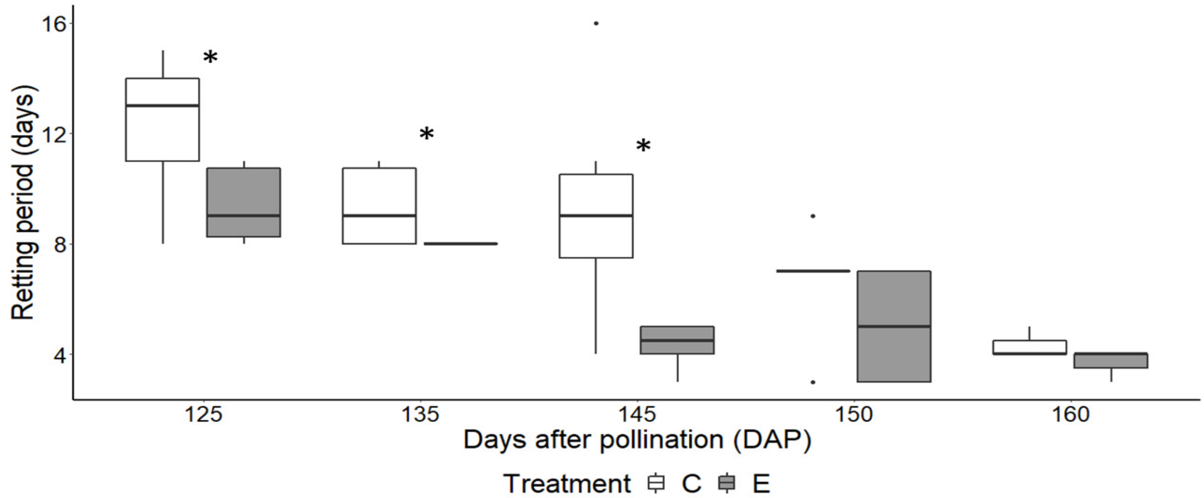

Figure 4. The boxplots represent median values, 25th–75th percentile of retting period (days) between ethephon-treated (E) and control (C) FFB across FFB ripeness. Significant difference in retting period between E and C are denoted by asterisk (*) based on the Kruskal–Wallis test at the $p = 0.05$ threshold. Eight FFB harvested at 135 DAP were retted at 8 days. Seven FFB harvested at 150 DAP completed retting process at 7 days, with 2 outliers retted at 3 days and 9 days.

3.3. Effect of Posttreatment with Ethephon on Seed Germination and Culling at Nursery for 145 DAP

In this study, only FFB from 145 DAP were selected for seed germination and culling recording. This is based on the lowest retting period at 145 DAP with minimal loose fruits upon harvesting. The seed germination of ethephon-treated FFB recorded a median of 79% and control FFB recorded a median of 74% and showed no significant difference with $p = 0.8102$. Grass leaf (or narrow leaf) and juvenile were the major abnormalities culled from 3-month-old and 6-month-old seedlings. Again, the final culling percentage of the ethephon-treated (culling median = 19.98%) and the control (culling median = 15.29%) was not significantly different with $p = 1.000$.

4. Discussions

Oil palm as a perennial crop usually requires 12–20 years to complete a selective breeding cycle [24]. Consequently, improvement of oil palm planting material is much slower than annual crops. This is partly the result of infrequent replanting with typical 25 years per cycle in oil palm. The recent advancement of genomics and molecular breeding in oil palm, however, is expediting the breeding progress to produce high-yield planting materials such as GenomeSelect™ to bring improvements to oil yield in existing fields without further expansion and clearance of forest land [24,25]. Moreover, the rapid introduction of elite planting materials motivates early replanting for the oil palm industry to take advantage of better oil yield and to reduce labor shortage, especially for FFB harvested from tall palms. For instance, Sime Darby Plantation is accelerating 5% annual replanting with the GenomeSelect™ material to fully cover its 0.32 million hectare of total land bank in Malaysia by 2023 [26]. A similar trend in oil palm industry is foreseeable. Hence, oil palm seed producers need to improve their production efficiency and seed quality to cope with the high demand for seeds.

In this study, natural fruit abscission was found to initiate at 145 DAP and peaked within 150–160 DAP, inferring full FFB ripeness. The primary abscission zone of a ripe oil palm fruit is sensitive to ethylene, which induces cell separation and abscission in the abscission zone [27]. Therefore, FFB harvest for seed production should be carried out before 150 DAP for minimal loose fruits on the ground or being caught in the axil of lower fronds, which can also lead to seed lot contamination. The findings coincide with the SDPSAS current standard operating procedure. A seed garden is usually planted with *dura* palms and only those with high yield performance are certified as mother palms based on MS 157:2005 (Malaysian Standard, 2005). Recovery of loose fruits in the seed garden may pose a risk of mixing with open-pollinated *dura* seeds derived from the same mother palms or neighboring nonmother palms, leading to *dura* contamination. Planting of thick-shelled *dura* contaminants can immediately lead to a 25% reduction in oil yield compared to the commercial thin-shelled DxP hybrids and the impact remains throughout the 20–25 years of economic life of the commercial palm [28]. To safeguard seed purity, *SHELL* and SNP-based legitimacy assays are now available to detect and remove the *dura* contaminants as early as the seed stage [29,30], but preventing the 10% loose fruits through early FFB harvest before 150 DAP is clearly more effective to completely avoid the risk of genetic contamination and seed loss. Mesh netting can be a good solution to secure the loose fruits during FFB harvest. Still, the method requires more manpower and eventually incur unnecessary production cost.

A major challenge was to enhance efficiency of fruit shedding from underripe FFB younger than 150 DAP. The fruits were not ready to abscise from FFB during the underripe stage and it would require a prolonged FFB retting. Without ethephon-treatment, the underripe FFB spent a median of 9 days to complete the retting process at 145 DAP and recorded the longest retting with a median of 13 days at 125 DAP. Oil palm FFB usually start to rot due to fungal and maggot infestation after a week of retting (Supplementary Figure S1). In such conditions, mycelia have enough time to grow over the seeds and penetrate through the germ pores to the surface of the testa near to the embryo, affecting respiration activity during seed germination [31]. Our target, hence, is to keep the retting period shorter than a week to avoid the fungal infection. Applying high mechanical shedding force is possible to forcibly remove fruits from the spikelets, but it also increases the risk of seeds cracking. Again, this would expose the kernel (endosperm) to microbial colonization, causing further complications with disease control in the subsequent seed processes. Even worse is the 10-month effort of seed production and monetary loss.

Many studies have shown that the application of exogenous ethylene can expedite ripening and fruit abscission in various fruit crops such as banana [32], tomato [33], apple [34], persimmon [35] and even oil palm [18,36]. However, the previous studies in oil palm were mainly focused on the response to ethephon of commercial FFB in the oil mill without knowing their actual fruit ripeness. We close the gap with similar results in this study by evaluating seed maturity, viability and damage. Ethephon-induced fruit abscission started as early as 72-h posttreatment on underripe FFB at 125 DAP and fruit abscission peaked at 145 DAP with 50% loose fruits. For the untreated control bunches, the peak of fruit abscission coincided with the typical 150-DAP ripeness, as expected. This suggests that FFB retting at 145 DAP can be shortened significantly from 9 days to 4 days, enabling a mechanical shedding method e.g., vibration, which was tested by Ismail and colleagues in the oil mill to minimize seed damage [37]. By consolidating the assayed parameters, we found that around 145 DAP is the optimum FFB ripeness for ethephon treatment. The 145-DAP FFB produced less than 1% seed loss upon harvest in a seed garden and only required a median of 4 days of retting, compared to 10–14 days of conventional interval. Apart from excessive 9.9% seed loss and risk of seed legitimacy, ethephon treatment on ripe FFB (>150 DAP) was found to be expendable because the endogenous ethylene probably had sufficed the requirement for fruit ripening and abscission during seed production [11,20].

In early studies, possible roles of ethephon in dormancy breaking in oil palm [38] and macaw palm [39] were reported. Loblolly pine seeds were found to be sensitive to higher ethephon concentration at 10% (v/v), which significantly reduced 40% of seed germination and increased culling beyond 10% [40]. To further understand the effect of ethephon residues on the treated oil palm FFB, the germinated seeds tested in this study were sown and monitored throughout the prenursery (3 months) and main nursery (9 months) stages. The result, however, indicated that the ethephon treatment did not significantly affect germination and culling in oil palm because the concentration used was 200 times lower than the reported concentration in loblolly pine seeds. More importantly, the observed percentage of culling was still within the normal range i.e., about 25% [41]. This is possibly due to the physical barrier of endocarp (or shell) preventing excessive exogenous ethylene released from ethephon to reach the embryo [42]. Grass leaf and juvenile spotted seedling palms are the major abnormal phenotypes at the prenursery and main nursery based on the industry standard in MPOB-created Code of Practice (CoP) 1001:2015 [43–45]. The levels of these “abnormalities” were also in concordance with a previous study reported by United Plantations Bhd. [46] with or without ethephon. These “abnormalities” are mostly resulted from bad planting, such as deep or inverted planting or from insufficient water supply [47].

5. Conclusions

Ethephon was found to be effective in expediting fruit abscission (50%). This study also has provided evidence that the ethephon treatment can be a powerful method to improve the current commercial seed production of oil palm with minimal seed loss (0.27%) and reduced retting period from 9 days to 4 days, provided it is applied on the optimal FFB ripeness, which is around 145 DAP. Furthermore, the use of ethephon has no obvious adverse effect on germination and culling at nursery stages. The assayed oil palm seedlings have been field planted and a further evaluation of ethephon effect on FFB yield could be conducted when the yield recording is completed after 6 years. This study has paved a way for a future study on ethylene gas which is cheaper and leaves fewer residues as an alternative for commercial seed production, as has been widely used in fruit industry.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/horticulturae7100380/s1, Figure S1: Harvested FFB retted up to 8 days without ethephon treatment. Visible bunch rot and fungal growth was observed on FFB after a week of retting.

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