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Original Article

Effect of fruit age and post-harvest maturation storage on germination and seedling vigor of wood apple (*Feronia limonia* L. Swingle)

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Abstract

Wood-apple is commonly propagated generatively using seed. Seed commonly is obtained from the waste of mature wood-apple with 3-6 days of post-harvest maturation storage. The aims of this study were to investigate the effect of post-harvest maturation storage on various fruit age on wood-apple seed germination and seedling growth. This experiment was arranged by using Completely Randomized Design (CRD) with two factors i.e. fruit age and the duration of post-harvest maturation storage. The first factor was fruit age of four levels i.e. 6 months after anthesis (MAA), 7 MAA, 8 MAA, and mature fruit exactly detached from the tree, hereafter the fallen mature fruit. The second factor was the duration of post-harvest maturation storage which consist of three levels i.e. 0 days of post-harvest maturation storage (DOP) (without storage), 3 DOP, and 6 DOP. An Analysis of Variance followed by Duncan Multiple Range Test with 5% significant level was used. The results showed that post-harvest maturation storage and fruit age affected the seed germination and seedling growth. Germination and seedling emergence increased with the increase of fruit age and post-harvest maturation storage duration. The highest seed germination and seedling emergence were occurred from seed which came from fallen mature fruit either with or without post-harvest maturation storage. Six days of post-harvest maturation storage on fruit aged 8 MAA significantly increased the percentage of germination and the emergence of wood-apple seedling which was similar to the percentage of seed germination from fallen mature fruit.

Keywords: Germination, post-harvest maturation storage, seed, wood-apple

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Introduction

Wood-apple (*Feronia limonia* L. Swingle) is a fruit plant from *Rutaceae* family originated from South India and distributed over Southeast Asia, including Indonesia (Widiati, 2010). In Indonesia, wood-apple generally used as the main ingredient of foods and drinks. This plant grows well in coastal area of Indonesia i.e. Sumatera, Java, Madura, Bali, and West Nusa Tenggara (Jones, 1992). Unfortunately, information on the cultivation of wood-apple is not sufficient and the conservation status of this plant belongs to endangered status (Yulistyarini et al., 2000). The most important factors in plant cultivation is the availability of high quality seed and seedlings at the sufficient amount and time. Wood-apple is commonly propagated generatively using seed. However, the information of high quality wood-apple seed production is still limited.

One important factor that determines the quality of the seed is the maturity levels. Seed reaches their maximum vigor at their physiological maturity (Murniati et al., 2008). Seed harvested when the fruits reach their maturity will produce plant with better vigor and seed storage ability. Vigor and dry seed weight were some of the properties of physiological maturity. Seed in their physiological maturity have complete food storage to support the growth of seedling (Copeland and McDonald, 1985).

Wood-apple fruit used for food was that those already fallen from their mother plant and stored for three until six days (Murrinie et al., 2017). During the post-harvest maturation storage, there was a change in flesh color and texture from white and hard into brown and soft with unique smell. Seed commonly used for propagation come from wood-apple fruit waste, which mean the fruit has been stored before extraction, suggesting the change of seed properties during post-harvest maturation storage.

Post-harvest maturation storage was a common activity in the temporary storage or seed conservation period (Zanzibar et al., 2009). Schmidt (2000) stated that temporary storage was aimed to make seed reach their optimum potency before the next handling. Sadjad (1994) reported that there were several conservation period i.e. temporary storage period before the seeds were stored, during harvest, after processing and before planting. Usually this period was short, like when seed are on delivery process and waiting for soil preparation and planting.

Murniati et al. (2008) stated that seeds from post-harvest maturation storage fruits show the same signs as those with priming treatment. Papaya seeds harvested from fruit with 30-40% yellow color then followed by 4 days of post-harvest maturation storage have maximum growth potency, seed germination, germination speed, and rise of first count germination, which is the same as control treatment where the fruits were harvested when fruit reach 80-90% yellow color. These findings indicated the maturity of seed was increased during post-harvest maturation storage and fruit maturity. Zanzibar and Mokodompit (2007) stated that the principle of priming was to activate the internal resources of seeds and supported by an external resources to maximize the growth of seedling. Information of the duration of post-harvest maturation storage and the optimum fruit harvesting age for seeds is still unknown. The exact harvesting age followed by post-harvest maturation storage should result in seeds with high viability. This study was aimed to find out the effect of post-harvest maturation storage on different ages of fruit to wood-apple seed viability.

Material and Methods

This study was conducted on March-December 2015 at Seed Technology Laboratory Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia. Wood-apple fruits used in this study originated from Dasun Vilage, Lasem District, Rembang City, Indonesia. The experiment was arranged using Completely Randomized Design (CRD) factorial with two factors. The first factor was fruit age with four levels i.e. 6 months after anthesis (MAA), 7 MAA, 8 MAA, and mature fruit exactly detached from the tree (hereafter called the fallen mature fruit). The second factor was the duration of post-harvest maturation storage with three levels i.e. 0 days of post-harvest maturation storage (DOP) (without storage), 3 DOP, and 6 DOP.

This study was conducted periodically when fruit aged 6,7, 8 MAA and the fallen mature fruit when the age was 8.25-8.75 MAA. Post-harvest maturation storage was conducted at room temperature (27-28 ºC), at the end of post-harvest maturation storage seeds were extracted from each fruit by washing it with water until clean. Surface drying and air drying were then conducted for 12 hours (Sirisena, 1998). Each treatment consisted of fifty seeds and replicated four times. Total seeds that used for each treatment in this study were 200.

The observations were made on: seed electrical-conductivity (mS), seed moisture content, percentage of germination (%), seedling emergence (%), height of seedling (cm), root length (cm), stem diameter (cm), seedling fresh weight (g), and seedling vigor index. Seed electrical-conductivity (mS) was measured by soaking 25 wood-apple seeds on 100 ml aquades for 20 hours on room temperature then the water electro-conductivity was measured using conductometer HI 8819. Seeds moisture content was measured by using oven method on 105 ºC temperature for 16 hours (Sutopo, 1985). The percentage of germination was observed at 21 days after planting (DAP) by counting number of germinated seeds with 3 mm radicle. Percentage of seedling emergence was counted by dividing the number of seedling emerged by the total of seeds planted multiplied by 100, seedling dry weight was measured by drying seedling on oven with temperature 60 ºC for 3 × 24 hours until seedling reach their dry weight. Seedling Vigor Index (VI) measured following equation bellow (Adenikinju, 1987):

$$VI = \frac{\log N + \log H + \log R + \log G}{\log T}$$

VI was the vigor index whereas N for number of leaves, H for the height of seedling (cm), R for the dry weight of seeds (g), G for the diameter of stem (mm), and T for the age of seedling (weeks).

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) of 5% level of confidence with R Statistics Software version 3.1.1.

Results and Discussion

Analysis showed significant interaction between fruit age and the duration of post-harvest maturation storage to the percentage of germination on 21(DAP). Fruit aged 7 and 8 MAA, duration of post-harvest maturation storage showed significant differences, but no significant differences were found on fruit aged 6 MAA and fallen mature fruit. The highest percentage of germination was found on seeds from fallen mature fruit either with or without post-harvest maturation storage, then the lowest percentage of germination was found on fruit 6 MAA (Figure 1).

Seeds from fruit aged 6 and 7 MAA show very low germination rate (30-50%) even when the fruit were stored. Seeds from fruit aged 8 MAA followed by six days post-harvest maturation storage significantly increase the percentage of germination compared to non stored fruits and show the same result with seeds from fallen mature fruit.

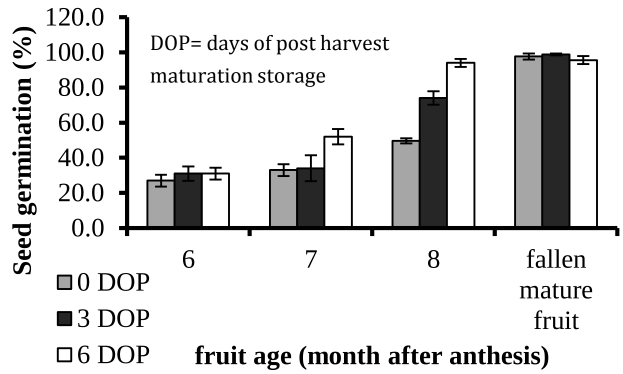


Figure-1: Percentage of wood-apple seed germination at 21 days after planting on duration post-harvest maturation storage and fruit age levels

The emergence of seedling also showed significant interactions between the duration of post-harvest maturation storage and fruit age (Table 1). Seeds from fruit aged 7 and 8 MAA followed by post-harvest maturation storage significantly increase the number of emerged seedling. Post-harvest maturation storage did not affect seeds from fruit aged 6 MAA and fallen mature fruit. Seeds from fruit aged 6 MAA gave the lowest percentage of seedling emergence. Seeds from fallen mature fruit gave the highest percentage. Seeds from fruit aged 8 MAA followed by 6 days of post-harvest maturation storage gave the same percentage of seedling emergence as fallen mature fruit.

Table-1: The emergence of wood-apple seedlings 3 weeks after planting on the duration of post-harvest maturation storage and fruit age levels

Age of fruit (months after anthesis)	Seedling emergence 3 weeks after planting (%)			Mean
	0 days of post-harvest maturation storage	3 days of post-harvest maturation storage	6 days of post-harvest maturation storage	
6	17.33 e[1)]	27.33 e	23.78 e	22.81
7	18.00 e	31.33 de	82.67 b	44.00
8	42.00 d	63.00 c	87.00 ab	64.00
Fallen mature fruit	96.67 a	94.00 ab	94.67 ab	95.11
Mean	43.50	53.92	72.03	(+)[2)]

Note:[1)] Number followed by same letter on row or column indicates no significant differences with DMRT 5%
[2)] Indicates an interactions between fruit age and the duration of post-harvest maturation storage

Post-harvest maturation storage was aimed to increase the speed of fruit maturation, especially in climacteric fruit. Wood-apple belongs to climacteric fruit as shown by the continuity of fruit maturation even after the fruit has fallen from their mother plant. During post-harvest maturation storage, there were changes in seeds properties together with the flesh of the fruit because seeds were the part of the fruit, so their development will follow each other. Murniati et al. (2008) stated that fruit harvesting before physiologically maturity followed by post-harvest maturation storage were expected to result in seeds with high viability and vigor as seeds from mature fruit.

Wahab (1990) reported the postponement in seeds extraction and post-harvest maturation storage will affect the seeds viability in eggplant. Postponement of seeds extraction from eggplant fruit for 2-6 days when fruit aged 47days after anthesis (DAA) resulted in seed with viability potential higher than seeds that came from directly extracted in eggplant fruit. Postponement in fruit a aged 53 DAA for 2-6 days result in same viability potential and initial vigor with seeds from directly extracted fruit. Sanchez et al. (2003) reported that *Capsicum annuum* L. seeds harvested from fruit aged 50 DAA colored red follows by post-harvest maturation storage for 7-14 days result in higher germination rate than fruit with no post-harvest maturation storage and fruit aged 30 DAA with green color. This result suggests the occurrence of in-situ priming in seeds during post-harvest maturation storage progress. Zanzibar et al. (2009) stated that priming can increase seeds potency on several conditions. This treatment results in the same effect with post-harvest maturation storage which can improve seeds germination, storage ability, and seedling growth. This result was supported by Schmidt (2000) who stated that post-harvest maturation storage was the example of temporary storage to optimize the seeds until they reach their optimum potency until the next treatment were conducted. Those results suggest that priming was occurred on wood-apple seeds while the fruit was stored.

Zanzibar and Mokodompit (2007) stated that the concept of priming was to activate both the internal and external resources of the seeds to optimize the growth of seedling. A study conducted by Widayati (1999) showed that osmotic priming with PEG 6000 on soybean seeds increasing the seeds germination and the uniformity of seeds growth compared to the control treatment. Muray and Wilson (1987) stated that osmotic priming regulate the amount of water and the rate of seed imbibition. The control of seed imbibition suggested to recover the integrity of cell membrane. A study conducted by Armstrong and McDonald (1992) on soybean showed that the seed cell membranes leakage were lower on seeds treated with priming compared to the control. Smaller cell leakage make less organic and an organic compound came out from cell, so seed can utilize compound for their growth. Seed treated with priming had better germination rate and growth uniformity compared to the control treatment. This statement was supported with the result of electrical conductivity (EC) of wood-apple seeds that showed the post-harvest maturation storage treatment made the lower EC value as the indicator of less membrane leakage (Table 2).

Tabel-2: Value of wood-apple seeds electrical conductivity on post-harvest maturation storage and fruit age treatment

Fruit age (months after anthesis)	Electroconductivity value (mS)			Mean
	0 days of post-harvest maturation storage	3 days of post-harvest maturation storage	6 days of post-harvest maturation storage	
6	18.48 f[1)]	6.96 e	2.06 b	9.17
7	6.58 e	3.87 d	1.68 ab	4.04
8	3.80 d	2.80 c	1.42 a	2.67
Fallen mature fruit	1.16 a	1.23 a	1.22 a	1.20
Mean	7.51	3.72	1.60	(+)[2)]

Note:[1)] Number followed by same letter on row or column indicates no significant differences with DMRT 5%
[2)] Indicates an interactions between fruit age and the duration of post-harvest maturation storage

Table 2 showed that there was an interaction between fruit age and post-harvest maturation storage to EC. Post-harvest maturation storage on fruit aged 6,7, and 8 MAA decreased the EC, suggesting that it will reduce the damaged of seeds cell membrane from fruit which did not reach their maturity yet. Seeds EC decreased as the fruit age increase. Post-harvest maturation storage did not affect EC on the seeds originated from fallen mature fruit, because seeds had already reached their maturity and their cell membrane had already completed their development. Research conducted by Adjisir (2000) on cocoa showed that seeds harvested at the young age have cell membrane that was not well-developed. Seeds that came from over mature fruit was more susceptible to organic compound leakage due to greater membrane damage.

On fruit aged 6 and 7 months, post-harvest maturation storage can even reduce the EC and repair the structure of cell membrane. The growth of seeds embryo was not well-developed yet, so the germination ability and seedling emergence is still low. Seeds from fruit aged 8 MAA has reach their maturity stage. Followed by post-harvest maturation storage, the leakage of cell membrane will be reduced, so seeds can utilize the organic and anorganic compound for germination and seedling emergence as seeds from fallen mature fruit. This result indicates that post-harvest maturation storage can reduce the cell membrane leakage.

Study conducted by Irawati et al. (1997) supports this finding. The study indicated that there were negative correlation between the ability of seed germination and EC due to organic compound. The content of sugar, protein, and lipid on water with soaked seeds increased as the longer storage of the seeds and result in higher EC. Endang et al. (2001) reported that the longer duration of storage, the concentration of an organic compound such as N, P, K, Ca, and Mg on soaked seeds water and EC will increase but seeds growth and weight of seeds will decrease. The exudate concentration and EC were positively correlated, meanwhile seeds growth and seedling weight were negatively correlated.

Widayati (1999) reported that seeds with osmotic priming treatment were able to utilize more P better than seeds without osmotic priming treatment. P on the seeds is mostly present in organic form while in the inorganic form the number is very small. There were organic compounds present in seeds that contain P like nucleic acid, phospolipid, nucleotide and fitin (Mayer and Mayber, 1982). Fitin has the most P storage on the seeds, for about 80% of total P content of the seeds. Fitin was complex of calcium, magnesium, potasium from inositol hexaphospate acid (fitat acid). In addition, fitin also contains Fe, Mn, Cu, and often Potassium (Bewley and Black, 1986). Fitat acid on the seeds were the important source of P during germination progress before seedlings can absorb P from the environment (Mayer and Mayber, 1982). Fitin content decreased significantly during seed germination, suggesting that fitin was the most important storage of P in seedling. Widayati (1999) adds that on soybean, fitat acid content as the product of hydrolysis of fitin by fitase enzyme shows the significant increase from first until third day, then reduce significantly on fourth day. Seeds that grow on osmotic pressure showed the presence of more fitat acid compared to seeds that grow in optimum conditions. This result indicates that seeds cannot maximize the utilization of fitat acid, so there were less energy for seeds germination. Seeds that are already recovered for their vigor and given invigoration treatment are supposed to increase their activity of hydrolytic enzyme, including fitase as fitin hydrolyzing agent. According to Williams (1970) the esterification progress from ADP to ATP was done by fitat acid hydrolysis by fitase enzyme. Fitat acid content on cotyledon of soybean seedling with lower osmotic priming treatment showed that fitat acid effectively utilized for the formation of energy (Widayati, 1999). Also, the activity of fitat degenerating enzyme also higher, then the fitat acid left remain little. If the P released from fitat acid was higher, it suggested that the ATP content of seeds increased, so the germination rate will increase. Therefore, post-harvest maturation storage on fruit aged 7 and 8 month will result in high seed germination and seedling emergence compared to the seeds from non post-harvest maturation storage treatment.

On seeds from fruit aged 8 months, duration of post-harvest maturation storage significantly increase the percentage of seed germination and seedling emergence. According to Zanzibar et al. (2009) the longer period of seed conservation will increase the capacity of germination. Then the longer duration of post-harvest maturation storage, percentage of germination and seedling emergence will increase. Seeds from fallen mature fruit does not affect by post-harvest maturation storage treatment to their percentage of seed germination and seedling emergence.

Table-3: Height, root length, stem diameter, fresh weight, dry weight, and vigor index of seedling on 6 weeks after planting under post-harvest maturation storage and fruit age treatment

Treatment		Height (cm)	Root length (cm)	Stem diameter (mm)	Fresh weight (g)	Dry weight (g)	Seedling Vigor Index
Post-harvest maturation storage (days)	0	5.55 b[1)]	4.45 b	1.31 b	0.2728 b	0.0639 b	4.45 b
	3	5.80 b	4.47 ab	1.33 ab	0.3050 b	0.0686 b	4.54 ab
	6	6.01 a	4.83 a	1.37 a	0.3388 a	0.0783 a	4.62 a
Fruit age (Months after anthesis)	6	5.13 r	3.62 q	1.27 q	0.2301 s	0.0487 s	4.21 s
	7	5.53 q	4.57 p	1.29 q	0.2787 r	0.0652 r	4.42 r
	8	5.85 q	4.97 p	1.38 p	0.3277 q	0.0799 q	4.67 q
	Fallen mature fruit	6.62 p	5.17 p	1.40 p	0.3855 p	0.0890 p	4.86 p
		(-)[2)]	(-)	(-)	(-)	(-)	(-)

Note:[1)] Number followed by same letter on row or column indicates no significant differences with DMRT 5%
[2)] Indicates an interactions between fruit age and the duration of post-harvest maturation storage

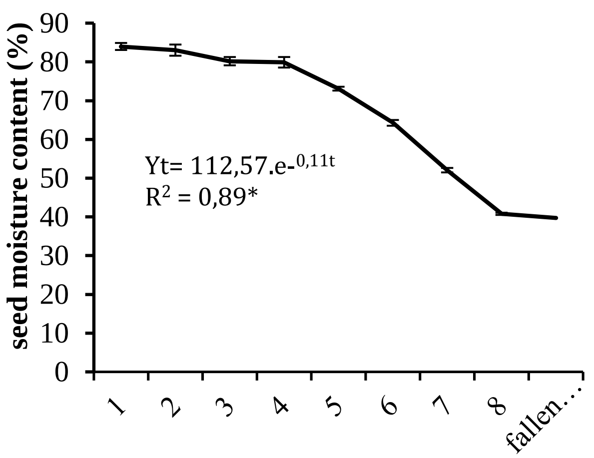
This phenomenon suggests that seeds have already reached their maturity and made their viability to reach maximum with 97.71% of seed germination and 96.67% of seedling emergence. This result supports the result of study conducted by Widayati (1999) on soybean seeds with high viability (the percentage of germination 90-98%) which showed that the recovery of the vigor by osmotic priming did not significantly affect the vigor. Seeds with good viability have a good organelle conditions so the activity of enzyme that cause the osmotic priming treatment was not effective. Seedlings aged 6 WAP show that there was no interaction between post-harvest maturation storage and the age of fruit. Individually, post-harvest maturation storage significantly affect seedling height, diameter, fresh weight, and dry weight and also the vigor index. Fruit age significantly affects which seedling height, root length, stem diameter, fresh weight, and dry weight and significantly affect seedling vigor index (Table 3). Table 3 shown that the longer of fruit post-harvest maturation storage will result in better seedling growth and seed post-harvest maturation storage for 6 days gave better seedling growth. Fruit age treatment show that the increase of fruit age will make better seedling growth. Generally, growth parameter of seedling is significantly higher on seedlings than that which came from seeds from fallen mature fruit. [Table 3]

Post-harvest maturation storage treatment individually affected all parameters of seedling growth. According to Murniati et al. (2008) fruit with post-harvest maturation storage would make the seeds treated like being primed. This would increase the viability of wood-apple seeds that already been stored in comparison with the non stored seeds. The study conducted by Dias et al. (2006) showed that short duration of tomato fruit storage for fruit harvested on green stage and stored until the pericarp has already colored red can increase the quality of seed physiology. Tilden cit. Gardner et al. (1991) observed that priming process (water taken slowly and controlled) will affect the strength of plasma membrane, less electrolyte loss, and increase in germination and seedling growth. Therefore, wood-apple seeds that have already been stored inside fruit will show better seedling growth than those without post-harvest maturation storage. Post-harvest maturation storage from 6 days will gave better seedling growth better than the other treatment. According to Zanzibar et al. (2009), more conservation period will increase the capacity of germination and finally increase seedling growth.

Overall, seedling growth parameter was highly affected by age of fruit harvesting. Seeds harvested at the age of 6,7, and 8 MAA will gave lower seedling growth compared to seeds harvested from fallen mature fruit. Seedling came from seeds of fallen mature fruit had a better growth ability than the other. Seeds from fallen mature fruit have fully developed embryo and perfectly formed their structure, especially in seed membrane integrity that cause less membrane leakage as shown by small EC value from seeds of fallen mature fruit. The less leakage happens because of membrane damage, thus the more substrate can be used for seed germination and initial growth.

The seeds harvested before the fruit reached maturity and fell off from the tree, the formation of embryo structure and membrane have not been fully developed and the accumulation of food storage inside the seeds still did not reach their maximum capability. It suggested that after fruit passing age 8 MAA, period of maturation is still on-going, the ongoing synthesis process ended when the fruit fell off from their tree. Process of seed drying still took place until fruit reach their maturity and fell from their mother plant which was shown by decrease of seed moisture content as shown in Figure 2. This evidence suggested that wood-apple maturity reached when fruit fell from their tree. On mature condition, food storage inside fruit and seed have already been fulfilled. At that time seeds had already acquired maximum food storage that was able to be used to produce the next plant with good vigor (Bewley and Black, 1986). Therefore, seedling from seeds that came from fallen mature fruit gave seedling growth better than the other fruit age treatment. [Figure 2]

Figure-2: Wood-apple seed moisture content on various fruit age



Following the seedling growth, seedling index vigor was increasing in line with the fruit age. Seedling index vigor from fallen mature fruit was significantly higher than those from the other fruit age. This result was relevant with a study conducted by Nautiyal et al. (2010) on peanut whichrevealed that the seed germination and seeds vigor index (SVI) were affected by seed maturity. The highest seed germination and SVI occured on mature seeds (brown colored inside pod), followed by seeds that have already passed the maturity period (black colored inside pod), controlled treatment (without inside pod color category), and the lowest on seeds before maturity (white colored inside pod). A study by Kartika and Ilyas (1994) reported that Jogo Bean seeds harvested on 36 days after flowering or when reaching maturity, followed by drying process both by sunshine or artificial result in maximum seeds vigor. Sutopo (1985) said that the determination of exact harvesting date is very important in order to obtain seeds with maximum viability, and result in healthy, strong, and high yielding plant. Fruit harvested before reaching their maturity did not have high viability.

Conclusion

Post-harvest maturation storage and fruit age affected seed germination and seedling emergence in wood-apple. Germination and seedling emergence increased in accordance with the length of post-harvest maturation storage duration and fruit age. The highest seed germination and seedling emergence were obtained from fallen mature fruit at age 8.25-8.75 MAA either with or without post-harvest maturation storage. Six days of post-harvest maturation storage at fruit aged 8 MAA significantly increased the percentage of seed germination and seedling emergence the same as germination percentage from fallen mature fruit. No significant interactions was found between the duration of post-harvest maturation storage with wood-apple fruit age to wood-apple seedling growth parameter. Six days of post-harvest maturation storage gave the highest seedling growth individually. Seeds from fallen mature fruit gave the highest seedling growth.

Contribution of Authors

Murrinie ED: Conceived idea, conducted experiment and write up of article
Yudono P: Contributed in data analysis and write up of article
Purwantoro A: Contributed in data collection and write up of article
Sulistyaningsih E: Contributed in data collection and write up of article

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