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Effects of naphthalene acetic acid (NAA) on the fruit growth, chlorophyll, maturity index and sugar content in water apple

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Abstract
The experiment was carried out to investigate the effect of NAA on the growth and development of water apple fruits (*Syzygium samarangense*). Three concentrations of GA_3 at 6, 12, 18 mg/l and water control were applied using swabbing technique. The number of buds and fruit setting were increased at 6 mg/l NAA concentration as compared to the other treatments. It also decreased the premature fruit dropping. It was found that the application of NAA at 6mg/l increased fruit length and diameter. In addition to that fruit growth, maturity development and per fruit weight were higher at NAA at 6mg/l compared to other concentrations and water control. With regard to fruit quality, the application of NAA at 6mg/l increased TSS (soluble sugar), besides, acid conten (pH) were higher in treated fruits than water control. From this study it can be seemed that 6mg/l NAA was showed better results than other concentrations and water control.

Keywords: water apple, NAA, growth, maturity development, sugars

Introduction

Water apple (*Syzygium samarangense*) fruit keeps a significant role in fulfilling the demand of nutrition in Asian countries. The fruit is extensively cultivated and widely grown throughout the Malaysia as well as other Asian countries mainly as small scale gardener *(*Zen-hong *et al*, 2006).

Fruit development and repining have been considered as the most important phenomena in agriculture and fruit production. Idea to develop fruit growth was very old and increase of yield or weight using horticultural practices were reported by many researches. Some of the old used techniques were the pruning, hormone application by spray of trees increased fruit growth and development (Savage and Cowart, 1942; Elfving and Forshey, 1976).

Phytohormone is a natural compound synthesized by plant cell with a very low concentration, then translocated to another plant tissue where it causes physiology responses (Romanov, 2002; Gaspar *et al*., 2003; Galston *et al*., 1980; Salisbury and Ross, 1992). Phytohormones contribute in a large range of phenomena that occur during the growth, and the development of plants (Taiz and Zeiger, 1998).

Spraying of plant growth hormone or chemicals is a traditional method. Nowadays Environmental Scientists do not suggest for using this technique too because of the pollution of the air in the environment, water and human health and also not cost effective (Miller, 2004; Tashkent, 1998). Dipping technique has been developed for the fruit growth and quality development instead of spray method due to not affecting environment and cost effective and can control the liquid effluent much easier (Probert, 2009). Asano *et al*. (2001) used dipping methods instead of spray and found better effects in grape fruit. An innovative technique swabbing method has been developed because of using small quantity to get more output compared to spray and dipping methods. Swabbing method does not create any droplet and spray drift which caused by spray and dipping method also. Hossain *et al*. (2007) developed swabbing technique and resulted excessive flowering in peach plants. They also reported that swabbing method enhanced early flowering (blooming) by dwarfing plant growth while ABA (abscisic acid) was applied to the bark in peach plant The size of the fruit can be affected by certain horticultural cultural practices, such as application of plant growth hormones. Gibberellic acid (GA_3) has been shown to increase fruit set and growth in apples, pears (Weaver, 1972). A spray of GA_3 at 50 mg/l using 5 weeks AFB reduced fruit dropped in 'Huaizhi' (Ji *et al*., 1992), and a spray of GA_3 at 50–100 mg/L at full bloom also enhanced fruit retention and fruit size in 'Early seedless' and 'Calcuttia' litchiin India (Singh and Lal, 1980). It has been reported that the spraying of auxins prevented the senescence of fruits presumably by maintaining the cell turgidity at the zone of abscission, which prevents the synthesis of hydrolytic enzymes, such as cellulase, which hydrolyze cell walls (Onguso *et al*. 2004).

The deep-red colored fruits are popular, factors influencing red color has become important for investigators. Gibberellic acid has been shown to increase fruit set and growth in clementine orange Van Rensburg *et al*. (1996)**.** Choi *et al*. (2002) reported that spraying GA_3 increased the fruit size and firmness in cherry fruits**.** In addition to this El-Sese (2005) working on Balady mandarin trees reported that treatment with GA_3 increased the yield of fruits**.** GA_3 increased fruit firmness, soluble solids and fruit weight (Basak, *et al*. 1998).

Very little scientific information is available and known about the growth and development of jambu air fruits. A search in the

Thomson-Reuters and Scopus database revealed only a few articles reporting on its chemical constituents as cited above. In this project the growth and development as well as the pre and Post harvest characteristics of the tree and fruits were investigated and documented with the expectation it led to better quality fruits, which would benefit our local farmers. The following objectives were for the studies:

1. To investigate the effectiveness of swabbing method using NAA.
2. To investigate the various physical & biochemical characteristics of the fruits
3. To develop larger fruits with better taste and good color using NAA.
4. To reduce fruit dropping

Materials and Methods

This study was carried out in a private orchard located at a commercial farm in Banting, Malaysia, 20 30N, 1120 30E and 1028 N, 1110 20 E at an elevation of about 147 ft from sea level.

Plant materials

Twelve years old water apple trees (wax jambu) were selected for the study. The trees were spaced at 20.25 m2 (square pattern). Tree to tree distance was 4.5 m and row to row distance was 4.5 m. 12 trees were used in the study. Three trees were used for each treatment.

Five branches from each tree were used for each unit. All the insects and diseases infected branches were removed before the experiment launched. Sixty uniform branches of the same length, diameter and number of leaves were maintained from the twelve trees for the experiment. Field was maintained properly and irrigation was done when necessary. Pesticides were applied once at growing season.

Weeding was done at one month interval. Plant hormone was applied in the sunny day. Fertilizer was applied at the rate of 15-15-15% (N-P-K) yearly (Hossain *et al*., 2004).

Treatment Setting and Design of experiment

The five selected uniform branches n swabbed with 6, 12 and 18 mg/L NAA and water (control) in three plants. Five branches were considered as replication per tree, total 60 branches. 15 fruits were selected in each branch to make swabbing instate of spray.

Total number of fruit was 15×15=225 per treatments [n= (10×15) for fruit and n=15 for branch]. The design used in the experiment was Completely Rendomized Design (CRD). The swabbing method was applied to the branches once a week starting from bud formation stage to flower opening stage (blooming) and continued until fruit set stage.

Swabbing technique

In this work we have applied a new technique called swabbing (Figure 1).

This method consists to swab PBRs with wetting cotton and forceps without any contamination of fruits.

This method was applied successfully followed by Hossain *et al*. (2007), where aqueous solutions of growth iregulator were applied by swabbing two-to-three times with cotton wool held with forceps.

Fig 1: Swabbing, by cotton applied, at bud flower and flower blooming stage of water apple by using NAA

Measurement of Physiological Parameter

Total number of bud

The total number of buds was determined when bud size was 0.8-1.0 mm. Number of buds grown in 60 cm selected branch were counted before the opening of the flower bud. Percentage bud drop was calculated by dividing the total number of buds before anthesis minus the number of buds at anthesis with the total number of buds before anthesis. Flower initiation was reported at the beginning of the experimental and counted the flower initiation at 60 cm of the selected branches. Blooming percentage were calculated by the bloomed bud divided by total number of buds then multiply the result by hundred.

Fruit setting (%)

Percentage fruit setting were calculated from tagged branches of the experimental trees immediatly after anthersis. The number of flowers bud and total number of fruit set were counted before and after anthesis. Fruit set percentages were calculated using the following formula:

Fruit setting (%) =Total number of fruit set/ Total number of flower bud x100

Fruit dropping (%)

Fruit dropping percentage was determined from tagged branches on the experimental tree by counting the number of initial fruit and the total number of fruit immediately after anthesis. 35 days of anthesis fruit drop percentage was calculated using the following formula:

Fruit dropping (%) = Number of fruits at final harvest/ Total number of initial fruit x100

Fruit length, diameter and fruit growth

Fruit length, fruit diameter, fruit growth was measured weekly with digital caliper (Japan, Model). For fruit growth measurement

10 fruits per selected branch were tagged after anthesis until the fruit harvested. Final length and diameter were measured immediately after harvest.

Fruit maturity (Observing color development)
The surface color of each tagged fruit was determined at three different points of the fruit using a standard color chart (Minolta, Osaka, Japan) and expressed the percentage of maturity (peel color).

Fruit volume
Fruits were kept in the scaled glass water for 2 minutes, after that volume was measured by this visual observation of water level in the scaled glass.

Volume = Initial level of water–Final level of water

Juice volume (ml)
Volume was measured by this visuals observation of juice level in the scaled glass.

Chlorophyll content (Represented by SPAD unit)
Chlorophyll content in leaves of treatment branches was determined using a Minolta SPAD meter and measured usually after 1.5 month of treatment application. SPAD value of the leaves were expressed the chlorophyll content.

Yield
Yield per treatment was recorded by weighing the total number of fruits per treatment at the time of harvesting. Fruits were harvested after ripening

Measurement of Biochemical Parameters
Fruit grinding (Collection of fruit juice)
Three fruit were selected randomly from each branch. Total of 3×15=45 fruits were ground separately for each treatments. Total of 180 fruits (4×45) were used for 4 treatments. Fruit was cut into pieces and blender machine was used for grinding. The juice was centrifuged and supernatant (Clear juice) was collected and it was placed in airtight glass bottles, stored in an ice filled cooler and transported to the laboratory to keep at cold temperature (4±1 °C) for biochemical analysis.

Total soluble solid (TSS) content
Total soluble solids (TSS) content in the fruits were evaluated at 25ºC with abbe Refractometer. TSS were expressed with % Brix. A hand-held refractometer (Atago ATC-1, 32-10 Honcho, Itabashi-ku, Tokyo 173- 001, Japan) was used from 2010 and a digital refractometer (Atago PR-101) was used for TSS determinations. A few drops of juice were kept on the refractometer prism surface (Figure 7) and reading was collected from skin pad.

PH of fruit juice: Sample Preparation
Immediately after harvest, fruit were clean, washed and dried of surface water with fan. Then the fruit are blended and fruit juices are kept in glass bottle.
All fruit juice samples were first allowed to equilibrate to room Temperature (25°C) before pH determination. PH was measured using a Microprocessor pH meter (Hanna Instrument). Before measurement of pH the Microprocessor pH meter was calibrate properly.

Statistical Analysis
The data were plotted and analyzed using MSTAT statistical software. One way ANOVA was applied to evaluate the significant difference in the parameters studied in the different treatments. Least significant difference (Fisher's protected LSD) was calculated, following significant F-test (p=0.05). Standard error (SE) was measured by Excel.

Results
The effects of NAA on bud number and dropping of water apple fruits are shown in Table 1. Number of bud has been increased with increase the NAA concentration. Bud number for control brunches (pollinated fruit) reached 54. Whereas, bud number for 12 ppm NAA treated brunches showed maximum compared to others NAA treated brunches. Bud dropping has been increased with increase the NAA concentration too. On the other hand, the fruit development started from the beginning of fruit setting or initial fruit growth to until maturity stage. Assessments of fruit development are based on the measurement of fruit size and weight from its initial growth to maturity stage. It is well documented that plant hormone, NAA has a distinct characteristics to control fruit set and fruit development. Table 2 showed that the effects of NAA at different concentrations on the induction of fruit setting and final fruit size. Fruit setting was extended by applying NAA (6, 12 and 18 ppm) at initial developmental stage. Application of 12 ppm NAA showed the highest fruit setting compared to the other treatments. However, fruit dropping was increased applying NAA (12 and 18 ppm) by 17% and 16%, respectively, compared to the control. Fruit length and wide of water apple were greatly enhanced as a result of the activity of NAA. In the present study, it was observed that the best result was exhibited by 12 ppm NAA among the different concentrations of NAA.
Taking into account of all data from the experiment, yield per branch of water apple was 455 g in control, whereas yield per branch of water apple was significantly high *viz*, 489, 517 and 493 g in treated braches with NAA (Table 3). However, fruit weight was significantly increased in the case of 12 ppm NAA per branch. As a result, fruit volume and juice content also was increased by the application of 12 ppm NAA.
NAA can enhance fruit set and it can be applied early in the growing phage to prevent abscission of flower buds. The effect of the NAA treatments on the increase of fruit maturity was evaluated by measuring fruit length and width from first to eight harvest weeks. Thus, in the first week, the fruit length and width were not significant and subsequently, fruit length and width showed difference at different weeks and significant difference was observed especially in the 8th week (Fig. 1 and 2).

Table 1: Effects of different treatments of NAA on bud number and bud dropping of water apple fruits. Values are means ± S.E. (Different alphabets mark significant differences, P < 0.05 by LSD)

Treatments	Bud number	Bud dropping
Control	54.0±0.57d	29.0±0.57d
NAA 6 ppm	57.3±0.33c	41.3±0.88c
NAA 12 ppm	60.6±0.33a	51.3±0.33b
NAA 18 ppm	59.6±0.33ab	55.3±0.33a

Table 2: Effects of different concentration of NAA on fruit growth parameters, fruits setting, Fruit dropping and Fruit wide. Values are means ± S.E. (Different alphabets mark significant differences, P < 0.05 by LSD)

Treatments	Fruit setting (%)	Fruit dropping (%)	Fruit length (mm)	Fruit wide (mm)
Control	29.6±0.33d	34.6±0.88c	58.1±0.02d	31.5±0.02d
NAA 6 ppm	37.3±0.33c	32.6 ±0.33cd	60.1±0.01bc	32.5±0.01c
NAA 12 ppm	48.6±0.33a	42±0.57a	63.6±0.03a	37.3±0.32a
NAA 18 ppm	44.6±0.33b	39.3±0.66b	61.1±0.01b	34.2±0.02b

Table 3: Effects of different treatments of NAA on fruit yield, fruit weight, fruit volume and juice volume of water apple. Values are means ± S.E. (Different alphabets mark significant differences, P < 0.05).

Treatments	Fruit yield (g/branch)	Fruit weight (g/fruit)	Fruit volume (ml/fruit)	Juice volume (ml/100g)
Control	455±1.8d	50.6±0.24d	51.7±0.2d	64.2±0.85d
NAA 6 ppm	489.1±7bc	53.7±0.23c	54±0.15c	66.5±0.34c
NAA 12 ppm	517.7±4.4a	63.3±0.2a	63.6±0.2a	72.7±0.34a
NAA 18 ppm	493.3±1.5b	60.2±0.14b	61.8±0.5b	70±0.15b

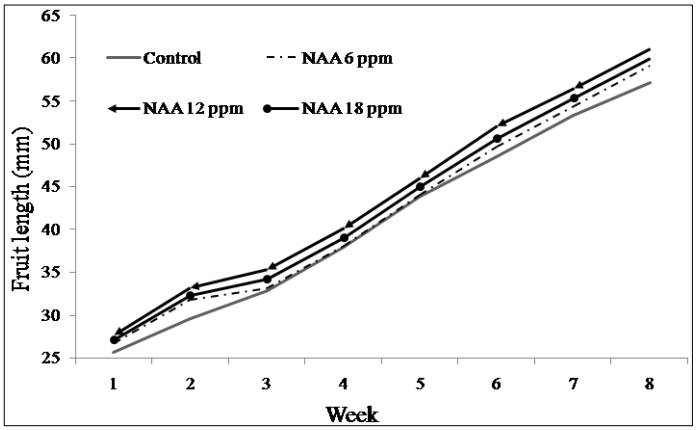

Fig 2: Fruit growth (Length)/week as influenced by different concentration of NAA.

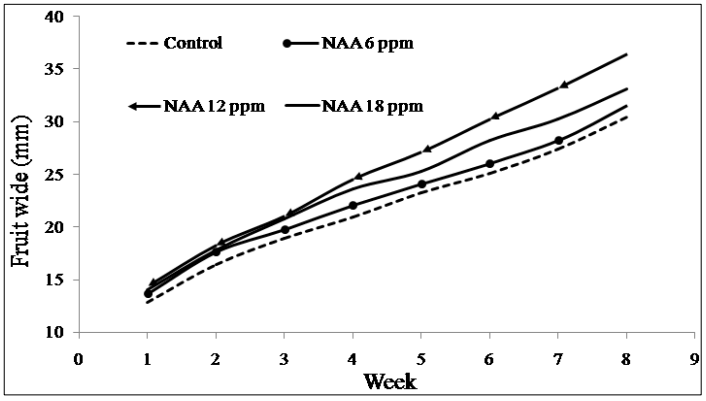

Fig 3: Fruit wide per week as influenced by different concentration of NAA

The chlorophyll (represented by SPAD value) content showed a significant difference among the applied hormone treatments (NAA) and control leaves. The highest amount of chlorophyll content was observed in the 12ppm NAA treated branches leaves. The accumulation of chlorophyll was lower in plants which undertaken as control (Fig. 3). It was observed that higher concentration of NAA (18 ppm) had the lower chlorophyll content in water apple leaves.

Potassium content was higher in NAA treated branches fruits than in control and potassium content was reduced by high concentration of NAA. This was the effective mechanism for increasing potassium content in fruits. The potassium content was higher in 12 ppm NAA treated fruits than other concentrations (Fig. 4).

Total flavonoid content of mature fruits was measured at the end of the experiment, where the content was 50% higher in treated fruits than in untreated fruit. The maximal total flavonoid content was obtained in 12 ppm NAA treated fruits (Fig. 5).

Total soluble Sugar (TSS) content was affected significantly by the application of different concentrations of NAA (Fig. 6). The highest sugar content was observed by 12 ppm NAA concentration, through affecting the metabolism of high physiological process which led to increase sugar content in fruits. The 6 and 18 ppm NAA concentrations resulted significant reduction of sugar content in fruits. Hence, it was observed that 12ppm NAA was the optimal concentration for water apple fruits to maintain the highest soluble sugar content.

In addition, inverted sugar and fructose content were improved by all NAA treatments. Highest increament in inverted sugar was recorded by 12 ppm NAA. Both of inverted sugar and fructose were reduced by higher concentration of NAA (18ppm). There was a similar increasing trend were observed by the same concentration in the case of both inverted sugar and fructose (Fig. 7 and 8).

Furthermore, it was noted that there was almost a similar difference in morphological and biochemical observation. The remarkable effect by different NAA concentrations on fruit color what was assessed by measuring anthocyanin content of untreated fruit was almost same as untreated fruits. It was observed that the biochemical (anthocyanin) content was showed same trend as harvest color level (maturity) and the effects decreased as the NAA concentration was increased (Fig 9). Consequently, color and maturity were earlier in treated fruit than in untreated fruits.

In addition, color and pH are the most important harvest parameter in fruit harvest index because of adjusting juice pH, it is dependent extensively on the fruits color (Tehrani *et al*., 2011; Moneruzzaman *et al*., 2008) [16, 15]. Regarding the effects of NAA treatment on pH value, no significant differences were found between 6 and 18 ppm NAA concentrations. The control showed significantly difference from all other treatments. Meanwhile, 12ppm NAA treatment showed a highest pH value which was significantly different from other treatments. The difference among pH values was showed as the result of effective hormonal activity (Fig 10 and 11).

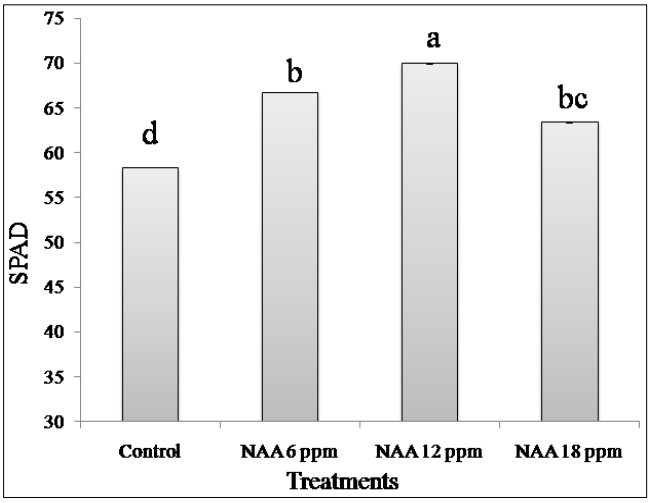

Fig 4: Effect of different NAA treatments on leaf chlorophyll content (SPAD) of water apple frui

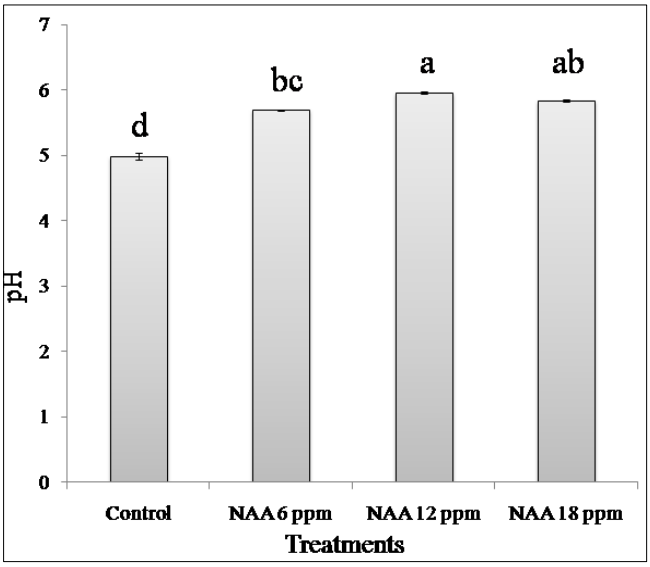

Fig 5: Effect of different treatments of NAA on acidity or pH content of water apple fruit juice.

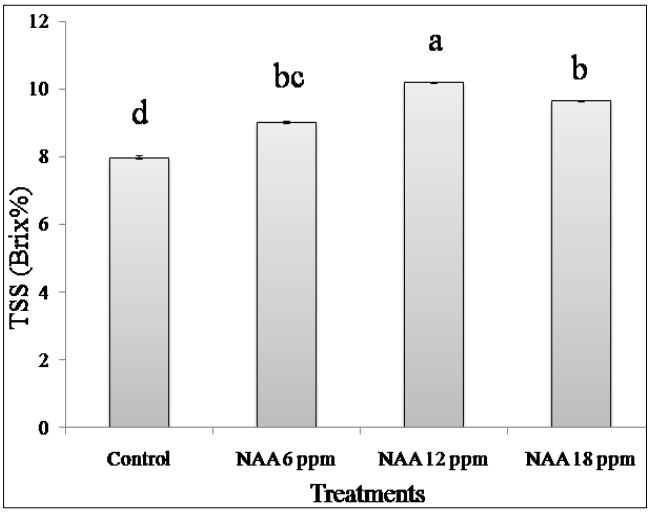

Fig 6: Effect of different treatments of NAA on Total soluble solids (TSS) content of water apple fruit

Discussion

Naphthalene acetic acid (NAA) is an organic compound which is a plant hormone of the auxin family. The impacts of NAA hormone on a plant often depend on the stage of the plant's development and the concentration. NAA have been reported to raise flowers and fruits dropping off trees before maturation (Chang and Chen, 2001) [10]. Similar results have been observed in this study where initial bud and fruits dropping increased by 60% in NAA than in control. Brent *et al.,* (1995) [9] also reported that when NAA was sprayed on young buds or fruits such as apple and olive, some of the buds and fruits dropped off so that the remaining fruits could grow larger. Consequently, fruits dropping were stopped until the maturity stage. The effects of this hormone on a plant often showed better activity than any other plant hormone in case of bud and fruit dropping. Many researchers have recommended that maximum fruits were sensitive to ethylene in the young stages of development or middle maturity stages (Yoko *et al*., 2006) [12]. For that reason, a lot of fruits were dropped at the young stage before maturity. Elgar *et. al.,* (2003) [1] reported that NAA might reduce abscission more successfully, as well as ethylene production might be neutralized by NAA in the young stage. Chang and Chen (2001) [10] also showed that NAA delayed flower abscission. In this study, therefore fruit setting was significantly increased by NAA. Application of 12ppm NAA resulted in significantly higher fruit length and wide than pollinated control. It was observed that growth of NAA treated fruits closely related to changing carbohydrate level. However, the carbohydrate content among the treated fruits varied with the enzymatic activity. This is resembled to the work done by Agusti *et al* (2002) [8] on citrus fruits. They described that NAA hormone stimulated cell elongation by stimulating naturally produced hormone, cytokinin which has an ability to increase the cell dimension. Application of the NAA increased final fruit quality such as size, color and juice content without causing any fruit damage. The effectiveness of NAA depended on the concentration (12ppm) applied in this study and many researchers mentioned that it also depended on seasons and periods of application (Issam, 2010) [13]. When NAA was applied at initial fruit stage, final fruit diameter and distribution of fruit color showed a significantly increment in fruits. Therefore, fruit maturation and harvest time were earlier in treated branches than in untreated branches. In general, it was showed that NAA induced fruit set and development in varieties of fruit crops, but very few research has been carried on water apple in this concern. Swelling of NAA successfully induced fruit development from initial fruit setting to maturity stage.

In the present study, it was observed that the treatments of NAA significantly improved fruit size, weight, and biochemical content of water apple. The improvement of fruit quality and fruit management could possibly be resulted of enzymatic action of NAA (Chang and Chen, 2001) [10]. The variable difference in juice content, TSS and maturity was observed among the different concentrations. That was probably due to the various activity levels of different concentrations of NAA (Saifuddin *et al*., 2009) [14]. It could be changed in cell wall of fruits and leaves in different way. The action of NAA might have been increased soluble carbohydrates in the fruits than the other parts of plant. As a result, the fruit volume and juice content of treated fruit was increased at the time of fruit setting and reached a maximal level in fruit maturity stage.

NAA is also known to enhance hydrolysis of TSS, starch and sucrose into glucose which can increase the fruit volume. The additional sugars in the fruits may increase the osmotic potential in cell wall, thus improved their ability to absorb more nutrients and maintain their turgidity. NAA increased anthocyanin or color because of addition hydrolysis of TSS which added color ingredient in fruit cell. The higher sugar uptake and the simultaneous extra uptake of water would enhance the cellular turgor pressure, which might effect in greater fruits expansion.

The prominent organic acids in fruits such as malic, succinic and fumaric acid control the fruits acidity. In general, green or early stages fruits contain comparatively more organic acid than maturity stage (Amoros *et al*. 2004) [2]. At the fruit maturity stage, organic acid and other compounds turn into sugar, fructose and glucose substance. Consequently, total fruit acidity decreased with increase of fruit volume or maturity. Significant differences were found in the case of acid contents among the untreated and treated fruits.

Yoko *et al.* (2006) [12] reported that the leaf tended to have its stomata closure during NAA stress. The stomata adjustment might send a chemical signal towards abscission zone and therefore, NAA might protect ethylene production through ACC path. Consequently, fruit setting or number of fruit was higher in NAA than control. This is meant that NAA might be effective in blocking ethylene activity.

It is well documented that the key role of potassium is to act as a catalysts for many enzymatic processes and to regulate osmotic potential in cell (Bussakorn *et al*., 2003) [18]. Translocation of carbohydrates in plant cells can be increased in presences of NAA hormone (Han *et al*., 1995) [17]. In addition, higher K allows more enzymatic effects to take place and maintain higher TSS and glucose content in fruits allowing the cell to maintain growth. Therefore, higher fruit volume and juice content was observed in NAA treated branches.

Actually flavonoids considerate a large group of phenolic compounds that are synthesized various enzymatic steps. Flavonoids might work as protecting the fruits from excess light, defense against pathogens, and attracting for pollinator. Flavonoids also may contribute to maintain the water apple quality such as its taste, color and bitterness or texture like other fruits (Amiot *et al*. 1997) [1]. Flavonoid biosynthesis in tissue may be accumulated extensively in presences of phenolic compounds.

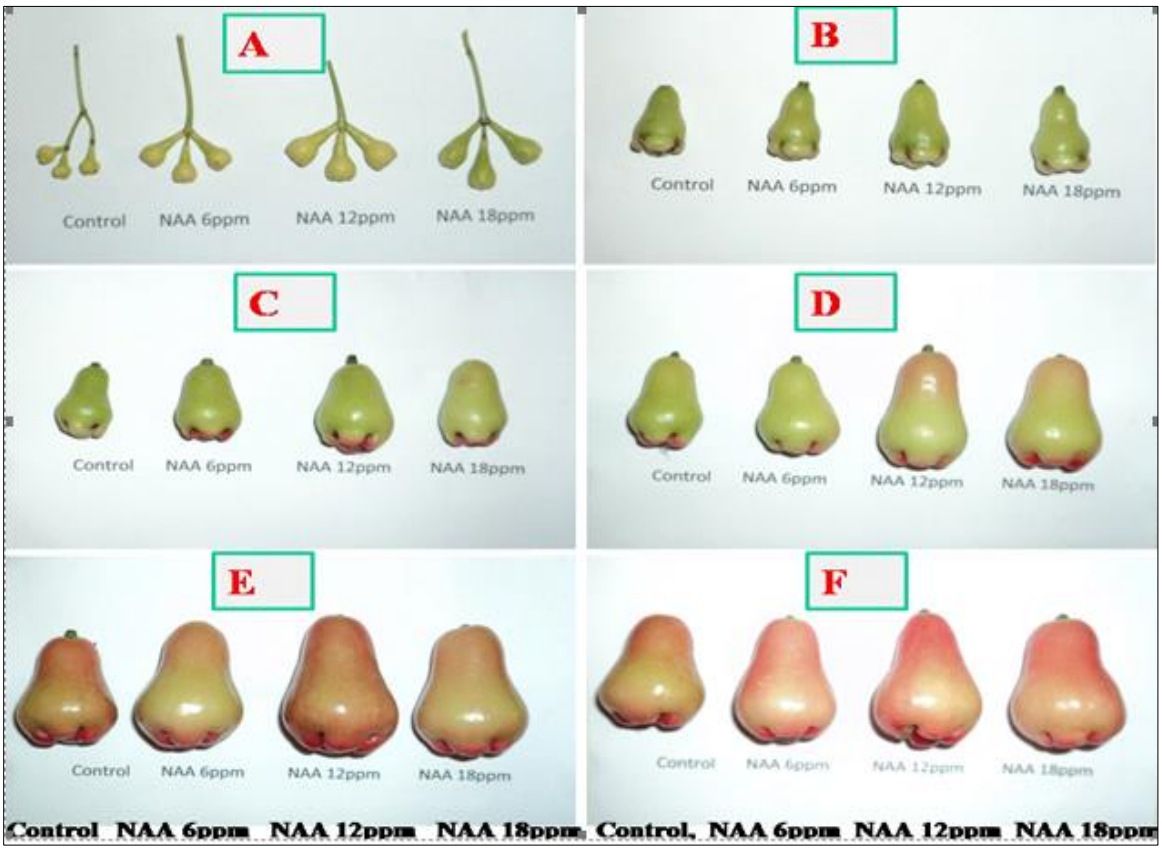

Fig 7: Photgraph shows the effect of different concentrations of NAA on water apple bud and fruit setting and development.

According to the pH content anthocyanin pigments that may appear as a red, purple, or blue tint. They belong to a parent class of molecules called flavonoids, synthesized via the phenylpropanoid pathway (Raghvendra *et al*., 2011) [19]. Anthocyanins found in all tissues of higher plants, including leaves and maximal was found in flowers and fruits. Hydrocarbons what can be produced via photosynthesis and laterally might be converted into flavonoids and their derivatives compound is anthocyanins. Generally fruits contain many compounds such as anthocyanins, chlorophyll, carotenoids, and flavonols which can be combined together to make its color formation. The most important composite for the red coloration in water apple are the anthocyanins, situated specially in fruit skin. The anthocyanin can rise more than 4 times during the ripening stage. Figure 1 showed that the biochemical which led to anthocyanin production. Most important steps in this

biochemical process were to increase the availability of sugars and the activity of the enzyme in presence of K content. These impacts combined with physiological and environmental factors speed up cell activity to raise the net color as well (Brouillard and Dangles, 1994) [4].

Therefore, the commercial value of water apple is depended on fruit size and color. The application of 12ppm NAA at the onset of fruit setting has been found to be effective in water apple fruit, increasing fruit diameter by 10%, and yield by 20% per tree compared with other NAA concentration. However, it was observed that each concentration of NAA significantly increased yield and fruit size (Fig. 1) compared with control fruit. The aforementioned effects could be due to an increase of cell enlargement (Ohmiya, 2000) [6]. This might function via encouragement of leaf chlorophyll allowing more photosynthesis and carbohydrate accumulation. According to other findings, NAA and other auxins, such as 3, 5, 6-TPA, have been begun to have a similar effect on fruit growth (Ortola *et al*., 1991) [7].

Conclusion

Finally it can be seemed that the result of application of NAA on fruit development and biochemical variations has been observed in this study and the outcome varies considerably depending on the NAA concentration. The treatments of 12ppm NAA increased yield, number of fruits and size. The production of larger fruit and early maturation by the application of NAA, would be greater due to the economic advantage. Having observation of the physico-chemical properties of treated water apple, it can be concluded that NAA hormone may be applied to enhance the fruit quality, such as colour, maturity index, Sugar and acid contents.

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