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Carbohydrate, flavonoid, anthocyanin, total phenol, chlorophyll and mineral (K^+) content development of wax apple fruit as affected by CPPU and NAA using swabbing technology

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Abstract: The study was conducted to investigate the effect of CPPU(2-Chloro-4-pyridyl-phenylurea) and NAA (Naphthaleneacetic acid) on the pigments, total phenol, flavonoid and mineral (K^+) content in wax apple fruit. CPPU concentrations were 10, 15 and 20ppm (Expt.1) and 6, 12, 18ppm NAA were applied coming after swabbing technique (Expt.2). In Expt.1, the lowest fruit weight, fruit size and chlorophyll content was observed in control treatment. However, the highest fruit weight, fruit size and chlorophyll (SPAD) content was found in 15ppm CPPU. The most effective concentration was 15ppm CPPU for the earlier fruit maturity (color development) compared to other concentrations. Moreover, flavonoid, fructose, inverted sugar, total phenol and K^+ were higher in 15ppm CPPU than control, 10 and 20ppm CPPU. In addition, anthocyanin was found increasing trend while developing the fruit maturity represented by color development. In Expt.2, chlorophyll was higher in 15ppm CPPU than control, 6, 18ppm NAA. Furthermore, flavonoid, fructose, inverted sugar, total phenol and K^+ content were higher in 12ppm NAA than control, 6 and 18ppm NAA. Besides, the maximum anthocyanin was found in 12ppm NAA. Finally it seemed that 15ppm CPPU and 12ppm NAA were the best concentration for fruit growth and biochemical contents development in wax apple.

Keywords: CPPU, NAA, total phenol, flavonoid, anthocyanin, nutrient content.

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Desenvolvimento do conteúdo de carboidratos, flavonoides, antocianinas, fenóis totais, clorofila e minerais (K+) de maçã de cera afetada por CPPU e NAA usando tecnologia de zaragatoa

Resumo - O estudo foi conduzido para investigar o efeito de CPPU(2-Cloro-4-piridil-fenilureia) e NAA (ácido naftalenoacético) sobre o teor de pigmentos, fenóis totais, flavonoides e minerais (K+) em frutas de maçã de cera. As concentrações de CPPU foram 10, 15 e 20ppm (Expt.1) e 6, 12, 18ppm NAA foram aplicadas após a técnica de swab (Expt.2). No Expt.1, o menor peso do fruto, tamanho do fruto e teor de clorofila foi observado no tratamento controle. No entanto, o maior peso de fruto, tamanho de fruto e conteúdo de clorofila (SPAD) foi encontrado em 15ppm CPPU. A concentração mais eficaz foi de 15 ppm CPPU para a maturidade precoce da fruta (desenvolvimento da cor) em comparação com outras concentrações. Além disso, flavonoides, frutose, açúcar invertido, fenóis totais e K+ foram maiores em 15ppm CPPU do que o controle, 10 e 20ppm CPPU. Além disso, a antocianina apresentou tendência crescente ao desenvolver a maturidade do fruto representada pelo desenvolvimento da cor. No Expt.2, a clorofila foi maior em 15ppm CPPU do que no controle, 6, 18ppm NAA. Além disso, os teores de flavonoides, frutose, açúcar invertido, fenóis totais e K+ foram maiores em 12ppm NAA do que no controle, 6 e 18ppm NAA. Além disso, o máximo de antocianina foi encontrado em 12ppm NAA. Finalmente, parece que 15ppm CPPU e 12ppm NAA foram a melhor concentração para o crescimento do fruto e desenvolvimento do conteúdo bioquímico em maçã de cera.

Termos de indexação: CPPU, NAA, fenol total, flavonoide, antocianina, teor de nutrientes.

Introduction

Wax apple (*Syzygium samarangense*) is a common fruit in Malaysia as well as other Asian countries, besides it is grown in European, American and African countries (HOSSAIN, 2013). The fruit is widely cultivated and grown throughout Malaysia mainly as small scale gardener ranging from 1 to 5 ha with its hectare average estimated at 1500 ha in 2005 (ZEN-HONG et al., 2006). Fruit development and ripening technologies have been considered as the most important phenomena in fruit production. Many researchers reported that development of fruit growth technique was very old and traditional in horticulture. Some of the traditional techniques were the pruning, hormone application by spraying of trees increased fruit growth and development (HOSSAIN et al, 2007, ONGUSO et al., 2006).

Spraying of plant growth regulators or chemicals has been used for a long time as a traditional technique. 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; HOSSAIN et al., 2015). 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 could control the liquid effluent much easier (PROBERT, 2009, HOSSAIN et al., 2017). Asano et al. (2001) used dipping methods instead of spray and exhibited better effects in grape fruit and also reported that there was some pollution occurred due to the remaining droplet.

An innovative swabbing technique has been developed due to the using less quantity to get more output compared to the spraying and dipping techniques. It was reported that swabbing technique did not create any droplet and spray drift which caused by spraying and dipping techniques (ONGUSO et al., 2006). Hossain et al., (2007) developed swabbing technique and resulted excessive flowering in peach trees. They also reported that swabbing technique enhanced early flowering (blooming) by dwarfing tree growth while ABA (abscisic acid) was applied to the ring shaped bark in peach trees. It has been exhibited that the spraying of auxins prevented the senescence of fruits presumably by maintaining the cell turgidity at the zone of abscission, which prevented the synthesis of hydrolytic enzymes, such as cellulase, which hydrolyzed cell walls (ONGUSO et al., 2004, HOSSAIN et al., 2017).

Gibberellic acid (GA_3) has been shown to increase fruit set and growth in clementine orange (HOSSAIN et al., 2019). Choi et al., (2002) observed that spraying GA_3 increased the fruit size and firmness in cherry fruits. In addition to that El-Sese, (2005) conducted an experiment on Balady mandarin trees and reported that GA_3 increased the yield of fruits. GA_3 increased fruit firmness, soluble solids and fruit weight (HOSSAIN et al., 2019). The phenolic content of edible fruits are useful since the role of these factors play in health and disease chemoprevention have been widely reported. The leaves of samarangense have shown the presence of ellagitannins, proanthocyanidins, flavanones, flavonol glycosides (HOSSAIN, 2013), anthocyanidins (KUO et al., 2004), triterpenoids, chalcones and volatile terpenoids (HOSSAIN, 2013; WONG; LAI, 1996, HOSSAIN et al., 2019). Very little scientific information is available and known regarding the growth and quality development of wax apple fruit by growth regulators except CPPU and NAA. In this study the growth and development as well as the pre and post harvest characteristics of the fruits were investigated and documented with the expectation it led to better quality fruits, which would benefit for the fruit growers. The following objectives were undertaken:

i. To investigate the effectiveness of swabbing technique using CPPU and NAA.

ii. To investigate the various biochemical characteristics like chlorophyll, total phenols, flavonoid, anthocyanin, K^+ content, fructose and inverted sugar content of the fruits using CPPU and NAA.

iii. To develop a good color using CPPU and NAA.

Materials and Methods

Study area and Plant materials:

This study was carried out in a private orchard located at a commercial farm in Banting, Malaysia. Twelve years old water apple trees were selected for the study. The trees were spaced at 20.25 m^2. Tree to tree distance was 4.5 m and row to row distance was 4.5 m. Twelve trees were used in this study. Three trees were used for each treatment. Five branches from each tree were used for each unit. Sixty uniform branches of the same length, and diameter were maintained from the twelve trees for the experiment.

Tree Management:

Trees were maintained properly and irrigated when necessary. Pesticides were applied once at growing season. Weeding was done at one month interval. Plant growth regulators were 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 for expt. 1

Five selected uniform branches were swabbed with 10, 15 and 20ppm (mg/L) CPPU and water (control) in three trees. Five branches were considered as replication per tree. Fifteen buds were selected in each branch to swab instate of spray. The design used in the experiment was Completely Randomized Design (CRD). The swabbing technique was applied to the branches once a week starting from bud formation stage to flower opening stage (blooming) and continued until fruit set stage.

Treatment setting and Design of experiment for expt. 2

Five selected uniform branches were swabbed with 6, 12 and 18ppm (mg/L) NAA and water (control) in three trees. Five branches were considered as replication per tree. Branches per tree, bud number per branch and experimental design were same as expt. 1 mentioned above.

Swabbing technique

This method consists of swab with growth regulator using wetting cotton and forceps without any contamination of buds. This method was applied successfully followed by Hossain et al., (2007), where aqueous solutions of growth regulators were applied by swabbing two-to-three times.

Chlorophyll content in leaves of treated branches was measured using a SPAD meter (Minolta-502, Japan) usually after 1.5 month of treatment application. SPAD-502 determined the relative amount of chlorophyll by measuring the absorbance of the leaf.

Measurement of biochemical parameters

Fruit harvesting and grinding

Three fruit were selected randomly and harvested from each branch then stored in an ice filled cooler and transported to the laboratory to keep at cold temperature (4°C) for biochemical analysis. Total of 3×15=45 fruits were ground separately for each treatments. Fruit was cut into pieces and blended. The juice was centrifuged and supernatant was collected and it was placed in airtight glass bottles.

Fruit color measurement

The surface color of fruit was measured at three different parts of the fruit like proximal end, distal end and middle portion using a standard color chart (Konica Minolta, Osaka, Japan) and expressed as percentage of color.

Total phenols

The total phenolic content of water apple fruits was determined by using the Folin-Ciocalteu assay (SINGLETON; ROSSI, 1965). Folin-Ciocalteau (FC) colorimetry was based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The intensity of light absorption at that wavelength was proportional to the concentration of phenols.1ml of fruit juice, gallic acid calibration standards and folin-Ciocalteau (FC) reagent were stored in the dark and discarded until reagent became visibly green. Sodium carbonate solution (100-ml) were used in a volumetric flask. Spectrophotometer was set to 765 nm. A reagent blank was prepared. 1 ml of Folin – Ciacalteu's phenol reagent was also added to the mixture. The solution was diluted with distilled water and mixed then incubated at room temperature. The absorbance against reagent blank was determined with an UV-Vis Spectrophotometer Lambda 5 and expressed as mg gallic acid equivalent (GAE)/100g fresh weight.

Total flavonoid

Total flavonoid content was determined by the aluminum chloride colorimetric assay (ZHISHEN et al., 1999). An aliquot (1 ml) of extract (0.5 g dried shredded peel in 50 ml 80% aqueous MeOH) or standards solution of quercetin (3, 6, 14 mg/ml) was added to 10 ml volumetric flask containing 4 ml dd H_2O. 0.3 ml 5% $NaNO_2$ was added in the flask. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At the 6th min, 2 ml 1M NaOH solution was added and the total volume was made up to 10 ml with d H_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg Catechin equivalents (CE)/100g fresh mass.

Total anthocyanin content

Total anthocyanin contents of the hydrophilic extracts were determined by the pH-differential method described by Rodriguez-Saona et al. (1998). The matured wax apples were harvested and the crude extract was prepared in the following manner. The wax apple was washed thoroughly with distilled water at room temperature (28 °C). The pigment from peels was extracted with water using food processor (Singer, FP-450). The pigment extract was filtered to remove the fibrous particles and then it was centrifuged at 10,000 rpm for 5 min to remove the tiny suspended solid particles. The color extract was then stored at 4°C in the refrigerator and used for the analysis then anthocyanin content was determined.

K^+ content

Fruit juice was taken for each treatment. Then 3 drops of the supernatant liquid of centrifuged juice (4000 rpm for 10 min) were dropped onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA), on a sampling paper placed on the sensor. The reading in ppm was taken from the display pad after it stabilized (30 to 43 sec).

Fructose and inverted sugar

Centrifuged wax apple juice was used for fructose and inverted sugar determination. Fructose and inverted sugar were evaluated at 25°C with Atago 8469 digital handled refractometer (Atago Co. LTD., Tokyo, Japan) and expressed as percentage.

Statistical analysis

The data were plotted and analyzed using MSTAT statistical software. Least significant difference (Fisher's protected LSD) was calculated, following significant F-test (p=0.05).

Results

Experiment 1

Fruit weight was the highest in 15ppm CPPU concentration (Table 1). However, fruit length was higher in 20ppm CPPU concentration than other concentrations and control (Table 1). Chlorophyll content (photosynthetic yield represented by SPAD unit) was higher in CPPU treated leaves than the control leaves. The chlorophyll content was higher in 15ppm CPPU treated leaves than other concentrations of CPPU (Table 1). Fruits color was measured after harvest. The influence of different concentrations of CPPU on color was easily observed in Table 1. The results showed that 15ppm CPPU had greatly influenced the treated fruits by increasing color. The potassium content of mature fruit was measured at the end of the experiment. As can be seen in Table 1, the potassium content was 50% higher in all CPPU treated branches fruits than in control. The maximum potassium content was obtained in 15ppm CPPU treated fruits. Figure1 showed the swabbing technique by cotton wool at bud flower and flower blooming stage of wax apple fruit by applying CPPU and NAA.

Table 1 - Effects of different concentration of CPPU on wax apple weight, size and chlorophyll content. Different alphabets mark significant differences, P < 0.05 by LSD.

Treatments	Fruit weight (g)	Fruit size (length, cm)	Chlorophyll content (SPAD)	Color % (Pigment)	K+ content (mg/g)
Control	58.7±0.19d	5.5±0.11b	58.2±0.14d	90.1±0.18a	16.6±0.17d
CPPU 10ppm	60.9±0.36c	5.7±0.12ab	61.5±0.13b	92.8±0.16b	31.5±0.16c
CPPU 15ppm	64.2±0.09a	6.3±0.11a	62.6±0.12a	95.6±0.15d	34.3±0.15a
CPPU 20ppm	62.6±0.03b	6.5±0.12a	60.5±0.10 c	94.4±0.17c	33.2±0.15b

Figure1 - Photographs show the swabbing technique by cotton wool at bud flower and flower blooming stage of wax apple fruit by applying CPPU and NAA.

Bud stage Flower stage

The results showed the total phenol and fructose contents in fruit varied considerably among the treatments (Figure 2 and 3). The 15ppm CPPU concentrations showed higher phenol and fructose content than other concentrations of CPPU. The lowest phenol and fructose content was obtained in 20ppm CPPU treated fruit among all CPPU concentrations. The inverted sugar concentration did not show the significant difference between 10 and 20ppm CPPU concentrations. Only 15ppm CPPU treated fruit obtained more inverted sugar than other treatments (Figure 4). Result shown in Figure 5 and Figure 6 that the fruit flavonoid and anthocyanin contents of wax apple were significantly increased by maturity development (fruit color percent at different stage) increased. There was a strong correlation between color percent developed and flavonoid (R^2=0.828) as well as anthocyanin content (R^2=0.792). Figure 7 showed the different fruit maturity stages (green, light green, light red, red and deep red) represented by developing color as affected by CPPU at different concentrations.

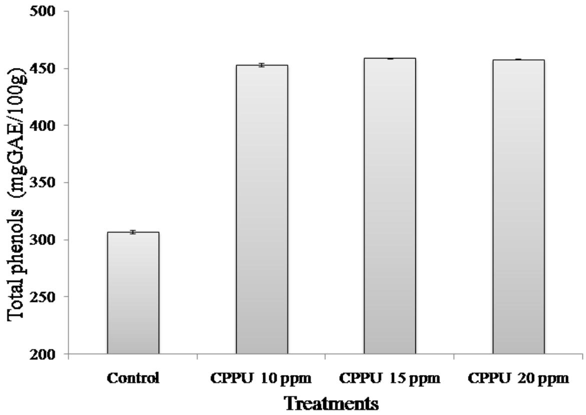

Figure 2 - Total phenols content as affected by different treatments applied to water apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD).

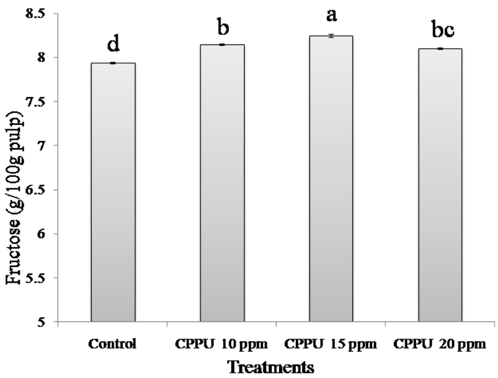

Figure 3 - Effect of different CPPU treatments on fructose content (Different alphabets mark significant differences, P < 0.05 by LSD.

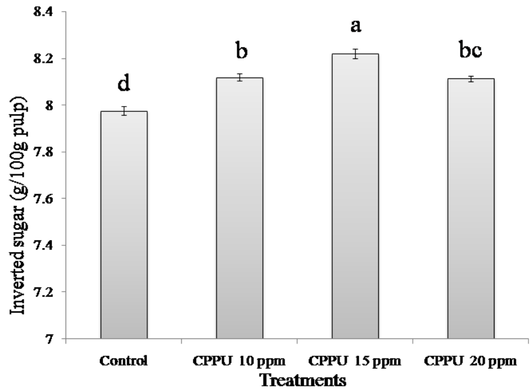

Figure 4 - Effect of different CPPU treatments on inverted sugar content (Different alphabets mark significant differences, P < 0.05 by LSD).

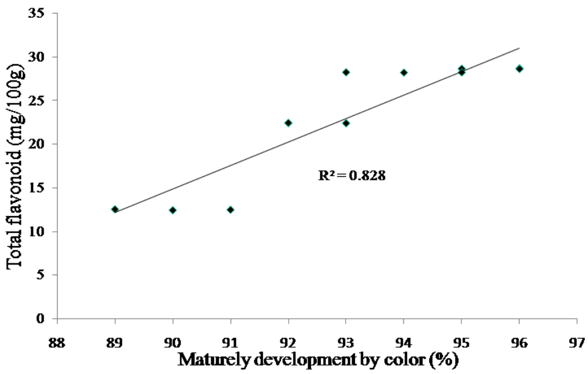

Figure 5 - Correlation between fruits maturely development by color (%) and total flavonoid content.

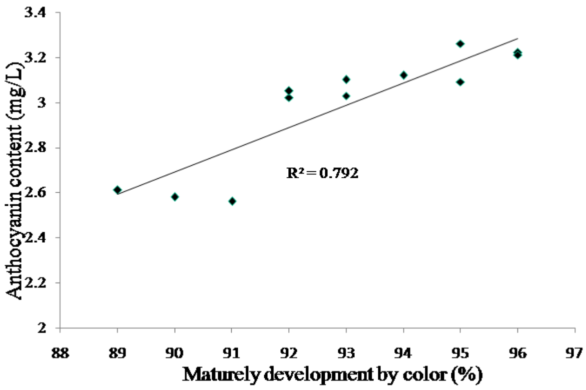

Figure 6 - Correlation between fruits maturely development by color (%) and anthocyanin content.

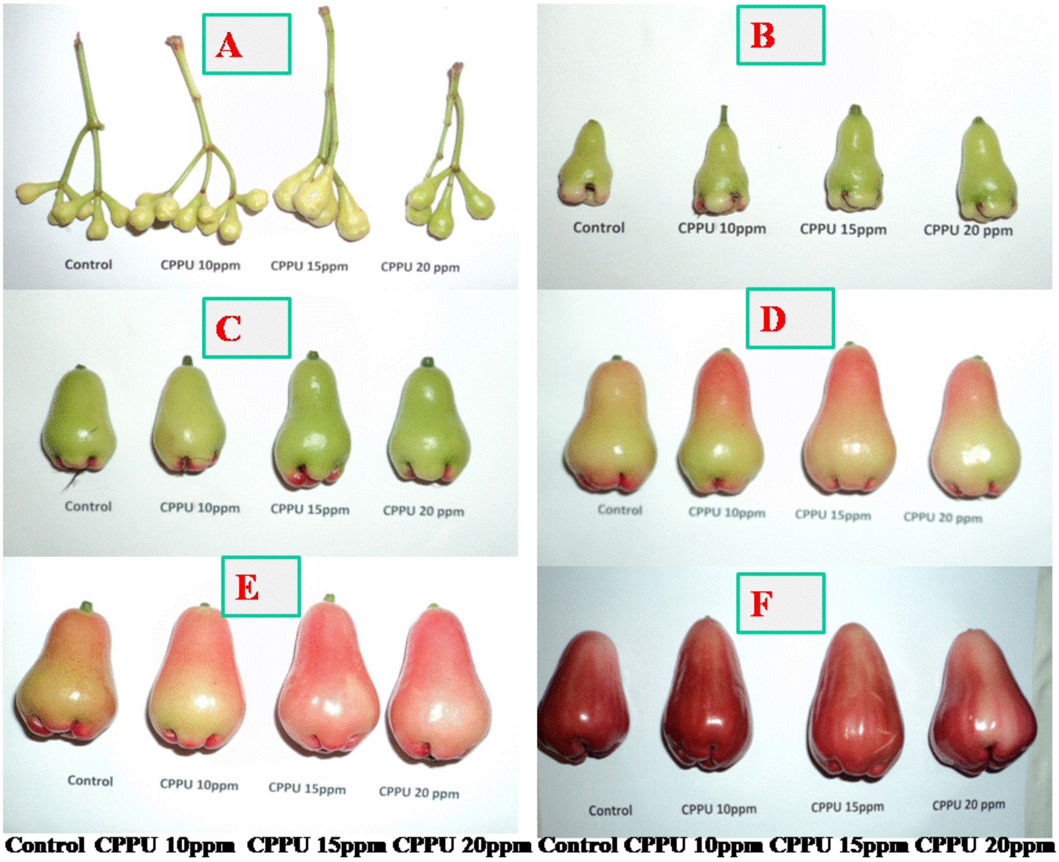

Figure 7 - Photgraph shows the effect of different concentrations of CPPU on water apple fruits, (A): Initial budding**,** (B): Green stage, (C): light Green stage, (D): Light red, (E): Red and (F): Deep red or harvesting stage.

Experiment 2

The chlorophyll content was higher in 12ppm NAA treated leaves than other concentrations of NAA (Table 2). The results showed that 12ppm NAA had greatly influenced the treated fruits by increasing fruit color .Potassium content was higher in NAA treated fruits than in control and potassium content was reduced by high concentration of NAA (Table 2). Total flavonoid content of mature fruits was measured at the end of the experiment, where the content was greatly higher in treated fruits than in untreated fruit (Figure 8). The maximal total flavonoid content was obtained in 12ppm NAA treated fruits (Figure 8). In addition, inverted sugar and fructose content were improved by all NAA concentrations (Figure 9 and 10). Highest inverted sugar was recorded by 12ppm NAA concentration. 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 (Figure 9 and 10).

Table 2 - Effects of different concentration of NAA on wax apple size. Different alphabets mark significant differences, P < 0.05 by LSD.

Treatments	Chlorophyll content (SPAD)	Color % (Pigment)	K+ content (mg/g)
Control	59.9±0.12c	91.5±0.16d	15.1±0.15d
NAA 6ppm	67.5±0.10b	94.4±0.17c	27.5±0.14c
NAA 12ppm	70.8±0.14a	97.8±0.15a	36.2±0.16a
NAA 18ppm	63.1±0.13bc	95.5±0.18b	32.4±0.17b

Furthermore, the remarkable effect by different NAA concentrations on fruit antioxidant what was assessed by measuring anthocyanin content. It was observed that the anthocyanin content was higher in 12ppm NAA treated fruits than other concentrations and control fruit (Figure 11). Regarding the effects of NAA treatment on total phenol content, the control showed significantly difference from all other treatments. Meanwhile, 12ppm NAA treatment showed the highest total phenol content which was significantly different from other treatments (Figure 12). Figure 13 showed the different fruit maturity stages as affected by NAA at different concentrations.

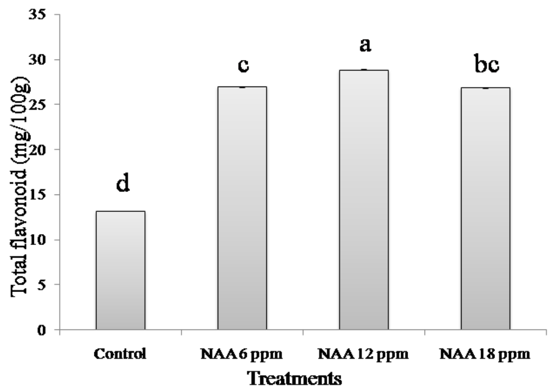

Figure 8 - Total flavonoid content in different treatments of NAA of wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD).

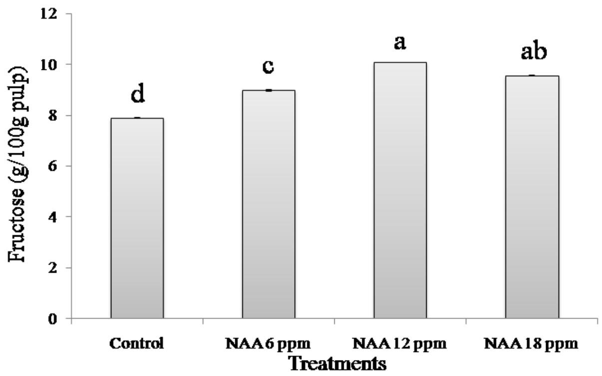

Figure 9 - Effect of different NAA treatments on fructose content (Different alphabets mark significant differences, P < 0.05 by LSD).

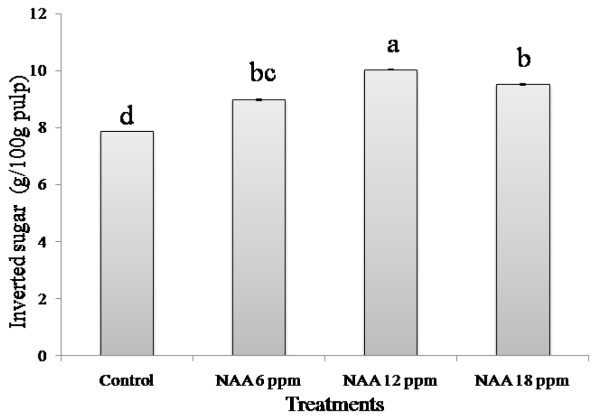

Figure 10 - Effect of different NAA treatments on inverted sugar content (Different alphabets mark significant differences, P < 0.05 by LSD).

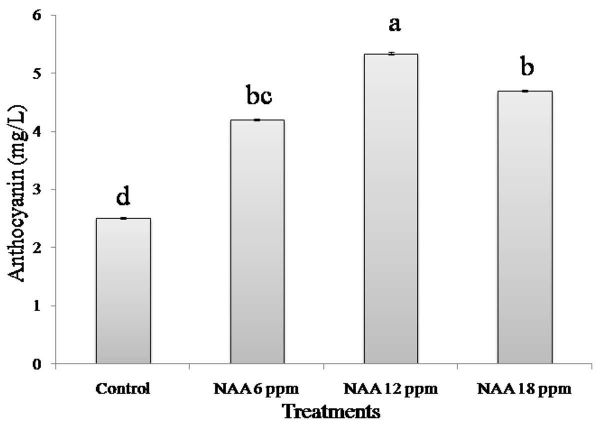

Figure 11 - Effect of different NAA treatments on anthocyanin content (Different alphabets mark significant differences, P < 0.05 by LSD).

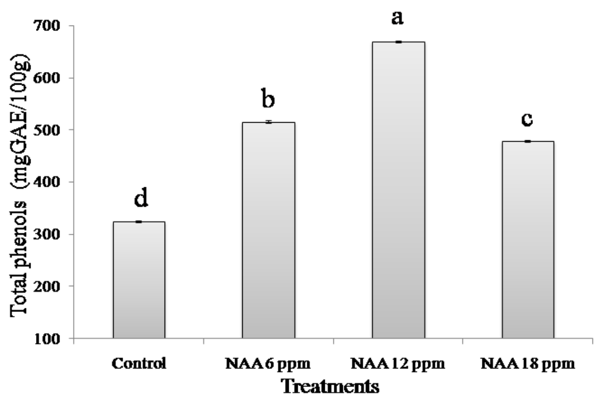

Figure 12 - Total phenols content as affected by different treatments applied to water apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD).

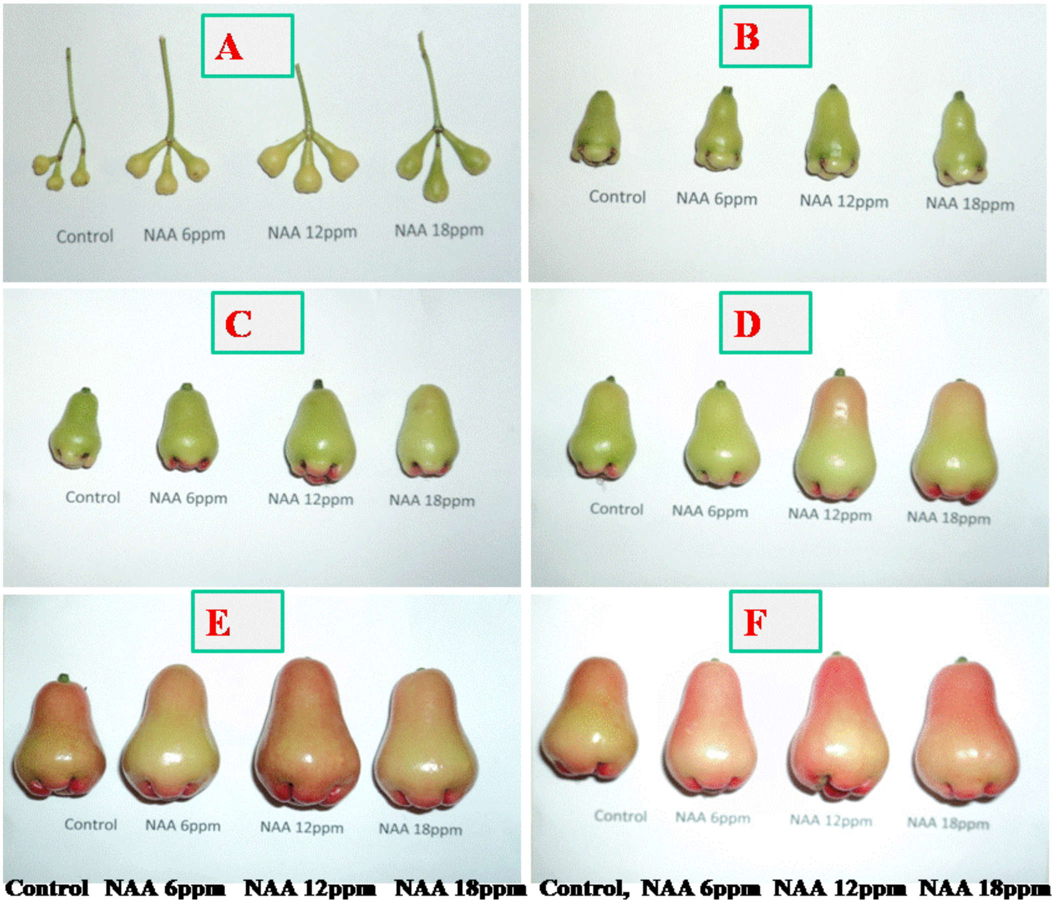

Figure 13 - Photgraph shows the effect of different concentrations of NAA on water apple bud and fruit setting and development. (A): Initial budding**,** (B): Green stage, (C): light Green stage, (D): Light red, (E): Red and (F): Deep red or harvesting stage.

Discussion

Experiment 1

It can be discussed that application of 15ppm CPPU resulted in significantly higher fruit length than control. It was observed that growth of CPPU 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) 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.

Photosynthetic pigment (chlorophyll) of treated leaf was substantially increased by CPPU. This enhancement might be resulted to more photosynthesis taken place in treated leaf and fruit enlargement is mostly dependent on the input of excess water, minerals and assimilates from other parts of plants into fruits. According to Johnson et al., (1992), most of the essential substance on which fruit growth depends on the translocation from the leaf and stem in the fruit through the xylem and phloem. Lewis et al., (1996) presented that CPPU applications accelerated fruit ripening showing higher SPAD value of treated leaf than the control leaves in kiwifruit. Results showed that the potassium content was higher in 15ppm CPPU treated fruit whereas control fruit produced the lowest amount of potassium. As mentioned above fruit quality depended on the level of total soluble solids (TSS) contents what could generally improve with increasing fruit maturity and color. Total soluble sugar (TSS) included the sucrose, glucose and fructose as well as many organic acids and soluble substances. In this study, increase of TSS in wax apple possibly due to the hydrolysis of starch to soluble sugars such as glucose, sucrose and fructose (SOLTANI et al., 2010). Kader et al. (2002) stated that fruit consumer preferred the total soluble sugar (TSS) contents in mature stage. The effect of CPPU on total soluble sugar content of fruit has been positively addressed in a number of studies (LEWIS et al., 1996; ANTOGNOZZI, et al., 1996).

Flavonoids are the most essential plant pigments for fruit coloration (maturity development) in fruits skin and commonly known for its antioxidant activity. Therefore, flavonoids may contribute as fruit quality instead of not only color but also flavor, bitterness or texture (LIN; TANG, 2007). In present study, the composition of flavonoids in different fruit varies greatly due to the different CPPU concentrations. Similar results were described by Winkel-Shirley (2001) and it was refereed that the few enzymes were involved in flavonoid's metabolism and this flavonoid's pathway were regulated by plant hormones. Woolley et al., (1992) found that CPPU stimulated both cell expansion and cell division in fruit tissue. As can be seen, the improvement of the wax apple peel color with maturity was the result of massive accumulation of anthocyanin content. Zhang et al., (2008) described that this was also because of chlorophyll degradation during the maturing period (HOSSAIN; MIZUTANI, 2008). Additionally, CPPU application. The promotion on physical characters as well as the early of fruit maturity could be a result of increasing fructose and inverted sugars.

Experiment 2

In the present study, the application of 12ppm NAA at the onset of fruit setting has been found to be effective in water apple fruit, increasing chlorophyll content. This might cause the 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).

It was observed that the treatments of NAA significantly improved biochemical content of wax apple. The improvement of fruit quality and fruit management could possibly be resulted of enzymatic action of NAA (CHANG; CHEN, 2001). The variable difference in inverted sugar, fructose and maturity was observed among the different concentrations. That was probably due to the various activity levels of different concentrations of NAA (BRENT et al., 1995). NAA increased anthocyanin or antioxidant compount 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). 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. 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). Translocation of carbohydrates in plant cells can be increased in presences of NAA hormone (HAN et al., 1995). 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 (AMIOT et al., 1997). Flavonoids also may contribute to maintain the water apple quality such as its taste, color and bitterness or texture like other fruits.

Anthocyanin content that may appear as a red, purple, or blue tint. They belong to a molecules called *flavonoids* which synthesized via the *phenylpropanoid* pathway (RAGHVENDRA et al., 2011). Anthocyanin found in all tissues of higher plants, including leaves and maximal was found in flowers and fruits. Generally fruits contain many compounds such as anthocyanins, chlorophyll, carotenoids, and flavonols which can be combined together to make its color formation.

Conclusion

Application of CPPU and NAA had generally impact to improve fruit quality in wax apple. It can be concluded that maximum fruits weights, length, biochemical content such as total inverted sugar and fructose and other biochemical content were observed in the 15ppm CPPU and 12 ppm NAA treated fruits. The results suggested that application of CPPU and NAA could increase the fruit biochemical content. The best results with regard to fruit quality of water apple were obtained when CPPU was swabbed at 15ppm and 12 ppm NAA after bud initiation. These findings exhibited the effectiveness of 15ppm CPPU and 12ppm NAA in wax apple and it had a potential effect to develop fruit quality without any depressing features. In addition, an innovative technique swabbing method seems to be better than spray and dipping methods which does not create any droplet and spray drift and not pollutes the Environment.

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