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Fruit growth, TSS and pH content development of water apple as affected by N-2-chloro-4-pyridyl-N-phenylurea (CPPU)

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Abstract

The study was conducted to evaluate the effect of CPPU on the growth and fruit quality development of water apple fruits (*Syzygium samarangense*). Three concentrations of CPPU at 10, 15, 20 mg/l and water control were applied using swabbing technique. The number of buds and fruit setting were increased at 15 mg/l CPPU concentration as compared to the other concentrations (10 and 20mg/l). The premature fruit dropping was decreased. It was observed that the application of CPPU at 15mg/l increased fruit length and diameter. In addition to that fruit growth, maturity development and per fruit weight were higher at CPPU at 15mg/l compared to other concentrations and water control. With regard to fruit quality, the application of CPPU at 15mg/l increased TSS and pH content in the fruit compared to the other concentrations (10 and 20mg/l) and water control. From this study it can be seemed that 15mg/l CPPU was showed better results than other concentrations (10 and 20mg/l) and water control.

Keywords: water apple, CPPU, growth, pH, TSS

Introduction

Water apple is a common fruit in Malaysia as well as other USA and Asian countries. 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 1,500 ha (Tehrani *et al*, 2006) [15]. 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 (Saifuddin *et al*. 2009) [13]. Phytohormones contribute in a large range of phenomena that occur during the growth, and the development of plants (Hossain and Mizutani, 2009) [3].

Spray 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 (Hossain *et al*. 2007) [4]. 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 (Hossain *et al*. 2007) [4]. It was reported that dipping method was used instead of spray and found better effects in fruit (Onguso *et al*, 2006) [11, 12]. Swabbing method as an innovative technique 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) [4] 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 (Saifuddin *et al* 2009) [13]. 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) [5]. The deep-red colored fruits are popular, factors influencing red color has become important for investigators. The red color in wax apples (water apple) is believed to be influenced by several factors such as; leaf: fruit ratio (Wang, 1991) [18], sugars, position of fruits on the tree, fruit development stages, light and temperature (Shu *et al*., 2001).

In addition, it was reported that GA_3 increased fruit firmness, soluble solids and fruit weight (Soltani *et al*. 2010) [14]. Every year a lot of water apple fruit has been dropped in Malaysia. That is also one kind of issue to reduce the dropping fruit. The phenol content of edible fruits are useful since the role of these factors play in health and disease chemoprevention have been widely reported by Hossain *et al*. 2007 [4].

Few scientific information is available and known about the growth and development of water apple. In this project the growth and development as well as the pre and post harvest characteristics of the tree and fruits were investigated. The following objectives were for the studies:

1. To investigate the effectiveness of swabbing method to reduce fruit dropping using NAA.
2. To investigate the various physical & biochemical characteristics of the fruits

Materials and Methods

This study was conducted 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 apple) were selected for the study. The trees were spaced at 20.25 m2. Tree to tree distance was 4.5 m and row to row distance was 4.5 m. 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*., 2007) [4].

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. 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) [4], where aqueous solutions of growth regulator 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 bud number

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 immediately after anthesis. 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
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
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 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
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 (LSD) was calculated. Standard error (SE) was measured.

Results and Discussion
The impacts of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on bud number and dropping of water apple fruits are shown in Table 1. Number of bud has been increased at 15ppm CPPU concentration. In contrast, the maximum bud dropping was observed in 15ppm CPPU concentration and the minimum was observed in control. In Table 2, parameters of fruits growth and development are given (fruit length, fruits setting, fruit dropping and fruit width). The given parameters were investigated with the aim of monitoring the quality of water apple fruit. All concentrations showed better initiation of fruit setting than control in the experimental period. The results showed that fruit length and fruit width were significantly increased by CPPU compared to control. Fruit width was almost similar in 15 and 20ppm CPPU concentrations. The highest fruit length was observed in 15ppm CPPU as compared with control. It was clear that CPPU had a positive effect on fruit development compared to control. The influence of CPPU on fruit yield, weight and fruit volume was observed throughout the experiments. The most effective result was found to increase fruit yield and fruit weight by 15ppm CPPU treatment (Table 3). In the case of fruit volume, higher value was observed in 15ppm CPPU treatment than other treatments. Higher juice volume was also observed in 15ppm CPPU than other treatments. It was observed in Fig. 1 and Fig. 2 that fruit growth (Length and width) per week influenced greatly by different concentrations of CPPU.

One of the important qualities of fresh fruit is attributed with TSS content. The influence of different CPPU concentrations on TSS content of mature fruits was measured at the end of experiment. All CPPU concentrations were able to enhance the TSS content in mature fruit. The highest increament of TSS content was recorded in 15 ppm CPPU treated fruit. A little bit lower TSS content was found in the fruit treated with 20ppm CPPU than 10 and 15ppm CPPU treatments (Fig. 3).

Table 1: Effects of different treatments of CPPU 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	57.33±0.33d	31.0±0.57d
CPPU 10 ppm	60.3±0.33bc	41.6±0.33c
CPPU 15 ppm	63.6±0.33a	45.0±0.57a
CPPU 20 ppm	61.0±0.57b	43.33±0.33b

Table 2: Effects of different concentration of CPPU 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	31.0±0.33d	38.0±0.57ab	59.32±0.25d	34.2±0.03cd
CPPU 10 ppm	43.0±0.33c	36.6 ±0.57c	60.69±0.25c	35.5±0.04c
CPPU 15 ppm	44.0±0.33a	38.0±0.57a	65.01±0.04a	40.2±0.01a
CPPU 20 ppm	42.0±0.57b	35.6±0.33cd	63.76±0.03b	40.0±0.01ab

Table 3: Effects of different treatments of CPPU 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±3.3d	58.7±0.19d	60.0±0.1d	67.2±0.18d
CPPU 10 ppm	489±2.3c	60.9±0.36c	61.7±0.39bc	72.6±1.38c
CPPU 15 ppm	610±2.8a	64.2±0.09a	64.4±0.13a	78.9±0.38a
CPPU 20 ppm	584±7.6b	62.6±0.03b	62.8±0.02b	75.2±0.10b

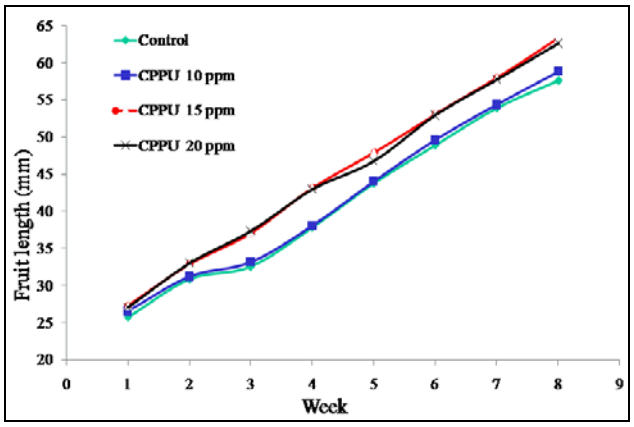

Fig 1: Fruit growth (Length)/week as influenced by different concentration of CPPU

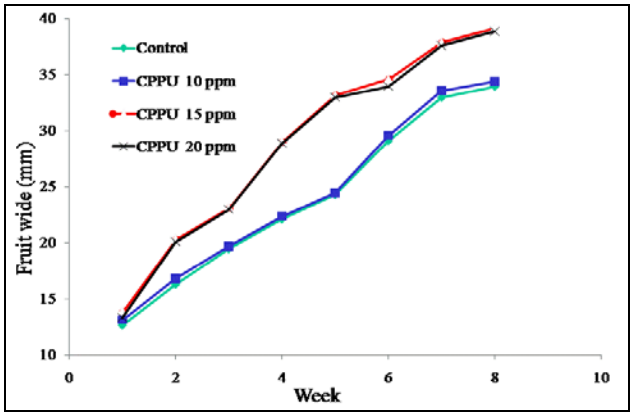

Fig 2: Fruit wide per week as influenced by different concentration of CPPU

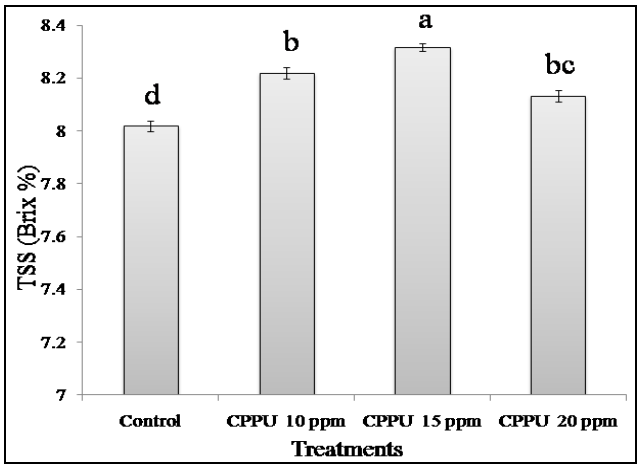

Fig 3: Effect of different treatments of CPPU on Total soluble solids (TSS) content of water apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD).

Fruits acidity level represented by pH value was affected significantly by the application of different concentrations of CPPU (Fig. 4). The highest pH value was observed in 15ppm CPPU concentrations. The 10 and 20ppm CPPU concentrations resulted significant reduction of pH value in fruit compared to other concentrations. Hence, it was observed that 15ppm CPPU was the optimum for water apple fruits to maintain the lowest acidity. Fig. 5 showed the fruit color at different concentration.

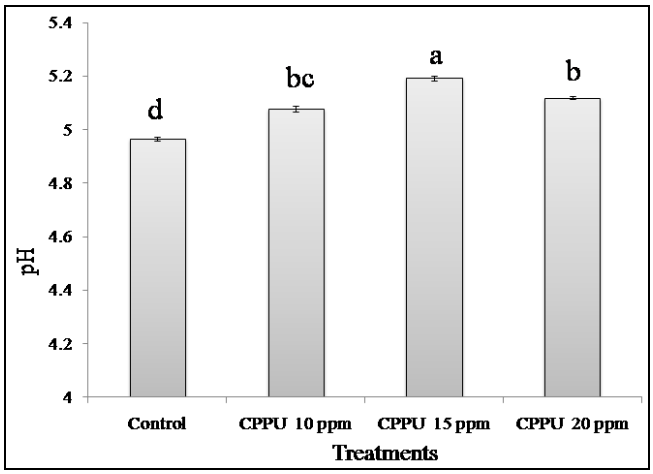

Fig 4: Effect of different treatments of CPPU on acidity or pH content of water apple fruit juice (Different alphabets mark significant differences, P < 0.05 by LSD).

Discussion

In this study, the application of CPPU of different concentrations has been executed to observe the fruit physical quality and biochemical content. Swabbing application of CPPU to the treated branches caused a significant increment of fruit quality.

Assessments of fruit quality are actually based on fruit developmental measurement such as, fruit growth, fruit setting and fruit nutrients. In this present work, the number of fruit per branch was higher in 20ppm CPPU than in control branches. This might be due to the deposition of satisfactory nutrients at treated brunches. Consequently, a sound fruit development was started from the onset of bud initiation and continued until maturity stage. Similar findings have been reported in other fruits by many researchers. Yang-Gyu and Woolley (2006) [17] stated that endogenous hormones and its balance in plants played a vital role in mobilization of produced nutrients into fruits. Table 2 showed that the physical improvement of fruits by different concentration of CPPU application. When fruit size (length and width) was plotted as function of CPPU concentration, the 15ppm CPPU

treated fruit was larger than the control fruit and other CPPU concentrations also increased yield. It was assumed that upgrading of water balance in fruits might enhance the juice content and overall fruit size. Therefore, the dynamic changes of internal fruits water levels during the period of fruit growth have been investigated by assessing the fruit length and diameter or biochemical content. Woolley *et al*. (1992) [16] assumed that application of CPPU might be regulated the plant physiological processes specially growth and development, organ formation and so on through the cell division and increasing cell volume, which attributed to comprehensive effects of others plant hormones. Huang *et al*. (1994) also hypothesized that fruit growth depended on the endogenous of all hormones in stimulating the growth of flesh tissues. Therefore, treatment of CPPU hormones played an imperative part in the growth and development of water apple fruits. The increase of percentage of fruit size, observed in this study and was found an agreement with reports from previous studies (Manabu *et al*., 2008) [9]. The results presented in the present work and supported the previous results (Antognozzi *et al*., 1996) [1] that fruit set and fruit growth in water apple depended on the presence of tolerable levels of endogenous CPPU. Caixi *et al*. (2007) [2] found that the CPPU, a synthetic cytokinin, was effective in enhancing Japanese pear fruit enlargement by stimulating cell division and/or cell expansion and also involved in improving fruit set.

The photosynthetic pigment 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) [6], 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) [7] presented that CPPU applications accelerated fruit ripening showing higher SPAD value of treated leaf than the control leaves in kiwifruit. In addition, fruit juice content, which was related to fruit size, was increased by CPPU application. Therefore, the different treatments produced significant differences in potassium content in fruit juice. Results showed that the potassium content of fruit juice 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. TSS is included the sucrose, glucose and fructose as well as many organic acids and soluble substances. In this study, increase of TSS in fruit possibly due to the hydrolysis of starch to soluble sugars such as glucose, sucrose and fructose (Onguso *et al*. 2006) [11, 12]. Hossain *et al*. (2004) [5] stated that fruit consumer preferred the TSS contents in mature stage. The effect of CPPU on TSS content of fruit has been positively addressed in a number of studies (Hossain *et al*, 2007) [4].

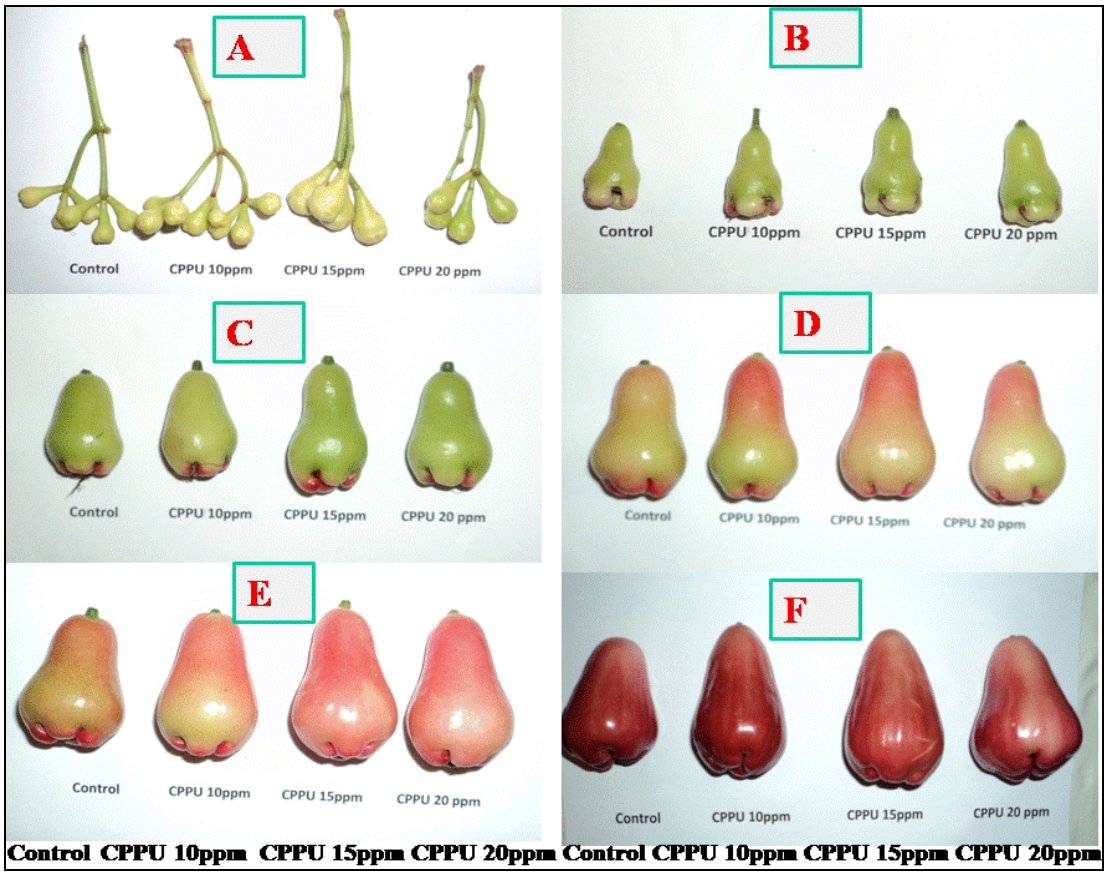

Fig 5: Photgraph shows the effect of different concentrations of NAA on water apple fruits, (A): Initial budding**,** (B): Green stage, (C): light Green stage, (D): Light red, (E): Red and (F): Deep red or harvesting stage.

Conclusion

The water apple growth and biochemical content in fruit were associated with treated conditions. The composition of these compounds may vary greatly on CPPU concentrations. It can be assumed that deep-colored fruits are TSS contents, anthocyanin-rich rich. Maximum fruits weights, length, biochemical content were observed in the 15ppm CPPU treated fruits. The best results with regard to yield and fruit quality of water apple were obtained when CPPU was swabbed at 15ppm after bud initiation. These findings exhibited the effectiveness of CPPU in water apple and it had a potential effect to develop yield and fruit quality without any depressing features.

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References

1. Antognozzi E, Battistelli A, Famiani F, Moscatello S, Stanica F, Tombesi A. Influence of CPPU on carbohydrate accumulation and metabolism in fruits of Actinidia deliciosa (A. Chec.). Sci Hort,1996:65:37-47.
2. Caixi Z, Ugyong L, Kenji T. Hormonal regulation of fruit set, parthenogenesis induction and fruit expansion in Japanese pear. Plant Growth Regulation,2007:55:231-240.
3. Hossain ABMS, Mizutani F, Onguso JM, Ali R Shereif, Yamada H. Inhibiting peach-trees growth with Abscisic acid, hinokitiol, and tropolone applied to partially ringed bark stress. Journal of the Horticultural Science and Biotechnology,2007:82(2):175-178.
4. Hossain ABMS, Mizutani F. Growth regulatory effect of cytokinin in peach trees as affected by ringed bark strip by using bioassay *in vitro*. Canadian Journal of Pure and Applied Sciences,2009:3(1):655-659.
5. Hossain ABMS, Mizutani F, Onguso JM, Yamada H. Effect of summer and winter pruning stress on the growth and fruit quality of peach over four year period. Journal of Applied Horticulture,2005:7(1):11-15.
6. Johnson RW, Dixon MA, Lee DR. Water relations of the tomato during fruit growth Plant, Cell and Environment,1992:15:947-953.
7. Lewis DH, Burge GK, Hopping ME, Jameson PE. Cytokinins and fruit development in the kiwifruit (Actinidia deliciosa): effects of reduced pollination and CPPU application. Physiol Plant,1996:98:187-195.
8. Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry,2007:101:140-147.
9. Manabu W, Hideyuki S, Masanobu M, Satoru S, Sadao K. Effects of Plant Growth Regulators on Fruit Set and Fruit Shape of Parthenocarpic Apple Fruits. J. Japan. Soc. Hort. Sci,2008:77(4):350-357.
10. Mohsen TA. Thinning Time and *Fruit* Spacing Influence on *Maturity*, Yield and fruit quality of peaches. Journal of Horticultural Science and Ornamental Plants,2010:3:79-87.
11. Onguso JM, Mizutani F, Sharif Hossain ABM, El-Shereif AR. Partial ringing stress and liquid nitrogen effects on shoot growth and fruit quality of peach. J. Applied Horticulture,2006:8(91):70-74.
12. Onguso JM, Mizutani F, Hossain ABMS, El-Shereif AR, Rutto KL. Effect of vertical root restriction with corrugated plastic sheets stress on growth and flower bud formation in young peach trees. Bulletin of the Experimental Farm Ehime university,2006:28:1-7.
13. Saifuddin M, Hossain ABMS, Normaniza O, Nasrulhaq Boyce A, Moneruzzaman KM. The effects of naphthaleneacetic acid and gibberellic acid in prolonging bract longevity and delaying discoloration of *Bougainvillea spectabilis*. Biotechnology,2009:8(3):343-350.
14. Soltani M, Alimardani R, Omid M. Prediction of banana quality during ripening stage using capacitance sensing system. Australian Journal of Crop Science,2010:4:443-447.
15. Tehrani M, Chandran S, Sharif Hossain ABM, Nasrulhaq-Boyce A. Postharvest physico-chemical and mechanical changes in *jambu air* (*Syzygium aqueum* Alston) Fruits. AJCS,2011:5(1):32-38.
16. Woolley DJ, Lawes GS, Cruz-Castillo JG. The growth and competitive ability of Actinidia deliciosa 'Hayward' fruit: carbohydrate availability and response to the cytokinin-active compound CPPU. Acta Hort,1992:297:467-475.
17. Yang-Gyu K, Woolley DJ. Effect of plant growth regulators and spear bud scales on growth of Asparagus officinalis spears. Scientia Horticulturae,2006:108:238-242.
18. Zhang W, Li X, Zheng J, Wang G, Sun C, Ferguson I, Chen K. Bioactive components and antioxidant capacity of Chinese bayberry (*Myrica rubra* Sieb. and Zucc.) fruit in relation to fruit maturity and postharvest storage. European Food Research and Technology, 2008:227:1091-1097.