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Growth, yield and quality responses to gibberellic acid (GA₃) of Wax apple *Syzygium samarangense* var. *Jambu air madu* fruits grown under field conditions

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A study was carried out to investigate the effects of gibberellic acid (GA₃) on the growth and development of the red *jambu air madu* fruits (*Syzygium samarangense*). Various horticultural parameters were monitored during two seasons of fruit growth between December, 2008 to December, 2009 with the application of three concentrations of GA₃ at 20, 50 and 100 mg/L. It was observed that the application of GA₃ at 50 mg/L increased fruit length and diameter. Furthermore, it enhanced faster fruit growth and color development in addition to increasing fruit number, weight and yield. It also decreased premature fruit dropping. However, spraying with 20 mg/L GA₃ increased the number of buds and fruit setting and reduced bud dropping before anthesis. With regard to fruit quality, the application of GA₃ at 50 mg/L increased total soluble solids (TSS), total sugar, total biomass and total flavonoids content in the fruits by 112, 97, 45 and 92% compared with the control treatment. In addition, anthocyanin content, total phenol and antioxidant activity was higher in GA₃ treated fruits. From this study, it can be concluded that spraying with 50 mg/L GA₃ once a week results in better yield and quality of *jambu madu* fruits under field conditions.

Key words: Gibberellin, growth, quality, wax apple, yield.

INTRODUCTION

The wax apple or jambu var. *jambu air madu* is a tropical fruit which belongs to the genus *Syzygium* in the family Myrtaceae and is fairly widely cultivated and grown throughout Malaysia, mainly as smallholdings ranging from 1 to 5 ha with its hectare age estimated at 1,500 ha in 2005 (Zen-hong et al., 2006). In Malaysia, there are still some problems with its fruit quality, namely, fruit dropping, small fruit size and less taste, despite the fact that, there is a great scope to developed wax apple fruit industry and possible earn huge amount of foreign capital by exporting to the other countries.

Fruits are pear shaped, often juicy, with a subtle sweet

taste and aromatic flavor. In Malaysia, the fruits of jambu air are eaten raw with salt or cooked as a sauce. Ninety per cent or more of the fruit is edible. The composition of wax apple per 100 g edible portion is: Water more

than 90%, protein 0.7 g, fat 0.2 g, carbohydrates 4.5 g, fibre 1.9 g, vitamin A 253 IU, vitamin B1 and B2 traces, vitamin C 8 mg, energy value 80 kJ/100 g (Wills et al., 1986). The fruit pulp of pink cultivar of *Syzygium* samarangense is a rich source of phenolics content, flavonoids and several antioxidant compounds (Shu et al., 2007). They also reported that edible fruits of *S.* samarangense represent potential benefits for human health because they are rich source of polyphenolic antioxidants.

It has been well documented that the size and quality of the fruits can be affected by certain horticultural cultural practices, such as the application of plant growth hormones (Guardiola, 1992). Gibberellic acid (GA₃) has been shown to increase fruit set and growth in clementine orange (Van Rensburg et al., 1996). Choi et al. (2002) reported that, spraying GA₃ increased the fruit size and

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Abbreviations: GA₃, Gibberellic acid; **DPPH**, 2, 2-diphenyl-1picryhydrazyl; **ABTS**, 2,2'-azino-bis(3-ethylbenzthiazoline-6sulphonic acid); **2,4-D**, 2,4-dichlorophenoxyacetic acid.

firmness in cherry fruits. In addition to this, EI-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). Although some references are available in literatures and effort have been made to control bud and fruit drop, stimulate fruit growth and development lastly increased the yield and quality improvement but there is no precise recommendation for the control dropping, enhanced growth and quality improvement in wax apple.

The objective of this study is to investigate the effects of spraying GA₃ on the growth, yield, development quality of *jambu air* fruits to further improve its potential as an agriculture produce.

MATERIALS AND METHODS

Experimental site

The study was carried out in an orchard located at the MARDI station orchard in Jalan Kebun, Klang and Banting, Selangor, Malaysia, 2°30N, 112°30E and 1°28 N, 111°20E at an elevation of about 45 m above sea level. Both areas under study has a hot and humid tropical climate. The soil in the orchards is peat with a mean pH of around 4.6 (Ismail et al., 1994). Experiments were conducted between December, 2008 to December, 2009. The first season of experiments were carried out from December, 2008 to May, 2009 and second seasons from August to December 2009.

Treatment application

Twelve years old wax apple were selected for the study. The trees were planted in a 14.5×14.5 ft hexagonal pattern and received the same horticultural management. wax apple cultivars, Jambu air madu, were used in the study. Four trees were used in the experiments for each season. Twenty four uniform branches (six branches per tree) of about the same length and diameter, and approximately the same number of leaves from four trees were selected for the experiments. The experiments consist of 4 treatments including control with six replications and a single uniform branches were sprayed with 20, 50 and 100 mg/L GA₃ and water (control) based on a completely randomized design (CRD) with six replications each once a week at the beginning of flower opening until maturation.

Measurement of physiological parameters

Percentage bud drop was calculated using the following formula:

For the determination of fruit setting percentage from tagged branches on the experimental tree, the following formula was used:

Fruit set (%) = $\frac{\text{Total number of fruitlets}}{\text{Total number of flowers}} \times 100$

Fruit dropping percentage was calculated at 35 days after anthesis

using the following formula:

The chlorophyll and carotene content of leaf and fruit was measured by methods describe by Hendry and Price (1993). Fruit length, fruit diameter and fruit growth was measured with the Vernier caliper. Percentage fruit color cover was estimated qualitatively based on the percentage of red color on the fruit. Average fruit weight and yield was determined by weighing and counting the total number of fruits.

Measurements of biochemical parameters

Total soluble solids (TSS) was evaluated at 25℃ with an Atago 8469 hand refractometer (Atago Co. LTD., Tokyo, Japan) and expressed as °Brix. pH was measured using a microprocessor pH meter (Hanna Instrument). K⁺ content of fruit juice was determined by using a Cardy potassium meter. Total soluble sugar was determined according to the phenol-sulphuric method by Dubois et al. (1956). The total phenolic content of wax apple fruits were determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen et al., 1999). Antioxidant capacity was determined via 2, 2-diphenvl-1picryhydrazyl (DPPH) as described by Tadolini et al. (2000) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay according to Miller and Rice-Evans (1997). Total anthocyanin contents of the hydrophilic extracts were measured by the pHdifferential method described by Rodriguez-Saona et al. (1999).

Statistical analysis

The experimental design was a completely randomized design (CRD) with six replications. Uniform branches of about the same length, diameter and approximately, the same number of leaves were selected for the experimental units. The data obtained from the two seasons were pooled and analyzed using MSTAT statistical software. One way analysis of variance (ANOVA) was applied to evaluate the significant difference in the parameters studied in the different treatments. Least significant f test (p = 0.05).

RESULTS AND DISCUSSION

Number of buds and bud dropping

As shown in Table 1, 50 mg/L GA_3 treated branches produced highest number of buds amounting to about 60 buds per branch. This was followed by 100 and 20 mg/L GA_3 . Control branches showed the lowest number of buds, in the region of 53 buds per branch, although the difference was not statistically significant. Morton (1987) working on oranges reported similar findings.

Initial bud drop is a serious problem in fruit production. Endogenous hormones and their balance play a modulating role in the mobilization of nutrients to the developing organs and can influence the longevity of a bud (Almedia et al., 2004). As shown in Table 1, GA_3 at a lower concentration of 20 mg/L exhibited the lowest bud

Treatment (mg/L)	Number of buds	Bud drop (%)	Fruit set (%)	Fruit drop (%)	Yield (kg)	Average fruit weight (g)
Control	53 ± 3.21 ^a	65 ± 8.66^{a}	27 ± 4.40 ^c	52 ± 6.00^{a}	0.31 ± 0.1 ^d	45 ± 2.17 ^c
GA3 20	55 ± 3.21 ^a	29 ± 4.80 ^a	35 ± 3.84 ^b	32 ± 4.40^{b}	0.49 ± 0.1 ^c	49 ± 2.57 ^b
GA3 50	60 ± 8.66^{a}	35 ± 2.18 ^a	35 ± 3.84 ^b	45 ± 2.89 ^b	1.02 ± 0.2^{a}	63 ± 2.2 ^a
GA3 100	59 ± 4.91 ^a	36 ± 4.58^{a}	69 ± 3.0^{a}	48 ± 6.42^{b}	0.79 ± 0.1 ^b	52 ± 2.97 ^a

Table 1. Effects of different treatments of GA3 on number of buds, bud dropping, fruit setting and pre harvest fruit dropping and yield of wax apple.

Means (\pm S.E) within the same column followed by the same letter, do not differ significantly according to LSD test at α =0.05.

drop, averaging about 29 followed by 100 and 50 mg/L, respectively, in order of least bud dropped. Control branches recorded around 65 bud drop. Almost two times the number of bud dropped in untreated branches compared with the 20 mg/L treated branch, although the difference was not statistically significant.

Fruit setting and fruit dropping

It is well documented in the literature that gibberellic acid is used widely in horticultural crops for improving fruit set (Taylor and Knight, 1986). Data in Table 1 shows that, fruit setting was almost 2.6 times more in 50 mg/L treated branches compared with control branches. All the GA₃ treated branch posted significantly higher fruit setting values compared with the control which recorded about 27% fruits set per branch. Similar findings have been reported in apple, pear and seedless grape; gibberellic acids (GAs) have been shown to increase fruit set and growth (Zabadal and Dittmer, 2000). Recently, Davies and Zalman (2006)reported that. 2.4dichlorophenoxyacetic acid (2,4-D) and GA₃ significantly increased the total number of fruits, the fruit weight per plant by reducing pre-harvest fruit drop in orange. As can be seen in Table 1, control branches showed the highest number of fruit dropped (52%), with the least percentage of fruit dropping observed (32%) in 20 mg/L treated branches followed by 50 and 100 mg/L GA₃ treatments. All the GA₃ treated branches posted significantly higher fruit setting values compared with the control. The results found are in agreement with the findings of Almeida et al. (2004) who observed that, GA₃ significantly reduce the fruit drop in citrus fruits.

Yield

As shown in Table 1, all the GA_3 treated branches in this study yielded higher fruit weight than the untreated control. The yield on a fruit weight basis was almost 5 times higher in the treated branches compared with the control. From the results, it can be seen that 50 mg/L GA_3 treated branch produced the highest yield followed by 100 and 20 mg/L treatment. It was found to be statistically significant between the treatments and control. Sayed et al. (2004) reported that fruit weight, peel thickness and fruit diameter of Valencia oranges were increased due to spraying with GA_3 . Our results were found to be in agreement with that of Saraswathi et al. (2003) who observed that, GA_3 significantly influenced the fruit weight as well as yield in mandarin.

Fruit growth (Length and diameter)

The results showed that all the GA_3 treated branch exhibited higher fruit growth rate from the first week till the 7th week, with regard to fruit length and diameter (Figures 1 and 2). At the 3rd week of observation, fruit length was 4.90 and 5.0 cm in 50 and 100 mg/L GA₃, whereas it was 2.43 cm in control. Singh and Lal (1980) observed that, spraying GA₃ once at full bloom to some Indian cultivars of litchi with 50 and 100 mg/L of GA₃ were effective in improving fruit size

Fruit diameter was 3.26 and 3.06 cm in 50 and 100 mg/L GA₃ treated fruits, whereas it was 1.76 cm in control fruits. This growth trend was observed through the whole fruit developmental period until the harvesting period. From the results, it can be seen that all the treated fruits grew at a faster rate and were larger than the untreated control fruits. Stern and Gazit (2003) also described a similar trend in Yu Her Pau' litchi over two years in Taiwan. They also hypothesize that sprays of GA₃ during stage I of fruit growth would increase fruit and aril weight. At the 3rd and 7th week of observation, fruit growth (length and diameter) was found to be significant between the treatments and control.

Color-development of wax apple

Figure 3 shows that fruit color development was greatly enhanced by the GA_3 treatments used in this study, with the 50 and 100 mg/L GA_3 , treated fruits exhibited the greatest percentage color cover from day 14 till 28. Furthermore, it was observed that on day 14 (after anthesis) the red color of the fruits had already started to

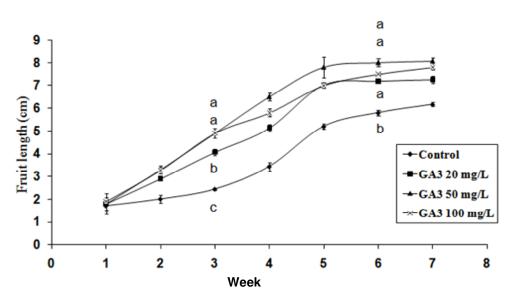


Figure 1. Fruit growth (length)/week as influenced by different concentrations of GA₃ (n= 6).

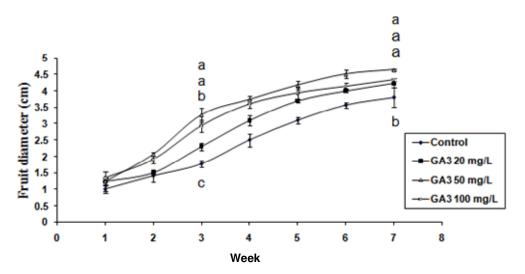


Figure 2. Fruit growth (diameter)/week as affected by different concentrations of GA₃ (n= 6).

show in the treated branches compared with the control fruits, which only started coloring one week later. At the 28th day of observation, the 50 mg/L treated fruits showed more or less 95% red color whereas, control was only 35%. From the figure it can be seen that significant difference was observed in peel color development between different GA_3 treatments and control.

Leaf chlorophyll content

As shown in Table 3, the chlorophyll readings in leaves from all the treated branches was the highest in the 20 mg/L GA₃ treated branch, followed by control and 50 mg/L GA₃, whereas GA₃ 100 mg/L treated branch

showed the lowest value. This suggests that chlorophyll synthesis was enhanced by lower GA_3 concentrations and higher concentration of GA_3 showed a negative effect on chlorophyll synthesis. Our results is supported by the finding of Lim et al. (2003) who reported that mepiquat chloride and GA_3 alone or combined, increased leaf area and chlorophyll content in apple.

Fruit juice and k⁺ content

Fruit juice content, which is related to fruit size is an extremely important parameter in industrial processing of fruits size. Fruit size in turn depends on genetic characteristics and cultural practices such as application

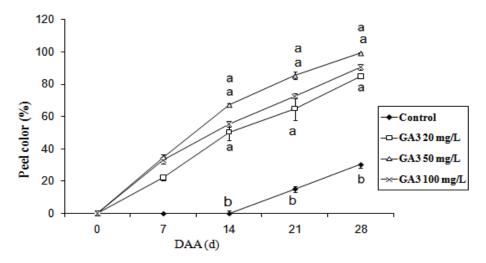


Figure 3. The effect of different treatments of GA_3 on color cover (%) of jambu madu fruits after anthesis. DAA (d): Day after anthesis (n= 6).

Table 2. Effects of different treatments of GA3 on juice con	ent, K+, TSS (°Brix), Total sugar and flavonoids and total phenols
content of wax apple fruit.	

Treatment (mg/L)	Juice MI/100g	K+ content (mg/kg)	TSS (°Brix)	Total sugar (g/100g)	Flavonoids (mg/100g)	Total phenols (mg GAE/100g)
Control	69 ± 0.66 ^b	15.3 ± 1.8 ^b	5.63 ± 0.3 ^b	3.32 ± 0.71 ^b	12.5 ± 0.5 ^c	311±21.64 ^c
GA3 20	80 ± 2.30^{a}	31.3 ± 7.5 ^ª	10.8 ± 0.6^{a}	6.16 ± 0.23 ^a	24.4 ± 2.0^{b}	589 ± 51.38 ^b
GA3 50	81 ± 2.08^{a}	22.0 ± 7.5 ^a	11.9 ± 0.9 ^a	6.57 ± 0.40 ^a	24.0 ± 1.3 ^b	535 ± 32.35 ^b
GA3 100	80 ± 1.15 ^a	28.0 ± 7.2 ^a	10.5 ± 1.3 ^a	6.21 ± 0.02^{a}	36.9 ± 4.7 ^a	752 ± 99.50 ^a

Means (±S.E) within the same column followed by the same letter, do not differ significantly according to LSD test at $\dot{\alpha}$ =0.05,

Table 3. Effects of different treatments of GA3 on chlorophyll, carotenoid and anthocyanin content of wax apple fruit...

Treatment (mg/L)	Chlorophyll a(mg/L)	Chlorophyll b(mg/L)	Chlorophyll a+b(mg/L)	Chlorophylla fruit (mg/L)	Carotenoid (µg/g)	Anthocyanin (mg/L)
Control	3.27 ± 0.36 ^a	2.28 ± 0.14 ^a	5.56 ± 0.51 ^a	0.63 ± 0.06^{a}	5.97 ± 0.24 ^b	1.43±0.07 ^c
GA3 20	3.72 ± 0.62^{a}	2.41 ± 0.27^{a}	6.14 ± 0.89 ^a	0.44 ± 0.02^{b}	10.58 ± 0.36 ^a	4.02±0.13 ^b
GA3 50	3.28 ± 0.29 ^a	2.17 ± 0.06^{a}	5.46 ± 0.34 ^a	$0.24 \pm 0.04^{\circ}$	11.32 ± 0.20^{a}	5.60 ±0.17 ^a
GA3 100	2.84 ± 0.30^{b}	1.92 ± 0.31 ^b	4.77 ± 0.62^{b}	0.26 ± 0.02^{b}	10.35 ± 0.12 ^a	4.60±0.23 ^b

Means (\pm S.E) within the same column followed by the same letter, do not differ significantly according to LSD test at α =0.05.

of plant growth regulators. Table 2 shows that the highest amount of juice (81 ml) was observed in 50 mg/L GA₃ treated fruits followed by 20 and 100 mg/L treatments with a juice percentage of 80 and 80, respectively. These values were statistically significant. The lowest percentage (69 ml) of juice was found in the control treatment. Mathew and Davis (2002) reported that the application of gibberellic acid at flowering and preharvest significantly increased the juice percentage in various citrus species. The different treatments produced significant differences in the case of K⁺ content in treated and non-treated fruits (Table 2). Results showed that the K⁺ content of fruit juice was higher in 20 mg/L treated fruits followed by 100 and 50 mg/L GA₃ treated fruits, whereas control produced the lowest value, however, statistical differences do not exist.

Total soluble solids (TSS)

TSS are considered an important quality parameter of any fruit. It has been reported that, plant growth regula-

tors can change the TSS content of fruits. Significant variations between the fruits of different GA₃ treatments were recorded with respect to TSS content in the fruit pulp. As can be seen in Table 2, highest TSS value(11.88 °Brix) was observed in 50 mg/L GA₃ treated fruit followed by 20 and 100 mg/L GA₃ with a TSS value of 10.76 and 10.50 (Table 2) while, the minimum TSS (5.63) was recorded in control treatment. The TSS (°Brix) results were found to be similar with that of Huang and Huang (2005) who reported that application of growth regulators like auxin and gibberellins can significantly increased the total soluble contents of the fruit in citrus species. Clayton et al. (2006) also reported that GA₃ sprays increased fruit soluble solids in sweet cherry.

Total soluble sugar

It is well known now that plant growth regulators can play a role in increase in the sugar content in fruits. In this study as shown in the Table 2, fruits of different treated branches produced a significant difference in total sugar content. The highest sugar content of 6.56 g was recorded in 50 mg/L GA₃ treated fruits, followed by 100 and 20 mg/L treatments which recorded a sugar content of 6.21 and 6.16 g, respectively, whereas, untreated control fruits showed the lowest sugar content of 3.32 g. These results are similar to the results of Wang et al. (2004) who found that, application of 2,4-D, GA₃ and some other growth regulators increased the sugar contents in various mandarin and sweet orange cultivars.

Total flavonoids and total phenols

These experiment and one on total flavoniod was carried out to try and correlate color development and antioxidant activity with the presence and content of phenols and flavonoids in the fruits. However as reported, the relationships between these compound and the two parameter is not straight forward. Many fruits and vegetables are high and rich in flavonoid content. Flavonoids impart color and taste to flowers and fruits and it is estimated that humans consume between a few hundred milligrams and one gram of flavonoid everyday (Pietta, 2000). From the results, it was observed that fruits of different treatments produced significant differences in flavonoid content. As can be seen from Table 2, 100 mg/L GA₃ treated fruits had the highest (36.95) flavonoids content followed by the 20 and 50 mg/L GA₃ treatments which recorded values of 24.41 and 24.00, respectively, while the control fruits produced the lowest flavonoid content. The application of different concentrations of GA₃ had a significant effect on the total phenolic content of wax apple fruits (Table 2). Fruits from 100 mg/L GA₃ treated branches exhibited the highest amount (752 mg) of phenols followed by 20 and 50 mg/L

treated fruits with phenols content of 589 and 435 mg, respectively. Control fruits showed the lowest (311 mg) phenols content.

Color versus TSS content of fruit and total phenolics versus antioxidant activity

As shown in Figure 4, it was observed that fruit peel color has a strong relationship (R^2 = 0.98) with TSS (°Brix) content of fruit juice. TSS content increased simultaneously with peel color of fruits. From the results with 50 mg/L GA₃ treatment, it was observed that fruit peel color correlated with its TSS content. Similar results were described by Mustafa et al. (1995). They reported that fruit color had positive correlation with TSS in cranberries. Figure 5 shows the relationship between antioxidant capacity and the phenolic contents of *S. samarangense* fruit studied. A high correlation between the total phenolic content and DPPH measurements was observed (R^2 = 0.949).

Chlorophyll, carotenoid and anthocyanin content in the fruit

It is well documented in literature that during ripening, the skin of fruits changes from green to a different brighter color. The most obvious change which take place is the degradation of chlorophyll and is accompanied by synthesis of other pigments usually either the anthocyanin or carotenods. In this study, it was observed that chlorophyll loss gradually took place with the GA₃ application at color turing stage (Table 3). Similar results were reported by Perez et al. (1993). They reported that, the plant growth regulators methyl jasmonate promoted the chlorophyll degradation of the skin golden delicious apple fruit. As can be seen from the results in Table 3, 50 mg/L GA₃ treated fruits showed the highest carotene content followed by 20 and 100 mg/L treated fruits, whereas control fruits showed the lowest value then, carotene content decreased with increasing GA₃ concentration. Anthocyanin pigments are responsible for the red, purple and blue colors of many fruits, vegetables, cereal grains and flowers and as a result, research on anthocyanin pigments has intensified recently because of their possible health benefits as dietary antioxidants (Ronald, 2001). The application of various concentration of GA₃ had a significant effect on the anthocyanin content of wax apple fruits (Table 3). The anthocyanin content of fruits showed a strong correlation with the GA₃ concentrations applied. Results showed that anthocyanin content in fruits treated with up to 50 mg/L treatment was high, but thereafter decreased. The highest amount of anthocyanin was observed in 50 mg/L treated fruits followed by 100 and 20 mg/L treatment, whereas untreated control fruits showed the lowest anthocyanin

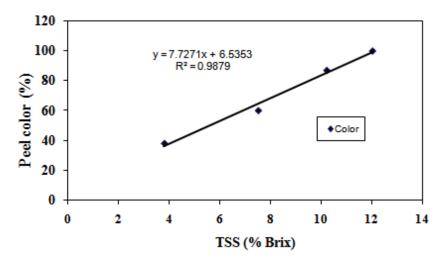


Figure 4. Correlation between peel color and TSS (°Brix) of wax apple fruits affected by 50 mg/L GA₃.

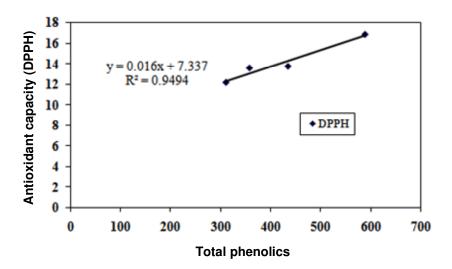


Figure 5. Regression analysis between total phenolics and antioxidant capacity (DPPH) in wax apple fruits.

content. These results concur with the findings of Roussos et al. (2009) who observed that anthocyanin content in strawberry fruit increased significantly when the plants were treated with GA_3 hormone.

Conclusion

From the stated results, it can be concluded that the application of GA_3 had an improving effect on the status of the wax apple tree. GA_3 treatment increased the bud number, stimulated the fruit growth, enhanced color development and finally, increased the yield. Furthermore, GA_3 also reduced bud and fruit dropping and increased the chlorophyll content of leaves. With regard to fruit quality, GA_3 treatment increased amount of

juice, increased the biomass, TSS and total sugar content. Anthocyanin content and antioxidant activity via the DPPH assay was also observed in GA₃ treated fruits. GA₃ treated fruit also showed increased K⁺ and carotenoid content. From the results, it can be concluded that 20 mg/L GA₃ should be recommended before anthesis and 50 mg/L GA₃ should be applied after anthesis for enhancing the growth, development and improving the quality of wax apple under field conditions.

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