THE INFLUENCES OF SHADING, FRUIT THINNING, PLANT GROWTH PROMOTER AND INHIBITOR ON MALAYSIAN WAX APPLE (*SYZYGIUM SAMARANGENSE*) FRUIT DEVELOPMENT AND QUALITY

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INSTITUTE OF GRADUATE STUDIES UNIVERSITY OF MALAYA KUALA LUMPUR

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE DOCTOR OF PHILOSOPHY

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2014

DECLARATION

In the name of Allah, the Most Gracious, the Most Merciful

I hereby declare that all the work in this thesis is the results of my own data, effort, and observation and all references sited have been acknowledged. I also affirm that this thesis has never been submitted for any other degree somewhere else.

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Field of Study: Fruit Physiology

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DEDICATION

This dissertation is dedicated to my beloved mother, wife, and children. Their unconditional love, support, and encouragement throughout this academic research journey have meant everything to me.

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ABSTRACT

Currently, there are limited documented reports on effects of shading and thinning horticultural practice and plant growth regulators (PGRs) on wax apple (Syzygium samarangense) var. 'jambu air madu' growth, development, and quality. PGRs and horticultural practices are important means used in fruit production throughout the world. These means regulate several physiological and biochemical aspects of growth, development, yield and nutritional quality of fruits. In this study, five experiments were conducted to improve the potential of wax apple fruits as follows; applications of gibberellic acid (GA₃) (30, 60 and 90 ppm) applied via xylem injection (an innovative method), application of naphthalene acetic acid (NAA) (30, 60 and 90 ppm) through fruit swabbing method, application of abscisic acid (ABA) (50, 100, 150 ppm) applied through swabbing method at the 4th,5th,6th week of fruit development, fruit thinning regimes at 10 fruits/branch (F/B), 15 F/B, 20 F/B and control treatment (un-thinned fruit), and lastly shading branches treatments at 50%, 70% levels shade and without shading (control). Various physiological and biochemical parameters were monitored during successive seasons of fruit growth between 2010 to 2012 at commercial farm located in Bating, Selangor, Malaysia. The results showed that thinning treatment (at 10 F/B and 15 F/B) enhanced fruit growth, increased fruit length, diameter, and weight. The highest fruit weight was observed in 10F/B treatment followed by 15 F/B treatment whereas the lowest weight was recorded in the control treatment. However, fruit thinning treatments had negative impacts on final yield. The highest yield was obtained in 20 fruits/branch treatment followed by the control treatment and 15 F/B treatments whereas the lowest yield was recorded in 10 F/B treatment. Regarding fruit quality parameters, thinning at 10 F/B and 15 F/B increased slightly fruit juice percentage, pH, total soluble solids (TSS), inverted sugars, fructose, sugar acid ratio (TSS/TA), total flavonoids, total phenol, K⁺ content and anthocyanin content in fruits. On the other hand, thinning treatment at 10 F/B and 15 F/B decreased

vi

titratable acidity (TA) in fruits. In addition, thinning did not significantly affect leaf chlorophyll content (SPAD) value. The effect of shading experiment on wax apple fruit growth and quality showed that; all shading treatments (50% and 70% shade) decreased significantly pH, TSS, inverted sugars, and fructose, sugar acid ratio (TSS/TA), total flavonoids, total phenol, K^+ content and anthocyanin content and increased TA in the fruits. Although, there was no significant difference among all shading treatments in total phenol, the shading treatment had slightly non-significant less phenol content when compared to the control. Shading treatment adversely affected fruit growth, weight, bud drop, fruit drop, and yield. The highest weight was recorded in the control treatment whereas the lowest weight was observed in 70% shading treatment. All shading treatments increased significantly the bud and fruit drop when compared to the control treatment. The lowest branch yield observed in 70% shade treatment followed by 50% shade treatment. In addition, shading reduced slightly (non-significant) the SPAD value. The application of GA₃ via xylem injection enhanced fruit growth, increased fruit length, diameter colour, and weight. GA₃ also increased fruit set and reduced bud and fruit drop. With regard to fruit quality, the application of GA₃ increased fruit juice percentage, pH, TSS, inverted sugars, fructose, TSS/TA ratio, total flavonoids, total phenol, K^+ , and anthocyanin content in the fruits. On the other hand, GA₃ decreased TA in fruits, and did not affect SPAD value. Swabbing with NAA treatment enhanced fruit growth, fruit size, weight, and fruit set. NAA also reduced bud and fruit drop. NAA treatment increased TSS, inverted sugar, fructose, total phenol, total flavonoids, K⁺ and anthocyanin content in fruit, however, NAA treatment did not affect SPAD value. Swabbing fruit with ABA at relatively advance stage of fruit development, increased slightly fruit drop, but did not significantly affect yield, fruit size, and weight. In addition, all ABA treatments enhanced significantly anthocyanin accumulation in fruit. ABA at 150 ppm increased TSS, inverted sugar, fructose, TSS/TA ratios, and pH, and

reduced TA, but did not affect significantly K^+ , total phenols, and total flavonoids content in fruit. ABA treatments did not affect significantly the SPAD value.

In conclusion, GA₃ treatment via xylem injection at 90 ppm concentration, NAA swabbing treatment at 90 ppm concentration, ABA swabbing treatment at 150 ppm concentration, and fruit thinning treatments at 10F/B and 15 F/B levels resulted in better fruit quality parameters of wax apple fruits under field conditions. However, thinning treatment at 10F/B severely lowered the branch yield. Therefore, a good balance between fruit quality and yield should be taken into consideration in wax apple fruit commercial production. All shading treatment (50 and 70%) had negative effect on fruit development and quality. Thus, maintaining a good sunlight exposure for wax apple tree is essential to improve wax apple fruit quality and yield.

ABSTRAK

Tanaman buah-buahan berkait rapat dengan sejarah manusia dan ia adalah satu bahagian penting dalam diet manusia, budaya, perubatan dan amalan kerohanian. Buah buahan didapati menjadi sumber vitamin antioksidan, dan mineral, yang sangat baik. Buah jambu air madu wax epal buah-buahan yang semakin popular di Asia. Buah wax epal kepunyaan genus Syzygium, spesies samarangense, keluarga Myrtaceae. Buah wax epal ditanam secara meluas di seluruh Malaysia pada skala yang kecil, di mana cuaca adalah sangat sesuai untuk pengeluaran sepanjang tahun. Sebagai sektor pertanian di Malaysia yang semakin berkembang sejak beberapa tahun kebelakangan ini, terdapat permintaan yang tinggi untuk menghasilkan buah-buahan yang berkualiti tinggi. Buat masa ini, laporan yg didokumenkan mengenai kesan teduhan dan amalan penipisan hortikultur dan penggalak pertumbuhan tumbuhan pada perkembangan, pertumbuhan dan parameter kualiti. Pengawal selia pertumbuhan tumbuhan dan amalan hortikultur adalah cara terpenting yang digunakan dalam pengeluaran buah-buahan di seluruh dunia. Ini beerti mengawal beberapa fisiologi dan biokimia dari aspek kualiti pertumbuhan perkembangan, hasil dan nutrisi buah-buahan. Oleh itu, lima eksperimen percubaan dalam kajian ini, telah dijalankan untuk meningkatkan potensi buah wax epal seperti berikut; aplikasi asid gibberellic (GA₃) pada 30, 60 dan 90 ppm yang digunakan melalui suntikan xilem (satu kaedah baru yang diperkenalkan), aplikasi naftalena asetik asid (NAA) pada 30, 60 dan 90 ppm melalui kaedah 'fruit swabbing', aplikasi asid absisc (ABA) pada 50, 100, 150 ppm kaedah 'fruit swabbing' pada buah perkembagan ke-4, 5 dan 6, rejim penipisan buah pada 10 buah-cabangan (F / B), 15 F / B, 20 F/B dan kawalan rawatan (un-menipis buah), dan akhir sekali rawatan teduhan cawangan pada aras naungan 50%, 70% dan tanpa naungan (kawalan). Pelbagai parameter fisiologi dan biokimia telah dipantau semasa musim berturut-turut pertumbuhan buah-buahan antara tahun 2010 hingga 2012 pada ladang komersial yang terletak di Banting, Selangor,

Malaysia. Hasil kajian menunjukkan bahawa rawatan penipisan (pada 10 F/B dan 15 F/B) didapati pertumbuhan buah-buahan meningkat, panjang buah, diameter dan berat juga meningkat. Berat buah tertinggi diperhatikan dalam rawatan 10F/B diikuti oleh rawatan 15 F/B manakala berat terendah dicatatkan dalam rawatan kawalan. Rawatan penipisan buah mempunyai kesan negatif ke atas hasil akhir buah wax epal. Hasil tertinggi diperolehi dalam rawatan 20 buah/cabangan diikuti oleh rawatan kawalan dan rawatan 15 buah /cabangan manakala hasil terendah dicatatkan di rawatan 10 buahbuahan/ cabangan. Mengenai parameter kualiti buah-buahan pula, penipisan buah pada 10 F/B dan 15 F/B meningkat kan sedikit peratusan jus, pH, jumlah pepejal larut (TSS), gula terbalik, fruktosa, nisbah asid gula, jumlah flavonoid, jumlah fenol, kandungan K^+ dan kandungan antosianin dalam buah-buahan. Sebaliknya rawatan, penipisan pada 10 F/B dan 15 F/B mengurangkan TA dalam buah-buahan. Di samping itu, penipisan tidak mempengaruhi secara ketara nilai SPAD. Kesan cahaya matahari secara langsung dan percubaan teduhan pada pertumbuhan buah wax epal dan parameter kualiti menunjukkan bahawa semua rawatan naungan (50% dan 70% naungan) menurun dengan ketara pH, jumlah pepejal larut (TSS), gula terbalik, dan nisbah asid gula fruktosa (TSS/TA), jumlah flavonoid, jumlah fenol, kandungan K^+ dan kandungan antosianin dan meningkat kan TA dalam buah-buahan. Walaupun, tiada perbezaan yang ketara di antara teduhan di jumlah fenol, rawatan teduhan mempunyai kandungan fenol yang tidak apabila dibanding kan dengan rawatan kawalan. Rawatan teduhan memberi kesan yang teruk pada pertumbuhan dan, berat buah, penurunan putik, keguguran buahbuahan dan hasil. Berat tertinggi dicatatkan dalam rawatan kawalan manakala berat terendah diperhatikan dalam rawatan naungan 70%. Semua rawatan teduhan meningkat secara sigifnkan bagi keguguran putik dan buah-buahan apabila dibandingkan dengan rawatan kawalan. Hasil cabangan terendah diperhatikan dalam rawatan naungan 70% diikuti oleh rawatan naungan 50%. Di samping itu, mengurangkan sedikit (tidak ketara) nilai SPAD. Rawatan GA₃ melalui suntikan xylem didapati meningkatkan tumbesaran, panjang, garis pusat, warna dan berat buah. GA_3 juga meningkatkan penghasilan buah serta mengurangkan keguguran putik dan buah. Dari perspektif kualiti buah, GA₃ didapati meningkatkan peratusan jus buah, pH, TSS, gula terbalik, fruktos, nisbah TSS/TA, jumlah flavonoids, jumlah phenol, K⁺ dan kandungan anthocyanin dalam buah. Walau bagaimanapun, GA₃ didapati mengurangkan kandungan TA dalam buah tetapi tidak memberi sebarang kesan ke atas kandungan SPAD. Rawatan GA₃, meningkat tumbesaran, saiz, berat dan penghasilan buah juga meningkat. NAA telah mengurangkan keguguran putik dan buah. Rawatan NAA telah meningkatkan TSS, gula terbalik, fruktos, jumlah phenol, K^+ dan kandungan anthocyanin dalam buah tetapi tidak memberi kesan terhadap kandungan SPAD. Rawatan ABA pada tahap yang agak matang dalam perkembangan buah, didapati keguguran buah telah meningkat tetapi tidak ketara menjejaskan hasil penuaian, saiz dan berat buah. Selain itu, penggunaan ABA juga meningkatkan anthocyanin dalam buah. Rawatan ABA pada 150 ppm meningkatkan TSS, gula terbalik, fruktos, nisbah gula asid TSS/TA dan pH, mengurangkan TA tetapi tidak menjejaskan tahap K⁺, jumlah phenol dan jumlah kandungan flavonoids dalam buah. Rawatan ABA juga tidak menjejaskan kandungan SPAD.

Kesimpulannya, penggunaan GA₃ melalui teknik suntikan xylem pada kepekatan 90 ppm, penggunaan NAA pada kadar kepekatan 90 ppm, ABA pada kadar kepekatan 150 ppm dan rawatan penipisan buah-buahan pada tahap 10F/B dan 15 F/B menghasilkan kualiti buah wax epal yang lebih baik bergantung kepada keadaan ladang. Walau bagaimanapun, rawatan penipisan pada tahap 10F/B mengurangkan penghasilan cabang yang sangat teruk Oleh itu, keseimbangan yang baik antara kualiti buah-buahan dan hasil harus diambil kira dalam pengeluaran segara komersial buah wax epal. Semua rawatan teduhan (50% dan 70%) mempunyai kesan negatif terhadap perkembangan

buah-buahan dan parameter kualiti. Oleh itu, mengekalkan pendedahan secara langsung cahaya matahari yang adalah penting untuk meningkatkan kualiti dan hasil, buah pokok wax epal.

TABLE OF CONTENT

ADATION	ii
ECLARATION	11
RIGINAL LITERARY WORK DECLARATION	. 111
EDICATION	iv
CKNOWLEDGEMENTS	. V
BSTRACT	. vi
BSTRAK.	ix
IST OF TABLES	xix
IST OF FIGURES	xxi
BBREVIATION	xxiv

CHAPTER 1

GENERAL INTRODUCTION AND OBJECTIVES

 1.1.1 Chapter 1: General Introduction and Objectives	2 2 nt 2 1 2
 1.1.2 Chapter 2: Review of Literature. 1.1.3 Chapter 3: The Effect of Shading on Wax Apple Fruit Development and Quality. 1.1.4 Chapter 4: The effect of fruit thinning on wax apple fruit quality and development. 1.1.5 Chapter 5: Effects of Xylem Injection with GA₃ on Wax Apple Fruit Quality. 1.1.6 Chapter 6: Application of ABA to Enhance Wax Apple Fruit Quality. 1.1.7 Chapter 7: The effects of NAA swabbing on Wax Apple Fruit Quality. 1.1.8 Chapter 8: General Discussion and Conclusions. 1.2 GENERAL INTRODUCTION 1.2.1 Fruit Importance. 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	2 nt 2 1 2
 1.1.3 Chapter 3: The Effect of Shading on Wax Apple Fruit Developmed and Quality. 1.1.4 Chapter 4: The effect of fruit thinning on wax apple fruit quality and development. 1.1.5 Chapter 5: Effects of Xylem Injection with GA₃ on Wax Apple Fruit Quality. 1.1.6 Chapter 6: Application of ABA to Enhance Wax Apple Fruit Quality. 1.1.7 Chapter 7: The effects of NAA swabbing on Wax Apple Fruit Quality. 1.8 Chapter 8: General Discussion and Conclusions. 1.2 GENERAL INTRODUCTION 1.2.1 Fruit Importance. 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	nt 2 1 2
 1.1.4 Chapter 4: The effect of fruit thinning on wax apple fruit quality an development. 1.1.5 Chapter 5: Effects of Xylem Injection with GA₃ on Wax Apple Fruit Quality. 1.1.6 Chapter 6: Application of ABA to Enhance Wax Apple Fruit Quality. 1.1.7 Chapter 7: The effects of NAA swabbing on Wax Apple Fruit Quality. 1.1.8 Chapter 8: General Discussion and Conclusions. 1.2 GENERAL INTRODUCTION 1.2.1 Fruit Importance. 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	1 1 2
 1.1.5 Chapter 5: Effects of Xylem Injection with GA₃ on Wax Apple Fruit Quality. 1.1.6 Chapter 6: Application of ABA to Enhance Wax Apple Fruit Quality. 1.1.7 Chapter 7: The effects of NAA swabbing on Wax Apple Fruit Quality. 1.1.8 Chapter 8: General Discussion and Conclusions. 1.2 GENERAL INTRODUCTION 1.2.1 Fruit Importance. 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	-
 1.1.6 Chapter 6: Application of ABA to Enhance Wax Apple Fruit Quality. 1.1.7 Chapter 7: The effects of NAA swabbing on Wax Apple Fruit Quality 1.1.8 Chapter 8: General Discussion and Conclusions. 1.2 GENERAL INTRODUCTION	. 3
 1.1.7 Chapter 7: The effects of NAA swabbing on Wax Apple Fruit Quality 1.1.8 Chapter 8: General Discussion and Conclusions. 1.2 GENERAL INTRODUCTION 1.2.1 Fruit Importance. 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	. 3
 1.1.8 Chapter 8: General Discussion and Conclusions. 1.2 GENERAL INTRODUCTION	3
 1.2 GENERAL INTRODUCTION 1.2.1 Fruit Importance. 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	. 3
 1.2.1 Fruit Importance. 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	4
 1.2.2 Classification of wax apple. 1.2.3 Botanical Description. 1.1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	. 4
 1.2.3 Botanical Description. 1.1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	5
 1.1.4 Wax Apple Respiration Pattern. 1.2.5 The Chemical Compositions of Wax Apple. 1.2.6 Optimal Growing Conditions of Wax Apple. 	7
1.2.5 The Chemical Compositions of Wax Apple1.2.6 Optimal Growing Conditions of Wax Apple	8
1.2.6 Optimal Growing Conditions of Wax Apple	. 8
	. 8
1.2./ Soil	. 10
1.2.8 Therapeutic Uses of Wax Apple	. 10
1.2.9 Economic Values	. 11
1.3 OBJECTIVES	12
1.3.1 Research Gap	12
1.3.2 Specific Research Objectives	

CHAPTER 2:

REVIEW OF LITERATURE

Fruit Quality	14
Effect of Fruit Shading	16
Effect of Fruit Thinning	21
Plant Growth Regulators	24
Effects of Abscisic Acid (ABA)	25
Effect of Gibberellic Acid (GA ₃)	31
Effects of Naphthaleneacetic Acid (NAA)	38
	Fruit Quality Effect of Fruit Shading Effect of Fruit Thinning Plant Growth Regulators Effects of Abscisic Acid (ABA) Effect of Gibberellic Acid (GA ₃) Effects of Naphthaleneacetic Acid (NAA)

CHAPTI	ER 3:	
THE EF	FECT OF SHADING ON WAX APPLE FRUIT DEVELOPMENT	
AND QU	JALITY	
3.1	INTRODUCTION	. 44
3.2	MATERIALS AND METHODS	. 45
3.2.1	Experimental site	. 45
3.2.2	Applications	.45
3.2.3	Total Anthocyanin Content.	46
3.2.4	The Total Phenolic Content (TPC).	.47
3.2.5	Total flavonoid content (TFC).	.47
3.2.6	Fructose and Inverted Sugar	.48
327	Total Soluble Solid (TSS) Content	48
328	Titrable Acidity and TSS/TA Ratio	48
329	nH Measurement	48
3 2 10	Bud Dronning (%)	<u>49</u>
3 2 11	Fruit Setting (%)	49
3 2 1 2	Fruit Dropping (%)	. 19
3 2 1 3	The Chlorophyll Content (SPAD value) 50
3.2.13 3.2.14	Fruit Size and Development	.50
3.2.14	Fruit Weight	50
3.2.13 2 2 15	Fiult Weight	50
3.2.13	Fiult Ylela	.30
3.2.10	Fiuld Juice	. 30
3.2.17	Measurement of The K Content in Fruit Juice	. 51
3.2.18	Statistical Analysis.	.31
3.3	RESULTS AND DISCUSSION	52
3.3.1	Anthocyanin Content.	. 52
3.3.2	Total Phenois Content (TPC)	.52
3.3.3	Measurement of pH	. 53
3.3.4	Total Flavonoid Content (TFC)	. 53
3.3.5	K' Content	. 54
3.3.6	Total Soluble Solids (TSS)	. 54
3.3.7	Titratable Acidity (TA) and TSS/TA Ratio	. 55
3.3.8	Inverted sugar	. 55
3.3.9	Fruit Fructose content	. 56
3.3.10	Chlorophyll Content (SPAD)	.56
3.311	Fruit Juice	. 57
3.3.12	Fruit Length	. 57
3.3.13	Fruit Width	58
3.3.14	Bud Drop (%)	.59
3.3.15	Fruit Set (%)	. 60
3.3.16	Fruit Drop (%)	. 60
3.3.17	Fruit Weight (g)	. 61
3.3.18	Branch Yield	.61
3.4	CONCLUSION	63
CHAPTI	E R 4:	
THE E	FFECT OF FRUIT THINNING ON WAX APPLE FRUIT	Г
DEVELO	OPMENT AND QUALITY	

4.1INTRODUCTION.694.2MATERIALS AND METHODS.714.2.1Experimental Site.714.2.2Applications.71

4.2.3	Total Anthocyanin Content	
4.2.4	The Total Phenolic Content (TPC)	71
4.2.5	Total Flavonoid Content (TFC)	71
4.2.6	pH Measurement	
4.2.7	Measurement of the K ⁺ Content in Fruit Juice	72
4.2.8	Total Soluble Solids (TSS) content	
4.2.9	Titrable Acidity and TSS/TA Ratio	72
4.2.10	Fructose and Inverted Sugar	72
4.2.11	The Chlorophyll Content (SPAD value)	
4.2.12	Fruit Juice	
4.2.13	Fruit Length and Width	72
4.2.14	Fruit Weight	
4.2.15	Branch Yield	
4.2.16	Statistical Analysis	
4.3	RESULTS AND DISCUSSION	
4.3.1	Anthocyanin Content	73
4.3.2	The Total Phenolic Content (TPC)	
4.3.3	Total Flavonoid Content (TFC)	74
4.3.4	Measurement of pH.	74
4.3.5	K ⁺ Content	75
4.3.6	Total soluble solids (TSS)	
4.3.7	Titratable acidity (TA) and TSS/TA ratio	
4.3.8	Inverted	76
	Sugar	
4.3.9	Fruit Fructose Content	
4.3.10	Chlorophyll Content (SPAD)	
4.312	Fruit Juice	
4.3.13	Fruit Length	
4.3.14	Fruit Width	
4.3.15	Fruit Weight	79
4.3.16	Branch Yield	81
4.4	CONCLUSION	83

CHAPTER 5:

EFFECT	'S OF XYLEM INJECTION WITH GA3 ON WAX APPLE FRU	IT
QUALIT	Y	
5.1	INTRODUCTION	89
5.2	MATERIALS AND METHODS	92
5.2.1	Experimental Site	92
5.2.2	Treatment Methods	92
5.2.3	Total Anthocyanin Content	93
5.2.4	The Total Phenolic Content (TPC)	93
5.2.5	Total flavonoid content (TFC)	93
5.2.6	Fructose and Inverted Sugar	93
5.2.7	Total Soluble Solid (TSS) Content	93
5.2.8	pH Measurement	93
5.2.9	Titrable Acidity and TSS/TA	94
	Ratio	
5.2.10	Bud Dropping (%)	94
5.2.11	Fruit Setting (%)	94
5.2.12	Fruit Dropping (%)	94
5.2.13	The chlorophyll Content (SPAD value)	94

5.2.14	Fruit Size and Development	
5.2.15	Fruit Weight	94
5.2.16	Fruit Yield.	
5.2.17	Fruit Juice (%)	
5.2.18	Measurement of the K ⁺ Content in Fruit Juice	
5.2.19	Statistical Analysis	
5.3	RESULTS AND DISCUSSION	
5.3.1	Anthocyanin Content	95
5.3.2	Total Phenols Content (TPC)	
5.3.3	Measurement of pH.	
5.3.4	Total Flavonoid Content (TFC)	
5.3.5	K ⁺ Content	
5.3.6	Total Soluble Solids (TSS)	
5.3.7	Titratable Acidity (TA) and TSS/TA Ratio.	
5.3.8	Inverted Sugar	99
5.3.9	Fruit Fructose Content	
5.3.10	Chlorophyll Content (SPAD)	100
5.3.11	Fruit Weight	
5.312	Fruit Juice	
5.3.13	Fruit Length	
5.3.14	Fruit Width	
5.3.15	Bud Drop	105
5.3.16	Fruit Set (%)	106
5.3.17	Fruit Drop (%)	
5.3.18	Branch Yield.	
5.4	CONCLUSION	108

CHAPTER 6:

APPLICATION OF ABA TO ENHANCE WAX APPLE FRUIT QUALITY		
6.1	INTRODUCTION	116
6.2	MATERIALS AND METHODS	119
6.2.1	Experimental Site	119
6.2.2	Applications	120
6.2.3	Total anthocyanin content	120
6.2.4	The Total Phenolic Content (TPC)	120
6.2.5	Total Flavonoid Content (TFC)	120
6.2.6	Fructose and Inverted Sugar	120
6.2.7	Total Soluble Solids (TSS) content	120
6.2.8	Titrable Acidity and TSS/TA Ratio	120
6.2.9	pH Measurement	120
6.2.10	Measurement of the K ⁺ Content in Fruit juice	121
6.2.11	The Chlorophyll Content (SPAD)	. 121
6.2.12	Fruit Length	121
6.2.13	Fruit Width	. 121
6.2.14	Fruit Weight	121
6.2.15	Fruit Juice	121
6.2.16	Fruit Dropping (%)	121
6.2.17	Fruit Yield	121
6.2.18	Statistical Analysis	121
6.3	RESULTS AND DISCUSSION	. 122
6.3.1	Anthocyanin Content	122

6.3.2	Total Phenols Content (TPC)	
6.3.3	Total Flavonoid Content (TFC)	
6.3.4	Measurement of pH	125
6.3.5	K ⁺ Content	126
6.3.6	Total Soluble Solids (TSS)	
6.3.7	Titratable Acidity (TA) and TSS/TA Ratio	
6.3.8	Inverted Sugar	
6.3.9	Fruit Fructose	
6.3.10	Chlorophyll Content (SPAD)	
6.3.11	Fruit Weight	
6.3.12	Fruit Width and Length	131
6.313	Fruit Juice (%)	
6.3.14	Fruit Drop (%)	
6.3.15	Branch Yield.	
6.4	CONCLUSION	134

CHAPTER 7:

THE EFF	ECTS OF NAA SWAPPING ON WAX APPLE FRUIT QUALIT	Y
7.1	INTRODUCTION.	141
7.2	MATERIALS AND METHODS	144
7.2.1	Experimental Site	144
7.2.2	Treatments	144
7.2.3	Total Anthocyanin Content	145
7.2.4	The total Phenolic Content (TPC)	145
7.2.5	Total flavonoid content (TFC).	145
7.2.6	Fructose and Inverted Sugar.	145
7.2.7	Total soluble Solids (TSS) Content	145
7.2.8	pH Measurement	145
7.2.9	Titratable Acidity and TSS/TA Ratio	145
7.2.10	Bud dropping (%)	145
7.2.11	Fruit Setting (%)	145
7.2.12	Fruit Dropping (%)	145
7.2.13	The Chlorophyll content (SPAD value)	145
7.2.14	Fruit Length and Width.	145
7.2.15	Fruit Weight	145
7.2.16	Fruit Yield	146
7.2.17	Fruit Juice	146
7.2.18	Measurement of The K ⁺ Content in Fruit Juice	146
7.2.19	Statistical Analysis	146
7.3	RESULTS AND DISCUSSION	147
7.3.1	Anthocyanin Content	147
7.3.2	Total phenols Content (TPC)	147
7.3.3	Total Flavonoid Content (TFC)	148
7.3.4	Measurement of pH	148
7.3.5	K ⁺ Content.	149
7.3.6	Total Soluble Solids (TSS)	149
7.3.7	Titratable Acidity (TA) and TSS/TA ratio	150
7.3.8	Inverted Sugar	. 151
7.3.9	Fruit Fructose Content	152
7.3.10	Chlorophyll Content (SPAD)	152
7.3.11	Fruit Weight	153
7.312	Fruit Juice	154

7.3.13	Fruit Length	
7.3.14	Fruit Width	
7.3.15	Bud Drop (%)	
7.3.16	Fruit Set (%)	
7.3.17	Fruit Drop (%)	
7.3.18	Branch Yield.	
7.4	CONCLUSION	

CHAPTER 8:

GENERAL DISCUSSIONS AND CONCLUSIONS

LIST OF TABLES

Table 1.1	Plant profile for <i>Syzygium samarangense</i> (Blume) Merr. and L.M. Perry. (USDA GRIN, 2011)	6
Table 3.1	Shading treatments	46
Table 3.2	Effects of different treatments of shading on anthocyanin, pH, K^+ , flavonoids and total phenols content of wax apple fruit under field condition	64
Table 3.3	Effects of different shading treatments on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, inverted sugar (°Brix) and fructose (°Brix) content of wax apple fruit under field condition.	65
Table 3.4	Effects of different shading treatments on SPDA, fruit weight, fruit juice, fruit length, and fruit width of wax apple fruit under field condition	66
Table 3.5	Effects of different shading treatments on bud drop, fruit set, fruit drop and branch yield of wax apple fruit	67
Table 4.1	Effects of different treatments of thinning on anthocyanin, pH, K+, total flavonoids and total phenols content of wax apple fruit under field condition.	85
Table 4.2	Effects of different treatments of thinning on total soluble Solids (TSS), titratable acidity (TA), TSS/TA ratio, inverted sugar (°Brix) and Fructose (°Brix) content of wax apple fruit under field condition.	86
Table 4.3	Effects of different treatments of thinning on chlorophyll content (SPAD), fruit weight, juice (%), shape (length and width) and branch yield of wax apple fruit	87
Table 5.1	Effects of different treatments of xylem injection with GA3 on anthocyanin, pH, K+, flavonoids and total phenols content of wax apple fruit under field condition	111
Table 5.2	Effects of different treatments of xylem injection with GA3 on titratable acidity (TA), total soluble solids (TSS), TSS/TA ratio, inverted sugar (°Brix) and fructose (°Brix) content of wax apple fruit under field condition.	112
Table 5.3	Effects of different treatments of xylem injection with GA3 on chlorophyll content (SPAD), fruit weight, juice (%), fruit length and fruit width of wax apple fruit under field condition	113

LIST OF TABLES (Cont.)

Table 5.4	Effects of different treatments of xylem injection with GA3 on bud drop, fruit drop, fruit set, and branch yield of wax apple	114
Table 6.1	Effects of different treatments of ABA swabbing on total anthocyanin, total phenols, pH, total flavonoids and K+ content of wax apple fruit.	137
Table 6.2	Effects of ABA treatment on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, inverted sugar and fructose of wax apple under field conditions.	138
Table 6.3	Effects of different treatments ABA on SPAD value, fruit length, fruit width, fruit weight, juice volume, fruit drop and branch yield of wax apple fruit under field condition	139
Table 7.1	Effects of different treatments of NAA on anthocyanin, pH, K+, flavonoids and total phenols content of wax apple fruit under field condition.	164
Table 7.2	Effects of different concentrations of NAA on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, inverted sugar (°Brix) and fructose (°Brix) content of wax apple fruit under field condition	165
Table 7.3	Effects of NAA on chlorophyll content (SPAD) fruit weight, juice (%), fruit length, fruit width of wax apple fruit under field condition.	166
Table 7.4	Effects of NAA on bud drop fruit drop, fruit set, and branch yield of wax apple fruit under field condition	167

LIST OF FIGURES

Figure 1.1	The wax apple Syzygium samarangense red cultivar grown in Malaysia, (a): tree (b): leaves, (c, d): flowers, (e, f): buds, (g, h): fruits	9
Figure 2.1	Abscisic acid (ABA) structure	28
Figure 2.2	Gibberellic acid (GA3) structure	32
Figure 2.3	Photograph illustrating the injection of the chemicals into tree trunk (Chemjet Trading Pty, Ltd, Australia, 2012)	36
Figure 2.4	Photograph illustrating the injection of chemicals into tree trunk (Shaaban, 2009)	37
Figure 2.5	Photograph showing injection of chemicals into tree (Shaaban, 2009)	37
Figure 2.6	Naphthalene acetic acid (NAA)	40
Figure 3.1	Selected branched covered with shade at the field	46
Figure 3.2	Effect of different Shading 50%, 70% shading and exposed fruit (without shading) on wax apple fruit appearance	53
Figure 3.3	Effect of different shading on wax apple leaves chlorophyll content (SPAD)	57
Figure 3.4	Fruit growth (length)/week as influenced by different shading	58
Figure 3.5	Fruit growth (width)/week as influenced by different shading	59
Figure 3.6	Yield (kg) of wax apple fruit as influenced by different. Shading	62
Figure 4.1	Effect of fruit thinning of wax apple on anthocyanin (mg/L)	73
Figure 4.2	Effect of thinning of wax apple; 10 fruits per branch, 15 fruits per branch, 20 fruits per branch and non-thinned as a control on fruit size.	80
Figure 4.3	Effect of fruit thinning of wax apple on fruit weight (g) under field condition	81
Figure 4.4	Effect of fruit thinning of wax apple on yield (kg) under field	0.2
Figure 5.1	Introducing a new method to inject plant growth regulators (GA ₃) into tree branch using small drill and injector.	82 93

LIST OF FIGURES (Cont.)

Figure 5.2	Correlation between TSS and anthocyanin content in GA ₃ treated wax apple	96
Figure 5.3	Effect of different GA3 treatment on wax apple leaves chlorophyll content (SPAD). Bars with different letter are significantly different	101
Figure 5.4	Effects of different GA ₃ injection treatments; 90, 60, 30 ppm GA ₃ and control on fruit size of wax apple fruits	102
Figure 5.5	Average fruit weight at harvest as influence by GA3 applications. Bars with different letter are significantly different	102
Figure 5.6	Fruit growth (length)/week as influenced by different applications of GA ₃ . Lines with same letter are not statically significant	104
Figure 5.6	Fruit growth (width)/week as influenced by different applications of GA ₃ . Lines with same letter are not statically significant.	105
Figure 5.7	The increase percentage of branch yield as influenced by different GA ₃ applications	107
Figure 6.1	Swabbing fruit treatments with ABA	120
Figure 6.2	Effect of ABA swabbing treatments on wax apple fruit colour development at week 6	123
Figure 6.3	Effect of ABA swabbing on anthocyanin content of wax apple under field condition	123
Figure 6.4	Effect of ABA swabbing treatment on total flavonoid content of wax apple under field condition	125
Figure 6.5	Correlation between TSS and anthocyanin (mg/100g) content of ABA treated wax apple fruit	127
Figure 7.1	Effect of different NAA treatment on wax apple leaves chlorophyll content (SPAD).	153
Figure 7.2	Effect of NAA swabbing treatments (at 30, 60, 90 ppm) on wax apple fruit size and colour	154
Figure 7.3	Fruit growth (length)/week as influenced by different application of NAA applications	155
Figure 7.4	Fruit growth (width)/week as influenced by different application of NAA applications	156

Figure 7.5Effect of NAA application on yield (kg) under field condition.....159

ABBREVIATION

А	absorbance
ABA	abscisic acid
ANOVA	analysis of variance
ATS	ammonium thiosulphate
C.V.	coefficient variation
cm	centimetre
CV	cultivar
DAA	days after anthesis
DAFB	days after full bloom
DAH	days after harvest
FB	full bloom
FW	fresh weight
g	gram
GA	gibberellic acid
hr	hour(s)
IAA	indole acetic acid
IBA	indole-3-butyric acid

ABBREVIATION (Cont.)

IPP	isopentenyl pyrophosphate
JA	jasmonic acid
Κ	degree kelvin
K^+	potassium
Kg	kilogramme
L/D	length/diameter
LSD	least significant difference
М	molarity
mg	milligram
mg/L	milligram per litre
min	minute(s)
mL	millilitre
mM	milli molar
mm	millimetre
mM	millimole
mol	moles
n	number of replicate
NAA	napthaleneacetic acid
NaOH	sodium hydroxide
PGR	plant growth regulator
ppm	parts per million
RNA	ribonucleic acid
S	second
SE	standard error of mean
SPSS	statistical procedures for social sciences

ABBREVIATION (Cont.)

suc	sucrose
Т	thymidine
TSS	total soluble solid
V	volume
v/v	volume per volume
w/v	weight per volume
WAFB	weeks after full bloom
μL	microliter
μmol	micromole
%	percent
°C	degree celsius

GENERAL INTRODUCTION AND OBJECTIVES

1.1 OUTLINES OF THE THESIS

1.1.1 Chapter 1: General Introduction and objectives

This chapter introduces the contextual sets and background for this thesis. It includes a background of wax apple fruit botany and scientific classifications, and a general overview of the impact of improving wax apple fruit quality on orchard profitability and consumers demand. This chapter also presents the objectives of this work.

1.1.2 Chapter 2: Review of Literature

This chapter covers the literature review of the topics under this thesis. It emphasizes the impact of several factors contribute to fruit quality and yield. This chapter covers the impact of shading, fruit thinning, plant growth promoter, and inhibitor on fruit's development and quality.

1.1.3 Chapter 3: The Effect of Shading on wax apple fruit development and quality

Light it is very important for fruit development and quality. This chapter focused on the effect of shading on wax apple fruit development and quality by using three levels of fruit shading technique under the experimental field conditions and development (first report on wax apple grown in Malaysia).

1.1.4 Chapter 4: The effect of fruit thinning on wax apple fruit quality and development

This chapter reports the impact of fruit thinning on wax apple fruit quality and development under the field conditions. This chapter highlights the relation between fruit thinning practice and the physical and chemicals quality components of wax apple fruit (first report on wax apple grown in Malaysia).

1.1.5 Chapter 5: Effects of xylem injection with GA₃ on wax apple fruit quality

In the effort of improving fruit quality, this chapter introduces a new technique of injection plant growth regulators into wax apple tree branches via xylem injection. This chapter reports the results of three levels of xylem injection with gibberellic acid (GA₃) on wax apple fruit development and quality parameters under the field's condition.

1.1.6 Chapter 6: Application of ABA to enhance wax apple fruit quality.

Fruit colour is one of the important factors the influence the fruit appearance. Fruit with full colour is more applied to consumer than less colour one. This chapter highlights the effect of applications of abscisic acid on wax apple fruit colour development at advance development stage.

1.1.7 Chapter 7: The effects of NAA swabbing on wax apple fruit quality

Fruit size is one of the restrictive factors in fruit marketing of fruit. This chapter reports the effect of different concentrations of Naphthalene Acetic Acid (NAA) on wax apple fruit size and other fruit quality parameters. The method used to apply NAA was swabbing the fruit.

1.1.8 **Chapter 8: General Discussion and Conclusions**

The last chapter focused on general discussion and conclusion points of this multi tasks work.

1.2 GENERAL INTRODUCTION

1.2.1 Fruit Importance

Fruit cultivation is closely linked to human history and it is an important part of human diet, culture, medicine, and spiritual practices. Fruit is found to be an excellent source of vitamins antioxidants, and minerals. Fruits are rich source of phenolic compounds. Martinez-Romero et al. (2007) indicated that fruits offer carbohydrates, organic acids, fiber, vitamins, lipids, and minerals (nutritional properties), as well as antioxidant compounds with health-benefits (functional properties). Phenolic compounds are important for their antioxidant properties, which allow them to acts as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Ronald, 2001). Moreover, Aruoma (2003) detailed that antioxidant compounds block the action of free radicals which have been associated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease and in the aging process. Fruits remain an important source of nutrients in many parts of the world, and offer advantages over dietary supplements because of low cost and wide availability (Kader et al., 2004). Fruit is strongly associated with human health. Thompson et al (1999) indicated that consumption of a diet that significantly increased vegetable and fruit intake from a diverse number of botanical families resulted in significant reductions in markers of oxidative cellular damage to DNA and lipids. High dietary intakes of fruit and vegetables are associated with reduced risks cardiovascular disease (John et al. 2002). Another similar study stated that there is an inverse association of fruit intake with the risk of cardiovascular disease and all-cause mortality in the general US population (Bazzano et al., 2002). Nevertheless, consuming more fruit can reduce the obesity. Tetens and Alinia (2009) mentioned that the potential role of fruit in preventing overweight and obesity is related

to their relatively low energy density, high content of dietary fiber, and associated increasing satiety effect.

As with other nations, Malaysia's population increases gradually and it is anticipated that the demand for local fruits to be increased as well. In this regard, the fruit industry has to be up to the challenge by applying efficient methods to increase the production and improve the quality of the fruit. Hence, there is a great window to develop wax apple fruit industry to earn a great amount of foreign capital by exporting to the other countries.

1.2.2 Classification of wax apple

The wax apple, (or locally called *jambu air*), is botanically identified as *Syzygium samarangense* (Blume) Merr and L. M. Perry (Table 1, USDA NRCS, 2011) (syns. *S. javanicum* Miq; *Eugenia javanica* Lam. in part; *E. alba* Roxb. (Morton, 1987) and it is a non-climacteric tropical fruit (Liao *et al*, 1983). It is origin from the Malay Archipelago (island group) (Nakasone and Paull, 1998). Depending on cultivars (variety), the colour of the fruit is usually pink, light-red, red, green, sometimes greenish-white, or cream-colored (Morton, 1987). In Malaysia there are three main cultivars (cv) of wax apple belongs to the species *samarangense* namely 'Jamba air madu'- red colour cultivar.

Kingdom	Plantae– Plants
Subkingdom	Tracheobionta- Vascular plants
Super division	Spermatophyta- Seed plants
Division	Magnoliophyta-Flowering plants
Class	Magnoliopsida– Dicotyledons
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae– Myrtle family
Genus	Syzygium P. Br. ex Gaertn - syzygium
Species	Syzygium samarangense (Blume) Merr. & L.M.
	Perry- syzygium

Table 1.1 Plant profile for *Syzygium samarangense* (Blume) Merr. and L.M. Perry. (USDA GRIN, 2011)

Common names

Wax apple	English
Semarang rose-apple	English
wax jambu	English
Java-Apfel	German
Cajuil de Surinam	Spanish
Makopa	Spanish
Javaäpple	Swedish

Syzygium is a genus of flowering plants that belongs to the family, Myrtaceae. The genus comprises about 1200 species (Tuiwawa *et al.*, 2013). High levels of variety occur from Malaysia to north eastern Australia, where many species are very poorly known and many more have not been described taxonomically (Morton, 1987). Some of the edible species of *Syzygium spp* are planted throughout the tropics worldwide. In

Malaysia, there are about three species belonging to the genus *Syzygium* which bear edible fruits, namely the water apple (*Syzygium aqua*), Malay apple (*Syzygium malaccense*) and wax apple (*S. samarangense*). The species *samarangense* apparently originated in Malaysia and other South-East Asian countries (Nakasone and Paull, 1998). It is widely cultivated and grown throughout Malaysia and in many neighbouring countries such as Thailand, Indonesia, and Taiwan. Currently in Malaysia, it is cultivated mainly as smallholdings areas ranging from 1 to 5 ha with its hectare estimated 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).

1.2.3 Botanical Description

Wax apple is a tree up to 15 m high, with short and crooked trunk, 25-50 cm diameter, regularly branched near the base (Orwa *et al.*, 2009). Wax apple tree has an open, wide spreading crown, and pinkish-gray, flaking bark (Morton, 1987). Shoot growth proceeds in flushes, which are more or less synchronous, depending on the environment. The juvenile period lasts for 3-7 years. Bearing of clonal trees starts after 3-5 years. Leaves opposite, elliptic to elliptic-oblong, 10-25 cm x 5-12 cm (Orwa et al., 2009). Wax apple commonly flower early or late in the dry season; the flowers appear to be self-compatible and the fruit ripens 40 to 50 days after anthesis Flowers are fragrant, yellowish-white. (Shü et al., 2001). Fruit is a berry, pear shaped, broadly pyriform, crowned by the fleshy calyx with incurved lobes, $3.5-5.5 \times 4.5-5.5$ cm, light red to white; fruit flesh is white spongy, juicy, aromatic, sweet-sour in taste. Seeds 0 to 2, mostly suppressed globose up to 8 mm in diameter (Morton, 1987). The size, shape, and colour of fruit are usually distinct characteristics for different cultivars in the same species (Galan, 1989). Only few cultivars of wax apple are available, which are exotic and perpetuated through vegetative methods of propagation (Morton, 1987). Fruits are pear shaped, often juicy, with a subtle sweet taste and aromatic flavour. In Malaysia, the

fruits of wax apple are eaten raw with salt or cooked as a sauce and about 90% or more of the fruit is edible. The skin of wax apple fruit is very thin and cannot be easily separated from the flesh. The tree fruits all year round (Morton 1987; Wu and Peter, 1994). Shü *et al.* (1998) found that fruit length and width, was found to be in a single sigmoid growth pattern while firmness decreases when fruits mature. Wax apple fruit is usually eaten fresh (Nakasone and Paull, 1998). Fruit is harvested when blossom-end is fully expanded and skin shows desired market colour (Gross *et al.*, 2004).

1.2.4 Wax Apple Respiration Pattern

Morton (1987) indicated that wax apple is a non-climacteric fruit and therefore, will not ripe after harvest. Wax apple has a very low respiration rate of 10 to 20 mg CO_2/kg h at 20°C, though they are highly perishable commodity100 (Liao *et al.*, 1983).

1.2.5 The Chemical Compositions of Wax Apple

The compositions of wax apple per 100 edible portion are: water which is more than 90%, protein 0.7 g, fat 0.2 g, carbohydrates 4.5 g, fiber 1.9, vitamin A 253 IU, vitamin B1 and B2 traces, vitamin C 8 mg, energy value 80 kJ/100 and the major carbohydrate constituents in wax apples juice are fructose, and sucrose (Moneruzzaman *et al.*, 2011).

1.2.6 Optimal Growing Conditions of Wax Apple

Wax apples fruits prefer warm temperatures for normal growth and development. Low temperatures hinder fruit growth and red colour development, while high temperatures accelerate fruit growth and ripening yet inhibit red colour development (Shü *et al.*, 2007). Hsiao (1996) found that wax apple plants grown under 35/25°C and 30/20°C had higher inflorescence percentage (more than 20%) than those under 25/15°C (5.2%). The Wax apple trees are tropical and cannot bear temperatures below 7°C, preferring temperatures is above 18°C (Kuo, 1995; Huang *et al.* 2005). Wax

apple tree growth is flowing cycle pattern. Hsu *et al.* (1996) indicated that growth of tropical evergreen fruit trees occurs in sequences of vigorous vegetative growth followed by phases of low vegetative growth, during which time flowers are often initiated. Therefore, controlling the vigour of shoots is one of the main concerns for sustaining high and steady yields. In addition, the water supply may not always be controllable due to the high occurrence of rainfall in tropical and subtropical regions.



Fig 1.1 The wax apple *Syzygium samarangense* red cultivar grown in Malaysia, (a): tree, (b): leaves, (c, d): flowers, (e, f): buds, (g, h): fruits.
1.2.7 Soil

According to Morton (1987), the soil must be fertile, or the crops will be small and the fruit quality poor. Good soil drainage system is required for normal tree growth and function. However, flooding decreases net carbon dioxide exchange rates, stomatal conductance, evaporation, and maximum quantum yield of PSII (Fv/Fm) (Shü *et al.*, 2007). The decreased physiological parameters improve after the emergence of new roots, which replace the functions of the injured root systems, on the trunk (Huang, 2003).

1.2.8 Therapeutic Uses of Wax Apple

In addition to nutritive value of wax apple, Wax apple leaves, roots, bark, and fruit all have potential medical applications. The juice extract from wax apple was beneficial for Type 2 diabetes (Shen et al., 2012). It has been reported to have antibacterial (Amor et al., 2004), antidiabetic (Nonaka et al., 1992) and immune stimulant compounds (Srivastava et al., 1995). Edema and Alaga (2012) indicated that the juice extracts from wax apple fruit showed significant (P < 0.05) antimicrobial activities against Escherichia coli, Salmonella typhi and Candida albicans, implying that the juices possess both antibacterial and antifungal properties. The leaves and seeds of Syzygium samaragene have antimicrobial activities against some microorganisms like Pseudomonas aeruginosa, Klebsiella pneumonia and Cryptococcus neoformans (Chandrasekaran and Vankatesalu, 2004). The fruit is used to treat high blood pressure and several inflammatory conditions, including sore throat, and can also be used as an antimicrobial, antiscorbutic, carminative, diuretic, and astringent (Khandaker et al., 2011) The flowers are astringent and used in Taiwan to treat fever and halt diarrhoea. Researchers have found their principal to be tannin. They also contain desmethoxymatteucinol, 5-O-methyl-4'-desmethoxymatteucinol, oleanic acid and Bsitosterol. They show weak antibiotic action against Staphylococcus aureus,

Mycobacterium smegmatis, and Candida albicans. (Morton, 1987). Villasenor et al. (1998) reported that the methanol extracts of S. samarangense leaves exhibited high antidiabetic activity.

1.2.9 Economic Values

Supapvanich *et al.* (2011) noted that wax apple has become a popular exotic fruit in western countries because the combination of apple-like crispness, watery sweet and low-acid taste and the aroma of roses. Wax apple is widely cultivated and grown throughout Malaysia and in many neighbouring countries such as Thailand, Indonesia and Taiwan and has become economically important (Srisaard, 2003; Vara-Ubol *et al.*, 2006). Currently in Malaysia, it is cultivated mainly as smallholdings areas ranging from 1 to 5 ha. With its hectarage at 1,500 ha in 2005 (Zen-hong *et al.*, 2006). As other nations, Malaysia population increases gradually and it is anticipated that the demand for local fruits to be increased as well. In this regard, the fruit industry has to be up to the challenge by improving the production efficiency and quality. Hence, there is a great window to develop wax apple fruit industry to earn a great amount of foreign capital by exporting to the other countries. This study aims to improve the potential of wax apple fruits that has increasingly gained more popularity in Malaysia and outside in recent years. Therefore, more research in wax apple will benefit both the local farmers and the Malaysian's agricultural sector.

1.3 OBJECTIVES

1.3.1 Research Gap

In Malaysia, little has been done to study wax apple fruit quality parameters in relation to horticultural practices such as thinning and shading, and plant growth regulators (PGRs). This project was conducted on local Malaysian red famous cultivar of wax apple var. 'Jambu Air Madu'. This study is a pioneer project aimed to develop better understanding of fruit quality parameters in relation to specific horticultural practices (thinning and shading) and plant growth regulators (PGRs) through series of field experiments. Little researches have been done to tackle the important aspects that contribute to fruit quality. This study introduced the first report on the effects of shading and thinning on wax apple fruit grown in Malaysia.

1.3.2 Specific Research Objectives

- 1.3.2.1 To study the effect of shading on wax apple fruit development and quality.
- 1.3.2.2 To improve wax apple fruit quality by fruit thinning treatments.
- 1.3.2.3 To develop larger fruit with better quality by using GA_3 through xylem injection (a new introduced method).
- 1.3.2.4 To study the effect of abscisic acid (ABA) on wax apple fruit colour through swabbing method at advance stage of fruit development.
- 1.3.2.5 To investigate the effect of naphthalene acetic acid (NAA) on wax apple fruit quality through swabbing method.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Fruit Quality

The development of fruit tissue characterizes the final stage of floral development and involves both cell division and cell expansion (O'Neill, 1997). Pharis and King (1985) indicated that fruit development generally is dependent on the interactions of five major classes of plant hormones (auxin, GAs, cytokinin, ABA, and ethylene). Carrari and Fernie (2006) noticed that the development and maturation of fruits has received substantial consideration because of both the distinctiveness of such processes to the biology of plants and the importance of these fruits as a component of the human diet.

Fruit's orchard profitability depends largely on average fruit quality. The components of external and internal fruit quality, i.e. colour, skin finish, fruit taste, size, total soluble solids (TSS) primarily determine whether a superior price is achieved. Fruit quality is the result of a complex interaction of agricultural management and environmental factors (Bertin *et al.*, 2000; Wu *et al.*, 2002). As fruit reach maturity, various physical and chemical changes take place. Generally, titratable acidity, firmness, and starch decline while total sugars increase (Mann and Singh, 1985). The agricultural practices play a significant role in fruit quality. Best prices are gained with big, crispy, thick-fleshed, juicy, sweet and deep red wax apple fruits (Shü *et al.*, 2007). Fruit quality is affected and improved by many factors, such as light, temperature, position on the tree, growing stage, leaf/fruit ratio, supplemental calcium and manganese applications.

The increase in global demand and competition for high quality fruit year after year requires the exploration for new scientific means to improve fruit yield and quality. Farmers as well need to maximize their profit to cope up with the increasing in production and maintenance price. Fruit's orchard profitability depends largely on average fruit quality.

Quality is defined as the degree of excellence or superiority. Fruit quality is generally reliant on the variety, the stage of maturity and on the climatic conditions during the growing period. Fruit quality is categorized into external and internal component. The components of external and internal fruit quality, i.e. colour, skin finish, fruit taste, size, shape, texture, firmness, nutrition, total soluble solids (TSS) primarily determine whether a superior price is achieved. Fruit quality is the result of a complex interaction of agricultural management and environmental Factors (Bertin et al., 2000; Wu et al., 2002, Arpaia, 2004). Tomala (1997) noted that fruit quality is associated with climatic and soil conditions, tree characteristics and cultivation practices. Appearance of fruit is critical parameter for marketing fruits. Kays (1999) indicated that the appearance of fresh fruits is a primary standard in making purchasing decisions. Muñoz-Robredo et al. (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. Product appearance is characterized by size, shape, form, colour, condition and absence of defects. Kader (1994) indicated that high consumer acceptance with high soluble solids concentration (SSC), acidity and SSC/acidity ratio in many commodities.

In addition, there are more factors involved in high consumption of commodities such as phenolic content (Robertson and Meredith, 1989), and volatiles compound in fruit (Romani and Jennings, 1971). Chang *et al.* (2003) stated that sucrose, glucose and fructose are important quality parameters that influence the anthocyanin biosynthesis in wax apple.

Fruit size, appearance, flavour, firmness and storability are of main interest for the fresh market. Accordingly, fruit growers must pay more attention to orchard management practices to achieve market demands in order to produce high quality fruit consistently at maximum yields. However, it is difficult to maximize all quality parameters concurrently, because of the interrelations between management practices and quality parameters. Therefore, a balanced practically compromise between quality and quantity must be achieved. The grower must find the correct point of this compromise, based on management skills, cultivars grown, cultural practices implemented and marketing goals (Link, 2000). The increase in fruit size in many fruit result from cell division, cell growth or a synchronized series of cell division and expansion (Yamaguchi *et al.*, 2004; Zhang *et al.*, 2006).

Therefore, it is vital to understand the principles of how fruit thinning, shading, and applications of plant growth regulators (ABA, NAA, and GA₃) improve quality parameters of wax apple fruit. Hence, the environmental factors including temperature, rain, and light are hard to manipulate under tropical filed condition.

2.2 Effect of Fruit Shading

High-density planting and intensive cultural systems are worldwide accepted practices in the modern fruit production especially where heavy cropping is demanded. As a result, excessive shade within and between trees can easily occur and affect fruit quality (Jackson, 1980).

Direct sunlight is one of the important factors affecting photosynthesis and growth and production and crop quality. However, shading is an agricultural practice often use to reduce direct sun heat reaching plant and plant parts and the practice also implemented as a prevention measure to reduce the sun burning (scald) of plant and plant parts tissue. Hamner *et al.* (1945) in earlier study pointed at the desirable effect of direct sunlight to fruit content and transferring plants from shade to sunshine increased

fruit vitamin C content by up to 66% and the reverse effect was observed when transferring plants from sunshine to shade. Light is one of the main factors responsible for anthocyanin synthesis in apple fruits (Chalmers and Faragher, 1977). Fruit quality is positively associated with photosynthetic photon flux densities (PPFD) within the canopies of several fruit species (Marini et al., 1991). Nishizawa et al. (2000) indicated that sunlight for netted melon plants during fruit maturation is significant not only for sucrose build up but also for the conservation of fruit firmness. Woolf et al. (1999) suggested that exposure of sun fruit to higher temperatures might result in increased water flow to these fruit leading to increased mineral accumulation in sun fruit, and possibly even higher accumulation in the exposed side of sun fruit. In addition, Azuma et al. (2012) indicated that the accumulation of anthocyanin in grape skin was dependent on both low temperature and light and dark treatment severely suppressed anthocyanin accumulation. Many researchers on fruit shading found that sunlightexposed fruits are generally rich in total soluble solids and show reduced titratable acidity, compared to non-exposed or canopy shaded (Koblet, 1984). Sunlight affects fruit colouring and quality. Kliewer and Dokoozlian (2005) indicated that there was a desirable effect of day temperature and light intensity on colouring of *Vitis vinifera* L. grapes. Hole and Scott (1981) pointed that fruit and seed weight per fruit and per plant were reduced by fruit shading in Pisum sativum L. cv. Feltham. In addition, there was a reduction in the total yield of plant. Li et al. (2010) suggested that lighting conditions could indirectly affect the capacity of biosynthesis and recycling of the levels of ascorbate (AsA) in young fruits of kiwi, and this regulation might occur via the interaction of signal from leaves and development of fruit. Crippen and Morrison (1986) found that sun exposure affected the compositional development the phenolic content of Cabernet Sauvignon berries. In generalization of shading on apple fruit quality, storage disorders, flowering and fruit set, Palmer (1997) indicated that shading apple decreased

fruit weight, fruit red colour, soluble solids (Brix), bitter pit, flower bud numbers, and fruit set. In the other hand, shading increase shrivels and fruit firmness in apple. One study indicated that the sun-exposed peel of apple fruit had higher xanthophyll cycledependent thermal dissipation and antioxidants of the ascorbate-glutathione pathway than the shaded apple peel (Ma and Cheng, 2003). Koyama and Goto-Yamamoto (2008) found that the anthocyanin concentrations In 'Cabernet Sauvignon' grapes fruit were significantly reduced when bunches were shaded during ripening, which was associated with the decreased transcription of several anthocyanin biosynthetic genes and transcriptional factors. Seeley et al. (1980) reported that shading reduced apple fruit sugar content, and similar result found by Chen et al. (1998) in 'Cox's Orange Pippin'. (Ma and Cheng, 2003). Another study suggested that sunlight exposure stimulates ripening-associated pigment changes in apple fruit (Solovchenko et al., 2006). Scientific investigation showed that sunlight influences transpiration and calcium accumulation in fruit of kiwi fruit Actinidia deliciosa var. deliciosa (Montanaro et al. 2006). Kiwi fruits grown under weak light intensity need more time to reach the commercial harvest stage with paler colour, smaller size, and poorer sensory characteristics compered to fruits grown under full light (Antognozzi et al., 1995). Awang and Atherton (1995) indicated that shading depressed the fruit dry weight but not fresh weight, resulting in fruits with higher moisture content. Morandi et al. (2011) suggested that the decrease in apple fruit growth rate under shading should be attributed to the reduction of canopy photosynthesis, rather than to a direct effect of shading on fruit sink strength. Yakushiji et al. (1997) indicated that low solar radiation for the primary fruit growing season decreases fruit size and delays fruit coloration of Japanese persimmon (Diospyros kaki L.). A recent study in apple fruit showed that shading during early fruit development reduced fruit growth and initiated fruit abscission in apple (Malus domestica) (Dash et al, 2012). In pineapple, Liu and Liu (2012) found that the shading decreased the fruit quality. Vaast et al. (2006) indicated that shade decreased coffee tree productivity by 18% but reduced alternate bearing. Shade positively affected bean size and composition as well as beverage quality by delaying berry flesh ripening by up to 1 month. Jeong et al. (2004) stated that the accumulation of anthocyanin in grapes fruit was suppressed by shading. Accumulation and compositional changes of flavonols, proanthocyanidins, and anthocyanins were measured in *Vitis vinifera* L. cv. Pinot noir in shaded and exposed treatments and the results showed fruit had a lower concentration of flavonols, anthocyanins, and proanthocyanidins in the shaded treatment (Cortell and Kennedy, 2006). Azuma et al. (2012) reported that shading severely suppressed anthocyanin accumulation. Shading experiments (45% and 90% shade) were carried out on whole vines and individual kiwifruit fruits (Actinidia deliciosa). All shaded vines showed higher fruit drop in comparison to un-shaded ones, even at only 45% shade and with both early and late treatments, leading to reduced productivity. Fruits on shaded vines showed reduced growth. Shortage of light negatively affected flesh firmness and soluble solids concentration. Fruit colour was only slightly affected by shading. Even 90% shade allowed fruits to develop their colour, with only a slight increase of peel paleness and a decrease of flesh colour. Fruit size can be affected by light intensity. In apple fruit cv 'Delicious', Doud and Ferree (1980) found that reducing light by 63% from tight cluster stage through to harvest reduced fruit weight. Sommelier result of the pervious study obtained by Robinson *et al.* (1983), they reported that as the solar exposure level was reduced, fruit weight of apple cv 'Delicious' decreased. In numerous studies, exposure of grape bunches to light significantly increased the accumulation of flavonoids and the expression of their biosynthesis genes, whereas shading reduced them (Cortell and Kennedy 2006, Fujita et al. 2006; Matus et al. 2009). Marini et al. (1991) in peach fruit (*Prunus persica* [L.] Batsch) indicated that shading branches for 20 to 0 or 44 to 0 days before harvest (DBH) altered the relationship between flesh

firmness and ground colour. Firmness declined as ground colour changed from green to yellow for fruit shaded 44 to 20 DBH, but firmness declined with little change in ground colour for fruit shaded 20 to 0 or 44 to 0 DBH. Woolf *et al.* (1999) found that there were significant differences in harvest quality and ripening between 'Hass' avocado fruit exposed to the sun on the tree (sun fruit) and of completely shaded fruit (shade fruit). Sun fruit had higher dry matter, and higher levels of potassium, calcium and magnesium. In addition, Sun fruit took longer to ripen than shade fruit, and the exposed side of sun fruit was firmer than the unexposed side. Demirsoy et *al* (2007) found that in 'Sweet Charlie' strawberry yield was significantly reduced by shading in the flower initiation period treatments.

Shading during the fruiting period reduced inflorescence number and yield. Constant shading (CS) significantly reduced all of the yield parameters. Fruit was the smallest by shading in the flower initiation period. Fruits in CS and in the fruiting period (FP) were the lowest in size. The amount of rejected fruit (deformed, rotten and small fruits) on plants shaded throughout FP was the highest while the least amount of total discarded fruit was from plants under direct sunlight. Woolf et al. (1999) indicated that the exposed side of 'Hass'avocado fruit to sun was lighter in colour, with a higher chroma and lower hue angle (more yellow) than the unexposed side of sun fruit and shade fruit. Moreover, Sun-fruit had higher oil content than shade fruit, with relatively little difference between exposed and shaded sides of sun fruit. Sun exposure increased the saturated fatty acid palmitic acid, and decreased the monounsaturated fatty acid. Dussi et al. (2005) found that canopy shading significantly decreased the amount of photosynthetically active radiation (PAR) available; they also reduced fruit colour, soluble solid content and flesh firmness, and the specific leaf weight (SLW). In addition, fruits at bottom of the canopy were lower in colour and soluble solid content of than that observed in higher canopy fruit. Morrison and Noble (1990) and Smart et

al. (1998) showed that shading decrease the accumulation of phenols in fruits. However, Anttonen *et al.* (2006) in strawberry found that there was no significant deference between control and shaded fruit in phenolic content. Callejón-Ferre *et al* (2009) reported that shading treatment reduced the pH reading in tomato fruit (*Solanum lycopersicum* L).

2.3 Effect of Fruit Thinning

Dennis (2000) noted that fruit thinning has been practiced for thousands of years for many purposes. The main reason behind thinning is that too many fruits per tree can result in small fruit size and poor quality. Fruit size and quality has become as important as total yield in the determination of the profitability of the fruit plantations.

The size of the fruit can be affected by certain horticultural practices, such as thinning. Link (2000) pointed that fruit size was directly related to thinning intensity. In addition to crop load, age of wood, flower bud quality, competition within clusters and canopy were important factors affecting the response to thinning. The amount of fruit left on a tree should be determined by the vigour and general condition of the tree (Williams and Edgerton, 1981).

It is well established that thinning fruit at certain time of fruit development increase fruit size. Zibordi *et al.* (2009) indicated that thinning can be accomplished by hand-removal of fruit, which is expensive, or via the application of phytochemicals that cause fruit drop. Abd El-Razek *et al.* (2010) showed that fruit thinning improves fruit quality on grapes. Ouma (2007) indicated that fruit thinning has become a common management practice to produce apples of high quality including a particular fruit size, coloration, firmness and soluble solids. Other research result showed that strawberry guava fruit thinning had a positive effect on the fruit quality of the natural cycle (Michels and Normand, 2004).

Fruit weight and diameter increase linearly with increasing the severity of thinning. Fruits from trees thinned at 80% bud had significantly higher soluble solids compare with those thinned at 20% and 50% of buds and flowers and in compare with control trees. However, fruits from 80% bud thinned trees had significantly higher soluble solids than control and 50% bud and flower thinning (Davarynejad et al., 2008). Koike *et al.* (2003) reported that there was a 14% increase in sugar levels in 'Fuji' apple fruit from hand-thinned trees compared with un-thinned trees. Yeshitela et al. (2004) found that thinning 'Sensation' mango Mangifera indica fruits to one and two fruits per panicle increased fruit number, weight, and yield per tree at harvest. Thinning 50% of the panicles increased fruit retention size and quality. Vaast et al. (2006) pointed out that at higher fruit loads; there is competition for carbohydrates among berries that strongly affects coffee bean size, biochemical composition and beverage quality. Day and DeJong (1999) indicated that thinning improves fruit size in nectarines, peaches, and plums. Moreover, flower bud thinning in kiwifruit cv. 'Allison' was more effective in improving fruit size and weight as compared with flower and fruitlet thinning. In addition, fruit quality, in terms of total soluble solids (TSS), acidity, total sugars, reducing sugars and ascorbic acid, was better in flower bud thinning treatments (Thakur and Chandel, 2004). Goffinet et al. (1995) pointed to the possible mechanism of thinning and the study indicated that thinning may stimulate fruit growth by influencing cell division (rate or duration), enhancing cell enlargement, producing more or proportionately more intercellular space (IS), or some combination of these processes. The severity of fruit thinning may vary from crop to crop and it is never the same for all fruit. For instance, in kiwifruit cv. 'Allison', thinning to retain six flower buds per fruiting shoot resulted in optimum thinning and maximum production of A-grade fruits of better quality with highest increase in net benefits over the control (Thakur and Chandel, 2004). Basak (2006) indicated that fruitlet thinning is one of the most efficient

and widely used methods of obtaining high quality apples. Smith et al. (1993) indicated that in Pecan fruit *Carya illinoinensis*, fruit thinning improved the kernel percentage, individual nut weight, and kernel grade. Hussein (1993) found that fruit size can be maximised through many practises including thinning of the fruit in 'Samany' dates palm. Bergh (1990) indicated that thinning apple fruit during the first 2 weeks from full bloom (WFFB) significantly increased the cell number in the cortical region of fruits of a similar size. Cell division ceased during the fourth WFFB. Fruit number per tree affected cell number, and consequently harvest size, significantly. Byers et al. (2003) indicated that flower and fruit tinning of apricot used commercially to maximize crop price by optimizing fruit size, colour and increase fruit quality. Burge et al. (1987) stated that reducing fruit numbers to less than 330 per meter of T-bar row increased yield of fruit and fruit size. Naor et al. (2008) pointed that the fruit size can be improved by thinning in apple fruit cv. Golden Delicious. Thinning led to significant increase in the fruit weight, size and flesh percentage. In addition, thinning improved the date palm fruit chemical properties as compared to un-thinning treatment (Mostafa and El Akkad, 2011). Guidoni et al. (2002) found that grape berry Vitis vinifera cv. 'Nebbiolo' weight and berry skin weight slightly increased following cluster thinning. Soluble solids and berry skin anthocyanin and flavonoids were more concentrated in berries from clusterthinned plants. Cluster thinning increased the concentrations of cyanidin-3-glucoside, peonidin-3-glucoside. In grape Vitis vinifera cv 'Sangiovese', Pastore et al. (2011) reported that cluster thinning increased the source/sink ratio from 0.6 to 1.2 m² leaf area per kg of berries and enhanced the sugar and anthocyanin content at harvest. Moreover, anthocyanins accumulated more rapidly in clusters- thinned's berries compared to the control. In addition, they found that cluster thinning introduced a large set of genes not usually expressed in the control treatment. In 'Royal Gala' and 'Braeburn' apple Malus domestica (Borkh.), McArtney et al. (1996) found that thinning could result in a very large improvement in mean fruit weight. Although thinning has a positive effect on fruit size, it decreases the total yield of the tree (Childers *et al.*, 1995; Goffinet *et al.*, 1995; Stover et al., 2001; Guak et al., 2002; Williams and Marini, 2002; Pastore et al., 2011). Westwood (1993) found that fruitlet thinning is an important part of the commercial production of quality apples. In addition, blossom or fruitlet thinning early in the season improved fruit size. Meland (2009) found that thinning in apple tree Malus domestica Borkh.cv 'Elsta' at full bloom (FB) gave a significantly lower final fruit set percentage than thinning at the 20-mm fruitlet stage. However, fruit weights and soluble solids contents (SSC) were significantly higher, and the background fruit colour improved when trees were thinned at FB. In some cases hand or physical thinning, prevent injury to tree leaves caused by applying chemicals thinner. Chemical thinners such as ammonium thiosulphate (ATS) or urea caused phytotoxic effects ranging from leaf yellowing to leaf burn and reduced apple photosynthesis by 6%-33% over 3-7 days (Ouma, 2007). Taghipour et al. (2011) indicated that hand thinning 'Gerdi' apricot fruit increased total soluble solids (TSS), total soluble solids to total acidity ratio (TSS/TA), pit weight and flesh to pit ratio. In 'Mut' apricot Prunus armeniaca growing in central Turkey. Son (2004) found that hand thinning at 30%, 50%, and 70% caused 1-2 days harvest earliness in comparison with control fruits. The biggest fruit were obtained from 70% hand thinning. The soluble solids (TSS) were greatest (13.53%) for 70% hand thinning.

2.4 Plant Growth Regulators

The term 'plant growth regulator' (PGRs) is usually employed for plant hormones or substances of similar effect that are administered to plants. PGRs include a large group of chemical compounds that can regulate plant growth. Hormones are substances naturally produced by plants, substances that control normal plant functions, such as root growth, fruit set and drop, growth and other development processes. There are five plant hormone categories; these are auxins, cytokinins, gibberellins, abscisic acid and ethylene (Wang and Irving, 2011). Hormones are substances naturally produced by plants. Plant growth regulators play multiple roles in the regulation of plant growth and development. Plant growth regulators (PGR) exist almost in all plant parts. Pharis and King (1985) indicated that fruit development generally is dependent on the interactions of five major classes of plant hormones (auxin, GAs, cytokinin, ABA, and ethylene). Atwell *et al.* (1999) stated that (PGRs) play a crucial role in fruit, seeds growth and development. Guardiola (1992) pointed that the application of plant growth hormones affected the size and quality of the fruits. PGRs have been used commercially in fruit production (under regulations) in many countries (Ritenour, 2012).

In This review of literature, we focused on plants growth regulators that have been used in our study; and these are abscisic acid (ABA), gibberellic acid (GA₃) and naphthaleneacetic acid (NAA).

2.4.1 Effects of Abscisic Acid (ABA)

Abscisic Acid (ABA) belongs to a class of metabolites known as isoprenoids, also called terpenoids and the level of abscisic acid (ABA) in any specific tissue in a plant is determined by the rate of biosynthesis and catabolism of the hormone (Nambara and Marion-Poll, 2005) (Fig 2.1).

In plants, ABA is a cleavage product of xanthophylls (especially of violaxanthin). ABA can be metabolized by glycosylation or by oxidation, to produce 8'-hydroxy-ABA that is converted into phaseic acid (Cutler and Krochko, 1999). ABA is a plant hormone plays a major role in various aspects of plant growth, development and adaption of environmental stresses as well as fruit development (Rock and Quatrano, 1995; Seo and Koshiba, 2002). ABA biosynthesis is a key element facilitating the elucidation of plant physiological characteristics (Mitsunori and Koshiba, 2002). A

plant's response to hormones is controlled by both the hormone concentration and the exposure time (Nicole *et al.*, 2010).

There are numerous scientific reports that have pointed out the various effects of ABA applications on fruit quality. Finkelstein et al. (2002) in their reviews, pointed that the inhibitory effects of ABA on growth have been documented as resulting from a combination of limited cell extensibility and inhibited cell division. Brenner (1989) suggested that ABA played a role in the accumulation of assimilates (nutrients) by strengthening sink activity in tissue. Giora (2012) reported that the phytohormone abscisic acid (ABA) affects a wide range of stages of plant growth as well as the plant's response to biotic and abiotic stresses and the management of ABA signalling in commercial crops holds favourable potential for improving crop yields. Zaharah et al. (2012) found that the exogenous application of 1.0 mM ABA accelerated ethylene production, respiration rate, fruit skin colour development and softening as well as rheological properties of pulp (hardness, springiness, cohesiveness, chewiness, adhesiveness and stiffness) compared to the control. It has been reported that treatments of ABA increase the anthocyanin content in grape skins (Ban et al., 2003) and improved the colour and quality of the grapes (Cantín *et al.*, 2007). Spraving 500 mg. liter⁻¹ abscisic acid (ABA) on fruit after 90 days of full bloom (DAFB) enhanced sugar accumulation in 'Hakuho' peach fruit (Kobash et al., 1999). Peppi et al. (2008) found that the application of abscisic acid at veraison to Crimsom seedless increased fruit colour within a week of treatment. Peppi and Fidelibus (2008) indicated that ABA's primary effect is to increase anthocyanin content of 'Flame Seedless' grapes. Yozo et al. (2006) indicated that exogenous abscisic acid (ABA) could induce anthocyanin synthesis and chlorophyll senescence in regenerating torenia shoots on the medium containing a low concentration of sucrose (1.5%). Jeong *et al.* (2004) found that the accumulation of anthocyanins was enhanced by ABA treatment. ABA enhanced the

mRNA accumulation of VvmybA1, a putative regulatory gene of anthocyanin biosynthesis of grape, and all the tested enzyme genes of the anthocyanin biosynthetic pathway. Göktürk and Harmankaya (2005) stated that changes in ABA levels were closely related to the onset of ripening in grape beery. Leung and Giraudat (1998) reported that ABA can promotes seed maturation and germination, and act as a signalling molecule when plants are under stresses such as drought, high salinity, cold and microbial infections. It also considered that ABA plays an important role in fruit ripening, stimulates sugar accumulation in fruits, thus improving fruit and quality (Xia et al., 2000). Jeong et al. (2004) found that the accumulation of anthocyanin in grapes fruit was enhanced by ABA treatment at veraison stage (veraison represents the transition from berry growth to berry ripening). ABA also participates in the initiation of ripening and related changes in grape development (Devtieux-Belleau et al., 2007). Exogenous ABA to mango fruit promoted fruit colour development and softening during ripening compared with the control (Zaharah et al., 2012). Vendrell and Buesa (1989) found that injection apple fruit with 1 mM ABA increased the respiration and ethylene production compared to controls treated with water or not treated, and ripening was advanced. This effect was noticeable in the younger fruits. Setha (2012) reported that ABA concentration is very low in unripe fruit, but it increases as a fruit ripens, so it is therefore assumed that ABA plays an important role for regulating the rate of fruit ripening.



Fig. 2.1. Abscisic Acid (ABA) structure.

Lacampagne et al. (2009) reported that ABA regulates enzymes involved in tannin and flavonoid biosynthesis. Exogenous application of ABA significantly stimulated the biosynthesis of ethylene and hastened the fruit ripening (Chen and Zhang, 2000). Quiroga et al. (2009) study, showed no significant change observed with ABA treatment in number of internodes, shoot length, leaf area, leaf water potential at midday, photosynthesis, and stomatal conductance. Zhang et al. (2008) reported that the development of the red pigmentation with maturity in wax apple colour is the result of accumulation of anthocyanin content and chlorophyll degradation. Yong-ging et al. (2009) stated that the accumulation of endogenous ABA during late period of peach Prunus persica L.'Hakuho' fruit in development triggered peach fruit ripening, and the peak of endogenous ABA content initiated the fruit ripening process. The treatment of exogenous ABA promoted ripening and senescence process of detached peach fruits. Peppi et al. (2006) found that applications of ABA hormone on fruit improved the colour of 'Flame Seedless' and 'Redglobe' grapes. Cantin et al. (2007) showed that ABA-treated grapes were characterized by superior appearance both in berries and clusters' rachises compared to ethephon-treated and control grapes. However, firmness, berry weight, decay incidence, and shatter remained unaffected by the treatments. Smith et al. (1995) reported the positive relationship between ABA concentration and rapid fruit growth in kiwifruit, and they suggested that ABA may have a role in the allocation of assimilates to fruit. In addition, the study showed that changes in water content of

kiwifruit followed closely the stages of fruit growth. Peppi *et al.* (2008) reported that application of ABA at version maximized anthocyanin accumulation, but later applications of ABA also increased pigmentation and, at the same time, reduced the fruit firmness of 'Crimson Seedless' grapes. In kiwifruit, Nakano *et al.* (1997) reported that ABA treatment at 100 or 250 ppm to individual fruit before entering growth stage III, promoted fruit coloration and slightly increased fruit growth. Moreover, the respiration rise, which occurred simultaneously with the onset of growth stage III, was advanced in ABA treated fruit.

ABA was shown to stimulate sugar metabolism and accumulation in fleshy fruits (Richings et al., 2000; Pan et al., 2005). Post-harvest applications of ABA accelerate ripening, and this effect was mediated by endogenous ethylene synthesis and induction of enzymes involved in anthocyanin accumulation (Jiang and Joyce, 2003). Wang et al. (2001) found that the concentrations of ABA in the peel and aril of litchi fruit Litchi chinensis, increased around 62 days after full bloom. This increase was followed by an accumulation of sugars and anthocyanins. These results suggest that ABA play a role in fruit maturation. The study suggested that ABA was more closely associated with fruit maturity than ethylene. Jiang and Joyce (2003) found that ABA treatment enhanced strawberry fruit colour and softening. Treatment with 10^{-5} or 10^{-4} mol ABA l⁻¹ stimulated ethylene production, Anthocyanin and phenolic contents and PAL activity increased during storage, but more rapidly in ABA treated fruit. Moreover, the study suggested that ABA might play a role in strawberry fruit colour development during ripening through up-regulation of ethylene production and PAL activity. Anderson *et al.* (2008) found increase in several phenolic compounds and colour in 'Cabernet Sauvignon' and 'Merlot' grapes following the application of exogenous ABA. These responses were influenced by both timing and concentration of ABA. Application of ABA to 'Cabernet Sauvignon' fruit at veraison stage of fruit development with 200 and 400 ppm ABA resulted in significant increases of several phenolic components including flavon-3-ols and flavones. Application of ABA to 'Merlot' grapes showed mixed results. Cantin et al. (2007) found that ABA application at 150 μ L L⁻¹ permitted grapes to be harvested 10 d before non-treated fruit (control), and fruits treated with 300 μ L L⁻¹ ABA attained marketable quality 30 days before control fruit. Early harvest was possible because the ABA applications induced more rapid colouring of the grapes, and though total yield was not affected by ABA. ABA also doubled packable yields by improving the colour of the grapes. ABA-treated grapes were characterized by superior appearance in both berries and clusters' rachises compared to control grapes. ABA applications did not alter other quality attributes such as firmness, berry weight, decay incidence, and shatter. The study concluded that ABA is an effective alternative to ethephon for enhancing the colour and maintaining postharvest quality of 'Crimson Seedless' grapes. ABA sprayed on to leaves promotes growth in *Ilex paraguariensis* plants (Sansberro et al., 2004). Deluca et al. (2007) and Giribaldi et al. (2007) indicated that ABA biosynthesis involved in ripening berries. Application of ABA on grape clusters not only enhance the anthocyanin content of the skins (Hiratsuka et al. 2001; Peppi et al., 2006), it also promotes earlier colour development compared to untreated grapes (control) (Gagne' et al., 2006). Ban et al. 2003 and Jeong et al. (2004) found that the change in colour development is due to an increase in the expression of UFGT (UDP-glucose: flavonoid-3-O-glycosyltranferase encoding an enzyme specific to the anthocyanin pathway) and VvMYBA1 (encoding a transcriptional regulator controlling anthocyanin biosynthesis) genes.

Currently, there is no report available on the effects of ABA on fruit growth and colour development and ripening behaviour of wax apple under the field conditions.

2.4.2 Effect of Gibberellic Acid (GA₃)

Gibberellic Acid (GA₃) is a plant hormone with an empirical formula of $C_{19}H_{22}O_6$ (Figure 2.2). When purified, it is a white to pale-yellow solid. Davies (1995) and Crozier *et al.* (2000) reported that gibberellins are tetracyclic diterpenoid acids that play a significant role in a number of developmental and physiological processes in plants. It should be emphasized that gibberellins are known to interact with other hormones (Cohen *et al.*, 2001). Looney (1993) Indicated that gibberellins application are used on several crops to improve fruit appearance and internal condition but they also have potential to reduce pesticide usage. In addition , GA is used commonly in commercial crops production for enhancing fruit set and also to control some physiological diseases such as cracking of pomegranate fruit (Sepahi, 1986) and litchi (Sharma and Dhillon, 1986) apple fruit russeting (Taylor and Knight, 1986) and to hinder flowering of *Prunus* species (Coneva and Cline, 2006; Lenahan *et al.*, 2006). Kappel and MacDonald (2002) stated that growers in British Columbia, Canada, and the US Pacific Northwest used gibberellic acid (GA₃) to improve fruit quality of sweet cherries (*Prunus avium* L.).

Many scientific reports pointed that the role of gibberellins in plants include seed germination, seedling emergence, stem and leaf growth, floral induction and flower and fruit growth (Sponsel, 2002; King and Evans 2003). Earlier study on gibberellic (GA₃) showed that it has been used commonly in horticultural crops for enhancing fruit set (Taylor and Knight, 1986). Raven *et al.* (1992) indicated that gibberellins have been found to stimulate plant growth by increasing the extensibility of cell walls and accordingly allowing cell expansion.



Fig. 2.2 Gibberellic acid (GA₃) structure.

In early studies, Looney and Lidster (1980) indicated that the GA₃ application about 4 weeks before harvest on 'Van' and 'Lambert' sweet cherries reduced postharvest surface marking and pitting. Pitting was reduced more steadily than was visible bruising. GA₃ treated fruits were larger and firmer when harvested. In addition, mesocarp tissue of treated fruits was higher in alcohol-insoluble solids and in dry weight and contained less N/unit of fresh or dry weight. Looney (1993) showed that gibberellins (GA) are used on numerous crops to improve fruit appearance and internal condition but they also have potential to reduce pesticide practice. Thakur et al. (1996) showed that the acidity of tomato fruits was reduced when the plant was sprayed with GA₃. It has been reported that GA₃ application increased sweet cherry fruit firmness, soluble solids and fruit weight (Basak et al., 1998). Applying GA3 at 100 ppm on gooseberry plants one week after transplanting the seedlings resulted in plants producing significantly largest number of fruits number of branches and plant height (Wanyama et al., 2006). Saha et al. (2009) indicted that in tomato (Lycopersicon esculentum Mill.), maximum yield and vitamin C was obtained with the application of 40 ppm GA₃. Katiyar *et al.* (2008) reported that GA₃ foliar application at 90 ppm gave maximum fruit size and specific gravity and the lowest fruit acidity. In addition, GA₃ application had a positive effect on the postharvest life of guava fruits and maintained

various physio-chemical attributes at the desired level of consumer acceptance until 60 days of storage. In 'Rose Scented' litchi, higher fruit quality attributes were reported with GA₃ at 40 ppm followed by GA₃ at 20 ppm over the control. Reduced fruit cracking was also observed in trees, which were sprayed with GA₃ (Mishra *et al.*, 2012). Choi et al (2004) showed that sweet cherry (Prunus avium L.) fruit interacted to gibberellic acid (GA₃) application by a delay in ripening date and increased firmness at maturity. Khandaker *et al.* (2012) reported that GA₃ and auxin treatments significantly increased the TSS (Brix) content of wax apple. In addition, GA₃ treatment had a positive effect on the juice content of wax apple fruits. Wahdan et al. (2011) indicated that application of GA₃ significantly increased the TSS, sugar acid ratio TSS/TA, and total sugar content of mango cv. 'Succary Abiad'. Pozo (2008) reported that gibberellin accumulation in citrus ovaries has been proposed as an activating signal for fruit set in citrus. Davies and Zalman (2006) stated that GA_3 significantly increased the total number of fruits, the fruit weight per plant by reducing pre-harvest fruit drop in orange. Part et al. (2008) obtained the same results in jujube, they reported that GA₃ treatment increased the growth characteristic and total flowering. Synthetic auxin increases absolute juice content in citrus fruits, through simultaneous increases in fruit size and juice content from pulp (Xiao et al., 2005). Almeida et al. (2004) pointed that application of GA₃ at early stages of fruit growth reduced significantly the fruit drop in citrus fruits. Application of gibberellic acid in combination with hand pollination increased fruit set percentage pulp/seed ratio, average fruit weight and size in various mandarin and sweet orange cultivars (Ibrahim and Simbel, 1991). Choi et al. (2004) pointed that the optimal GA₃ concentrations applied to grape fruit varies with cultivar and year, and number of applications. In grape, the range of concentration can be established between 160 and 240 mg L^{-1} GA₃ as a single application. However, Korkas et al. (1999) recommended 320 mg L GA₃ to increase bunch weight and 650 mg L^{-1}

GA₃ to increase berry weight in 'Sultanina' seedless grape, while Bhujbal and Chaudhari (1973) recommended concentration of 100 mg L⁻¹ and Dass and Radhawa (1972) recommended 75 mg L^{-1} . Casanova *et al.* (2009) indicated that gibberellic acid (GA₃) application improves berry size of 'Emperatriz' seedless grape, and the response controlled by the phonological stage of vine at application date and on the concentration applied. From berry fruit set to 21 days later, 80 mg L^{-1} GA₃ increased berry weight by 50% up to -90%. Ji et al. (1992) found that application of GA₃ at 50 mg/l applied 5 weeks after full blooming reduced fruit drop in 'Huaizhi' compared with controls. Several studies have shown that GA₃-treated sweet cherry fruits were firmer, larger, and heavier compared with controls (Choi et al., 2002; Kappel and MacDonald, 2002; Horvitz et al., 2003; Clayton et al., 2006). In pear fruit cv. 'Punjab Beauty', Singh and Sharma (2005) indicated that GA₃ application at 20 ppm improved fruit set, yield, and TSS (%) of fruit juice. Singh and Lal (1980) reported that application of GA₃ at 50-100 mg/L at full bloom improved fruit retention and fruit size in 'Early seedless' and 'Calcuttia' litchi in India. Davies and Zalman (2006) stated that the application of and GA₃ and 2, 4-D significantly reduced the preharvest fruit drop and improved the total number of fruits at the time of harvest and fruit weight per plan in some citrus species. Foliar application of various levels of GA_3 (5, 50, 100 and 500 ppm) to young grapefruit fruitlets just after fruit set increased the fruit weight, peel thickness, juice content and taste (Berhow, 2000). Canli and Orhan (2009) reported that Sweet cherry fruit cv '0900 Ziraat' treated with 20 and 25 ppm GA₃ were significantly larger than the fruit treated with 15 ppm GA₃. Treatment with the optimum concentration of GA₃ (25 ppm), in two different locations, produced fruit with 13.4% and 14.1% larger weight and 38% and 25% higher firmness. GA_3 applications (5, 10, 15, 20 and 25 ppm) significantly (p < 0.05) increased the fruit weight, delayed the harvest date and decreased the fruit cracking in the '0900 Ziraat' sweet cherry cultivar (Yildirim and Koyuncu, 2010).

Göktürk and Harmankaya (2005) found that the number of seeds per grape berry was significantly linked with endogenous gibberellins concentration. Al-Najdawi et al. (2007) indicated that Spraying 15 ppm GA_3 on strawberry after three weeks of planting increased TSS, ascorbic acid, anthocyanin, and TSS/TA ratio, and a decrease in TA in fruits. Asrey et al. (2001) stated that application of GA₃ at 400 ppm enhanced the yield significantly when compared to control in muskmelon. Nakano et al. (1997) investigated the effect of gibberellic acid (GA₃) on persimmon fruit respiration in relation to fruit growth and maturation. GA₃ was applied 5 times during growth stage II at 100 ppm to whole branches bearing fruit. Results showed that GA treatment delayed fruit colouring and softening and retarded final swell at growth stage III. Fruit respiration rate was reduced by GA₃ treatment. Furthermore, the respiration rise, which occurred concurrently with the onset of growth stage III was retarded in GA₃ treated fruit. In tomato, Masroor et al. (2006) found that GA₃ applications (10⁻⁴, 10⁻⁶, and 10⁻⁸ M) gave the higher values for both fruit number and fruit yield per plant and the maximum lycopene content of fruits when compared to the control. In Japanese pear, Zhang et al. (2005) found that application of GA₃₊₄ at 20–30 mg resulted in a marked increase in pedicel diameter and bigger fruit at harvest. In addition, the histological studies of fruit revealed that GA₃ treatment increased the cell size of the mesocarp but not the cell number and core size. The study concluded that larger fruit size resulting from GA_{3+4} (at 20–30 mg) application during the period of rapid fruit growth caused an increase in the cell size of the mesocarp and increased carbon partitioning to the fruit. Zahedi et al. (2013) reported that grape fruits cultivars 'Perlette' and 'Yaghuti' treated with GA3 (0 and 50 mg/l) had the highest cluster weight, fruit diameter, length, volume and L/D ratio.

A high rate of productivity in current agriculture requires applying chemicals such as fertilizers, pesticides, or growth regulators (PGR). Chemicals are typically applied to the soil or via foliar application. Even though these old-style application methods have great rewards, they are sometimes unsuccessful, often unacceptable in urban areas, and they increase water and air pollution. Injection is an alternative method for introducing chemical compounds into trees (Pinkas *et al.*, 1973; Reil and Beutel, 1976; Sterrett and Creager, 1977). Injection through tree trunk (Fig 2.3, 2.4, 2.5) was implemented in early researches, mainly to inject chemical to prevent certain disease or to test the infection with blight. For instance, study done by Graham *et al.* (1983) was to investigate the blight disease through syringe injection tests. Moreover, a low-pressure, trunk-injection method was introduced into olive trees in the study done by (Navarro *et al.*, 1992).



Fig. 2.3 Photograph illustrating the injection of the chemicals into tree trunk (Chemjet Trading Pty, Ltd, Australia, 2012).



Fig. 2.4 Photograph illustrating the injection of chemicals into tree trunk (Shaaban, 2009).



Fig. 2.5 Photograph showing injection of chemicals into tree (Shaaban, 2009).

2.4.3 Effects of Naphthaleneacetic Acid (NAA)

NAA is a synthetic plant hormone in the auxin family (Fig 2.4). Khandaker et al. (2012) reported that the application acetic acid (NAA) on wax apple fruit significantly reduced titratable acidity and increased total sugar and carbohydrate content compared to the control. Iqbal et al. (2009) reported that guava fruit yield was significantly increased by NAA applications at 45 ppm and 60 ppm. NAA application increased TSS, total sugars, and ascorbic acid contents except acidity, which was reduced. However, Fruit drop exhibited negative correlation with all other characteristics except acidity whereas pulp seed ratio had positive correlation with TSS, total sugars and ascorbic acid contents and negative, association with acidity. Amorós et al. (2004) showed that the application of naphthalene acetic acid on loguat trees (Eriobotrya japonica Lindl) cv. 'Algerie' at 25 and 50 mg l-1 (NAA-25, NAA-50) when fruit were at 50 and 30% of their final size increased the total production significantly with respect to control trees, due to an increase in fruit size. A significant delay in maturity of loquat fruit NAA treated trees was observed when treatments were applied at 50% of final fruit size. However, when the auxin treatments were applied at 30% of final fruit loguat size, fruit precocity increased. Thus, these treatments accelerated fruit growth and maturation, without any undesirable effect on nutritive and organoleptic properties of loquat fruit. Dutta and Banik (2007) reported that GA₃ and NAA application before flowering, followed by three weeks after fruit setting, significantly increased fruit length, diameter and fruit weight and ultimately crop yield. In another study done by Yuan and Carbaugh (2007) stated that NAA possibly enhance background colour development and fruit softening. Son (2004) Found that in Mut apricot *Prunus armeniaca* growing in central Turkey, application of naphthalene acetic acid (NAA) at 10, 20, and 30 ppm significantly increased fruit weight. The biggest fruit were obtained from 20 ppm NAA; the soluble solids (TSS) were greatest (13.53%) for 20 ppm NAA. El-Shewy (1999) reported that 50 mg/L NAA and 50 mg/L GA₃ at full bloom and three months after the first spray were most effective treatments in reducing preharvest fruit drop as well as fruit seed contents in guava. Dubay et al. (2002) reported that NAA application on guava cv. Allahabad Sufeda at 250 mg/L concentration resulted in higher yield and quality. Yaday (2002) indicated that NAA at 60 mg/L improved TSS, total sugars and vitamin-C (ascorbic acid) contents in guava fruit. Agusti et al., 2000 showed that the application of NAA 15 days after full bloom of loquat fruit, increased fruit diameter by 10%, and yield by 20% per tree compared to the control. NAA has also been shown to promote maturation (softening and anthocyanin formation) in mesocarp discs of peach fruit (Ohmiya, 2000). Guinn and Brummett (1993) pointed that the application of NAA on leaves increased the net photosynthetic rate due to increase in stomatal aperture, which facilitates more CO₂ conductance. Ortolá et al. (1991) found that naphthaleneacetic acid (NAA) when applied shortly after the end of the June drop, it increases the growth rate of Satsuma mandarin fruit (*Citrus*) unshiu Marc.), and that results in a bigger fruit size at harvest without any adverse reduction in yield. Wismer et al. (1995) reported that the increase in fruit size in 'Empire' apple fruit (Malus domestica Borkh.) by application of NAA was due the enlargement in fruit cell size and was not due to the acceleration rate of cell division. Bal and Randhawa (2007) stated that the ber fruit size increased to maximum with 20 and 30 ppm NAA and the fruit yield per tree was significantly improved with NAA treatments. In addition, TSS was slightly improved with different NAA treatments and the total acids were decreased under all the NAA treatment. Application of NAA on Cabernet Sauvignon grapes at veraison suppresses all the genes of the anthocyanin biosynthesis pathway (Jeong et al., 2004).



Fig. 2.6 Naphthaleneacetic acid (NAA).

The average fruit weight was increased with different NAA treatments and it was higher with NAA 20-30 ppm. The fruit yield per tree was significantly enhanced with NAA treatments. TSS was slightly enhanced with different NAA while the total acids were decreased under all the NAA treatments. Haidry et al. (1997) studied the effect of naphthalene acetic acid (NAA) on fruit drop, yield, and quality in mango, cultivar 'Langra'. The study stated that application of NAA in any concentration significantly reduced the fruit drop, but the dose of 20 ppm NAA highly minimized the fruit drop at all stages of its development and gave maximum fruit retention at maturity. Moreover, NAA applications as foliar spray resulted in increase of total soluble solids (21.73-22.22%) and sugar contents (16.81-16.86%) whereas acidity decreased significantly (0.31%) but minimum total soluble solids (20.09%), sugars (15.04%) and increased acidity (0.34%) were noticed in the control fruits. Moustafa *et al.* (1993) indicated that naphthalene acetic acid (NAA) application on date palm trees increased fruit weight, dimensions, flesh weight percentage and total soluble solid and reduced fruit ripening. Mohammed and Shabana (1980) obtained the same results and they indicated that preharvest application of NAA at 10, 20, 40, or 60 ppm to immature 'Zahdi' date palm fruit at 15 to 16 weeks after pollination influenced fruit size, quality, and ripening. Applications of NAA at 40 or 60 ppm increased fruit size, weight, volume, pulp to seed

ratio and moisture content and delayed ripening by at least one month compared with the controls. TSS was not significantly altered. Given et al. (1988) reported that the application of 1-naphthalene acetic acid (NAA) into intact premature strawberry fruit through the peduncle hindered their subsequent ripening, as measured by the accumulation of anthocyanin, loss of chlorophyll and decrease in firmness. Other study on Japanese plum (*Prunus salicina*) has showed that application of 30 mg l^{-1} naphthaleneacetic acid (NAA) $(0.3\% \text{ Amigo}^{TM})$ at the beginning of pit-hardening, when fruitlet diameter was ca. 22 mm, caused an appreciable and significant increase in fruit size (Stern et al., 2007). Belakbir et al. (1998) found that NAA application at flower initiation, followed by two additional applications at 30-day intervals on bell pepper (*Capsicum annuum* L.) produced the highest yield of marketable fruit. In addition, treatments did not affect fruit firmness compared to the control. Jeong et al. (2004) stated that the accumulation of anthocyanin in grapes fruit suppressed by NAA treatment. Surányi (1986) found that 25-75 mg l⁻¹ of NAA for 'Hungarian Best' apricots and 50-100 mg l⁻¹ of NAA for 'Rose Apricot' was the best concentrations to improve fruit size and quality. In tomato fruit, NAA application at 25 ppm improved the number of fruits/plant, fruit weight/plant, total soluble solid (TSS), vitamin C and yield. Foliar spray of NAA at 60 ppm gave the maximum TSS content, had a favourable effect on the postharvest life of guava fruits, and maintained various physico-chemical attributes at the desired level of consumer acceptance until 60 days of storage (Katiyar *et al.*, 2008). In date palm (Phoenix dactylifera L. cv. Kabkab), Hesami and Abdi (2010) stated that trunk injection with NAA at 100 mg/L significantly increased bunch weight, improved physical properties (fruit weight, height, diameter and size and flesh weight percentage) compared with water treated bunches and other treatments. However, total soluble solid, total and reducing sugars were decreased significantly in fruit juice by NAA treatments compared with the control. NAA alone showed the lowest total soluble solid, total and reducing sugars.

From the literature covered above, it is clear that fruit growth and quality are very complex processes involving many factors. Moreover, horticultural practices such as shading, thinning, and plant growth regulators play an essential role in fruit growth and development. From this, it can be anticipated that plant growth regulators; ABA, GA₃, NAA, and horticultural methods can manipulate the fruit growth, yield and fruit quality.

This study, examines a new approach of applying GA₃ via injection and swabbing fruit with ABA and NAA, in addition to applying horticultural shading and thinning practices in order to reach a better understanding of growth and quality of wax apple fruit.

THE EFFECT OF SHADING ON WAX APPLE FRUIT DEVELOPMENT AND QUALITY

3.1 INTRODUCTION

In the modern fruit production, high-density planting and intensive cultural systems are worldwide accepted practices. However, with such practices, excessive shade within and between trees can easily occur and affect fruit quality (Jackson, 1980). Light it is very important for fruit development and quality. For instance, natural shading was found to reduce concentrations of soluble solids in grape fruit (Crippen and Morrison, 1986). Moreover, shading within trees has been shown to reduce fruit quality in many fruit species, including peach, grapes, and raspberries (Palmer, 1989). Fruit size, red skin colour, soluble solids concentration are all reduced by shading (Barritt *et al.*, 1987). The tree spacing and canopy structures (height, width, shape, and leaf density) control total light interception, dry matter production, and thus potential yield (Robinson and Lakso, 1991). Woolf *et al.* (1999) suggested that fruit under direct sunlight, exposed to higher temperature and that might result in increased water flow to these fruit, leading to increased mineral accumulation.

The wax apple is a tropical fruit and widely cultivated in Malaysia. However, there are still some problems with its fruit quality, namely, fruit drop, small fruit size and less colour and taste, despite the fact that, there is a great scope to develop wax apple fruit industry and huge amount of foreign capital can be earned by exporting to the other countries. Nevertheless, little attention has been given in Malaysia to study wax apple fruit quality parameters in relation to shading.

This chapter focuses on the effect of shading on wax apple fruit development and quality by using three levels of fruit shading (50%, 70%, non-shade) under experimental field conditions. This project is a pioneer project aimed to develop better understanding of fruit quality parameters in relation to shading in the field. This is the first report on the effect of shading on wax apple fruit grown in Malaysia.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Site

The study was carried out in a commercial orchard located at Banting, Selangor, Malaysia, 2°30N, 112°30E and 1°28 N, 111°20E at an elevation of about 45 m above sea level. The area under study has a hot and humid tropical climate. The soil in the orchard is peat with a mean pH of around 4.6 (Ismail *et al.*, 1994). The pH of the soil was neutralized by adding poultry manure and organic fertilizers before launching the experiments. The experiment was carried out between September 2010 to January 2011.

3.2.2 Applications

Thirteen years old, wax apple trees were selected for the study. The trees were planted in a 4.2×4.2 m hexagonal pattern and received the same agricultural management; fertilization and pruning. Three trees were selected for each treatment (shading) and nine trees used in the experiments. Forty-five uniform branches in all directions (five branches per tree) of about the same length and diameter from nine trees were selected and tagged for the experiments. Selected branches in each experimental tree were covered completely with agro commercial shading net (Agriculture Sunshade net, 50% and 70%, Tengfei, made of HDPE PE, Alex Manufacturer (ISO), Selangor, Malaysia) at bud emerging as follows: treatment 1 (T1): 50% shading T2, 70% shading, T3: without shading as a control. The experiments consist of three treatments including control with three replications and five sub-replications (15 replicates for each treatment). A single tree was taken as an experimental unit (Table 3.1 and Fig.3.1).
Table 3.1 Shading treatments.

Treatment	Shading		
T1	50% shading		
T2	70 % shading		
Т3	Without shading as a control		



Fig.3.1 Selected branches covered with shade net at the field.

3.2.3 Total Anthocyanin Content

Total anthocyanin content of the extracts was measured by the pH-differential method described by Rodriguez-Saona *et al.* (1999). Samples were diluted with two different solutions: potassium chloride (0.025 M), pH 1.0; and sodium acetate (0.4 M), pH 4.5. The pH was adjusted with concentrated hydrochloric acid. Samples were diluted to give an absorbance of <1.2 at 530 nm. Diluted samples were held for 15 min before measuring the absorbance. The absorbance was measured at 530 nm and 700 nm with distilled water as a blank. The absorbance difference between the pH 1.0 and pH 4.5 samples was calculated as follows:

 $A = (A_{530nm} - A_{700nm})_{pH1.0} - (A_{530nm} - A_{730nm})_{pH4.5}$

The monomeric anthocyanin pigment concentration was calculated using the following equation:

Monomeric anthocyanin pigment (mg/L)

= $(A \times MW \times DF \times 1000)/(\varepsilon \times 1)$

MW=449.2, $\varepsilon = 26,900$ and DF= Dilution factor

3.2.4 The Total Phenolic Content (TPC)

The total phenolic content of wax fruits were determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). An aliquot (1ml) of the extracts or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added in to a 25ml volumetric flask, containing 9 ml of distilled water. A reagent blank using H₂O was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na₂CO₃ solution was added to the mixture. The solution was diluted to a volume of 25 ml with double distilled water and mixed. After incubation for 90 min at room temperature, the absorbance against reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer Lambda 5. Total phenolic content of fruits was expressed as mg gallic acid equivalents (GAE)/100g fresh weight. All samples were analysed in triplicates

3.2.5 Total Flavonoid Content (TFC)

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen, *et al.* 1999). An aliquot (1 ml) of the extracts or a standard solution of catechin (20, 40, 60, 80 and 100 mg/L) was added to a double distilled water 10 ml volumetric flask containing 4ml of double distilled water. 0.3 ml of 5% NaNO₂ was added to the flask was added. After 5 min, 0.3 ml 10% AlCl₃ was added. At 6th min, 2ml 1M NaOH was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed well and the absorbance was measured against a reagent

blank at 510nm. The total flavonoid content of fresh fruit was expressed as mg catechin equivalents (CE)/100 g fresh mass. Triplicates samples were analysed.

3.2.6 Fructose and Inverted Sugar

A small amount of the homogenous mixture was centrifuged at $4000 \times g$ for 10 min and the clear supernatant was analysed for determination fructose, and inverted sugar and were evaluated at 25°C with Atago 8469 hand refractometer for fructose, and inverted sugar (Atago Co. LTD., Tokyo, Japan) and expressed as percentage.

3.2.7 Total Soluble Solid (TSS) content

A small fraction of the homogenous juice was centrifuged at $4000 \times g$ for 10 min and the clear supernatant was analysed for TSS. TSS was evaluated at 25°C with an Atago 8469 hand held refractometer (Atago Co. LTD., Tokyo, Japan) and expressed as °Brix.

3.2.8 Titrable Acidity and TSS/TA Ratio

A 10 ml of the wax apple juice prepared by direct homogenization was titrated with 0.1 N NaOH using phenolphthalein as indicator. Titratable acidity of the juice was defined as % citric acid. Sugar acid ratio of the wax apple juice was express as TSS/TA ratio.

$$TA (\%) = \frac{Nb \times Vb \times Ea \times df \times 100}{Vs}$$

where *Nb* is normality of the base, *Vb* is volume of the base, *Ea* is mill equivalent weight of citric acid, *Vs* is volume of sample, and df: dilution factor.

3.2.9 pH Measurement

For pH measurement, a small portion of the homogenous juice was centrifuged at $4000 \times g$ for 10 min and a small sample from the clear supernatant was analysed for pH using Microprocessor pH meter (Hanna Instruments).

3.2.10 Bud Dropping (%)

To determine the bud drop percentage from tagged branches on the experimental tree, the number of buds was counted and the bud drop percentage was calculated using the following formula:

= Total number of buds at initial stage – Buds before blooming Total number of buds at initial stage ×100

3.2.11 Fruit Setting (%)

For the determination of fruit setting percentage from tagged branches on the experimental tree, the number of flowers and total number of fruitlets were counted before and after anthesis. Fruit setting percentage was calculated using the following formula:

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

3.2.12 Fruit Dropping (%)

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

= Total number of fruitlets – Number of fruits in 35 days after anthesis Total number of fruitlets × 100

3.2.13 The Chlorophyll Content (SPAD value)

The chlorophyll content (SPAD) value was measured with a SPAD meter (SPAD 502 Chlorophyll Meter, Konica Minolta, Co. LTD., Japan) at the 5th week fruit growth period. For measuring the chlorophyll content, 15 reading from randomly selected leaves for each treatment were taken.

3.2.14 Fruit Size and Development

The development of 45 fruits per treatment was monitored weekly from the day of treatment until harvest, measuring the length and diameter of each fruit on the selected branches using a vernier caliper. Average fruit size was determined by measuring the length and diameter of 25 fruits per treatment tree randomly with the help of a vernier caliper at harvest.

3.2.15 Fruit Weight

Average fruit weight was determined by weighing 25 fruits per treatment tree on a digital electric balance (UWE-ESP) and the average weight was calculated.

3.2.15 Fruit Yield

Yield per treatment was recorded by weighing and counting the total number of fruits per treatment at the time of harvesting.

3.2.16 Fruit Juice

The fruit juice of each harvested fruit was extracted and weighed and average juice weight was calculated separately for each treatment. The average juice percentage per fruit was obtained from the following formula:

Juice Percentage = $\frac{\text{Juice weight per 100 g fruit}}{100 \text{ g fruit weight}} \times 100$

50

3.2.17 Measurement of the K⁺ Content in Fruit Juice

The K^+ content of the fruit juice was determined by using a Cardy Potassium meter immediately after harvesting the fruits, 15 fruits from each treatment were taken for K^+ measurement. 3 to 5 juice drops of the supernatant liquid 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).

3.2.18 Statistical Analysis

The experimental design was a completely randomized design (CRD) with three replications and 5 sub replications. A single tree was taken as an experimental unit. The data were plotted and analysed using PASW (SPSS) statistical software, release 18. One-way analysis of variance (ANOVA) was applied to evaluate the significant difference in the parameters studied in. Least significant difference (Fisher's protected LSD) was calculated, following significant F test ($p \le 0.05$).

3.3 RESULTS AND DISCUSSION

3.3.1 Anthocyanin Content

Leaf shading might play a key role in the compositional changes in fruit from shaded shoots Crippen and Morrison (1986). Shading 50% and 70% reduced anthocyanin significantly compared to the control. However, there is no significant difference between shading 50% and 70% in anthocyanin content (Table 3.2). The highest anthocyanin content was 2.64 ± 0.36 (mg/L) recorded in control treatment (without shading) followed by shading at 50% with anthocyanin content 2.43 ± 0.3 (mg/L) and the lowest anthocyanin content was 2.28 ± 0.30 (mg/L) recorded at 70% shading. The result is in agreement with the results of Cortell and Kennedy (2006) and Jeong *et al.* (2004). In addition, Azuma *et al.* (2012) similarly, reported that that shading severely suppressed anthocyanin accumulation in grape skin reported similar results.

3.3.2 Total Phenols Content (TPC)

Bravo (1998) stated that interest in phenolic compounds has increased intensely over recent years since they exist in mostly all plants and are therefore part of our food. Result in Table 3.2 showed that there was no significant difference among all treatments. However, the shading treatment had slightly non-significant less phenol content compared to the control (Table 3.2). The lowest phenol content was recorded at 70% shading with 354.67±13.52 mg GAE/100g followed by 50% shading with 356.67±11.85 mg GAE/100g. The highest phenol content was 361.47±11.06 mg GAE/100g recorded at the control treatment. Our result is in agreement with Cui *et al* (2012) where they observed that shade treatment decreased anthocyanin and total phenolic contents in maize. Similar results were also reported by Smart *et al.* (1998) and Morrison and Noble (1990). However, Anttonen *et al.* (2006) found that there was no significant deference between control and shaded fruit in phenolic content in Strawberry.

3.3.3 Measurement of pH

Study result showed that all shaded treatments had lower pH values compared to the un-shaded fruit (control). The highest significant pH was recorded at control with 4.9 pH. However, there were no significant differences between 50% and 70% shading treatment in pH measurement (Table 3.2). The lowest pH was 4.43 recorded in 70% shading treatment. The result is in agreement with the finding of Callejón-Ferre *et al.* (2009). In addition, our result is in agreement with Smart *et al.* (1985) study, which, indicated that shaded canopy microclimates increased potassium concentration and juice pH of grape berries. Rojas-Lara and Morrison (1998) indicated that grapes from heavily shaded vines had the highest pH.



Fig 3.2 Effect of different shading 50%, 70% shading and exposed fruit (without shading) on wax apple fruit appearance.

3.3.4 Total Flavonoid Content (TFC)

This study showed that flavonoid affected by shading. Flavonoid content was significantly higher in control treatment (fruit exposed to direct sun light) compared to 50% and 70% shading (Table 3.2). Moreover, the study revealed that there was a significant difference between 50% and 70% shading treatment in flavonoid content and it is clear that shading treatment depressed the accumulation of flavonoid in fruit. The highest flavonoid content was recorded in control at 13.66 ± 1.09 (mg/100g), followed by 50% shading at 11.16 ± 1.15 (mg/100g) and least flavonoid content was recorded in 70%

shading at 10.19 ± 1.22 (mg/100g) (Table 3.2). Our results was in agreement with Cortell and Kennedy (2006) finding which indicated that in grape *Vitus vinifera* L. cv. Pinot noir, shaded fruit had a lower concentration of flavonols, anthocyanins, and proanthocyanidins in the shaded treatment. In addition, Ristic et *al.* (2007) indicated that the most drastic effect of shading berries was at the level of flavonols, which were lowered to trace amounts.

3.3.5 K⁺ Content

Results showed that fruits in control treatment had a significantly higher K^+ content when compared to all shading treatments. There was no significant difference between shading at 50% and 70%. However, 50% shading had slightly higher K^+ then 70% shading treatment. The highest K^+ content was 14.56±0.73 (mg/kg) at control treatment followed by 50% shading at 12.25±0.86 (mg/kg) and 70% shading at 11.76±0.88 (mg/kg) (Table 3.2). Our result is in agreement with Smart *et al.* (1985) where they indicated that the shaded canopy microclimates increased potassium concentration and juice pH of grape berries.

3.3.6 Total Soluble Solids (TSS)

Results showed in Table 3.3 indicated that all shading treatments reduce TSS significantly when compared to control treatment. The highest TSS was 9.38 ± 0.33 (°Brix) recorded in control treatment followed by 50% shading at 8.83 ± 0.38 (°Brix) and 8.72 ± 0.23 (°Brix) was recorded in 70% shading. However, there was no significant difference between 50% and 70% shading treatment. Our result is in agreement with Koblet, (1984) who found that sunlight-exposed fruits are generally richer in total soluble solids when compared to non-exposed or canopy shaded. Smart *et al.* (1998) also indicated that increasing shade markedly reduced sugar, phenol, and anthocyanin concentrations in Cabernet Sauvignon fruits. Chorti *et al.* (2010) indicated that shading reduced total soluble solids and anthocyanin accumulation in Nebbiolo grapes.

3.3.7 Titratable acidity (TA) and TSS/TA ratio

Result showed that all shading treatments increase TA significantly when compared to control treatment (Table 3.3). However, there was no significant difference in TA between 50% and 70% shading treatment. Control treatment had the lowest TA followed by 50% and 70% shading at $0.78\pm0.03\%$, $0.82\pm0.04\%$, and $0.83\pm0.03\%$ respectively (Table 3.3). The results are in agreement with (Koblet, 1984). In addition, Smart *et al.* (1998) indicated that increasing shade markedly increased levels of titratable acidity in Cabernet Sauvignon fruits.

The TSS/TA ratio in control treatment was significantly higher when compared to all shading treatment, however; there was no significant difference in TSS/TA ratio between 50% and 70% shading. The highest TSS/TA ratio was 12.09 \pm 0.70 recorded in control treatment followed by 50% at 10.87 \pm 0.93 and the lowest TSS/TA ratio was 10.53 \pm 0.56 recorded in 70% shading treatment (Table 3.3).

3.3.8 Inverted Sugar

The study result showed that all shading treatment had inverse effect in inverted sugar when compared to control treatment (Table 3.3). However, there was no significant difference between 50% and 70% shading treatment. Fruit Exposed to direct sunlight (control) had the highest inverted sugar content at 9.09 ± 0.25 (°Brix) followed by 8.75 ± 0.28 (°Brix) recorded in 50% shading. The lowest inverted sugar was 8.57 ± 0.25 (°Brix) recorded in 70% shading treatment. Form the result it can be suggested that in order for better fruit quality, fruit must be exposed to direct sunlight (Table 3.3). Smart *et al.* (1998) indicated that increasing shade markedly reduced sugar concentration in Cabernet Sauvignon fruits.

3.3.9 Fruit Fructose Content

Watson *et al.* (2002) indicated that shading had a significant effect on sucrose concentrations of strawberry .As showed in table 3.3, the exposed fruit to direct sunlight in control treatment had the heights fructose content when compared to all shading treatment. Shading fruit reduced significantly the fructose content in fruit. Fructose content of 8.78 ± 0.30 (°Brix) was recorded in control treatment followed by 50% shading at 8.59 ± 0.36 and the least fructose content was recorded in 70% shading treatment. The result is in agreement with Watson *et al.* (2002) study, which indicated that sucrose concentration in strawberry decreased significantly by shading treatments.

3.3.10 Chlorophyll Content (SPAD)

Light intensity promotes alterations in the structural organization of the leaves, resulting in leaves of sun and shade (Cutter, 1978). In our study, the chlorophyll content was determined by Minolta SPAD meter. The SPAD values in Table 3.4 and Fig 3.2 showed that shading treatments slightly lowered chlorophyll content when compared to the control (un-shaded). However, there were no significant differences among all treatments. It appeared that shading did not affect notably the chlorophyll content in the leaves, and the slight effect was due to covering the branch in certain time during the experiment. Hence, the leaves in covered branched had reached the maturity before treatments. Therefore, shading treatment did not alter their structure or their chlorophyll content. This study is in agreement with the Paiva *et al.* (2003) who reported that fully mature leaves showed no change in their structure and photosynthetic capacity when transferred to shading. Similar result also obtained by Sims *et al.* (1992).



Fig 3.3 Effect of different shading on wax apple leaves chlorophyll content (SPAD).

3.3.11 Fruit Juice

From Table 3.4, the percentage of fruit juice per 100 g was similar in all treatments and they did not differ significantly. The percentage of fruit juice in control treatment was 74.15 ml, 74.34 \pm 0.42 ml in 50% shading treatment and it was 74.20 \pm 0.59 ml in 70% shading treatment. However, the fruit juice percentage was non- significantly a little higher in 50% shading. This result is in agreement with Jlfon and Syvertsen (2001) study, which indicated that juice yield of sweet orang fruit cv. 'Hamlin' was not affected by shade treatments. It can be suggested that shading branches bearing mature leaves did not alter the percentage of fruit juice.

3.3.12 Fruit Length

Fruit length significantly affected by shading treatments (Table 3.4 and Fig.3.3). All shading treatment decreased the fruit length when compared to the control treatment. The fruit length in control treatment was 60.30 ± 3.52 mm while they were 55.10 ± 3.55 mm and 52.63 ± 3.16 mm in 50% shading and 70% shading treatments

respectively. All shading treatments reduced the fruit length by about 9%. In addition, shading treatments slowed down significantly wax apple fruit growth over time (Fig 3.4). The study result is in agreement with Dash *et al.* (2012) study, which indicated that severe shading, reduces early fruit growth in apple by decreasing cell production and expansion in apple and with Morandi *et al.* (2011) study, which indicated that shading decreases the growth rate of young apple fruit, by reducing their phloem import. It can be suggested that in order to maximize fruit growth, fruits must receive appropriate sun light-exposure.



Fig 3.4 Fruit growth (length)/week as influenced by different shading.

3.3.13 Fruit Width

Fruit width significantly altered by shading treatments (Table 3.4 and Fig.3.5). All shading treatment decreased the fruit width when compared to the control treatment. The fruit length in control treatment was 38.93 ± 2.40 mm while they were 32.96 ± 2.48 mm and 30.56 ± 2.57 mm in 50% shading and 70% shading treatments respectively. Shading treatments slowed down significantly wax apple fruit growth over time (Fig.3.5). The study result is in agreement with Dash *et al.* (2012) study, which

indicated that severe shading, reduces early fruit growth in apple by decreasing cell production and expansion in apple and with Morandi *et al.* (2011) study, which indicated that shading decreases the growth rate of young apple fruit, by reducing their phloem import. It can be suggested from this result that sun light exposure is essential to maximize fruit growth in wax apple fruit.



Fig 3.5 Fruit growth (width)/week as influenced by different shading.

3.3.14 Bud Drop

As shown in Table 3.4, all shading treatments increased significantly bud drop when compared to the control treatment. 50% shading treatment increased bud drop by about 16% followed by 70% shading treatment at about 23% when compared to control treatment. Control treatment yielded the best result in bud drop when compared to all shading treatments. From the result, buds on all shading treatments aborted development and dropped before flowering. It seemed that proper sunlight exposure was essential for bud development. The result obtained in this study is in agreement with Bartolini *et al.* (2013) finding in apricot (*Prunus armeniaca* L.).

3.3.15 Fruit Set

Fruit se severely affected by all shading treatments as shown in Table.3.5 All fruits in shading treatments exhibited reduction in fruit set when compared to the control treatment. The fruit set was $45.53\pm2.95\%$ in control treatment followed by $42.00\pm2.85\%$ at 50% shading treatment. The lowest fruit set percentage was recorded in 70% shading treatment at $37.07\pm3.17\%$. This result confirmed the positive effect of sunlight radiation on wax apple fruit set. It can be suggested that it is important to maintain a good exposure to sun light on all tree branches during flowering and fruit development in wax apple. This result is agreement with the study by Roper *et al.* (1993) which indicated that early shading reduced fruit set in cranberry. In addition, Byers *et al.* (1991) indicated that low light levels could reduce fruit growth and set.

3.3.16 Fruit Drop

As shown in Table 3.5, shading had a negative effect on fruit drop. Fruit drop increased with all shading treatments when compared to the control treatment. The highest percentage of fruit drop observed in 70% shading treatment followed by 50% shading and the control treatment being the least, $50.20\pm4.04\%$, $45.47\pm3.66\%$ and $48.67\pm3.64\%$ respectively. However, the difference between the control and 50% shading treatment was non-significant. It is clear from the study that light level during flowering and subsequent fruit growth and development is essential. This study is in agreement with the work done by Biasi *et al.* (1995) which indicated that all shaded kiwifruit vines showed higher fruit drop in comparison to un-shaded ones. In addition, Marini *et al.* (1991) indicated that shading 'Biscoe' peach (*Prunus persica* [L.] Batsch) increased fruit drop. Similar result obtained by Zibordi *et al.* (2009) which indicated that shading during the early stages of fruit development caused fruit drop.

3.3.17 Fruit Weight (g)

All shading treatments had negative effect on fruit weight. All shading treatments resulted in smaller and lighter fruits when compared to non-shading treatment (control) (Table 3.4). The highest significantly weight was recorded in the control treatment followed by 50% and 70 % shading treatments, 44.37 ± 2.06 g, 37.04 ± 2.65 g, 34.74 ± 2.43 g respectively. The reduction of fruit weight caused by 50% shading was about 16%, and the reduction caused by 70% shading was about was 22% when compared with the control. The study result is in agreement with Dash *et al.* (2012) study, which indicated that severe shading reduces early fruit growth in apple by decreasing cell production and expansion in apple, and with Morandi *et al.* (2011) study, which indicated that shading decreases the growth rate of young apple fruit, by reducing their phloem import. From this result, it can be suggested that shading reduced fruit growth in wax apple fruit.

3.3.18 Branch Yield

There were significant differences among all treatments. Shading treatments had negative impacts on final yield in wax apple fruit. The highest yield was obtained in the control treatment followed by 50% shading treatment and 70 shading treatment, 0.82 ± 0.10 kg, 0.55 ± 0.09 kg, 0.45 ± 0.04 kg respectively (Table 3.5). The reduction in branch yield was about 33% in 50% shading and about 45% in 70% shading when compared with the control. From the length and width discussed previously, it is clear the fruit growth affected by shading. This effect in turn resulted in smaller fruits and consequently affected the branch final yield. The study result is in agreement with Dash *et al.* (2012) study, which indicated that severe shading reduces early fruit growth in apple by decreasing cell production and expansion in apple and with Morandi *et al.* (2011) study, which indicated that shading decreases the growth rate of young apple

fruit, by reducing their phloem import. It can be suggested that to maintain good fruit growth, it is required to expose fruits and branches to appropriate sunlight.



Fig 3.6 Yield (kg) of wax apple fruit as influenced by different shading.

3.4 CONCLUSION

Shading reduced significantly anthocyanin content in fruits. The highest anthocyanin was recorded in control followed by shading at 50%. There was no significant difference among all treatments in total phenols contents. Shading treatments lowered pH when compared to the control treatment. Flavonoid and K^+ contents were significantly higher in control treatment when compared to all shading treatments. Shading treatments reduced TSS and TSS/TA ratio significantly when compared to control treatment. The highest TSS and TSS/TA ratio were recorded in control treatment followed by 50% and 70% shading. Shading reduced TA significantly when compared to control treatment. However, there was no significant difference between 50% and 70% shading treatment. Shading treatments reduced significantly inverted sugar and Fructose when compared to control treatment. Fruit in control treatment had the heights fructose and inverted sugar contents when compared to all shading treatments. No significant differences were observed in chlorophyll content among all treatments. Fruit weight, length and width decreased significantly in all shading treatments when compared with control. Fruit growth in shading treatments slowed down significantly over time. The percentages of fruit juice per 100 g were similar in all treatments. Shading increased significantly bud drop and fruit drop when compared to the control treatment. The lowest fruit set percentage was recorded in 70% shading followed by 50% shading. Shading treatments resulted in smaller and lighter fruits weight when compared to the control. Fruit yield was significantly higher in the control. It seemed that wax apple fruit is very well adapted to tropical climate where it subjected to full exposure to sunlight.

It can be concluded that it is very important to maintain a good exposure to sun light on all tree branches during flowering and fruit development to improve wax apple fruit quality and yield

63

Shading	Anthocyanin (mg/L)	Total phenols (mg GAE/100g)	рН	Flavonoid (mg/100g)	K ⁺ (mg/kg)
Control	2.64±0.36a	361.47±11.06a	4.90±0.28a	13.66±1.09a	14.56±0.73a
Shade 50%	2.43±0.37ab	356.67±11.85a	4.72±0.36ab	11.16±1.15b	12.25±0.86b
Shade 70%	2.28±0.30b	354.67±13.52a	4.43±0.26b	10.19±1.22c	11.76±0.88b

Table 3.2 Effects of different shading treatments on anthocyanin, pH, K⁺, flavonoids and total phenols contents of wax apple fruit under field condition.

Table 3.3 Effects of different shading treatments on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, inverted sugar (°Brix) and fructose (°Brix) content of wax apple fruit under field condition.

TA (%)	TSS (°Brix)	TSS / TA ratio	Inverted sugar (°Brix)	Fructose (°Brix)
0.78±0.03b	9.38±0.33a	12.09± 0.70a	9.09±0.25a	8.78±0.30a
0.82±0.04a	8.83±0.38b	10.87±0.93b	8.75±0.28b	8.59±0.36ab
0.83 ±0.03a	8.72±0.23b	$10.53 \pm 0.56b$	8.57±0.25b	8.38±0.33b
	TA (%) $0.78\pm0.03b$ $0.82\pm0.04a$ $0.83\pm0.03a$	TA (%)TSS (°Brix)0.78±0.03b9.38±0.33a0.82±0.04a8.83±0.38b0.83±0.03a8.72±0.23b	TA (%)TSS (°Brix)TSS / TA ratio0.78±0.03b9.38±0.33a12.09± 0.70a0.82±0.04a8.83±0.38b10.87±0.93b0.83±0.03a8.72±0.23b10.53± 0.56b	TA (%)TSS (°Brix)TSS / TA ratioInverted sugar (°Brix) $0.78\pm0.03b$ $9.38\pm0.33a$ $12.09\pm0.70a$ $9.09\pm0.25a$ $0.82\pm0.04a$ $8.83\pm0.38b$ $10.87\pm0.93b$ $8.75\pm0.28b$ $0.83\pm0.03a$ $8.72\pm0.23b$ $10.53\pm0.56b$ $8.57\pm0.25b$

Shading	SPAD value	Fruit weight (g)	Fruit juice per 100 (g)	Fruit length (mm)	Fruit width (mm)
Control	61.46±7.79a	44.37±2.06a	74.15±0.71a	60.30±3.52a	38.93±2.40a
50% Shade	58.15±7.22a	37.04±2.65b	74.34±0.42a	55.10±3.55b	32.96±2.48b
70% Shade	57.97±6.72a	34.74±2.43c	74.20±0.59a	52.63±3.16b	30.56±2.57c

Table 3.4 Effects of different shading treatments on SPDA, fruit weight, fruit juice, fruit length, and fruit width of wax apple fruit under field condition.

Treatments	Bud drop (%)	Fruit Set (%)	Fruit drop (%)	Branch yield (Kg)
Control	48.53±2.90c	45.53±2.95c	45.47±3.66b	0.82±0.10a
50% Shade	56.20±2.93b	42.00±2.85b	48.67±3.64b	0.55±0.09b
70% Shade	59.93±3.94a	37.07±3.17a	50.20±4.04a	0.45±0.04c

Table 3.5 Effects of different shading treatments on bud drop, fruit set, fruit drop and branch yield of wax apple fruit.

THE EFFECT OF FRUIT THINNING ON WAX APPLE FRUIT DEVELOPMENT AND QUALITY

4.1 INTRODUCTION

The wax apple is a tropical fruit which belongs to the genus Syzygium in the family Myrtaceae and is 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 drop, small fruit size and less colour and 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. The wax apple become an increasingly popular fruit in the tropical region where it can fetch a price of up to 3 USD per kilogramme and has the potential to bring great benefits to local farmers and the country's economy (Khandaker et al., 2012). Dennis (2000) noted that fruit thinning has been practiced for thousands of years for many purposes. The main reason behind thinning is that too many fruits per tree can result in small fruit size and poor quality (Crisosto *et al.*, 1995). Fruit size and quality has become as important as total yield in the determination of the profitability of the fruit plantations. The size of the fruit is affected by certain horticultural practices, such as thinning. Link (2000) pointed that fruit size was directly related to thinning intensity. In addition to crop load, age of wood, flower bud quality, competition within clusters and canopy were important factors affecting the response to thinning. It is well established that thinning fruit at certain time of fruit development increase fruit size. Zibordi et al. (2009) indicated that thinning can be accomplished by hand-removal of fruit, which is expensive, or via the application of phytochemicals that cause fruit drop. Abd El-Razek et al. (2010) showed that fruit thinning improves fruit quality on grapes. Fruit thinning has become a common management practice to produce apples of high quality including a Particular fruit size, coloration, firmness, and soluble solids (Ouma, 2007). Other research result showed that strawberry-guava fruit thinning had a positive effect on the fruit quality of

the natural cycle (Michels and Normand 2004). Fruit weight and diameter increase linearly with increasing the severity of thinning (Davarynejad *et al.*, 2008).

This study reports the impact of fruit hand thinning on wax apple fruit quality and development under the field conditions. This chapter highlights the relationship between fruit thinning practice and the physical and chemicals quality components of wax apple fruit. The main goal of this study is to enhance fruit size with better taste through different hand thinning levels.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Site

The study was carried out in a commercial orchard located at Banting, Selangor, Malaysia, 2°30N, 112°30E and 1°28 N, 111°20E at an elevation of about 45 m above sea level. The area under study has a hot and humid tropical climate. The soil in the orchard is peat with a mean pH of around 4.6 (Ismail *et al.*, 1994). The pH of the soil was neutralized by adding poultry manure and organic fertilizers before launching the experiments. The experiment was carried out between January 2011 to April 2011.

4.2.2 Applications

Thirteen years old wax apple trees were selected for the study. The trees were planted in a 4.2×4.2 m hexagonal pattern and received the same agricultural management; fertilization, pruning and thinning of excess bud and fruits. Three trees were selected for each treatment and twelve trees used in the whole experiments. Sixty uniform branches in all directions (five branches per tree) of about the same length and diameter from twelve trees were selected for the experiments. The experiments consist of four treatments including control with three replications and five sub-replications (15 replicates for each treatment). A single tree was taken as an experimental unit. Fruits in selected branches subjected to the following thinning treatment; Control (un-thinned fruits), thinning to 10 fruits/branch, 15 fruits/branch and 20 fruits/branch. The fruit thinning has been done after 35 days of anthesis.

4.2.3 Total Anthocyanin Content

(Same as described in Chapter 3).

4.2.4 The Total Phenolic Content (TPC)

(Same as described in Chapter 3).

4.2.5 Total Flavonoid Content (TFC)

(Same as described in Chapter 3).

4.2.6 pH Measurement

(Same as described in Chapter 3).

4.2.7 Measurement of the K⁺ Content in Fruit Juice

(Same as described in Chapter 3).

4.2.8 Total Soluble Solids (TSS) Content

(Same as described in Chapter 3).

4.2.9 Titrable Acidity and TSS/TA Ratio

(Same as described in Chapter 3).

4.2.10 Fructose and Inverted Sugar

(Same as described in Chapter 3).

4.2.11 The Chlorophyll Content (SPAD value)

(Same as described in Chapter 3).

4.2.12 Fruit Juice

(Same as described in Chapter 3).

4.2.13 Fruit Length and Width

(Same as described in Chapter 3).

4.2.14 Fruit Weight

(Same as described in Chapter 3).

4.2.15 Branch Yield

(Same as described in Chapter 3).

4.2.16 Statistical Analysis

(Same as described in Chapter 3).

4.3 RESULTS AND DISCUSSION

4.3.1 Anthocyanin Content

All thinning treatments; 10 fruits/branch, 15 fruits/branch and 20 fruits/branch increase anthocyanin significantly when compared to the control (Table 4.1 and Fig. 4.1). The highest anthocyanin content was recorded in 10 fruits/branch and it was 4.26 ± 0.31 (mg/L) followed by thinning at 15 fruits/branch with anthocyanin content 3.70 ± 0.30 (mg/L) and 20 fruits/branch with anthocyanin content at 3.07 ± 0.44 the lowest anthocyanin content was 2.79 ± 0.40 (mg/L) recorded at the control treatment. This result is in agreement with Guidoni *et al.* (2002) who found that berry skin anthocyanins in *Vitis vinifera* cv. 'Nebbiolo' was more concentrated in berries from cluster-thinned plants.



Fig. 4.1 Effect of fruit thinning of wax apple on anthocyanin content (mg/L). Bars with different letters are significantly different.

4.3.2 Total Phenols Content (TPC)

Regarding the total phenol content, all thinning treatments showed a significant increase in total phenols (Table 4.1). The highest phenol content was recorded at 10 fruits/branch with $386.53\pm20.30 \text{ mg GAE}/100\text{g}$ followed by 15 fruits/branch at $362.20\pm27.61 \text{ mg GAE}/100\text{g}$ and $358.60\pm27.08 \text{ mg GAE}/100\text{g}$ in 20 fruits/branch treatment. The lowest phenol content was $346.33\pm17.46 \text{ mg GAE}/100\text{g}$ recorded at the control treatment (Table 4.1). Our result is in agreement with Roussos *et al.* (2011) which they that total phenol concentration increased with thinning apricot fruit (*Prunus armeniaca* L.). Tardaguila et *al.* (2008) indicated that phenolic concentration (both grapes berry and berry fresh weight basis), was higher in fruit from the thinned vines compared with the control (un-thinned).

4.3.3 Total Flavonoid Content (TFC)

Result showed that flavonoid affected by thinning fruit. Flavonoid content was significantly higher in 10 fruits/branch (Table 4.1). However, the study revealed that there were no significant differences between 15 fruits/branch and 20 fruits/branch. It is clear that thinning depressed the accumulation of flavonoid in fruit. The highest flavonoid content was recorded in 10 fruits/branch at 16.40 ± 1.55 (mg/100g), followed by 15 fruits/branch at 15.09 ± 2.32 (mg/100g) and least flavonoid content was recorded in control treatment at 14.51 ± 2.66 (mg/100g). Our result is in agreement with Guidoni *et al.* (2002) finding which indicated that in grape berry *Vitis vinifera* cv. 'Nebbiolo', flavonoids were more concentrated in berries from cluster-thinned plants.

4.3.4 Measurement of pH

Study result showed that all thinning treatments had higher pH value compared to the control. The highest significant pH was recorded at 10 fruits/branch treatment with 4.97±0.41 pH. The lower pH values were recorded at control and 20 fruits/branch treatments with 4.69 ± 0.32 in the control and 4.64 ± 0.29 in 20 fruits/branch treatment However, there were no significant differences among control treatment, 15 fruits/ branch and 20 fruits/branch treatment (Table 4.1). Our result is in agreement with Abdur Rab *et al.* (2012) finding that indicated that thinning decreased the acidity of apricot fruit pulp resulting in pH decrease.

4.3.5 K⁺ Content

Results showed that fruits in 15 fruits/branch had a significantly higher K⁺ when compared to all other treatments (Table 4.1). However, there was no significant difference among 15 fruit/branch, 20 fruits/branch and control. Thus, there was a tendency to accumulate more K⁺ in 15 and 20 fruits/branch treatments when compared to the control treatment. The highest K⁺ concentration was recorded in 10 fruits/branch at 17.75±0.92 (mg/kg) followed by 15 fruits/branch at 16.41±0.85 (mg/kg) and 16.29±0.96 (mg/kg) was recorded in 20 fruits/branch treatment. The least K⁺ concentration 16.21±0.92 (mg/kg) was recorded in the control. This result supported by the finding of Kok (2011) study, which indicated that thinning grape treatments increased the K⁺ content when, compared to the control treatment (un-thinned).

4.3.6 Total Soluble Solids (TSS)

Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. Results showed in Table 4.2, indicated that that 10 fruits/branch and 15 fruits/branch treatment increased TSS content significantly when compared to control treatment. However, there was no significant difference in TSS content between control treatment and 20 fruits/branch thinning treatment. The highest TSS was 9.87 ± 0.36 (°Brix) recorded in 10 fruits/branch treatment followed by 15 fruits/branch at 9.62 ± 0.36 (°Brix), 9.34 ± 0.26 (°Brix) was recorded in 20 fruits/branch

treatment, and the lowest TSS content was 9.29 ± 0.42 (°Brix) recorded in control treatment. This result is in agreement with Abdur Rab *et al.* (2012) study, which indicated that thinning treatment of apricot increased total soluble solids (TSS) in fruit.

4.3.7 Titratable Acidity (TA) and TSS/TA Ratio

Result showed that all thinning treatments reduced TA when compared to control treatment (Table 4.2). However, there was no significant difference in TA content among 20 fruits/branch and 15 fruits/branch and control treatments. 10 fruits/branch treatment had significantly the lowest TA followed by 15 fruits/branch, 20 fruits/branch and the control, 0.80 ± 0.5 , 0.76 ± 0.4 , 0.77 ± 0.5 , 0.79 ± 0.4 , respectively. Regarding the TSS/TA ratio, from result, the thinning treatment increased the TSS/TA ratio. The highest TSS/TA ratio was recorded in 10 fruits/branch at 13.11 \pm 0.82 followed by 15 fruits/branch at 12.52 \pm 1.01, 20 fruits/branch at 11.81 \pm 0.68 and the least TSS/TA ratio was 11.59 \pm 0.95 recorded in the control treatment. This result is in agreement with El-Boray *et al.* (2012) and Abdur Rab *et al.* (2012) where they reported that thinning treatment reduced titratable acidity (TA) and increased TSS/TA ratio in fruit.

4.3.8 Inverted Sugar

The study result showed that all thinning treatments increased significantly inverted sugar content in fruit when compared to control treatment (Table 4.2). However, there was no significant difference between 15 fruits/branch and 20 fruits/branch treatments in inverted sugar content. Fruit thinned to 10 fruits/branch had the highest inverted sugar content at 9.48 ± 0.45 (°Brix) followed by 9.19 ± 0.27 (°Brix) recorded in 15 fruits/branch treatment and 9.09 ± 0.29 (°Brix) was recorder in 20 fruits/branch. The lowest inverted sugar content was 8.87 ± 0.35 (°Brix) recorded in the control treatment. This result is in agreement with Abdur Rab *et al.* (2012) study,

which indicated that thinning treatment of apricot increased inverted sugar in fruit. From the result it can be stated that adequate fruit thinning result in better fruit quality

4.3.9 Fruit Fructose Content

As showed in Table 4.2, all fruit thinning treatments had higher fructose content when compared to the control treatment. 10 fruits/branch had the heights fructose content when compared to all thinning treatments. Un-thinned fruits in control treatment showed significantly lower fructose content when compared to all thinning treatments. Fructose content of 9.35 ± 0.32 . (°Brix) was recorded in 10 fruits/branch treatment, followed by 15 fruits/branch treatment at 9.04 ± 0.23 . (°Brix), 8.97 ± 0.34 . (°Brix) was recorded in 20 fruits/branch and the least fructose content was recorded in control at 8.77 ± 0.44 (°Brix). This result is in agreement with Zhou *et al.* (2000) and Abdur Rab *et al.* (2012) where they indicated that thinning treatment increased fructose. It can be stated that reasonable thinning result in more accumulation of fructose in fruit and that leads to a better fruit quality.

4.3.10 Chlorophyll Content (SPAD)

Datt (1999) indicated that foliar chlorophyll content is a good indicator of plant stress and therefore of the potential for plant carbon dioxide uptake and growth. In our study, the chlorophyll content was determined by Minolta SPAD meter. The SPAD values in Table 4.3 showed that there were no significant differences among all treatments in SPAD values. The SPAD values were 61.20±5.67 in control treatment, 61.89±6.03 in 15 fruits/branch treatment 63.80±5.61 in 20 fruits/branch treatment, and 64.33±4.73 in 10 fruits/branch treatments (Table 4.3). However, there was a slight nonsignificant increase in SPAD value with decrees fruit load in branch. Wünsche *et al.* (2000) found that percentage tree light interception increased linearly as crop load decreased. It appeared from this result that fruit thinning treatment did not affect significantly the chlorophyll content in the leaves since there was no stress imposed on tree.

4.3.11 Fruit Juice

From Table 4.3, the percentage of fruit juice per 100 g was similar in all treatments and they did not differ significantly. The percentage of fruit juice recorded in control treatment was 73.76 ± 1.97 ml, and it was 74.09 ± 1.92 ml in 10 fruits/branch treatment and it was 73.91 ± 1.92 ml in 15 fruits/branch and it was 73.96 ± 1.92 ml in 20 fruits/branch treatment. This result is in agreement with Gonkiewicz *et al.* (2011) study, which, stated that content of the fruit juice after harvest was not affected by fruit thinning treatment. However, the fruit juice percentage was non significantly a little higher in 10 fruits/branch. This result is in agreement with study done Sawale *et al.* (2001) which indicated that less percentage of juice was found to be associated with maximum number of fruits.

4.3.12 Fruit Length

Fruit length significantly affected by thinning 10 fruits/branch and 15 fruits/branch treatments (Table 4.3 and Fig.4.2). All thinning treatments showed increase in the fruit length when compared to the control. However, there was no significant difference in fruit length between 20 fruits/ brans and control treatments. The fruit length in control treatment was 61.24±2.96 mm while they were 71.92±2.62 mm, 65.36±2.11 mm and 65.36±2.11 in 10 fruits/branch, 15 fruits/branch and 20 fruits/branch treatments respectively. It seemed that fruit length increased as the severity of thinning increased. This result with Link (2000) study, which pointed that fruit size, was directly related to thinning intensity. In addition, this study result is in agreement with Day and DeJong (1999) study, which indicated that thinning, improves fruit size in nectarines, peaches, and plums.

4.3.13 Fruit Width

Fruit width significantly affected by thinning 10 fruits/branch and 15 fruits/branch treatments (Table 4.3 and Fig.4.2). All thinning treatments showed increase in the fruit width when compared to the control. However, there was no significant difference in fruit width between 20 fruits/ brans and control treatments. The fruit width in control treatment was 39.26±2.47 mm while they were 49.35±1.71 mm, 44.39±2.05 mm and 39.99±2.47 in 10 fruits/branch, 15 fruits/branch and 20 fruits/branch treatments respectively. It seemed that fruit width increased as the severity of thinning increased. This result with Link (2000) study, which pointed that fruit size, was directly related to thinning intensity. In addition, this study result is in agreement with Day and DeJong (1999) study, which indicated that thinning, improves fruit size in nectarines, peaches, and plums.

4.3.14 Fruit Weight

Dennis (2000) noted that fruit thinning has been practiced for thousands of years for many purposes. Link (2000) pointed that fruit size was directly related to thinning intensity. All thinning treatments had positive effect on fruit weight. All thinning treatments resulted in bigger and heavier fruits when compared to non-thinned treatment (control) (Table 4.3, Fig 4.2 and Fig 4.3). The fruit weight increased significantly in 10 fruits/branch and 15 fruits/branch thinning treatments when compared with control (un thinned fruit). However, there was no significant difference between 20 fruits/branch and control treatment and both had almost the same average fruit weight. Average Fruit weight in control treatment was 44.75±3.65 g, in 20 fruits/branch was 49.38±2.90 g, in 15 fruits/branch was 60.20±3.39 g, and in 10 fruits/branch being the highest was 70.61±3.75 g. The increase in fruit weight was about 57% with 10 fruits/branch and about 34% with 15 fruits/branch treatment when compared to the control. However, there was a slight increase (about 10%) in fruit weight obtained with 20 fruits/branch thinning treatment when compared with the control. This result is in agreement with the result showed by Yeshitela *et al.* (2004) which found that thinning 'sensation' mango *Mangifera indica* fruits to one and two fruits per panicle increased fruit number, weight, and yield per tree at harvest. Additionally, in apricot cv. 'Trevett', Abdur Rab *et al.* (2012) found that thinning treatment increased significantly fruit weight.



Fig 4.2 Effect of thinning of wax apple; 10 fruits per branch, 15 fruits per branch, 20 fruits per branch and non-thinned as a control on fruit size at harvest under field condition.



Fig 4.3 Effect of fruit thinning of wax apple on fruit weight (g) under field condition. Bars with different letters are significantly different.

4.3.15 Branch Yield

There were significant differences among all treatments (Table 4.3 and Fig 4.4). Thinning fruit treatments had negative impacts on final yield in wax apple fruit. The highest yield was obtained in 20 fruits/branch treatment followed by the control treatment, 15 fruits/branch treatment and 10 fruits/branch treatment being the lowest in final yield; 0.99 ± 0.06 kg, 0.97 ± 0.12 kg, 0.90 ± 0.05 kg and 0.71 ± 0.04 kg respectively (Table 4.3 and Fig 4.4). However, fruit thinning increased size, enhanced colour and fruit quality and that generally compensate the reduction in final yield. This study is in agreement with Abdur Rab *et al.* (2012) study on apricot cv. 'Trevett', which found that thinning treatment increased significantly fruit weight but decreased the yield. In addition, Rettke (2005) indicated that thinning reduced yield. Son (2004) and Meland, (2009) stated that thinning treatments in apple increased fruit size, enhanced fruit quality and that usually compensate the yield reduction. From the previous discussion in
this study, it can be suggested that the best treatment in final yield and fruit quality was 15 fruits/branch.



Fig 4.4 Effect of fruit thinning of wax apple on yield (kg) under field condition. Bars with different letters are significantly different.

4.4 CONCLUSION

Our results (Tables 4.1, 4.2, and 4.3) showed that all thinning treatments increased anthocyanin significantly when compared to the control. The highest anthocyanin content was recorded in 10 fruits/branch. All thinning treatments showed a significant increase in total phenols. Result showed that all thinning treatments had higher pH value compared to the control. The highest significant pH was recorded at 10 fruits/branch treatment. Flavonoid content in fruit affected by thinning treatments. Flavonoid content was significantly higher in 10 fruits/branch. Results showed that fruits in 15 fruits/branch treatment had significantly a higher K^+ when compared to all treatments. Results showed that 10 fruits/branch and 15 fruits/branch treatments increased TSS content significantly when compared to control treatment. However, there was no significant difference in TSS content between control treatment and 20 fruits/branch treatment. All thinning treatments reduced TA when compared to control However, there were no significant differences in TA content among 20 fruits/branch and 15 fruits/branch and control treatments. The highest TSS/TA ratio was recorded in 10 fruits/branch followed by 15 fruits/branch and 20 fruits/branch. The least TSS/TA ratio was in the control treatment. All thinning treatments increased significantly inverted sugar content in fruit when compared to control treatment. However, there was no significant difference between 15 fruits/branch and 20 fruits/branch treatments. All fruit thinning treatments had higher fructose content when compared to the control. The 10 fruits/branch had the heights fructose content when compared to all thinning treatments. There were no significant differences among all treatments in leaves chlorophyll content (SPAD values). The fruit weight increased significantly in 10 fruits/branch and 15 fruits/branch thinning treatments when compared with control (un thinned fruits). However, there was no significant difference between 20 fruits/branch and control treatment and both had almost the same average fruit weight. The

percentage of fruit juice per 100 g was similar in all treatments and they did not differ significantly. Fruit length significantly affected by 10 fruits/branch and 15 fruits/branch treatments. All thinning treatments showed increase in the fruit width when compared to the control. However, there was no significant difference in fruit width between 20 fruits/branch and control treatments. All thinning treatments had positive effect on fruit weight. All thinning treatments resulted in bigger and heavier fruits when compared to the control. Thinning fruit treatments had negative impacts on final yield in wax apple fruit. The highest yield was obtained in 20 fruits/branch treatment followed by the control treatment, 15 fruits/branch treatments and 10 fruits/branch treatments being the lowest in final yield.

Theoretically, the best treatment in terms of fruit quality was 10 fruits/branch. However, 10 fruits/branch resulted in sever reduction in the final yield. Practicality, to compromise the reduction in final yield observed in 10 fruits/branch, it can be suggested that the best treatment in final yield and fruit quality (together) was 15 fruits/branch.

Thinning Treatment	Anthocyanin Total Phenols		nЦ	Total Flavonoid	K^+
Thinning Treatment	(mg/L)	(mg GAE/100g)	рп	(mg/100g)	(mg/kg)
Control	2.79±0.40d	346.33±17.46b	4.69±0.32b	14.51±2.66 b	16.21±0.92b
10 Fruits/Branch	4.26±0.31a	386.53±20.30a	4.97±0.41a	16.40±1.55 a	17.75±0.92a
15 Fruits/Branch	3.70±0.30b	362.20±27.61ab	4.80±0.38ab	15.09±2.32ab	16.41±0.85ab
20 Fruits/Branch	3.07±0.44c	358.60±27.08b	4.64±0.29b	14.73±2.31 b	16.29±0.96b

Table 4.1 Effects of different treatments of thinning on anthocyanin, pH, K⁺, total flavonoids and total phenols content of wax apple fruit under field condition.

Thinning Treatment	TA (%)	TSS (°Brix)	TSS/TA Ratio	Inverted sugar (°Brix)	Fructose (°Brix)
Control	0.80 ±0.5a	9.29±0.42b	11.59±0.95b	8.87±0.35c	8.77±0.44c
10 Fruits/Branch	$0.76 \pm 0.40b$	9.87±0.36 a	13.11±0.82a	9.48±0.45a	9.35±0.32a
15 Fruits/Branch	0.77±0.5ab	9.62±0.39a	12.52±1.01a	9.19±0.27ab	9.04±0.23ab
20 Fruits/Branch	0.79± 0.4a	9.34±0.26b	11.81± 0.68b	9.09±0.29b	8.97±0.34b

 Table 4.2
 Effects of different treatments of thinning on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, inverted sugar (°Brix) and fructose (°Brix) content of wax apple fruit under field condition.

Thinning	SPAD	Fruit weight	Branch yield	Juice fruit per	Fruit length	Fruit width
Treatment	Value	(g)	(kg)	100 (g)	(mm)	(mm)
Control	61.20±5.67a	44.75±3.65c	0.97±0.12a	73.76±1.97a	61.24±2.96c	39.26±2.47c
10 Fruits/Branch	64.33±4.73a	70.61±3.75 a	0.71±0.04c	74.09±1.92a	71.92±2.62a	49.35±1.71a
15 Fruits/Branch	61.89±6.03a	60.20±3.39b	0.90±0.05b	73.91±1.92a	65.36±2.11b	44.39±2.05b
20 Fruits/Branch	63.80±5.61a	49.38±2.90c	0.99±0.06a	73.96±1.92a	62.06±2.85c	39.99±2.47c

Table 4.3 Effects of different treatments of thinning on chlorophyll content (SPAD), fruit weight, juice (%), shape (length and width) and branch yield of wax apple fruit.

EFFECTS OF XYLEM INJECTION WITH GA₃ ON WAX APPLE FRUIT QUALITY

5.1 INTRODUCTION

The wax apple 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 drop, small fruit size and less colour and 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. The wax apple fruits are pear shaped, often juicy, with a subtle sweet taste and aromatic flavour. In Malaysia, the fruits of jambu air are eaten raw with salt or cooked as a sauce and more than ninety per cent of the fruit is edible. It has been reported that wax apple become an increasingly popular fruit in the tropical region where it can fetch a price of up to 3USD per kilogramme and has the potential to bring great benefits to local farmers and the country's economy (Khandaker et al., 2012). The term 'plant growth regulator' (PGRs) is usually employed for plant hormones or substances of similar effect that are administered to plants. PGRs include a large group of chemical compounds that can regulate plant growth. Hormones are substances naturally produced by plants, substances that control normal plant functions, such as root growth, fruit set and drop, growth and other development processes There are five plant hormone categories; these are auxins, cytokinins, gibberellins, abscisic acid and ethylene (Wang and Irving, 2011). Hormones are substances naturally produced by plants. Plant growth regulators play multiple roles in the regulation of plant growth and development. Plant growth regulators (PGR) exist almost in all plant parts. Pharis and King (1985) indicated that fruit development generally is dependent on the interactions of five major classes of plant hormones (auxin, GAs, cytokinin, ABA, and ethylene). Atwell et al. (1999) stated that (PGRs) play a crucial role in fruit, seeds growth and development. Gibberellic Acid

 (GA_3) is a hormone found in plants. Its chemical formula is $C_{19}H_{22}O_6$. When purified, it is a white to pale-yellow solid (Figure 2.2) Davies (1995) and Crozier et al. (2000) indicated that gibberellins are tetracyclic diterpenoid acids that play a significant role in a number of developmental and physiological processes in plants. It should be emphasized that gibberellins are known to interact with other hormones (Cohen et al., 2001). Kappel and MacDonald. (2002) stated that growers in British Columbia, Canada and the US Pacific Northwest use gibberellic acid (GA_3) to improve fruit quality of sweet cherries (Prunus avium L.). Looney (1993) Indicated that gibberellins application are used on several crops to improve fruit appearance and internal condition but they also have potential to reduce pesticide usage. In addition, GA is used commonly in commercial crops production for enhancing fruit set and also to control some physiological diseases such as cracking of pomegranate fruit (Sepahi, 1986) and litchi (Sharma and Dhillon, 1986) apple fruit russeting (Taylor and Knight, 1986) and to hinder flowering of Prunus species (Coneva and Cline 2006; Lenahan et al. 2006). Many scientific reports pointed at the role of gibberellins in plants include seed germination, seedling emergence, stem and leaf growth, floral induction and flower and fruit growth (Sponsel, 2002; King and Evans 2003). Earlier study on gibberellic (GA₃) showed that it has been used commonly in horticultural crops for enhancing fruit set (Taylor and Knight, 1986). Raven et al. (1992) indicated that gibberellins have been found to stimulate plant growth by increasing the extensibility of cell walls and thus allowing cell expansion.

In the effort of improving fruit quality by using new methods, this chapter introduces a new technique of injection plant growth regulators into wax apple tree branches via *Xylem Injection*. This chapter reports the results of three levels of xylem injection with gibberellic acid (GA_{3}) on wax apple fruit development and quality parameters under the field's condition. The goal of this study is to develop larger fruit

with better quality by using GA₃ through Xylem injection (a new introduced method to inject GA₃ directly into tree).

5.2 MATERIALS AND METHODS

5.2.1 Experimental Site

The study was carried out in a commercial orchard located at Banting, Selangor, Malaysia, 2°30N, 112°30E and 1°28 N, 111°20E at an elevation of about 45 m above sea level. The area under study has a hot and humid tropical climate. The soil in the orchard is peat with a mean pH of around 4.6 (Ismail *et al.*, 1994). The pH of the soil was neutralized by adding poultry manure and organic fertilizers before launching the experiments. Experiment was carried out between April 2011 to July 2011.

5.2.2 Treatment Methods

Thirteen years old, wax apple trees were selected for the study. The trees were planted in a 4.2×4.2 m hexagonal pattern and received the same agricultural management; fertilization, pruning and thinning of excess bud and fruits. Three trees were selected for each treatment and twelve trees used in the experiments. Sixty uniform branches (five branches per tree) of about the same length and diameter from twelve trees were selected for the experiments. The experiments consist of 4 GA_3 concentration including control (Control, 30 ppm, 60 ppm and 90 ppm GA₃) with three replications and five sub-replications (15 replicates for each treatment). A single tree was taken as an experimental unit. GA₃ injected into selected branches through small needle four times; first at full blooming (FB), second after 2 weeks of FB, third after 4 week of FB, the fourth application was after 6 week of FB. Method of GA₃ Xylem Injection is illustrated in Fig 5.1. A small drill was used to make a tiny whole in the branch to penetrate the hard tissues reaching to the core of branch, and then we used a suitable syringe with sharp needle. Needle inserted gently into the tiny whole gently, and then assigned GA₃ concentration injected by applying hand pressure for about 15-20 minute or until the 10-ml GA₃ solution injected successfully into the branches.



Fig 5.1 Introducing a new method to inject plant growth regulators (GA₃) into tree branch using small drill and injector.

5.2.3 Total Anthocyanin Content

(Same as described in Chapter 3).

5.2.4 The Total Phenolic Content (TPC)

(Same as described in Chapter 3).

5.2.5 Total Flavonoid Content (TFC)

(Same as described in Chapter 3).

5.2.6 Fructose and Inverted Sugar

(Same as described in Chapter 3).

5.2.7 Total Soluble Solid (TSS) Content

(Same as described in Chapter 3).

5.2.8 pH Measurement

(Same as described in Chapter 3).

5.2.9 Titrable Acidity and TSS/TA Ratio

(Same as described in Chapter 3).

5.2.10 Bud Dropping (%)

(Same as described in Chapter 3).

5. 2.11 Fruit Set

(Same as described in Chapter 3).

5.2.12 Fruit Dropping (%)

(Same as described in Chapter 3).

5.2.13 The Chlorophyll Content (SPAD value)

(Same as described in Chapter 3)

5.2.14 Fruit Size and Development

(Same as described in Chapter 3).

5.2.15 Fruit Weight

(Same as described in Chapter 3).

5.2.16 Fruit Yield

(Same as described in Chapter 3).

5.2.17 Fruit Juice

(Same as described in Chapter 3).

5.2.18 Measurement of the K⁺ Content in Fruit Juice

(Same as described in Chapter 3).

5.2.19 Statistical Analysis

(Same as described in Chapter 3).

5.3 RESULTS AND DISCUSSION

5.3.1 Anthocyanin Content

All GA_3 treatments (90, 60 and 30 ppm) had positive effect on anthocyanin accumulation and there was a correlation between TSS and anthocyanin (Fig 5.2). All GA_3 treatments enhanced anthocyanin concentration in wax apple fruit (Table 5.1). The highest anthocyanin content was 4.58±0.47 (mg/L) recorded in GA₃ treatment at 90 ppm followed by GA₃ at 60 ppm with anthocyanin content 3.96±0.31 (mg/L), followed by GA₃ treatment at 30 ppm with anthocyanin content 3.29 ± 0.45 (mg/L), and the lowest anthocyanin content was 2.77±0.40 (mg/L) recorded at the control treatment. This result is in agreement with Montero et al. (1998) study, which indicated that the anthocyanin content and PAL activity are enhanced by the exogenous treatment of GA_3 in strawberry. In addition, our result is in agreement with the study done by Ali et al. (2012) which indicated that application of GA_3 enhanced anthoryanin and photosynthetic pigments in *Hibiscus sabdariffa* L., and with Roussos et al. (2009) who found that anthocyanin content increased significantly by GA₃ treatment in strawberry. Similar results obtained by Weiss and Halevy (1989) indicated that when young green corollas were detached and grown in a sucrose medium, GA₃ induced colouring pigmentation.

5.3.2 Total Phenols Content (TFC)

Regarding the total phenol content, all GA₃ treatments showed a significant increase in total phenols when compared to the control treatment (Table 5.1). The highest phenol content was recorded in 90 ppm GA₃ treatment at 464.27 \pm 36.02 GAE/100g followed by 60 ppm GA₃ treatment at 419.20 \pm 27.61 GAE/100g, followed by 30 ppm GA₃ treatment at 408.93 \pm 28.69 GAE/100g. The lowest phenols content was recorded in the control treatment at 349.33 \pm 17.46 GAE/100g. This result supported by Raifa *et al.* (2005) study, which indicated that total phenols increased by GA₃ treatment

in *Hibiscus sabdariffa* L. and with Khandaker *et al.* (2012) who indicated that GA_3 application increase phenol content in wax apple fruit.



Fig 5.2 Correlation between TSS and anthocyanin content in GA₃ treated wax apple.

5.3.3 Measurement of pH

Study result showed that all GA₃ treatments had higher pH value compared to the control (Table 5.1). The highest significant pH value was recorded at 90 ppm GA₃ treatment with 5.25 ± 0.22 pH followed by 60 ppm GA₃ treatment with 5.04 ± 0.32 pH. Lower pH values were observed in 30 ppm GA₃ treatment and the control; 4.94 ± 0.35 and 4.93 ± 0.34 respectively. However, there were no significant difference between the control and 30 ppm GA₃ treatment. This result is agreement with Khandaker *et al.* (2012) who indicated that GA₃ application increased pH value in wax apple fruit. In addition, Thakur *et al.* (1996) showed that the acidity of tomato fruits was reduced when the plant was sprayed with GA_{3.} Similar result obtained by Varma (1991) who observed significant decreased in acidity under the gibberellic acid treatments.

5.3.4 Total Flavonoid Content (TFC)

This study showed that flavonoid affected by GA₃ treatment. Flavonoid content was significantly higher in 90 ppm GA₃ followed by 60 ppm GA₃ treatments (Table 5.1). However, the study revealed that there was no significant difference between 30-ppm GA₃ treatment and the control. It is clear that GA₃ treatments increased the accumulation of flavonoid in fruit. The highest flavonoid content was recorded in 90 ppm GA₃ treatment at 18.75±2.36 (mg/100g), followed by 60 ppm GA₃ treatment at 16.16±2.69 (mg/100g). Lower flavonoids content were recorded in 30 ppm GA₃ and control treatment at 13.59±2.70 (mg/100g) and 12.66±2.54 (mg/100g) respectively. Our result is in agreement with Khandaker *et al.* (2012) who indicated that GA₃ application increased the flavonoid content.

5.3.5 K⁺ Content

Results showed that all treatments with GA₃ had a significantly higher K⁺ when compared to control treatment (Table 5.1). However, there was no significant difference between 60 ppm and 30 ppm GA₃ treatment. The highest K⁺ concentration was recorded in 90 ppm GA₃ treatment at 18.25±0.92 (mg/kg) followed by 60 ppm GA₃ treatment at 17.41±0.85b (mg/kg) and 30 ppm GA₃ treatment at 17.29±0.96. The lowest K⁺ concentration was recorded in the control treatment at 15.22±0.89 (mg/kg). This result is in agreement with Zuo (2006) who indicated that GA₃ could significantly enhance K⁺ content in strawberry fruit. Similar result obtained by Alamgir and Kutube (2000) indicated that GA₃ treatment increased K⁺ content in wheat (*Triticum aestivum* L.) seedlings. In addition, our result is in agreement with Shomeili *et al.* (2011) study, which indicated that exogenous gibberellic acid (GA₃) increased the K⁺ contents in sugarcane plants. Hence, K^+ is important in vegetative growth and in the production of good quality fruit (Ozbun *et al.*, 1967).

5.3.6 Total Soluble Solids (TSS)

Total soluble solid (TSS) is a main component of fruit quality that contributes to the sweetness of fruit. Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. Results in Table 5.2, indicated that 90 ppm and 60ppm GA₃ treatments significantly increased the TSS in fruits when compared to the control and 30 ppm GA₃ treatment. However, there was no significant difference between 30 ppm GA₃ and the control treatments. In addition, there was no significant difference in TSS between 90 ppm and 60 ppm GA₃ treatments. The highest TSS was recorded in 90 ppm GA₃ treatment at 9.92 ± 0.27 (°Brix), followed by 60 ppm treatment at 9.83 ± 0.34 (°Brix). The lowest TSS content was 9.23 ± 0.29 (°Brix) recorded in control treatment, followed by 30 ppm GA₃ treatment at 9.60 ± 0.29 (°Brix). This result is in agreement with Wahdan *et al.* (2011) study who indicated that application of GA₃ significantly increased soluble solids in sweet cherry fruit (Basak *et al.*, 1998).

5.3.7 Titratable Acidity (TA) and TSS/TA Ratio

Fruit acidity is main factor contributes to fruit taste. Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. Result showed that 90 ppm and 60 ppm GA₃ treatments significantly reduced TA when compared to control treatment (Table 5.2). However, there was no significant difference in TA between 30 ppm GA₃ treatment and the control. In addition, there was no significant difference between 90 ppm GA₃ and 60 ppm GA₃. The highest TA was observed in the control treatment at $0.81\pm0.05\%$ followed by 30 ppm GA₃ treatment at $0.79\pm0.04\%$. The lowest TA was observed in 90 ppm GA₃ treatment at $0.79\pm0.04\%$.

followed by 60 ppm GA₃ treatment. This result is in agreement with Reynolds and Savigny (2004) study, which indicated that in table grapes cv 'Sovereign Coronation', GA₃ treatments produced slight increases in °Brix and pH and decreases in titratable acidity (TA).

Regarding the TSS/TA ratio (the sweetness index), from the result (Table 5.2), the 90 ppm and 60 ppm GA₃ treatment increased significantly the TSS/TA ratio. However, there was no significant difference in TSS/TA ratio between 90 ppm GA₃ treatment and 60 ppm GA₃ treatment in TSS/TA ratio. In addition, there was no significant difference between 30 ppm GA₃ treatment and the control in TSS/TA ratio. The highest TSS/TA ratio was observed in 90 ppm GA₃ treatment at 13.45 \pm 0.86 followed by 60 ppm GA₃ treatment at 13.07 \pm 0.72. The lowest in TSS/TA ratio recorded in the control followed by 30 ppm GA₃ treatment at 12.42 \pm 0.73. The significant increases in TSS/TA ratio in 90 and 60 ppm GA₃ treatments were due their significant increases in TSS and decreases in TA. Our result is in agreement with Al-Najdawi *et al.* (2007) study, which indicated that Spraying 15 ppm GA₃ on strawberry after three weeks of planting increased TSS, ascorbic acid, anthocyanin, and TSS/TA ratio, and a decrease in TA in fruits.

5.3.8 Inverted Sugar

The study result showed that all GA₃ treatments increased significantly inverted sugar content in fruit when compared to control treatment (Table 5.2). However, there were no significant differences among 90 ppm, 60 ppm and 30 ppm GA₃ treatments in inverted sugar content. 90 ppm GA₃ treatment had the highest inverted sugar content at 9.78 ± 0.29 (°Brix) followed by 9.75 ± 0.35 (°Brix) recorded in 60 ppm treatment and 9.54 ± 0.26 (°Brix) was recorder 30 ppm GA₃ treatment. The lowest inverted sugar content sugar content was 9.13 ± 0.29 (°Brix) observed in the control treatment. Our result is in agreement with Saleem *et al.* (2008) study, which found that in 'Blood Red' Sweet

Orange, GA₃ treatments improved significantly reducing sugars (inverted sugar), nonreducing sugars and total sugars when compared to the control.

5.3.9 Fruit Fructose Content

As showed in Table 5.2, all GA₃ treatments (30 ppm, 60 ppm and 90 ppm GA₃) treatments had significant higher fructose contents when compared to the control treatment. However, there were no significant differences among all GA₃ treatments in fructose contents. Frits in the control treatment exhibited significantly lower fructose content when compared to all GA₃ treatments. The highest fructose content recorded in 90 ppm GA₃ treatment at 9.37±0.27 (°Brix) followed by 60 ppm GA₃ treatment at 9.35±0.38 (°Brix), followed by 30 ppm GA₃ treatment at 9.28±0.27 (°Brix) respectively. The lowest fructose content was 8.78 ± 0.30 (°Brix) was recorded in the control. It can be stated that GA₃ application improved the fructose content in wax apple fruit. Our result is in agreement with Saleem *et al.* (2008) study, which found that in 'Blood Red' Sweet Orange, GA₃ treatments improved significantly reducing sugars (inverted sugar), non-reducing sugars and total sugars when compared to the control.

5.3.10 Chlorophyll Content (SPAD)

Datt (1999) indicated that foliar chlorophyll content is a good indicator of plant stress and therefore of the potential for plant carbon dioxide uptake and growth. In our study, the chlorophyll content was determined by Minolta SPAD meter. The SPAD values in Table 5.3 showed that there were no significant differences among all GA₃ treatments in SPAD values. The SPAD values were 62.56 ± 5.42 in control treatment, 59.16 ± 5.93 in 30 ppm GA₃ treatment, 61.65 ± 4.05 in 60 ppm GA₃ treatment, and 60.58 ± 5.53 in 90 ppm GA₃ treatments (Fig. 5.3). It appeared from this result that GA₃ xylem injection treatment did not affect significantly the chlorophyll content in the leaves and the result obtained from the control was similar to those in all GA₃ treatments. Hence, in this study, GA₃ has not been applied to leaves.



Fig 5.3 Effect of different GA₃ treatment on wax apple leaves chlorophyll content (SPAD). Bars with different letters are significantly different.

5.3.11 Fruit Weight

GA₃ treatments had a positive effect on fruit weight. As shown in Table 5.3, Fig. 4.3 and Fig 5.4, fruit weight increased significantly by all GA₃ treatments when compared with control. The highest fruit weight was observed in 90 ppm GA₃ treatment at 67.19 ± 4.07 g followed by 60 ppm GA₃ treatment at 56.59 ± 3.93 g and 47.28 ± 3.15 g in 30 ppm GA₃ treatment. The lowest fruit weight was recorded in the control treatment at 43.74 ± 3.81 g. Our result is in agreement with Katiyar *et al.* (2008) study, which indicated that GA₃ foliar application at 90 ppm gave maximum fruit size and specific gravity and the lowest fruit acidity. Singh and Lal (1980) likewise, indicated that application of GA₃ at 50-100 mg/l at full bloom improved fruit retention and fruit size. Berhow (2000) indicated that Foliar application of various levels of GA₃ (5, 50, 100 and 500 ppm) to young grapefruit fruitlets just after fruit set increase the fruit weight, peel thickness, juice content and taste.



Fig 5.4 Effects of different GA₃ injection treatments; 90, 60, 30 ppm GA₃ and control on fruit size of wax apple fruits.



Fig 5.5 Average fruit weight at harvest as influence by GA₃ applications. Bars with different letters are significantly different.

5.312 Fruit Juice

From Table 5.3, the percentage of fruit juice per 100 g fruit was similar in 30 ppm GA_3 and 60 ppm GA_3 and the control treatments, and they did not differ

significantly. There was a significant difference in percentage of fruit juice recorded in 90 ppm GA₃ treatment when compared to all other treatments. However, the difference was very slim. The highest juice percentage was record in 90 ppm GA₃ treatment at 77.97 \pm 1.67 ml followed by 60 ppm GA₃ treatment at 76.641 \pm 1.60 ml and 30 ppm GA₃ treatment at 76.78 \pm 1.80 ml. the lowest fruit juice percentage among all treatments was recorded in the control treatment at 75.72 \pm 1.91 ml. Our result is in agreement with the finding by Davies *et al.* (1999) study which indicated that gibberellic acid applied to oranges at about colour break can increase juice yield 2-10% compared to non-treated fruit.

5.3.13 Fruit Length

GA₃ treatment had a positive effect in fruit length (Table 5.3). All GA₃ treatments showed increase in the fruit length when compared to the control. The highest fruit length was recorded in 90 ppm GA₃ at 73.92 \pm 3.36 mm followed by 60 ppm GA₃ treatment at 67.36 \pm 3.04, and 30 ppm GA₃ treatment at 64.06 \pm 3.21 mm. The lowest fruit length was observed in control treatment at 60.57 \pm 2.73. As shown in (Fig 5.5), GA₃ treatment at 60 and 90 ppm accelerated significantly wax apple fruit growth over time. However, GA₃ at 30 ppm had growth pattern similar to the control. Our result is in agreement with Katiyar *et al.* (2008) study, which indicated that GA₃ foliar application at 90 ppm gave maximum fruit size and specific gravity and the lowest fruit acidity. Singh and Lal (1980) also indicated that application of GA₃ at 50-100 mg/l at full bloom improved fruit retention and fruit size. Berhow (2000) indicated that foliar application of various levels of GA₃ (5, 50, 100 and 500 ppm) to young grapefruit fruitlets just after fruit set increase the fruit weight, peel thickness, juice content and taste. Coneva and Cline (2006) indicated also that GA₃-treated trees had larger fruit size mean and improved fruit size distribution the year after GA₃ application.



Fig 5.5 Fruit growth (length)/week as influenced by different applications of GA_{3.} Lines with same letter are not statically significant.

5.3.14 Fruit Width

Fruit width positively affected by GA₃ treatments (Table 5.3). 90 ppm GA₃ and 60 GA₃ treatments showed increases in the fruit width when compared to 30 ppm GA₃ and the control treatments. There was no significant difference between 90 ppm GA₃ treatment and 60 ppm GA₃ treatments. In addition, there was no significant difference between 30 ppm GA₃ and the control. The highest fruit width was recorded in 90 GA₃ ppm treatment at 44.87±1.96 mm followed by 60 ppm GA₃ treatment at 43.36±2.61. The lowest fruit width was observed in the control treatment at 37.65±2.85 mm followed by 30 ppm GA₃ applications at 60 and 90 ppm (Fig 5.6). However, fruit growth overtime in the control and GA₃ at 30 ppm was almost same. Our result is in agreement with Coneva and Cline (2006) study, which indicated that GA₃-treated trees had larger mean fruit size, and improved fruit size distribution the year after GA₃ application. In addition, Singh and Lal (1980) indicated that application of GA₃ at 50-100 mg/l at full bloom improved fruit size and retention.



Fig 5.6 Fruit growth (width)/week as influenced by different applications of GA₃. Lines with same letter are not statically significant.

5.3.15 Bud Drop (%)

Buban and Faust (1982) found that gibberellic acid (GA₃) is involved in control of flower bud development. GA₃ treatment had a positive effect in bud drop. As shown in Table 5.4, all GA₃ treatments reduced significantly bud drop when compared to untreated GA₃ branches (control). The highest significant reduction in bud drop was observed in 90 ppm GA₃ treatment at $38.93\pm2.46\%$, followed by 60 ppm GA₃ treatment at $39.73\pm3.77\%$ and 30 ppm GA₃ treatment at $43.60\pm1.96\%$. The highest bud drop was observed in the control treatment at $48.43\pm2.90\%$. Our result is in agreement with Jawanda *et al.* (1974) study which stated that all concentrations of GA₃ (5, 10, 20 and 50 ppm) brought about greater reduction in bud drop when applied at earlier stage of cluster development of Thompson Seedless grape (*Vitis vinifera* L.). In addition, Almedia *et al.* (2004) indicated that endogenous hormones and their balance play regulatory roles in the deployment of nutrients to the developing organs and can affect the longevity of a bud.

5.3.16 Fruit Set (%)

Almedia *et al.* (2004) indicated that endogenous hormones and their balance play regulatory roles in the deployment of nutrients to the developing organs. All GA₃ treatment had positive effects on fruit set. As shown in Table 5.4, GA₃ treatments increased significantly the fruit set in wax apple when compared to the control treatment. The highest fruit set was obtained by 90 ppm GA₃ treatment at $61.93\pm4.08\%$ followed by 60 ppm GA₃ treatment at $57.13\pm4.21\%$ and 30 ppm GA₃ treatment at $54.67\pm3.24\%$. The lowest fruit set was observed in the control treatment at $45.40\pm2.29\%$. This result is in agreement with Ogilvie et *al.* (1991) study which indicated that garden rose's fruit set was higher when GA₃ was applied to the stigma at the rate of 250 ppm ten days after pollination. In addition, on 'Le Conte' pear cultivar, Hegazi (2011) found that GA₃, treatment at 50, 100 and 150 ppm, improved fruit set and fruit characteristics when compared to the control.

5.3.17 Fruit Drop (%)

All GA₃ treatment had positive effect on fruit drop. GA₃ treatments reduced fruit drop significantly when compared to the control (increased fruit retention) (Table 5.4). The highest reduction in fruit drop was observed in 90 ppm GA₃ treatment $30.53\pm2.47\%$ at followed by 60 ppm GA₃ treatment at $37.20\pm2.08\%$ and 30 ppm GA₃ treatment at $41.53\pm3.7\%$. The lowest reduction in fruit drop was recorded in the control treatment at $46.20\pm3.7\%$. Our result is in agreement with Chen *et al.* (2006) study, which indicated that application of gibberellic acid (GA₃) on sweet orange significantly increased fruit retention. In addition, our result similarly in agreement with Al-Qurashi *et al.* (2012) study which indicated that the applications of GA₃ (100 and 150 ppm) significantly decreased fruit drop in 'Rothana' and 'Ghur' date palm cultivars.

5.3.18 Branch Yield

There were significant differences among all treatments (Table 5.4). All GA₃ treatments had significantly positive effects on branch yield. The highest yield was obtained by 90 ppm GA₃ treatment at 1.88 ± 0.18 kg followed by 60 ppm GA₃ treatment at 1.42 ± 0.14 kg and 30 ppm GA₃ treatment at 1.04 ± 0.13 kg. The lowest yield was observed in the control treatment at 0.80 ± 0.12 kg. The 90 ppm GA₃ treatment almost doubled the branch yield when compared to the control treatment. Our result is in agreement with Williamson *et al.* (1996) study which indicated that 250 ppm GA₃ sprays at 80-90% full bloom and again 10 days later increased fruit set and yield of 'Rabbiteye' blueberries (*Vaccinium ashei* Reade). In addition, yield in GA₃-treated plants were increased by about 100% over controls.



Fig 5.7 The increase percentages of branch yield as influenced by different GA₃ applications. Bars with different letters are significantly different.

5.4 CONCLUSION

All GA₃ treatments (90, 60 and 30 ppm) had positive effect on anthocyanin accumulation. GA₃ treatments enhanced anthocyanin concentration in wax apple fruit. The highest anthocyanin content was recorded in 90 ppm GA₃ treatment followed by 60 ppm and 30 ppm GA₃ treatments. All GA₃ treatments significantly increased the total phenols when compared to the control treatment. The highest phenol content was recorded in 90 ppm GA₃ treatment followed by 60 ppm GA₃ treatment and finally 30 ppm GA₃ treatment. The lowest phenols content was recorded in the control treatment at Study result showed that all GA₃ treatments had higher pH value compared to the control. The highest significant pH value was recorded at 90 ppm GA₃ treatment. Significantly, lower pH values were observed in 30 ppm GA₃ treatment and the control. This study showed that flavonoid affected by GA₃ treatment. Flavonoid content was significantly higher in 90 ppm GA₃ followed by 60 ppm GA₃ treatments. However, the study revealed that there was no significant difference between 30 ppm GA₃ treatment and the control. All GA₃ treatments had a significantly higher K^+ when compared to control treatment. However, there was no significant difference between 60 ppm and 30 ppm GA₃ treatments. Results indicated that 90 ppm and 60 ppm GA₃ treatments significantly increased the TSS in fruits when compared to the control and 30 ppm GA₃ treatments. However, there was no significant difference between 30 ppm GA₃ and the control treatments. In addition, there was no significant difference in TSS between 90 ppm and 60 ppm GA_3 treatments. The highest TSS was recorded in 90 ppm GA_3 treatment. The lowest TSS content was recorded in control treatment, followed by 30 ppm GA_3 treatment. Result showed that 90 ppm and 60 ppm GA_3 treatments significantly reduced TA when compared to control treatment. The highest TSS/TA ratio was observed in 90 ppm GA₃ treatment followed by 60 ppm GA₃ treatment. The lowest TSS/TA ratio was recorded in the control followed by 30 ppm GA₃ treatment. The significant increases in TSS/TA ratio in 90 and 60 ppm GA₃ treatments were due their significant increases in TSS and decreases in TA. All GA₃ treatments increased significantly inverted sugar content in fruit when compared to control treatment. All GA₃ treatments (30 ppm, 60 ppm and 90 ppm GA₃) treatments had significant higher fructose contents when compared to the control treatment. However, there were no significant differences among all GA₃ treatments in fructose contents. There were no significant differences among all GA₃ treatments in SPAD values. GA₃ treatments had a positive effect on fruit weight. The fruit weight increased significantly by all GA_3 treatments when compared with control. The highest fruit weight was observed in 90 ppm GA₃ treatment followed by 60 ppm GA₃ treatment and 30 ppm GA₃ treatment. The lowest fruit weight was recorded in the control treatment. The percentages of fruit juice per 100 g fruit were similar in 30 ppm GA₃ and 60 ppm GA₃ and the control treatments, and they did not differ significantly. There was a significant difference in percentage of fruit juice recorded in 90 ppm GA₃ treatment when compared to all other treatments. However, the difference was very slim. All GA₃ treatments showed increase in the fruit length when compared to the control. The highest fruit length was recorded in 90 ppm. Fruit width positively affected by GA₃ treatments. 90-ppm GA₃ and 60 ppm GA₃ treatments exhibited a significant increase in the fruit width when compared to 30 ppm GA₃ and the control treatments. All GA₃ treatments reduced significantly bud drop when compared to un-treated GA₃ branches (control). The highest significant reduction in bud drop was observed in 90 ppm followed by 60 ppm treatment. All GA₃ treatments increased significantly the fruit set in wax apple fruit when compared to the control. All GA₃ treatments had positive effect on fruit drop. GA₃ treatments reduced significantly fruit drop percentages when compared to the control. All GA₃ treatments had significantly positive effects on branch yield. The 90-ppm GA_3 treatment almost doubled the branch yield when compared to the control treatment. The highest yield was

observed in 90 ppm GA3 treatment followed by 60 ppm GA3 treatment. The lowest branch yield was recorded in the control treatment. Therefore, it can be concluded that 90 ppm GA₃ treatment via xylem injection, was the best treatment in teams of quality and yield.

GA ₃ Treatments	Anthocyanin mg/L	Total phenols (mg GAE/100g)	РН	Total Flavonoid (mg/100g)	K ⁺ (mg/kg)
Control	2.77±0.40 d	349.33±17.46 d	4.93±0.34b	12.66±2.54c	15.22±0.89d
30 ppm	3.29±0.45c	408.93±28.69 c	4.94±.35b	13.59±2.70c	17.29±0.96c
60 ppm	3.96± .31b	419.20±27.61 b	5.04±.32b	16.16±2.69b	17.41±0.85bc
90 ppm	4.58 ±.47a	464.27±36.02 a	5.25±.22a	18.75±2.36a	18.25±0.92a

Table 5.1 Effects of different treatments of xylem injection with GA₃ on anthocyanin, pH, K⁺, flavonoids and total phenols content of wax apple fruit under field condition.

Table 5.2	Effects of different treatments of xylem injection with GA3 on titratable acidity (TA), total soluble solids
	(TSS), TSS/TA ratio, inverted sugar (°Brix) and fructose (°Brix) content of wax apple fruit under field
	condition.

GA ₃ Treatments	TA (%)	TSS (°Brix)	TSS/TA ratio	Inverted sugar (°Brix)	Fructose (°Brix)
Control	0.81±0.05a	9.23±0.29b	$11.49 \pm 0.74b$	9.13±029b	8.78±0.30b
30 ppm	0.79 ±0.04a	9.60±0.29b	$12.42 \pm 0.73b$	9.54±0.26a	9.28±0.27a
60 ppm	$0.76 \pm 0.03b$	9.83±0.34a	13.07± 0.72a	9.75±0.35a	9.35±0.38a
90 ppm	$0.74 \pm 0.04 b$	9.91±0.27a	13.45±0.86a	9.78±0.29a	9.37±0.27a

GA ₃ Treatments	chlorophyll content (SPAD)	Fruit weight (g)	Juice per 100 gm fruit (ml)	Fruit length (mm)	Fruit width (mm)
Control	62.56±5.42a	43.74±3.81d	75.72±1.91b	60.57±2.73d	37.65±2.85b
30 ppm	59.16±5.93a	47.28±3.15c	76.78±1.80b	64.06±3.21c	38.66±2.59b
60 ppm	61.65±4.05a	56.59 ±3.93b	76.641±1.60b	67.36±3.04b	43.36 ±2.61a
90 ppm	60.58±5.53a	67.19 ±4.07a	77.97±1.67a	73.92±3.36a	44.87±1.96a

Table 5.3 Effects of different treatments of xylem injection with GA₃ on chlorophyll content (SPAD), fruit weight, juice (%), fruit length and fruit width of wax apple fruit under field condition.

GA ₃ Treatments	Fruit set (%)	Fruit drop (%)	Bud drop (%)	Branch yield (kg)
Control	45.40±2.29d	46.20±3.7d	48.43±2.90b	0.80 ±0.12d
30 ppm	54.67±3.24c	41.53±3.7c	43.60±1.96b	1.04 ±0.13c
60 ppm	57.13±4.21b	$37.20 \pm 2.08b$	39.73±3.77a	1.42 ±0.14b
90 ppm	61.93±4.08a	30.53±2.47a	38.93 ±2.46a	1.88 ±0.18a

Table 5.4 Effects of different treatments of xylem injection with GA₃ on bud drop, fruit drop, fruit set, and branch yield of wax apple.

APPLICATION OF ABA TO ENHANCE WAX APPLE FRUIT QUALITY

6.1 INTRODUCTION

The wax apple 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 drop, small fruit size and less colour and 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. It has been reported that wax apple become an increasingly popular fruit in the tropical region where it can fetch a price of up to 3 USD per kilogram and has the potential to bring great benefits to local farmers and the country's economy (Khandaker *et al.*, 2012).

ABA plays a major role in various aspects of plant growth, development and adaption of environmental stresses as well as fruit growth (Rock and Quatrano, 1995). Leung and Giraudat (1998) reported that ABA can promotes seed maturation and germination, and act as a signaling molecule when plants are under stresses such as drought, high salinity, cold and microbial infections. It is also consider that ABA plays an important role in fruit ripening, stimulates sugar accumulation in fruits, thus improving fruit and quality (Xia *et al.*, 2000). ABA also participates in the initiation of ripening and related changes in grape development (Deytieux-Belleau *et al.* 2007). It has been reported that treatments of ABA increase the anthocyanin content in grape skins (Ban *et al.*, 2003) and improved the colour and quality of the grapes (Cantín *et al.*, 2007). Lacampagne *et al.* (2009) reported that ABA regulates enzymes involved in tannin and flavonoid biosynthesis. Exogenous application of ABA significantly stimulated the biosynthesis of ethylene and accelerated the fruit ripening (Chan and Zhang, 2000). Abscisic Acid (ABA) belongs to a class of metabolites known as isoprenoids, also called terpenoids and the level of abscisic acid (ABA) in any specific

tissue in a plant is determined by the rate of biosynthesis and catabolism of the hormone (Nambara and Marion-Poll ,2005) (Fig 2.1). In plants, ABA is a cleavage product of xanthophylls (especially of violaxanthin). ABA can be metabolized by glycosylation or by oxidation, to produce 8'-hydroxy-ABA that is converted into phaseic acid (Cutler and Krochko, 1999). ABA is a plant hormone plays a key role in various aspects of plant growth, development and adaption of environmental stresses (Rock and Quatrano, 1995; Seo and Koshiba, 2002). There are numerous scientific reports pointed at various effects of ABA applications on fruit quality. Giora (2012) indicated that the phytohormone abscisic acid (ABA) affects a wide range of stages of plant growth as well as the plant's response to biotic and abiotic stresses. Management of ABA signalling in commercial crops holds favourable potential for improving crop yields. Zahrah et al. (2012) found that the exogenous application of 1.0 mm ABA accelerated ethylene production, respiration rate, fruit skin colour development and softening as well as rheological properties of pulp (hardness, springiness, cohesiveness, chewiness, adhesiveness and stiffness) compared to the control. In addition, exogenous application of ABA advanced accumulation of total sugars and reduction in total organic acids during fruit ripening compared to changes in control fruit. Peppi et al. (2008) found that the application of abscisic acid at veraison to Crimsom Seedless increased fruit colour within a week of treatment. Yozo et al. (2006) indicated that exogenous abscisic acid (ABA) could induce anthocyanin synthesis and chlorophyll senescence in regenerating torenia shoots on the medium containing a low concentration of sucrose (1.5%). It has been reported that treatments of ABA increase the anthocyanin content in grape skins (Ban et al., 2003) and improved the colour and quality of the grapes (Cantín *et al.*, 2007). Jeong *et* al. (2004) found that the accumulation of anthocyanins was enhanced by ABA treatments. ABA enhanced the mRNA accumulation of VvmybA1, a putative regulatory
gene of anthocyanin biosynthesis of grape, and all the tested enzyme genes of the anthocyanin biosynthetic pathway.

Fruit colour is one of the important factors the influence the fruit appearance. Fruit with full colour is more applied to consumer than less colour one. Currently, there is no report available on the effects of ABA on fruit growth, colour and quality parameters of wax apple fruit under the field conditions. This chapter highlights the effect of applications of abscisic acid through fruit swabbing on colour and quality parameters.

6.2 MATERIALS AND METHODS

6.2.1 Experimental Site

The study was carried out in a commercial orchard located at Banting, Selangor, Malaysia, 2°30N, 112°30E and 1°28 N, 111°20E at an elevation of about 45 m above sea level. The area under study has a hot and humid tropical climate. The soil in the orchard is peat with a mean pH of around 4.6 (Ismail *et al.*, 1994). The pH of the soil was neutralized by adding poultry manure and organic fertilizers before launching the experiments. The two season's experiments were carried out between September 2011 to December 2011.

6.2.2 Applications

Thirteen years old wax apple trees were selected for the study. The trees were planted in a 4.2×4.2 m hexagonal pattern and received the same agricultural management; fertilization, pruning and thinning of excess bud and fruits. Three trees were selected for each treatment and a total of twelve trees used in the experiments. Sixty uniform branches in all directions (five branches per tree) of about the same length and diameter from twelve trees were selected for the experiments. The experiments consist of 4 treatments including control with three replications and five sub-replications (15 replicates for each treatment). A single tree was taken as an experimental unit. The fruits of selected branches were swapped with 50, 100 and 150 ppm (abscisic acid) ABA and distilled water (control) at the 4th 5th and 6th week of fruit development (Fig 6.1). After harvesting the fruits, were kept in refrigerator in homogenous condition for biochemical analysis.



Fig 6.1 Swabbing fruit treatments with ABA.

6.2.3 Total Anthocyanin Content

(Same as described in Chapter 3).

6.2.4 The Total Phenolic Content (TPC)

(Same as described in Chapter 3).

6.2.5 Total Flavonoid Content (TFC)

(Same as described in Chapter 3).

6.2.6 Fructose and Inverted Sugar

(Same as described in Chapter 3).

6.2.7 Total Soluble Solids (TSS) Content

(Same as described in Chapter 3).

6.2.8 Titrable Acidity and TSS/TA Ratio

(Same as described in Chapter 3).

6.2.9 pH Measurement

(Same as described in Chapter 3).

6.2.10 Measurement of the K⁺ Content in Fruit Juice

(Same as described in Chapter 3).

6.2.11 The Chlorophyll Content (SPAD)

(Same as described in Chapter 3).

6.2.12 Fruit Length

(Same as described in Chapter 3).

6.2.13 Fruit Width

(Same as described in Chapter 3).

6.2.14 Fruit Weight

(Same as described in Chapter 3).

6.2.15 Fruit Juice

(Same as described in Chapter 3).

6.2.16 Fruit Dropping (%)

(Same as described in Chapter 3).

6.2.17 Fruit Yield

(Same as described in Chapter 3).

6.2.18 Statistical Analysis

(Same as described in Chapter 3).

RESULTS AND DISCUSSION

6.3.1 Anthocyanin Content

Anthocyanin pigments are responsible for the red, purple and blue colours of many fruits, vegetables, cereal grains and flowers and as a result, research on anthocyanin pigments has intensified recently because of their likely health benefits as dietary antioxidants (Ronald, 2001). Xie et al. (2011) pointed that anthocyanin biosynthesis in fruits becomes one feature of currently active research because colour is an important quality attribute for fruit. Kondo et al. (1991) and Sandhu et al. (2011) showed that ABA content is correlated with colour changes and pigment content in fruit. Usually, consumers favour fruits with red, blue or purple skin and flesh. ABA swabbing treatments of had a significant effect on anthocyanin content of wax apple fruits. The results displayed that the highest content of anthocyanin (3.86 mg/L) was recorded in 150 ppm ABA treatment followed 100 and 50 ppm treatment with anthocyanin content of 3.43 and 3.32 mg/L respectively. The lowest content of anthocyanin (2.82 mg/L) recorded in control fruits (Table 6.1, Fig 6.3). It was reported that exogenous application of ABA had a positive effects of on colour development and anthocyanin biosynthesis (Peppi et al., 2006). The mechanism by which ABA is adsorbed into the grape is not well understood. There could be two hypotheses: sprayed ABA penetrates through the skin, accumulates inside the grape and enhances the generation of phenolic compounds and ripening related changes, or exogenous ABA acts as a signalling agent that triggers the synthesis of endogenous ABA (Wheeler et al., 2009). Jiang and Joyce (2003) reported that treatment with ABA stimulated accumulation of anthocyanin and phenols as well as increased ethylene production. They also found that ABA treatments resulted in greater red colour development and softening of strawberry fruit in association with enhanced ethylene production.



Fig 6.2. Effect of ABA swabbing treatments on wax apple fruit colour development at week 6.



Fig 6.3 Effect of ABA swabbing on anthocyanin content of wax apple under field condition. Bars with different letters are significantly different.

6.3.2 Total Phenols Content (TPC)

Phenolic compounds are secondary metabolites produced by plants as a defence mechanism against various biotic and abiotic stresses. Phenolic compounds play an important role in colour and sensory characteristics of fruits and vegetables. The protective effects of these compounds against various chronic diseases such as cancer and cardiovascular diseases are well recognized among researchers and health conscious consumers (Arts and Hollman, 2005). Result showed in Table 6.1 indicated that there were no significant differences in total phenol among all treatments. The total phenols were in 150 ppm ABA, 100 ppm ABA, 50 ppm ABA and the control treatments as follows; 350.40±12.21 mg GAE/100g, 352.00±13.35 mg GAE/100g, 350.60±12.15 mg GAE/100g, 356.67±11.85 mg GAE/100g respectively. This result supported by the results of Buran *et al.* (2012), who reported that ABA delayed the ripening of blueberries, but did not affect total phenolic content, antioxidant capacity, or the content of individual phytochemicals in blueberries at harvest. In addition, Forney *et al.* (2009) indicated that ABA treatments had no significant effect on phenolic concentration or antioxidant capacity in late-harvested white cranberry fruit.

6.3.3 Total Flavonoid Content (TFC)

Gebhardt *et al.* (2005) stated that plant flavonoids are important in the diet because of their valuable effects on human health. ABA treatments by swabbing method at relatively advance stages of fruit development (at 4th, 5th, 6th week) had little or no effect on flavonoid content at harvest. Result shown in Table 6.1 and Fig 6.4, indicated flavonoid content in ABA treated fruits was little bit more than the control fruit, however, their differences were not statistically significant at p≤0.05. The flavonoid contents were in control treatment, 50 ppm ABA, 100 ppm ABA, 150 ppm ABA as follows; 13.37 ± 1.1 mg/100g, 14.14 ± 1.11 mg/100g, 13.41 ± 1.48 mg/100g, and 13.94 \pm 1.77 mg/100g respectively. This result is in agreement with Cheng *et al.* (2004) study, which indicated that external ABA could increase slightly the flavonoids, contents in ginkgo, leaves. In addition, Hao *et al.* (2010) indicated that ABA is involved in flavonoids accumulation in this plant.



Fig 6.4 Effect of ABA swabbing treatment on total flavonoid content of wax apple under field condition at harvest

6.3.4 Measurement of pH

Change in pH is directly related to change in acidity of fruits. Results showed ethat pH value of wax apple fruits significantly affected by 150 ABA treatments (Table 6.1). The highest pH value 5.04±0.31 was recorded in 150 ppm treated fruits, whereas, the lowest value 4.77.23 was found in control fruits. Result showed trend of increasing pH value in 100 ppm ABA and 50 ABA when compared to the control. However, the differences were not statically significant. The pH value in 50 ppm ABA and 100 ppm ABA were 4.79±0.28 and 4.79±.28 respectably. Zaharah and Singh (2012) study support this result, which indicated that ABA advanced accumulation of total sugars and reduction in total organic acids during mango fruit ripening compared to changes in control fruit.

6.3.5 K⁺ Content

Result shown in Table 6.1, indicated little increases in K⁺. However, they were not statically significant. The highest increase in K⁺ content was observed in 150 ppm ABA at 15.41 ± 1.161 mg/kg followed by 100 ppm ABA at 15.09 ± 1.01 mg/kg and 50 ppm ABA at 14.75 ± 1.13 mg/kg. The lowest K⁺ content among all treatments was observed in the control treatment at 14.59 ± 1.13 mg/kg. The result suggested that the exogenous ABA swabbing treatments at relatively advanced stage of wax apple fruit development had little or no effect in K⁺. Gurmani *et al.* (2006) indicated that ABA and CCC treated plants showed significant decrease in Na⁺ content but increased K⁺.

6.3.6 Total Soluble Solids (TSS)

Total soluble solid (TSS) is a main component of fruit quality that contributes to the sweetness of fruit. Muñoz-Robredo *et al.* (2011) stated that flavor composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids, and volatiles play a key role. Result in Table 6.2 indicated that there was a slight significant increase in TSS obtained by 150 ABA treatment. In addition, there were no difference in TSS among the 100 ABA, 50 ABA and the control. The highest TSS was recorded in 150 ABA treatment at 9.62 \pm 0.31 °Brix followed by 100 ABA treatment at 9.33 \pm 0.28 °Brix and 50 ABA treatment at 9.26 \pm 0.45 °Brix. The lowest TSS observed in the control treatment at 9.27 \pm 0.27 °Brix. The result showed that ABA treatments at relatively advance stage of fruit development had a slight effect on TSS. The result indicated a positive coloration between TSS and the anthocyanin (Fig 6.5). The TSS result is in agreement with Kojima *et al.* (1995) study, which indicated that ABA appears to play a role in the increase of the sugar concentration in citrus juice. In addition, Kobashi *et al.* (2001) indicated that ABA application to developing fruit increased sugar accumulation in the fruit. Peppi, *et al.* (2008) indicted that ABA applications at 150 and 300 mg/L on grapes advanced maturity slightly (higher Brix and lower TA) when compared to the control. Nakano *et al.* (1997) indicted that ABA application increased the accumulation of sugars in the pulp of persimmons.



Fig 6.5 Correlation between TSS and anthocyanin (mg/100g) content of ABA treated wax apple fruit.

6.3.7 Titratable Acidity (TA) and TSS/TA Ratio

Fruit acidity is main factor contributes to fruit taste. Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. 150 and 100 ppm ABA treatments, at relatively advance stage of fruit development reduced 127

significantly fruit acidity. However, there was no significant difference in TA between 50 ppm ABA and the control treatment in fruit TA. The greatest reduction in TA among all treatments was observed in 150 ppm ABA treatment at $0.74\pm0.40\%$ followed by 100 ppm ABA treatment at $0.75\pm0.30\%$. The highest TA was recorded in the control treatment at $0.79\pm0.40\%$ followed by 50 ppm ABA treatment at $0.78\pm0.40\%$. This result is in agreement with Kojima *et al.* (1995) study, which indicated that ABA treatment resulted in the increase in the glucose and fructose concentrations, but not in that of the organic acids in juice. Peppi *et al.* (2008) indicted that ABA applications at 150 and 300 mg/L on grapes advanced maturity slightly (higher Brix and lower TA) compared to the control. ABA appears to play a role in the increase of the sugar concentration in juice.

Regarding the TSS/TA ratio (the sweetness index), result in Table 6.2 indicated that the greatest TSS/TA ratio among all treatments was observed in 150 ppm ABA treatment at 13.02 ± 0.75 followed by 100 ppm ABA treatment at 12.41 ± 0.65 . The lowest TSS/TA ratio was recorded in the control treatment at 11.73 ± 0.85 followed by 50 ppm ABA treatment at 11.91 ± 0.73 . The elevated TSS/TA ratios in ABA treated fruit caused by the increasing sugar levels and reducing of the fruit acidity.

6.3.8 Inverted Sugar

Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. The study result in Table 6.2 showed that 150 ABA treatment increased significantly the inverted sugar content in wax apple fruit when compared to all other treatments. There were positive trend of increasing inverted sugar content by 100 and 50 ppm ABA treatments when compared to the control. However, trends were not statically significant. The highest inverted sugar contents observed in 150 ppm ABA treatment at 9.43 ± 0.36 °Brix followed by 100 ppm ABA at 9.13 ± 0.33 °Brix and 50 ppm

ABA treatment at 9.11±0.29 °Brix. The lowest inverted sugar content was observed tin the control treatment at 9.03±0.44 °Brix. The result is in agreement with Kojima *et al.* (1995) study, which indicated that ABA treatment resulted in the increase in the glucose and fructose concentrations, but not in that of the organic acids in juice. ABA Appears to play a role in the increase of the sugar concentration in juice. In addition, Kobashi *et al.* (2001) indicated that ABA application to developing fruit increased sugar accumulation in the fruit. Peppi *et al.* (2008) indicted that ABA applications at 150 and 300 mg/L on grapes advanced maturity slightly (higher Brix and lower TA) when compared to the control treatment (un treated). Nakano *et al.* (1997) indicted that ABA application increased the accumulation of sugars in the pulp of persimmons.

6.3.9 Fructose Content

Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. The study result in Table 6.2 showed that 150 ABA treatment increased significantly the fructose in wax apple fruit when compared to all other treatments. There were positive trend of increasing fructose content by 100 and 50 ppm ABA treatments when compared to the control. However, trends were not statically significant. The highest inverted sugar content observed in 150 ppm ABA treatment at 9.25 ± 0.34 °Brix followed by 100 ppm ABA at 9.15 ± 0.32 °Brix and 50 ppm ABA treatment at 9.09 ± 0.29 °Brix. The lowest fructose content was observed tin the control treatment at 9.05 ± 0.45 °Brix. The result is in agreement with Kojima *et al.* (1995) study, which indicated that ABA treatment resulted in the increase in the glucose and fructose concentrations, but not in that of the organic acids in juice. ABA Appears to play a role in the increase of the sugar concentration in juice. In addition, Kobashi *et al.* (2001) indicated that ABA application to developing fruit increased sugar accumulation in the fruit. The result is in agreement with Kojima *et al.* (1995) study, which indicated

129

that ABA treatments resulted in the increase in the glucose and fructose concentrations, but not in that of the organic acids in juice. ABA Appears to play a role in the increase of the sugar concentration in juice. In addition, Kobashi *et al.* (2001) indicated that ABA application to developing fruit increased sugar accumulation in the fruit. Peppi *et al.* (2008) indicted that ABA applications at 150 and 300 mg/L on grapes advanced maturity slightly (higher Brix and lower TA) but the change was not large enough to affect expected harvest date. Nakano *et al.* (1997) indicted that ABA application increased the accumulation of sugars in the pulp of persimmons.

6.3.10 Chlorophyll Content (SPAD)

Datt (1999) indicated that foliar chlorophyll content is a good indicator of plant stress and therefore of the potential for plant carbon dioxide uptake and growth. Leaf chlorophyll content (SPAD value) is an indicator of crop nitrogen as well as health status. In our study, the chlorophyll content was determined by Minolta SPAD meter. The SPAD values in Table 6.3 showed that ABA swabbing fruit treatments had no significant effects on chlorophyll content of leaves of wax apple. SAPD values were the in control treatment 62.79 ± 7.50 , in 50 ppm ABA 62.46 ± 8.47 , 100 ppm 60.33 ± 9.01 and in 150 ppm ABA 61.70 ± 6.30 . However, result showed that there were slight nonsignificant decreases in SPAD value with ABA treatments. Hence, the ABA treatments applied to fruits at relatively advance stage of fruit development. Moreover, there were no direct applications of ABA on leaves. Greene (2012) stated that extensive leaf yellowing and leaf abscission were observes after ABA applications, especially with the 250 mg·L⁻¹ and 500 mg·L⁻¹ in pear fruit.

6.3.11 Fruit Weight

Result in Table 6.3 showed that there were no differences in fruit weight among all treatments. However, there were slight non-significant increases tendency in fruit weight observed in the ABA treatments. The lowest fruit weight was recorded in the control treatment at 42.67 ± 3.38 g followed by 50 ABA treatments at 42.72 ± 3.04 g. The highest fruit weight was observed in 150 ABA treatment 43.46 ± 3.56 g followed by 100 ppm ABA treatment at 43.18 ± 2.55 g. The slight increase in fruit weight in ABA treatments could be related to a slight increase in fruit drop cause by ABA treatments. Hence, the ABA swabbing treatments subjected to fruit at relatively advance stage of fruit development. This result was supported by Lamsub *et al.* (2008) study, which indicated that S-ABA and ABA fertilizer 8 g/L application on peaches and apples increased the fruit weight by 5-14% relative to the control. In addition, Berhow (2000) stated that ABA treatments had little effect on fruit size or juice characteristics. Peppi *et al.* (2008) indicted that ABA berries showed higher skin weights compared to the control.

6.3.12 Fruit Length and Width

Result in Table 6.3 showed that ABA swabbing treatments at relatively advance stage of fruit development had no effect on fruit length and width. The fruit width in the control, 50 ppm ABA, 100 ppm ABA ,150 ppm ABA as follows; 39.48 ± 4.49 mm, 39.75 ± 3.43 mm 39.81 ± 4.15 mm 39.65 ± 3.27 mm respectively. The fruit length in the control, 50 ppm ABA, 100 ppm ABA ,150 ppm ABA as follows; 60.17 ± 5.02 mm, 60.20 ± 3.83 mm, 60.26 ± 3.48 mm, and 60.44 ± 4.30 mm respectively. This result is in agreement with Berhow (2000) who reported that ABA treatments (5, 25, and 50 ppm) had little effect on fruit size (length and width).

6.3.13 Fruit Juice (%)

Result showed that there were no significant differences in juice volume among all treatments; thus, there were a slight non-significant increase in juice volume observed in ABA treatments. The juice percentages in the control, 50 ppm ABA , 100 ppm ABA, and 150 ppm ABA as follows; $73.81\pm1.30\%$, $73.93\pm1.26\%$, $74.15\pm0.71\%$ and $74.43\pm1.08\%$ respectively (Table 6.3). The result is in agreement with the finding by Berhow (2000) who stated that ABA treatments had little effect on juice characteristics.

6.3.14 Fruit Drop

Almedia *et al.* (2004) indicated that endogenous hormones and their balance play regulatory roles in the deployment of nutrients to the developing organs. Result showed that ABA swabbing treatments increased slightly fruit drop (Table 6.3). Only in 150 ABA treatment fruit drop was significantly little higher when compared to all other treatments. The highest fruit drop was observed in ABA treatment at $48.13\pm3.18\%$ followed by 100 ABA treatment at $46.27\pm3.92\%$. The lowest fruit drop was recorded in the control treatment at $45.07\pm3.65\%$ followed by 50 ABA treatment at $45.20\pm2.68\%$. There were no significant differences in fruit drop percentage among 50, 100 ABA and the control treatments. This result is supported by Zhicheng et *al.* (1997) study, which indicated that the content of ABA in drop fruits was higher than that in normal longan fruits (*Dimocarpus longan* Lour. cv. 'Shuizhang').

6.3.15 Branch Yield

There were no significant differences among all treatments in yield (Table 6.3). However, result shoed that ABA swabbing treatments slightly reduced yield. The highest yield was recorded in the control treatment with 0.83±0.06 kg followed by 50 ppm ABA with 0.82±0.06 kg. The lowest yield was recorded in 150 ppm ABA with 0.79 ± 0.06 kg followed by 100 ppm ABA with 0.81 ± 0.07 kg. The non-significant reduction in branch yield could be due to the increases in fruit drop observed in ABA treatments in addition to the slight non-significant increases in weight observed in ABA treatment. result is in agreement with finding by Deyton *et al* (2010) study which indicated that 50 mg L⁻¹ ABA did not significantly affect yield or fruit quality of 'Niagara' grape (*Vitis labrusca*). However at higher ABA concentration, Contreras and Lagos (2012) study showed a significant reduction in fruit harvested per tree treated with 300 and 400 mg L⁻¹ ABA. In can be suggested that the slight reduction in yields is acceptable when looking to other beneficial effect on colour and other quality parameters.

6.4 CONCLUSION

ABA swabbing treatments had a significant effect on anthocyanin content of wax apple fruits. The results displayed that the highest content of anthocyanin was recorded in 150 ppm ABA treatment followed 100 and 50 ppm treatment respectively. The lowest content of anthocyanin recorded in control. Result indicated that there were no major significant differences in total phenol content among all treatments. ABA treatments by swabbing method at relatively advance stages of fruit development (at 4th, 5th, 6th week) had little or no effect on flavonoid content at harvest. Result indicated that flavonoid content in ABA treated fruits was little bit more than the control fruit; however, their differences were not statistically significant at $p \le 0.05$. Change in pH is directly related to change in acidity of fruits. Results showed that pH value of wax apple fruits significantly affected by 150 ABA treatments. The highest pH value was recorded in 150 ppm treated fruits, whereas, the lowest value was found in control fruits. Result showed trend of increasing pH value in 100 ppm ABA and 50 ABA when compared to the control. Result indicated little increases in K⁺ caused by ABA treatments. However, they were not statically significant. The highest increase in K⁺ content was observed in 150 ppm ABA followed by 100 ppm ABA and finally 50 ppm ABA. The lowest K^+ content among all treatment was observed in the control treatment. Result showed that ABA treatments at relatively at advance stage of fruit development had a slight effect on TSS. The result indicated that there was a slight significant increase in TSS obtained by 150 ABA treatment. In addition, there were no difference in TSS among the 100 ABA, 50 ABA and the control. The highest TSS was recorded in 150 ABA treatment followed by 100 ABA and 50 ABA treatments respectively. The lowest TSS observed in the control treatment. 150 and 100 ppm ABA treatments, at relatively advance stage of fruit development reduced significantly fruit acidity. However, there was no significant difference in TA between 50 ppm ABA and the control treatments. The greatest

reduction in TA among all treatments was observed in 150-ppm ABA treatment The highest TA was recorded in the control treatment followed by 50 ppm ABA treatment. Result showed that there were no significant differences in fruit weight among all treatments. However, there were slight non-significant increases trends in fruit weight observed in the ABA treatments. The lowest fruit weight was recorded in the control treatment at followed by 50 ABA treatments. The highest fruit weight was observed in 150 ABA treatment followed by 100 ppm ABA treatment The slight increase in fruit weight in ABA treatments could be related to a slight increase in fruit drop cause by ABA treatments. The study showed that 150 ABA treatment increased significantly the inverted sugar content in wax apple fruit when compared to all other treatments. There were positive trend of increasing inverted sugar content by 100 and 50 ppm ABA treatments when compared to the control. However, trends were not statically significant. The study showed that 150 ABA treatment increased significantly the fructose in wax apple fruit when compared to all other treatments. There were positive trend of increasing fructose content by 100 and 50 ppm ABA treatments when compared to the control. However, trends were not statically significant. Regarding SPAD values in, results showed that ABA swabbing fruit treatments had no significant effects on chlorophyll content of wax apple leaves. Result showed that there were no differences in fruit weight among all treatments. However, there were slight nonsignificant increases trends in fruit weight observed in the ABA treatments. Result in showed that ABA swabbing treatments at relatively advance stage of fruit development had no effect on fruit width. Similarly, result showed that ABA swabbing treatments at relatively advance stage of fruit development had no effect on fruit length. Result showed that there were no significant differences in juice volume among all treatments; thus, there were a slight non-significant increase in juice volume observed in ABA treatments. Results showed that ABA swabbing treatments increased slightly fruit drop.

Only in 150 ABA treatment fruit drop was significantly little higher when compared to all other treatments. There were no significant differences among all treatments in yield However, result showed that ABA swabbing treatments slightly reduced yield. The highest yield was recorded in the control treatment followed by 50 ppm ABA. The lowest yield was recorded in 150 ppm ABA followed by 100 ppm ABA.

From the above results, it can be suggested that ABA at the 100-150 ppm is beneficial to enhance fruit colour without adverse effects in other fruit quality parameters.

ABA	Total anthocyanin mg/L	Total phenols (mg GAE/100g)	рН	Total Flavonoid (mg/100g)	K ⁺ (mg/kg)
Control	2.82±0.33c	356.67±11.85a	4.77±23b	13.37±1.1a	14.59±1.13a
50 ppm	3.32±0.40b	350.60±12.15a	4.79±.28b	14.14±1.11a	14.75±1.13a
100 ppm	3.43±.60b	352.00±13.35a	4.94±35b	13.41±1.48a	15.09±1.01a
150 ppm	3.86±.53a	350.40±12.21a	5.04±.31a	13.94±1.77a	15.41±1.161a

Table 6.1 Effects of different treatments of ABA swabbing on total anthocyanin, total phenols, pH, total flavonoids and K⁺ content of wax apple fruit.

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

Treatment (ppm)	TA (%)	TSS (°Brix)	TSS/TA ratio	Inverted sugar (°Brix)	Fructose (°Brix)
Control	0.79±0.40b	9.26±0.45b	11.73±0.85b	9.03±0.44b	9.05±0.45b
ABA 50	0.78±0.40b	9.27±0.27b	11.91±0.73b	9.11±0.29b	9.09±0.29b
ABA 100	0.75±0.30a	9.33±0.28b	12.41±0.65a	9.13±0.33b	9.15±0.32b
ABA 150	0.74±0.40a	9.62±0.31a	13.02±0.75a	9.43±0.36a	9.25±0.34a

Table 6.2 Effects of ABA treatment on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio, inverted sugar and fructose of wax apple under field conditions.

 $\overline{\text{Means} (\pm \text{S.D.}) \text{ within the same column followed by the same letter do not differ significantly according to the LSD test at $\alpha=0.05$.}$

ABA	SPAD Value	Fruit length (mm)	Fruit width (mm)	Fruit weight (g)	Juice per 100 g fruit (ml)	(%)	Branch yield (kg)
Control	62.79±7.50a	60.17±5.02a	39.48±4.49a	42.67±3.38a	73.81±1.30 a	45.07±3.65b	0.83±0.06a
50 ppm	62.46±8.47a	60.20±3.83a	39.75±3.43a	42.72±3.04a	73.93±1.26a	45.20±2.68b	0.82±0.06a
100 ppm	60.33±9.01a	60.26±3.48a	39.81±4.15a	43.18±2.55a	74.15±0.71a	46.27±3.92b	0.81±0.07a
150 ppm	61.70±6.30a	60.44±4.30a	39.65±3.27a	43.46±3.56a	74.43±1.08a	48.13±3.18a	0.79±0.06a

Table 6.3 Effects of different treatments ABA on SPAD value, fruit length, fruit width, fruit weight, juice volume, fruit drop and branch yield of wax apple fruit under field condition.

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

THE EFFECTS OF NAA SWAPPING ON WAX APPLE FRUIT QUALITY

7.1 INTRODUCTION

The wax apple 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 drop, small fruit size and less colour and 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. Naphthaleneacetic acid (NAA) is a synthetic plant hormone in the auxin family (Fig 2.4). The application of acetic acid (NAA) on wax apple fruit significantly reduced titratable acidity and increased total sugar and carbohydrate content compared to the control. Idbal et al. (2009), Indicated that guava fruit yield was significantly increased by NAA applications at 45 ppm and 60 ppm. NAA application increased TSS, total sugars, and ascorbic acid contents except acidity, which was reduced. However, Fruit drop exhibited negative correlation with all other characteristics except acidity. Whereas pulp seed ratio had positive correlation with TSS, total sugars and ascorbic acid contents and negative association with acidity. Amorós et al (2004) indicated that the application of naphthalene acetic acid on Loquat trees (Eriobotrya japonica Lindl cv. Algerie) at 25 and 50 mg l-1 (NAA-25, NAA-50) when fruit were at 50 and 30% of their final size increased the total production significantly with respect to control trees, due to an increase in fruit size. A significant delay in maturity of loquat fruit NAA treated trees was observed when treatments were applied at 50% of final fruit size. However, when the auxin treatments were applied at 30% of final fruit size, loquat fruit precocity increased. Thus, these treatments accelerated fruit growth and maturation, without any undesirable effect on nutritive and organoleptic properties of loquat fruit Dutta and Banik (2007) reported that GA_3 and NAA application before flowering, followed by

three weeks after fruit setting, significantly increased fruit length, diameter and fruit weight and ultimately crop yield. In another study done by Yuan and Carbaugh (2007) stated that NAA possibly enhance background colour development and fruit softening. Son (2004) Found that in Mut apricot *Prunus armeniaca* growing in central Turkey, application of naphthalene acetic acid (NAA) at 10, 20, and 30 ppm significantly increased fruit weight. The biggest fruit was obtained from 20 ppm NAA; the soluble solids (TSS) were greatest (13.53%) for 20 ppm NAA. El-Shewy (1999) described that 50 mg/L NAA and 50 mg/L GA₃ at full bloom and three months after the first spray were most effective treatments in reducing pre harvest fruit drop as well as fruit seed contents in guava. Dubay et al. (2002) reported that NAA application on guava cv. Allahabad Sufeda at 250 mg/L concentration resulted in higher yield and guality. Yaday (2002) indicated that NAA at 60 mg/L improved TSS, total sugars and vitamin-C (ascorbic acid) contents in guava fruit. Agusti et al. 2000 showed that the application of NAA 15 days after full bloom of loguat fruit, increased fruit diameter by 10%, and yield by 20% per tree compared to the control. NAA has also been shown to promote maturation (softening and anthocyanin formation) in mesocarp discs of peach fruit (Ohmiya, 2000). Guinn and Brummett (1993) pointed that the application of NAA on leaves increased the net photosynthetic rate due to increase in stomatal aperture, which facilitates more CO₂ conductance. Ortolá et al. (1991) found that naphthaleneacetic acid (NAA) when applied shortly after the end of the June drop, it increases the growth rate of Satsuma mandarin fruit (Citrus unshiu Marc.), and that results in a bigger fruit size at harvest without any adverse reduction in yield. Wismer et al. (1995) assumed that the increase in fruit size in 'Empire' apple fruit (Malus domestica Borkh.) by application of NAA was due the enlargement in fruit cell size and was not due to the acceleration rate of cell division. Bal and Randhawa (2007) stated that the ber fruit size increased to maximum with 20 and 30 ppm NAA and the fruit yield per tree was significantly

improved with NAA treatments. In addition, TSS was slightly improved with different NAA treatments and the total acids were decreased under all the NAA treatments. Application of NAA on Cabernet Sauvignon grapes at veraison suppresses all the genes of the anthocyanin biosynthesis pathway (Jeong *et al.*, 2004).

Fruit size is one of the restrictive factors in fruit marketing of fruit. Currently, there is no available literature describing the effects of NAA fruit swabbing on fruit quality and on the physiochemical and phytochemical properties of wax apple under field conditions. This study reports the effect of different concentrations of Naphthalene Acetic Acid (NAA) on wax apple fruit size and other fruit quality parameters though swabbing method. The main objective of this study is to investigate the possible effect of naphthalene acetic acid (NAA) on wax apple fruit quality through swabbing method.

7.2 MATERIALS AND METHODS

7.2.1 Experimental Site

The study was carried out in a commercial orchard located at the Banteng, Selangor, Malaysia, 2°30N, 112°30E and 1°28 N, 111°20E at an elevation of about 45 m above sea level. The area under study has a hot and humid tropical climate. The soil in the orchard is peat with a mean pH of around 4.6 (Ismail *et al.*, 1994). The pH of the soil was neutralized by adding poultry manure and organic fertilizers before launching the experiments. The experiment was carried out between December 2011 to April 2012.

7.2.2 Treatments

Thirteen years old wax apple trees were selected for the study. The trees were planted in a 4.2×4.2 m hexagonal pattern and received the same agricultural management; fertilization, pruning and thinning of excess bud and fruits. Three trees were selected for each treatment and twelve trees used in the whole experiments. Sixty uniform branches in all directions (five branches per tree) of about the same length and diameter and same number of leaves from twelve trees were selected for the experiments. The experiments consist of four treatments including control with three replications and five sub-replications (15 replicates for each treatment). A single tree was taken as an experimental unit. Four times application of NAA were used in this study. First application was swabbing the flower bud at bud emerging, the second application was swabbing fruitlet after at 2 weeks of fruit development, the third application was swabbing fruit at 4th week of fruit development.

7.2.3 Total anthocyanin content

(Same as described in Chapter 3).

7.2.4 The Total Phenolic Content (TPC)

(Same as described in Chapter 3).

7.2.5 Total Flavonoid Content (TFC)

(Same as described in Chapter 3).

7.2.6 Fructose and Inverted Sugar

(Same as described in Chapter 3).

7.2.7 Total soluble Solids (TSS) Content

(Same as described in Chapter 3).

7.2.8 pH Measurement

(Same as described in Chapter 3).

7.2.9 Titrable Acidity and TSS/TA Ratio

(Same as described in Chapter 3).

7.2.10 Bud Dropping (%)

(Same as described in Chapter 3).

7.2.11 Fruit Setting (%)

(Same as described in Chapter 3).

7.2.12 Fruit Dropping (%)

(Same as described in Chapter 3).

7.2.13 The Chlorophyll Content (SPAD value)

(Same as described in Chapter 3).

7.2.14 Fruit Length and Width

(Same as described in Chapter 3).

7.2.15 Fruit Wight

(Same as described in Chapter 3).

7.2.16 Fruit Yield

(Same as described in Chapter 3).

7.2.17 Fruit Juice

(Same as described in Chapter 3).

7.2.18 Measurement of the K^+ content in fruit juice

(Same as described in Chapter 3).

7.2. 19 Statistical Analysis

(Same as described in Chapter 3).

7.3 RESULTS AND DISCUSSION

7.3.1 Anthocyanin Content

Xie et al. (2011) pointed that anthocyanin biosynthesis in fruits becomes one feature of currently active research because colour is an important quality attribute for fruit. Usually, consumers favour fruits with red, blue or purple skin and flesh. All NAA swabbing treatments had positive effect on Anthocyanin content (Table 7.1). The highest anthocyanin content was observe in the 90 ppm NAA swabbing at 4.28±0.27 mg/L treatment followed by 60 ppm NAA at 3.95±0.31 mg/L and 30 ppm NAA at 3.28 ± 0.45 mg/L. the lowest anthocyanin content was recorded at the control treatment at 2.76±0.40 mg/L. However, there was no significant difference between the 30 ppm NAA and the control treatments. Our result is in agreement with Ohmiya (2000) who indicated that anthocyanin formation at the surface of the discs of peach fruit was enhanced by high concentrations of NAA and light. In addition, Shulman and Lavee (1973) indicated that NAA application at concentrations up to 200 and 300 parts/ 10^6 caused anthocyanin accumulation but apparently as part of a general ripening induction in in green 'Manzanillo' Olives. Asano et al. (2002) indicated also that cultured leaf cells of Nyoho strawberry (Fragaria ananassa Duch.), produced 4 times more anthocyanin than fruit, with additions of 2.0 mg/l of 1-naphthalenacetic acid (NAA).

7.3.2 Total Phenols Content (TPC)

All NAA swabbing treatment exhibited increases in total phenol when compared to the control (Table 7.1). The highest phenol content was recorded in 90 ppm NAA treatment at 420.13 \pm 17.93 (mg GAE/100g) followed by 60 ppm NAA treatment at 386.20 \pm 27 (mg GAE/100g), followed by 30 NAA treatment at 368.07 \pm 27.79 (mg GAE/100g). The lowest phenol content was observed in the control treatment at 346.33 \pm 17.46 (mg GAE/100g). The result is in agreement with Nikolaeva *et al.* (2009)

study on tea plant, which indicated that, the content of total soluble phenolic compounds and flavans in the calli increased by 30% In the presence of NAA, on the average as compared with control. Moreover, North *et al.* (2012) indicated that NAA treatment increased significantly phenolic exudation in *Strelitzia reginae* plant. In addition, with Khandaker *et al.* (2012) also indicated that NAA application increases in phenol content in fruit.

7.3.3 Total Flavonoid Content (TFC)

Gebhardt *et al.* (2005) Plant flavonoids are important in the diet because of their valuable effects on human health. Table 7.1 showed that there were no significant differences among 60 ppm NAA, 30 ppm NAA and the control treatments. However, 90 ppm treatment exhibited a slight significant increase in Flavonoid content when compared to all other treatments. The highest Flavonoid content was recorded in 90 ppm NAA swabbing treatment at 15.91 ± 1.02 mg/100g followed by 60 ppm NAA treatment at 14.44 ± 1.51 mg/100g and 30 ppm NAA treatment at 14.03 ± 1.46 mg/100g. The lowest flavonoid content was recorded in the control treatment at 14.01 ± 1.90 mg/100g. Hence, NAA treatment was by swabbing fruit. Our result is in agreement with Khandaker *et al.* (2012) who indicated that NAA application increases in phenol and flavonoid content in fruit. Liu and Saxena (2009) indicated also that NAA induced significantly high antioxidant activity and flavonoids accumulate in the cell suspension cultures of S. medusa.

7.3.4 Measurement of pH

Study result showed that NAA increased the pH values (Table 7.1). The highest pH value was recorded in 90 NAA swabbing treatment at 5.15 ± 0.22 a followed by 60 ppm NAA treatment at 5.06 ± 0.29 , followed by 30 ppm NAA treatment at 4.93 ± 0.35 . The lowest pH value was observed in the control treatment. However, there was no

significant difference between 30 ppm NAA treatment and the control. In addition, there was no significant difference in pH value between 90 ppm NAA and 60 ppm NAA treatment. Our result is in agreement with Ouma and Rice (2001) study, which indicated that application NAA increases in pH, total soluble solids content, red colour percentage, return bloom and mean fruit weight of apple.

7.3.5 K⁺ content

As shown in Table 7.1, all NAA treatments caused an increased in K⁺ content when compared to the control. However, there were no significant differences in K⁺ content among all NAA swabbing treatments. The highest K⁺ content was observed in 90 ppm NAA treatment at 16.47 \pm 1.18 mg/kg followed by 60 ppm NAA treatment at15.96 \pm 0.85 mg/kg, followed by 30 ppm NAA treatment at 15.84 \pm 0.96 mg/kg. The lowest K⁺ content was recorded in the control treatment at 13.77 \pm 0.89 mg/kg. Our result is in agreement with Ouma and Rice (2001) study, which indicated that application NAA, reduced sodium (Na) and calcium (Ca) contents but increased potassium (K) content in apple juice.

7.3.6 Total Soluble Solids (TSS)

Total soluble solid (TSS) is a main component of fruit quality that contributes to the sweetness of fruit. Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. Results in Table 7.2, indicated that there were significant increases in TSS content recorded in 90 ppm NAA and 60 NAA treatments when compared to 30 ppm NAA the control treatments. Moreover, the 30 ppm NAA treatment had slightly higher TSS when compared to the control treatment, thus the difference was not statically significant, and that could be associated partially with the low dosage of 30 ppm NAA treatment. the highest TSS was recorded in 90 ppm treatment at 10.27 ± 0.27 °Brix followed by 60 ppm treatment at 9.83 ± 0.38 °Brix, followed by 30 ppm NAA treatment at $9.72\pm0.34b$ °Brix. The lowest TSS among all treatment was recorded in the control at 9.49 ± 0.44 °Brix. It can therefore, be suggested that the best treatment in TSS were 90 and 60 ppm NAA treatment. Our result is in agreement with Nawaz *et al.* (2008) study, which indicated that exogenous application of NAA increased significantly total soluble solids, reducing sugars and non-reducing sugars. In addition, Haidry *et al.* (1997) found that NAA as foliar spray on mango resulted in increase of total soluble solids and sugar contents whereas acidity decreased significantly when compared to the control treatment. Kassem *et al.* (2011) also indicated that NAA applications on Chinese jujube (*Ziziphus jujuba* Mill.) significantly increased in yield, fruit retention, flesh and seed weight, volume, length, diameter, shape index, TSS, maturity index, V.C, reducing, non-reducing and total sugars, moisture content and the percentage of large fruits.

7.3.7 Titratable Acidity (TA) and TSS/TA ratio

Fruit acidity is main factor contributes to fruit taste. Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. Result in Table 7.2 showed that NAA swabbing treatment decreased the titratable acidity (TA). The lowest significant TA was recorded in 90 ppm NAA treatment at 0.75 ± 0.04 percentage followed by 60 ppm NAA treatment at 0.77 ± 0.03 . The highest TA recorded in the control treatment at $0.81\pm0.05\%$ followed by 30 ppm NAA treatment at $0.77\pm0.03\%$. There was no significant difference between 90 ppm NAA treatment and 60 NAA treatment in TA. There was no significant difference between the control and 30 ppm NAA treatments. Our result is in agreement with Haidry *et al.* (1997) study which found that NAA as foliar spray on mango resulted in increase of total soluble solids and sugar contents whereas acidity decreased significantly when compared to the

control treatment, total soluble solids (TSS), total sugar content, and vitamin C in guava fruits.

Regarding the TSS/TA ratio (the sweetness index), result in Table 7.2 showed that the highest TSS/TA ratio was recorded in the 90 ppm NAA treatment followed by 60 ppm NAA treatment at12.86 \pm 0.67. The lowest TSS/TA ratio was recorded in the control treatment at 11.82 \pm 0.95 followed by 30 ppm NAA treatment at 12.19 \pm 0.85. However, there was no significant difference in TSS/TA between the control and 30 ppm NAA treatment. our result is in agreement with Our result is in agreement with Nawaz *et al.* (2008) study which indicated that exogenous application of NAA increased significantly total soluble solids, and with Haidry *et al.* (1997) study which found that NAA as foliar spray on mango resulted in significant decrease in acidity.

7.3.8 Inverted Sugar

Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. The study result in Table 7.2 showed that all NAA treatments increased the inverted sugar. The highest inverted sugar was observed in 90 ppm NAA treatment at 10.05 ± 0.33 °Brix followed by 60 ppm NAA treatment at 9.64 ± 0.39 °Brix and 30 ppm NAA treatment at 9.52 ± 0.34 °Brix. The lowest inverted sugar was recorded in the control treatment at 9.29 ± 0.45 °Brix. The difference between 30 ppm NAA treatment and the control was not statically significant. Iqbal *et al.* (2009) indicated that NAA has been shown to increase significantly fruit yield, total soluble solids (TSS), total sugar content, and vitamin C in guava fruits. Nawaz *et al.* (2008) indicated that exogenous application of NAA increased significantly total soluble solids, reducing sugars and non-reducing sugars.

7.3.9 Fruit Fructose Content

Muñoz-Robredo *et al.* (2011) stated that flavour composition in fruit has been defined as a complex attribute of fruit quality, in which the mix of sugars, acids and volatiles play a key role. As showed in table 7.2, the NAA swabbing treatments increase the accumulation of fructose in wax apple fruit. The highest significant increase in fructose was obtained in 90 ppm NAA treatment at 10.13 ± 0.34 °Brix followed by 60 ppm NAA treatment at 9.73 ± 0.37 °Brix. The lowest fructose was recorded in the control treatment at 9.39 ± 0.44 °Brix followed by 30 ppm NAA treatment at 9.61 ± 0.35 °Brix. However, the difference between 30 ppm NAA treatment and the control was not statically significant. Iqbal *et al.* (2009) indicated that NAA has been shown to significantly increase fruit yield, total soluble solids (TSS), total sugar content, and vitamin C in guava fruits Nawaz *et al.* (2008) indicated that Exogenous application of NAA significantly total soluble solids, reducing sugars and non-reducing sugars.

7.3.10 Chlorophyll Content (SPAD)

Datt (1999) indicated that foliar chlorophyll content is a good indicator of plant stress and therefore of the potential for plant carbon dioxide uptake and growth. In our study, the chlorophyll content was determined by Minolta SPAD meter. The SPAD values in Table 7.3 showed that there were no significant differences among all treatments. The SPAD values in all treatments were same. In 90 ppm NAA ppm treatment was 61.99±5.08, in 60 ppm NAA was 61.38±4.44, in 30 ppm NAA treatment was 60.58.5.53 and in the control was 60.16±5.48. NAA swabbing treatments did not affect the chlorophyll content in the leaves of wax apple fruits and that appeared to be due the method, which NAA applied in this study. Our result is in agreement with Percival and Gerritsen (1998) study, which indicated that NAA had no significant effect on chlorophyll fluorescence.



Fig 7.1 Effect of different NAA treatment on wax apple leaves chlorophyll content (SPAD).

7.3.11 Fruit Weight

All NAA swabbing treatments had a positive effect on fruit weight. As shown in Table 7.3, the highest significant fruit weight was observed in 90 ppm NAA treatment followed at 63.76±4.55 g followed by 60 ppm NAA treatment at 52.80±5.43 g. the lowest weight was recorded in the control treatment at 44.45±3.38c g followed by 30 ppm NAA treatment at 47.18±3.86 g. However, there was no significant difference between the control and 30 ppm NAA treatment. The difference in average fruit weight between the 90 ppm NAA and the control was about 20 g. it can be said that the NAA swabbing treatment increased substantially the fruit weight when compared to the control treatment. This result is in agreement with Kassem *et al.* (2001) who indicated that NAA applications on Chinese jujube (*Ziziphus jujuba* Mill.) significantly increased in yield, fruit retention, flesh and seed weight, volume, length, diameter, shape index, TSS, maturity index, V.C, reducing, non-reducing and total sugars, moisture content and the percentage of largest fruit.
In addition, Ouma and Rice (2001) indicated that application NAA increases fruit weight of apple. Nawaz *et al.* (2008) also indicated that exogenous application of NAA significantly increased fruit weight, juice percentage, and total soluble solids.



Fig 7.2 Effect of NAA swabbing treatments (at 30, 60, 90 ppm) on wax apple fruit size and colour.

7.312 Fruit Juice (%)

From Table 7.3, the percentage of fruit juice per 100 g in 90 ppm NAA showed a significant increase in fruit juice percentage when compared to all other treatments. The highest juice was obtained by 90 ppm NAA treatment at $75.26\pm1.04\%$ followed by 60 ppm NAA treatment at $74.78\pm1.45\%$. The lowest fruit juice percentage was recorded in the control treatment at $74.00\pm1.28\%$ followed by 30 ppm NAA treatment at $74.28\pm1.25\%$. Result showed the positive effect of NAA on fruit moistures. The increases in the percentage of fruit juice per 100 g in 60 ppm NAA and 30 ppm NAA were not statically significant when compared to the control treatment, thus the trends of increase was clear. This result is in agreement with Sawale *et al.* (2001) study, which indicated that exogenous application of NAA at 300, 350, 450 ppm significantly increased fruit juice percentage in Mandarin (*Citrus reticulata*, Blanco).

7.3.13 Fruit Length

All NAA swabbing treatments had a positive effect in fruit length (Table 7.3). There were substantial significant increases in fruit length in all NAA swabbing treatments. The highest fruit length was observed in 90 ppm NAA treatment at 67.06 ± 3.22 mm, followed by 60 ppm NAA at 63.96 ± 2.00 mm and 30 ppm NAA treatment at 61.20 ± 3.87 mm. Whereas the lowest fruit length was recorded in the control treatment at 57.97 ± 2.92 mm. As showed in (Fig 7.2), the fruit growth (length) over time was accelerated significantly by all NAA treatments. This result supported by Kassem *et al.* (2001) study which indicated that NAA applications on Chinese jujube (*Ziziphus jujuba* Mill.) significantly increased in yield, fruit retention, flesh and seed weight, volume, length, diameter, shape index, TSS, maturity index, V.C, reducing, non-reducing and total sugars, moisture content and the percentage of largest fruit.



Fig 7.3 Fruit growth (length)/week as influenced by different application of NAA applications. Lines with same letter are not statically significant.

7.3.14 Fruit Width

Fruit width positively affected by NAA treatments (Table 7.3). The fruit diameter increased significantly with all NAA treatments. The highest fruit width was observed in 90 ppm NAA treatment at 43.89 ± 2.37 mm followed by 60 ppm NAA at 42.92 ± 1.85 mm, however, the difference between both treatments was not statically different. The lowest width was recorded in the control treatment at 35.80 ± 2.04 mm followed by 38.46 ± 2.56 mm. As shown in (Fig 7.3), the fruit growth (width) over time was accelerated significantly by all NAA treatments. This result is in agreement with the finding by Kassem et *al.* (2001) who indicated that exogenous NAA applications on Chinese jujube (*Ziziphus jujube* Mill.) significantly increased in length, diameter, shape index, the percentage of largest fruit. Additionally, Alina (1998) indicated that the addition of NAA enhanced fruit size at harvest.



Fig 7.4 Fruit growth (width)/week as influenced by different application of NAA applications. Lines with same letter are not statically significant.

7.3.15 Bud Drop (%)

Almedia *et al.* (2004) indicated that endogenous hormones and their balance play regulatory roles in the deployment of nutrients to the developing organs. NAA swabbing treatments had positive effect on bud drop (Table 7.3). The highest reduction in bud drop was observed in 90 ppm NAA treatment at $38.20\pm3.20\%$ followed by 60 ppm NAA treatment at $39.33\pm2.92\%$. However, there was no significant difference in bud drop percentage between 90 and 60 ppm NAA treatments. The highest bud drop was recorded in the control treatment at 47.93 ± 2.86 percentage followed by 30 ppm NAA treatment at 43.33 ± 2.26 . This result is in agreement with McArtney *et al.* (2007) study, which indicated that NAA increased return bloom in apple (*Malus domestica*). In addition, Nagargoje et *al.* (2007) indicated that application of 100 ppm NAA at 50% flowering and at the pea stage of fruit development reduced significantly flower and fruit drop in sapota fruit, variety 'Kalipatti'.

7.3.16 Fruit Set (%)

The result showed that NAA had positive effect on fruit set in general (Table 7.4). The highest fruit set was obtained by 90 NAA treatment at $60.00\pm4.55\%$ followed by 60 ppm NAA treatment at $55.47\pm4.01\%$ and 30 ppm NAA treatment at $51.20\pm5.01\%$. The lowest fruit set percentage was recorded in the control treatment at $45.37\pm2.38\%$. Almedia *et al.* (2004) indicated that endogenous hormones and their balance play regulatory roles in the deployment of nutrients to the developing organs. This result is in agreement with Nagargoje *et al.* (2007) study which indicated that application of 100 ppm NAA at 50% flowering and at the pea stage of fruit development reduced significantly flower and fruit drop and increased fruit set and fruit retention in in sapota fruit, variety 'Alipatti'.

7.3.17 Fruit Drop (%)

Almedia et al. (2004) indicated that endogenous hormones and their balance play regulatory roles in the deployment of nutrients to the developing organs. NAA swabbing treatment treatments reduced fruit drop in wax apple fruit (Table 7.4). The greatest reduction observed in 90 ppm NAA treatment at 30.93±3.09% followed by 60 ppm NAA treatment at 36.47±2.32%. The highest fruit drop was observed in the control treatment at 45.51±3.03% followed by 30 ppm NAA treatment at 41.60±4.25%. This result is in agreement with Anthony and Cogging (2001) Indicated that that greatest reductions in fruit drop of citrus fruit were obtained by NAA spray concentrations in the 100 to 400 mg L¹ range. Iqbal et al. (2009) indicated that NAA significantly reduced preharvest fruit drop. Nawaz et al. (2008) indicated also that exogenous application of NAA significantly decreased preharvest fruit drop percentage, leading to increase in total number of fruits per plant, fruit weight, juice percentage, total soluble solids, acidity, vitamin-C, reducing sugars and non-reducing sugars. Our result also in agreement with Nagargoje et al. (2007) study, which indicated that application of 100 ppm NAA at 50% flowering and at the pea stage of fruit development reduced significantly flower and fruit drop and increased fruit set and fruit retention in in sapota fruit, variety 'Kalipatti'.

7.3.18 Branch Yield

There were significant differences among all NAA treatments. NAA had a positive effect in wax apple yield (Table 7.4 and Fig 7. 4). The greatest yield was obtained by 90 ppm NAA treatment at 1.77 ± 0.23 kg, followed by 60 ppm NAA treatment at 1.38 ± 0.20 kg. The lowest yield recorded in the control treatment at 0.83 ± 0.08 kg, followed by 30 ppm at 1.01 ± 0.16 kg. The increase yield was due the positive effect of NAA applications in fruit drop and set, in addition to the increase in fruit weight. This result is in agreement with Iqbal *et al.* (2009) study, which indicated

that NAA has been shown to increase significantly fruit yield, total soluble solids (TSS), total sugar content, and vitamin C in guava fruits. in addition, Nawaz *et al.* (2008) indicated that exogenous application of NAA significantly decreased preharvest fruit drop percentage, leading to increase in total number of fruits per plant, fruit weight, juice percentage, total soluble solids, acidity, vitamin-C, reducing sugars and non-reducing sugars. Belakbir *et al.* (1998) pointed that treatment with NAA produced the highest yield of marketable fruit.



Treatment with NAA

Fig 7.5 Effect of NAA application on wax apple yield (kg) under field condition.

7.4 CONCLUSION

The results showed that all NAA swabbing treatments had positive effect on anthocyanin content in wax apple fruit. The highest anthocyanin content was observed in the 90 ppm NAA treatment followed by 60 ppm NAA and 30 ppm NAA. The lowest anthocyanin content was recorded in the control. All NAA swabbing treatments exhibited increases in total phenol when compared to the control. The highest phenol content was recorded in 90 ppm NAA treatment followed by 60 ppm and 30 ppm NAA treatments. The lowest phenol content was observed in the control. Study result showed that NAA treatments increased the pH values. The highest pH value was recorded in 90 NAA swabbing treatment followed by 60 ppm NAA and 30 ppm NAA treatment respectively. The lowest pH value was observed in the control treatment. There were no significant differences among 60 ppm NAA, 30 ppm NAA and the control treatments in total flavonoid content However, 90 ppm treatment exhibited a slight significant increase in flavonoid content when compared to all other treatments. The highest Flavonoid content was recorded in 90 ppm NAA swabbing treatment followed by 60 ppm NAA treatment and finally 30 ppm NAA treatment. All NAA treatments increased K^+ content when compared to the control. However, there were no significant differences in K⁺ content among all NAA swabbing treatments. Results indicated that there were significant increases in TSS content recorded in 90 ppm and 60 ppm NAA treatments when compared to 30 ppm NAA and the control treatments. Moreover, the 30 ppm NAA treatment had slightly higher TSS when compared to the control treatment, thus the difference was not statically significant, and that could be associated partially with the low dosage of 30 ppm NAA fruit swabbing treatment. The highest TSS was recorded in 90 ppm treatment followed by 60 ppm treatment and finally by 30 ppm NAA. The lowest TSS among all treatments was recorded in the control NAA swabbing treatments decreased the titratable acidity (TA). The significantly lowest TA

was recorded in 90 ppm NAA treatment followed by 60 ppm NAA treatment. The highest TA recorded in the control. There was no significant difference between 90 ppm NAA treatment and 60 NAA treatments in TA. The study result showed that all NAA treatments increased the inverted sugar. The highest inverted sugar was observed in 90 ppm NAA treatment at followed by 60 ppm NAA treatment and finally 30 ppm NAA treatment. The lowest inverted sugar was recorded in the control treatment. The NAA swabbing treatments increased the accumulation of fructose in wax apple fruit. The highest significant increase in fructose was obtained in 90 ppm NAA treatment followed by 60 ppm NAA treatment. The lowest fructose was recorded in the control treatment followed by 30 ppm NAA treatment .However, the difference between 30 ppm NAA treatment and the control was not statically significant. Regarding SPAD values, results showed that there were no significant differences among all treatments. The SPAD values in all treatments were almost same. It was clear that NAA swabbing treatments did not affect the chlorophyll content in the leaves of wax apple fruits and that appeared to be due the method of applying NAA via swabbing method on fruit (no NAA applications were applied directly to wax apple leaves). All NAA swabbing treatments had a positive effect on fruit weight. The highest significant fruit weight was observed in 90 ppm NAA treatment followed by 60 ppm NAA treatment. The lowest weight was recorded in the control treatment followed by 30 ppm NAA treatment. However, there was no significant difference between the control and 30 ppm. NAA treatment. The percentage of fruit juice per 100 g significantly increased in 90 ppm NAA when compared to all other treatments. The highest juice percentage was obtained by 90 ppm NAA followed by 60 ppm NAA treatment. The lowest fruit juice percentage was recorded in the control treatment followed by 30 ppm NAA treatment respectively All NAA swabbing treatments had a positive effect in fruit length. There were substantial significant increases in fruit length in all NAA swabbing treatments. The highest fruit

length was observed in 90 ppm NAA treatment followed by 60 ppm NAA and finally 30 ppm NAA treatment whereas the lowest fruit length was recorded in the control treatment Fruit width positively affected by NAA treatments. The fruit diameter increased significantly with all NAA treatments. The highest fruit width was observed in 90 ppm NAA treatment followed by 60 ppm. However, the difference between 90 and 60 ppm treatments was not statically different. The lowest width was recorded in the control treatment followed 30 ppm NAA. The result showed that NAA swabbing treatments had positive effect on bud drop. The highest reduction in bud drop was observed in 90 ppm NAA followed by 60 ppm NAA treatment. However, there was no significant difference in bud drop parentage between 90 and 60 ppm NAA treatments. The highest bud drop was recorded in the control treatment followed by 30-ppm NAA treatment respectively. The result showed that NAA had positive effect on fruit set in general. The highest fruit set was obtained by 90 NAA treatment followed by 60 ppm NAA treatment and finally 30 ppm NAA treatment. The lowest fruit set percentage was recorded in the control treatment. All NAA swabbing treatments reduced fruit drop in wax apple fruit. The greatest reduction in bud drop was observed in 90 ppm NAA treatment followed by 60 ppm NAA treatment. The highest fruit drop was observed in the control treatment followed by 30 ppm NAA treatment. It appeared that swabbing wax apple fruit with NAA changed the hormonal balance, thus, reducing the percentages of fruit drop. NAA had a positive effect in wax apple yield. The greatest yield was obtained by 90 ppm NAA treatment followed by 60 ppm NAA treatment. The lowest yield was recorded in the control treatment followed by 30 ppm. Increasing yield by NAA swabbing treatments, appeared to be due the reduction of fruit drop, increasing the fruit weight and fruit set. These factors all together increased the yield in NAA treatments.

From the previous conclusion, the result suggested that the best NAA fruit swabbing treatment for wax apple grown under field condition in term of fruit quality and yield was 90 ppm NAA followed by 60 ppm NAA. Hence, there was no big difference between swabbing fruit with 30 ppm NAA and the control treatment

NAA Treatments	Anthocyanin mg/L	Total phenols (mg GAE/100g)	рН	Total Flavonoid (mg/100g)	K ⁺ (mg/kg)
Control	2.76±0.40cd	346.33±17.46d	4.79±0.29c	14.01±1.90b	13.77±0.89b
30 ppm	3.28±0.45c	368.07±27.79c	4.93±0.35bc	14.03±1.46b	15.84±0.96a
60 ppm	3.95±0.31b	386.20±27.61b	5.06±0.29ab	14.44±1.51b	15.96±0.85a
90 ppm	4.28 ±0.27a	420.13±17.93a	5.15±0.22a	15.91±1.02a	16.47±1.18a

Table 7.1 Effects of different treatments of NAA on anthocyanin, pH, K⁺, flavonoids and total phenols content of wax apple fruit under field condition.

(°Brix)
9.39±0.44c
0.61±0.35bc
9.73±0.37b
0.13±0.34a
).)

Table 7.2 Effects of different concentrations of NAA on total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio,inverted sugar (°Brix) and fructose (°Brix) content of wax apple fruit under field condition.

NAA Treatments	SPAD	Fruit weight (g)	Juice per 100 g/ fruit (ml)	Fruit length (mm)	Fruit width (mm)
Control	60.16±5.48a	44.45±3.38c	74.00±1.28b	57.97±2.92d	35.80±2.04c
30 ppm	60.58±5.53a	47.18±3.86c	74.28±1.25b	61.20±3.87c	38.46±2.56b
60 ppm	61.38±4.44a	52.80±5.43b	74.78±1.45ab	63.96±2.00b	42.92±1.85a
90 ppm	61.99±5.08a	63.76±4.55a	75.26±1.04a	67.06±3.22a	43.89±2.37a

Table 7.3 Effects of NAA on chlorophyll content (SPAD) fruit weight, juice (%), fruit length, fruit width of wax apple fruit under field condition.

NAA Treatments	Fruit set (%)	Fruit drop (%)	Bud drop (%)	Branch yield (kg)
Control	44.37±2.38d	45.51±3.03a	47.93±2.86a	0.83±0.08d
30 ppm	51.20±5.01c	41.60±4.25b	43.33±2.26b	1.01±0.16c
60 ppm	55.47±4.01b	36.47±2.32c	39.33±2.92c	1.38±0.20b
90 ppm	60.00±4.55a	30.93±3.09d	38.20±3.20c	1.77±0.23a

Table 7.4 Effects of NAA on bud drop fruit drop, fruit set, and branch yield of wax apple fruit under field condition.

GENERAL DISCUSSIONS AND CONCLUSIONS

8.1 GENERAL DISCUSSIONS

As outlined in Chapter 1, this chapter introduces the contextual sets and background for this thesis. It includes a background of wax apple fruit botany and scientific classifications, and a general overview of the impact of improving wax apple fruit quality on orchard profitability and consumers demand. This chapter also presents the objectives of this work. Chapter 2 covers the literature review of the topics under this thesis. It emphasizes the impact of several factors contribute to fruit quality and yield. Chapter 3 covers the impact of fruit shading, fruit thinning, plant growth promoter and inhibitor on fruit's development and quality. Light it is very important for fruit development and quality. Chapter 3 focused on the effect of shading on wax apple fruit development and quality by using three levels of fruit shading under the experimental field conditions. Chapter 4 reports the impact of fruit thinning on wax apple fruit quality and development under the field conditions (first report on wax apple in Malaysia). This chapter highlights the relation between fruit thinning practice and the physical and chemicals quality components of wax apple fruit. In the effort of improving fruit quality by using new methods, chapter 6 introduces a new technique of injection plant growth regulators into wax apple tree branches via Xylem Injection. Chapter 5 highlights the results of three levels of xylem injection with gibberellic acid (GA_3) on wax apple fruit development and quality parameters under the field's condition. Fruit colour is one of the important factors the influence the fruit appearance. Fruit with full colour is more applied to consumer than less colour one. Chapter 6 highlights the effect of applications of abscisic acid on wax apple fruit colour development at advance development stage. Fruit size is one of the restrictive factors in fruit marketing of fruit. Chapter 7 reports the effect of different concentrations of Naphthalene Acetic Acid (NAA) on wax apple fruit size and other fruit quality parameters. The method used to apply NAA was swabbing the fruit.

Our results (Tables 3.2, 3.3., 3.4, and 3.5) showed that 50% and 70% shading wax apple fruit under Malaysian climate conditions had negative effects on fruit physiological and biochemical characteristic. Shading branches bearing fruits increased bud and fruit drop, decreased fruit set lowered the anthocyanin content (colour), increased titratable acidity (TA), decreased sugar accumulation and decreased fruit growth rate and final yield. It seemed that wax apple fruit is very well adapted to tropical climate where it subjected to full exposure to sunlight.

Our results (Tables 4.1, 4.2., and 4.3) showed that all thinning treatments; 10 fruits/branch, 15 fruits/branch and 20 fruits/branch increase anthocyanin significantly when compared to the control. The highest anthocyanin content was recorded in 10 fruits/branch and it was 4.26±0.31 (mg/L).

All thinning treatments showed a significant increase in total phenols. Result showed that all thinning treatments had higher pH value compared to the control. The highest significant pH was recorded at 10 fruits/branch treatment with 4.97±0.41 pH. Flavonoid content in fruit affected positively by thinning treatments. Flavonoid content was significantly higher in 10 fruits/branch when compared to all other treatments. Results showed that fruits in 10 fruits/branch had a significantly higher K^+ when compared to all other treatments. Results showed that 10 fruits/branch and 15 fruits/branch treatments increased TSS content significantly when compared to control treatment. However, there was no significant difference in TSS content between control treatment and 20 fruits/branch thinning treatment. Result showed that all thinning treatments reduced TA when compared to control treatment. However, there was no significant difference in TA content among 20 fruits/branch and 15 fruits/branch and control treatments. The highest TSS/TA ratio was recorded in 10 fruits/branch at 13.11 ± 0.82 followed by 15 fruits/branch at 12.52 ± 1.01 , 20 fruits/branch at 11.81 ± 0.68 and the least TSS/TA ratio was 11.59±0.95 recorded in the control treatment. The study 170

result showed that all thinning treatments increased significantly inverted sugar content in fruit when compared to control treatment. However, there was no significant difference between 15 fruits/branch and 20 fruits/branch treatments in inverted sugar content. All fruit thinning treatments had higher fructose content when compared to the control treatment. 10 fruits/branch had the heights fructose content when compared to all thinning treatments. There were no significant differences among all treatments in leaves chlorophyll content (SPAD values). The fruit weight increased significantly in 10 fruits/branch and 15 fruits/branch thinning treatments when compared with control (unthinned fruits). However, there was no significant difference between 20 fruits/branch and control treatment and both had almost the same average fruit weight. The percentage of fruit juice per 100 g was similar in all treatments and they did not differ significantly. Fruit length significantly affected by 10 fruits/branch and 15 fruits/branch treatments. All thinning treatments showed increase in the fruit width when compared to the control. However, there was no significant difference in fruit width between 20 fruits/ brans and control treatments.

All thinning treatments had positive effect on fruit weight. All thinning treatments resulted in bigger and heavier fruits when compared to non-thinned treatment (control). Thinning fruit treatments had negative impacts on final yield in wax apple fruit. The highest yield was obtained in 20 fruits/branch treatment followed by the control treatment, 15 fruits/branch treatments and 10 fruits/branch treatments being the lowest in final yield. It can be suggested that the best treatment in final yield and fruit quality was 15 fruits/branch. All GA₃ treatments (90, 60 and 30 ppm) had positive effect on anthocyanin accumulation. All GA₃ treatments enhanced anthocyanin concentration in wax apple fruit. The highest anthocyanin content was 4.58 ± 0.47 (mg/L) recorded in GA₃ treatment at 90 ppm followed by GA₃ at 60 ppm with anthocyanin content 3.96 ± 0.31 (mg/L), followed by GA₃ treatment at 30 ppm with anthocyanin content

 3.29 ± 0.45 (mg/L). All GA₃ treatments showed a significant increase in total phenols when compared to the control treatment (Table 5.1). The highest phenol content was recorded in 90 ppm GA₃ treatment at 464.27±36.02 GAE/100g followed by 60 ppm GA₃ treatment at 419.20±27.61 GAE/100g, followed by 30 ppm GA₃ treatment at 408.93±28.69 GAE/100g. The lowest phenols content was recorded in the control treatment at 349.33±17.46 GAE/100g. Study result showed that all GA₃ treatments had higher pH value compared to the control (Table 5.1). The highest significant pH value was recorded at 90 ppm GA₃ treatment with 5.25 ± 0.22 pH. Lower pH values were observed in 30 ppm GA₃ treatment and the control; 4.94 ± 0.35 pH and 4.93 ± 0.34 pH respectively. This study showed that flavonoid affected by GA₃ treatment. Flavonoid content was significantly higher in 90 ppm GA₃ followed by 60 ppm GA₃ treatments (Table 5.1). However, the study revealed that there was no significant difference between 30 ppm GA₃ treatment and the control. All treatments with GA₃ had a significantly higher K⁺ when compared to control treatment (Table 5.1). However, there was no significant difference between 60 ppm and 30 ppm GA₃ treatment.

Results in Table 5.2, indicated that 90 ppm and 60 ppm GA₃ treatments significantly increased the TSS in fruits when compared to the control and 30 ppm GA₃ treatment. However, there was no significant difference between 30 ppm GA₃ and the control treatments. In addition, there was no significant difference in TSS between 90 ppm and 60 ppm GA₃ treatments. The highest TSS was recorded in 90 ppm GA₃ treatment at 9.92 ± 0.27 (°Brix). The lowest TSS content was 9.23 ± 0.29 (°Brix) recorded in control treatment, followed by 30 ppm GA₃ treatment at 9.60 ± 0.29 (°Brix). Result showed that 90 ppm and 60 ppm GA₃ treatment (Table 5.2). The highest TSS/TA ratio was observed in 90 ppm GA₃ treatment at 13.45 ± 0.86 followed by 60 ppm GA₃ treatment at 13.07 ± 0.72 . The lowest in TSS/TA ratio recorded in the control followed by 30 ppm GA₃ treatment at 13.07 ± 0.72 .

at 12.42 ± 0.73 . The significant increases in TSS/TA ratio in 90 and 60 ppm GA₃ treatments were due their significant increases in TSS and decreases in TA. The study result showed that all GA₃ treatments increased significantly inverted sugar content in fruit when compared to control treatment (Table 5.2). As showed in table 5.2, all GA_3 treatments (30 ppm, 60 ppm and 90 ppm GA₃) treatments had significant higher fructose contents when compared to the control treatment. However, there were no significant differences among all GA₃ treatments in fructose contents. Table 5.3, showed that there were no significant differences among all GA₃ treatments in SPAD values. GA₃ treatments had a positive effect on fruit weight. As shown in Table 5.3 the fruit weight increased significantly by all GA₃ treatments when compared with control. The highest fruit weight was observed in 90 ppm GA_3 treatment followed by 60 ppm GA₃ treatment and 30 ppm GA₃ treatment. The lowest fruit weight was recorded in the control treatment. From Table 5.3, the percentage of fruit juice per 100 g fruit was similar in 30 ppm GA_3 and 60 ppm GA_3 and the control treatments, and they did not differ significantly. There was a significant difference in percentage of fruit juice recorded in 90 ppm GA_3 treatment when compared to all other treatments. However, the difference was very slim. All GA₃ treatments showed increase in the fruit length when compared to the control. The highest fruit length was recorded in 90 ppm. Fruit width positively affected by GA₃ treatments Table 5.3. 90 ppm GA₃ treatment and 60 GA₃ treatments exhibited a significant increase in the fruit width when compared to 30 ppm GA₃ and the control treatment. As shown in Table 5.4, all GA₃ treatments reduced significantly bud drop when compared to un-treated GA₃ branches (control). The highest significant reduction in bud drop was observed in 90 ppm. As shown in Table 5.4, GA_3 treatments increased significantly the fruit set in wax apple when compared to the control treatment. All GA_3 treatments had positive effect on fruit drop. GA_3 treatments reduced fruit drop significantly when compared to the control (increased fruit retention) (Table 5.4). All GA₃ treatments had significantly positive effects on branch yield. The 90 ppm GA₃ treatment almost doubled the branch yield when compared to the control treatment. To improve wax apple quality parameters and yield, it can be suggested that 90 ppm GA₃ treatment.

Xie et al. (2011) pointed that anthocyanin biosynthesis in fruits becomes one feature of currently active research because colour is an important quality attribute for fruit. ABA swabbing treatments of had a significant effect on anthocyanin content of wax apple fruits. The results displayed that the highest content of anthocyanin (3.86 mg/L) was recorded in 150 ppm ABA treatment followed 100 and 50 ppm treatment with anthocyanin content of 3.43 and 3.32 mg/L respectively. The lowest content of anthocyanin (2.82 mg/L) recorded in control fruits (Table 6.1, Fig 6.3). Result showed in table 6.1 indicated that there were no significant differences in Total phenol among all treatments. The total phenols were in 150 ppm ABA, 100 ppm ABA, 50 ppm ABA and the control treatments as follows; 350.40±12.21 mg GAE/100g, 352.00±13.35 mg GAE/100g, 350.60±12.15 mg GAE/100g, 356.67±11.85 mg GAE/100g respectively. ABA treatments by swabbing method at relatively advance stages of fruit development (at 4th, 5th, 6th week) had little or no effect on flavonoid content at harvest. Result shown in Table 6.1, indicated flavonoid content in ABA treated fruits was little bit more than the control fruit, however, their differences were not statistically significant at $p \le 1$ 0.05. Change in pH is directly related to change in acidity of fruits. Results showed that pH value of wax apple fruits significantly affected by 150 ABA treatments (Table 6.1). The highest pH value 5.04±0.31 was recorded in 150 ppm treated fruits, whereas, the lowest value 4.77.23b was found in control fruits. Result showed trend of increasing pH value in 100 ppm ABA and 50 ABA when compared to the control. Result shown in Table 6.1, indicated little increases in K^+ . However, they were not statically significant. The highest increase in K^+ content was observed in 150 ppm ABA at 15.41±1.16 mg/kg

followed by 100 ppm ABA at 15.09±1.01 mg/kg and 50 ppm ABA at 14.75±1.13 mg/kg. The lowest K^+ content among all treatments was observed in the control treatment at 14.59±1.13 mg/kg. Result in Table 6.2 indicated that there was a slight significant increase in TSS obtained by 150 ppm ABA treatment. In addition, there were no difference in TSS among the 100 ppm ABA, 50 ppm ABA and the control. The highest TSS was recorded in 150 ABA treatment at 9.62±0.31 °Brix followed by 100 ABA treatment at 9.33±0.28 °Brix and 50 ABA treatment at 9.26±0.45 °Brix. The lowest TSS observed in the control treatment at 9.27±0.27 °Brix. The result showed that ABA treatments at relatively advance stage of fruit development had a slight effect on TSS. 150 and 100 ppm ABA treatments, at relatively advance stage of fruit development reduced significantly fruit acidity. However, there was no significant difference in TA between 50 ppm ABA and the control treatment in fruit TA. The greatest reduction in TA among all treatments was observed in 150 ppm ABA treatment at 0.74±0.40% followed by 100 ppm ABA treatment at 0.75±0.30%. The highest TA was recorded in the control treatment at 0.79±0.40% followed by 50 ppm ABA treatment at $0.78\pm0.40\%$ (Table 6.2). Result in Table 6.3 showed that there were no differences in fruit weight among all treatments. However, there were slight nonsignificant increases tendency in fruit weight observed in the ABA treatments. The lowest fruit weight was recorded in the control treatment at 42.67±3.38 g followed by 50 ABA treatments at 42.72±3.04 g. The highest fruit weight was observed in 150 ABA treatment 43.46±3.56 g followed by 100 ppm ABA treatment at 43.18±2.55 g. The slight increase in fruit weight in ABA treatments could be related to a slight increase in fruit drop caused by ABA treatments. The study result shown in Table 6.2 indicated that 150 ABA treatment increased significantly the inverted sugar content in wax apple fruit when compared to all other treatments. There were positive trend of increasing inverted sugar content by 100 and 50 ppm ABA treatments when compared to the control.

However, the trends were not statically significant. The study result in Table 6.2 showed that 150 ABA treatment increased significantly the fructose in wax apple fruit when compared to all other treatments. There were positive trend of increasing fructose content by 100 and 50 ppm ABA treatments when compared to the control. However, trends were not statically significant. The SPAD values in Table 6.3 showed that ABA swabbing fruit treatments had no significant effects on chlorophyll content of leaves of wax apple. Result in Table 6.3 showed that there were no differences in fruit weight among all treatments. However, there were slight non-significant increases tendency in fruit weight observed in the ABA treatments. The lowest fruit weight was recorded in the control treatment at 42.67±3.38 g followed by 50 ABA treatments at 42.72±3.04 g. The highest fruit weight was observed in 150 ABA treatment 43.46±3.56 kg followed by 100 ppm ABA treatment at 43.18±2.55 g. Result in Table 6.3 showed that ABA swabbing treatments at relatively advance stage of fruit development had no effect on fruit width. Similarly, result in Table 6.3 presented that ABA swabbing treatments at relatively advance stage of fruit development had no effect on fruit length. Result showed that there were no significant differences in juice volume among all treatments; thus, there were a slight non-significant increase in juice volume observed in ABA treatments. Result showed that ABA swabbing treatments increased slightly fruit drop (Table 6.3). Only in 150 ABA treatment fruit drop was significantly little higher when compared to all other treatments. There were no significant differences among all treatments in yield (Table 6.3). However, the result showed that ABA swabbing treatments slightly reduced yield. The highest yield was recorded in the control treatment with 0.83±0.06 kg followed by 50 ppm ABA with 0.82±0.06 kg. The lowest yield was recorded in 150 ppm ABA with 0.79±0.06 kg followed by 100 ppm ABA with 0.81±0.07 kg.

All NAA swabbing treatments had positive effect on anthocyanin content (Table 7. 1). The highest anthocyanin content was observe in the 90 ppm NAA treatment at 4.28 ± 0.27 mg/L, followed by 60 ppm NAA at 3.95 ± 0.31 mg/L and 30 ppm NAA at 3.28 ± 0.45 mg/L. The lowest anthocyanin content was recorded at the control treatment at 2.76±0.40 mg/L. NAA swabbing treatment exhibited increases in total phenol when compared to the control (Table 7.1). The highest phenol content was recorded in 90 ppm NAA treatment at 420.13±17.93 (mg GAE/100g) followed by 60 ppm NAA treatment at 386.20±27 (mg GAE/100g), followed by 30 NAA treatment at 368.07±27.79 (mg GAE/100g). The lowest phenol content was observed in the control treatment at 346.33±17.46 (mg GAE/100g). Study result showed that NAA increased the pH values. The highest pH value was recorded in 90 NAA swabbing treatment at 5.15±0.22 a followed by 60 ppm NAA treatment at 5.06±0.29, followed by 30 ppm NAA treatment at 4.93±0.35. The lowest pH value was observed in the control treatment. Table 7.1 showed that there were no significant differences among 60 ppm NAA, 30 ppm NAA and the control treatments. However, 90 ppm treatment exhibited a slight significant increase in Flavonoid content when compared to all other treatments. The highest Flavonoid content was recorded in 90 ppm NAA swabbing treatment at 15.91 ± 1.02 mg/100g followed by 60 ppm NAA treatment at 14.44 ± 1.51 mg/100g and 30 ppm NAA treatment at 14.03±1.46 mg/100g. As shown in Table 7.1, all NAA treatments caused an increased in K⁺ content when compared to the control. However, there were no significant differences in K^+ content among all NAA swabbing treatments. Results in Table 7.2, indicated that there were significant increases in TSS content recorded in 90 ppm NAA and 60 NAA treatments when compared to 30 ppm NAA and the control treatments. Moreover, the 30 ppm NAA treatment had slightly higher TSS when compared to the control treatment, thus the difference was not statically significant, and that could be associated partially with the low dosage of 30

ppm NAA treatment. the highest TSS was recorded in 90 ppm treatment at 10.27 ± 0.27 °Brix followed by 60 ppm treatment at 9.83 ± 0.38 °Brix, followed by 30 ppm NAA treatment at $9.72\pm0.34b$ °Brix. The lowest TSS among all treatment was recorded in the control at 9.49 ± 0.44 °Brix. Result in Table 7.2 showed that NAA swabbing treatment decreased the titratable acidity (TA). The lowest significant TA was recorded in 90 ppm NAA treatment at $0.75\pm0.04\%$ followed by 60 ppm NAA treatment at 0.77 ± 0.03 . The highest TA recorded in the control treatment at $0.81\pm0.05\%$ followed by 30 ppm NAA treatment at $0.77\pm0.03\%$. There was no significant difference between 90 ppm NAA treatment and 60 NAA treatment in TA.

The study result in Table 7.2 showed that all NAA treatments increased the inverted sugar. The highest inverted sugar was observed in 90 ppm NAA treatment at 10.05±0.33 °Brix followed by 60 ppm NAA treatment at 9.64±0.39 °Brix and 30 ppm NAA treatment at 9.52±0.34 °Brix. The lowest inverted sugar was recorded in the control treatment at 9.29±0.45 °Brix. As showed in table 7.2, the NAA swabbing treatments increase the accumulation of fructose in wax apple fruit. The highest significant increase in fructose was obtained in 90 ppm NAA treatment at 10.13±0.34 °Brix followed by 60 ppm NAA treatment at 9.73±0.37 °Brix. The lowest fructose was recorded in the control treatment at 9.39±0.44 °Brix followed by 30 ppm NAA treatment at 9.61±0.35 °Brix. However, the difference between 30 ppm NAA treatment and the control was not statically significant. The SPAD values in Table 7.3 showed that there were no significant differences among all treatments. The SPAD values in all treatments were same. In 90 ppm NAA ppm treatment was 61.99±5.08, in 60 ppm NAA was 61.38±4.44, in 30 ppm NAA treatment was 60.58±5.53 and in the control was 60.16±5.48. It was clear that NAA swabbing treatments did not affect the chlorophyll content in the leaves of wax apple fruits and that appeared to be due the method of using swabbing fruit. All NAA swabbing treatments had a positive effect on fruit weight. As

shown in Table 7.3, the highest significant fruit weight was observed in 90 ppm NAA treatment followed at 63.76±4.55 g followed by 60 ppm NAA treatment at 52.80±5.43 g. the lowest weight was recorded in the control treatment at 44.45±3.38 g followed by 30 ppm NAA treatment at 47.18±3.86 g. However, there was no significant difference between the control and 30 ppm NAA treatment. From Table 7.3, the percentage of fruit juice per 100 g in 90 ppm NAA showed a significant increase in fruit percentage when compared to all other treatments. The highest juice was obtained by 90 ppm NAA treatment at 75.26±1.04% followed by 60 ppm NAA treatment at 74.78±1.45%. The lowest fruit juice percentage was recorded in the control treatment at 74.00±1.28% followed by 30 ppm NAA treatment at 74.28±1.25%. All NAA swabbing treatments had a positive effect in fruit length (Table 7.3). There were substantial significant increases in fruit length in all NAA swabbing treatments. The highest fruit length was observed in 90 ppm NAA treatment at 67.06±3.22 mm followed by 60 ppm NAA at 63.96±2.00 mm and 30 ppm NAA treatment at 61.20±3.87 mm whereas the lowest fruit length was recorded in the control treatment at 57.97±2.92 mm. Fruit width positively affected by NAA treatments (Table 7.3.). The fruit diameter increased significantly with all NAA treatments. The highest fruit width was observed in 90 ppm NAA treatment at 43.89±2.37 mm followed by 60 ppm NAA at 42.92±1.85 mm, however, the difference between both treatments was not statically different. The lowest width was recorded in the control treatment at 35.80±2.04 mm followed by 38.46±2.56 mm. NAA swabbing treatments had positive effect on bud drop (Table 7.3). The highest reduction in bud drop was observed in 90 ppm NAA treatment at 38.20±3.20% followed by 60 ppm NAA treatment at 39.33±2.92%. However, there was no significant difference in bud drop parentage between 90 and 60 ppm NAA treatment. The highest bud drop was recorded in the control treatment at 47.93±2.86 percentage followed by 30 ppm NAA treatment at 43.33±2.26. The result showed that NAA had positive effect on fruit set in

general (Table 7.4). The highest fruit set was obtained by 90 NAA treatment at $60.00\pm4.55\%$ followed by 60 ppm NAA treatment at $55.47\pm4.01\%$ and 30 ppm NAA treatment at $51.20\pm5.01\%$. The lowest fruit set percentage was recorded in the control treatment at $45.37\pm2.38\%$. NAA swabbing treatment treatments reduced fruit drop in wax apple fruit (Table 7.4). The greatest reduction observed in 90 ppm NAA treatment at $30.93\pm3.09\%$ followed by 60 ppm NAA treatment at $36.47\pm2.32\%$. The highest fruit drop was observed in the control treatment at $45.51\pm3.03\%$ followed by 30 ppm NAA treatment at $41.60\pm4.25\%$. NAA had a positive effect in wax apple yield. The greatest yield was obtained by 90 ppm NAA treatment at 1.77 ± 0.23 kg, followed by 60 ppm NAA treatment at 0.83 ± 0.08 kg, followed by 30 ppm at 1.01 ± 0.16 kg.

8.2 GENERAL CONCLUSION

Our results (Tables 3.2, 3.3., 3.4, and 3.5) showed that 50% and 70% shading wax apple fruit under Malaysian climate conditions had negative effects on fruit physiological and biochemical characteristic. Shading branches bearing fruits increased bud and fruit drop, decreased fruit set lowered the anthocyanin content (colour), increased titratable acidity (TA), decreased sugar accumulation and decreased fruit growth rate and final yield. It seemed that wax apple fruit is very well adapted to tropical climate where it subjected to full exposure to sunlight.

Our results (Tables 4.1, 4.2., and 4.3) showed that all thinning treatments; 10 fruits/branch, 15 fruits/branch and 20 fruits/branch increase anthocyanin significantly when compared to the control. All thinning treatment showed a significant increase in total phenols. Result showed that all thinning treatments had higher pH value compared to the control. Flavonoid content was significantly higher in 10 fruits/branch. Results showed that fruits in 15 fruits/branch had a significantly higher K⁺ when compared to all other treatments. Results showed that 10 fruits/branch and 15 fruits/branch treatments increased TSS content significantly when compared to control treatment. However, there was no significant difference in TSS content between control treatment and 20 fruits/branch thinning treatment. Result showed that all thinning treatments reduced TA when compared to control treatment. However, there was no significant difference in TA content among 20 fruits/branch and 15 fruits/branch and control treatments. The study result showed that all thinning treatments increased significantly inverted sugar content in fruit when compared to control treatment. However, there was no significant difference between 15 fruits/branch and 20 fruits/branch treatments in inverted sugar content. All fruits thinning treatments had higher fructose content when compared to the control treatment. 10 fruits/branch had the heights fructose content when compared to all thinning treatments. There were no significant differences among

all treatments in leaves chlorophyll content (SPAD values). The fruit weight increased significantly in 10 fruits/branch and 15 fruits/branch thinning treatments when compared with control (un thinned fruits). However, there was no significant difference between 20 fruits/branch and control treatment and both had almost the same average fruit weight. The percentage of fruit juice per 100 g was similar in all treatments and they did not differ significantly. Fruit length significantly affected by 10 fruits/branch and 15 fruits/branch treatments. All thinning treatments showed increase in the fruit width when compared to the control. However, there was no significant difference in fruit width between 20 fruits/ brans and control treatments.

All thinning treatments had positive effect on fruit weight. All thinning treatments resulted in bigger and heavier fruits when compared to non-thinned treatment (control). Thinning fruit treatments had negative impacts on final yield in wax apple fruit. The highest yield was obtained in 20 fruits/branch treatment followed by the control treatment, 15 fruits/branch treatments and 10 fruits/branch treatments being the lowest in final yield.

Theoretically, the best thinning treatment in terms of fruit quality was 10 fruits/branch. However, 10 fruits/branch resulted in sever reduction in the final yield. Practicality, to compromise the reduction in final yield observed in 10 fruits/branch, thus, it can be suggested that the best treatment in final yield and fruit quality (together) was 15 fruits/branch.

All GA₃ treatments enhanced anthocyanin concentration in wax apple fruit. All GA₃ treatments showed a significant increase in total phenols when compared to the control treatment (Table 5.1). The highest phenol content was recorded in 90 ppm GA₃ treatment and the lowest phenols content was recorded in the control treatment. Study result showed that all GA₃ treatments had higher pH value compared to the control

(Table 5.1). This study showed that flavonoid affected by GA_3 treatment. Flavonoid content was significantly higher in 90 ppm GA_3 followed by 60 ppm GA_3 treatments (Table 5.1). However, the study revealed that there was no significant difference between 30-ppm GA_3 treatment and the control. All treatments with GA_3 had a significantly higher K⁺ when compared to control treatment (Table 5.1). However, there was no significant difference between 60 ppm and 30 ppm GA_3 treatment.

The best GA3 treatment that can be suggested based in the above conclusion is 90 ppm GA₃. However, more studies needed in this field.

ABA swabbing treatments had a significant effect on anthocyanin content of wax apple fruits. The results displayed that the highest content of anthocyanin was recorded in 150 ppm ABA treatment and the lowest content of anthocyanin recorded in control fruits (Table 6.1, Fig 6.3). Result showed in table 6.1 indicated that there were no significant differences in Total phenol among all treatments. ABA treatments by swabbing method at relatively advance stages of fruit development (at 4th, 5th, 6th week) had little or no effect on flavonoid content at harvest. Result shown in Table 6.1, indicated flavonoid content in ABA treated fruits was little bit more than the control fruit, however, their differences were not statistically significant at $p \le 0.05$. Change in pH is directly related to change in acidity of fruits. Results showed that pH value of wax apple fruits significantly affected by 150 ABA treatments (Table 6.1). The highest pH value was recorded in 150 ppm treated fruits, whereas, the lowest value was found in control fruits. Result showed trend of increasing pH value in 100 ppm ABA and 50 ABA when compared to the control. Result shown in Table 6.1, indicated little increases in K⁺. However, they were not statically significant.

The highest increase in K^+ content was observed in 150 ppm and the lowest K^+ content among all treatments was observed in the control treatment. Result in Table 6.2

indicated that there was a slight significant increase in TSS obtained by 150 ABA treatment. In addition, there were no difference in TSS among the 100 ABA, 50 ABA and the control. The highest TSS was recorded in 150 ABA treatment, and the lowest TSS observed in the control treatment. The result showed that ABA treatments at relatively advance stage of fruit development had a slight effect on TSS. 150 and 100 ppm ABA treatments, at relatively advance stage of fruit development reduced significantly fruit acidity. However, there was no significant difference in TA between 50 ppm ABA and the control treatment in fruit TA (Table 6.2).

Result in Table 6.3 showed that there were no differences in fruit weight among all treatments. However, there were slight non-significant increases tendency in fruit weight observed in the ABA treatments. The lowest fruit weight was recorded in the control treatment and the highest fruit weight was observed in 150 ABA treatment. The study result in Table 6.2 showed that 150 ABA treatments increased significantly the inverted sugar content in wax apple fruit when compared to all other treatments. There were positive trend of increasing inverted sugar content by 100 and 50 ppm ABA treatments when compared to the control. However, trends were not statically significant. The study result in Table 6.2 revealed that 150 ABA treatment increased significantly the fructose in wax apple fruit when compared to all other treatments. There were positive trend of increasing fructose content by 100 and 50 ppm ABA treatments when compared to the control. However, trends were not statically significant. The SPAD values in Table 6.3 showed that ABA swabbing fruit treatments had no significant effects on chlorophyll content of leaves of wax apple. Result in Table 6.3 showed that there were no differences in fruit weight among all treatments. However, there were slight non-significant increases tendency in fruit weight observed in the ABA treatments. Result in Table 6.3 displayed that ABA swabbing treatments at relatively advance stage of fruit development had no effect on fruit width. Similarly,

result in Table 6.3 presented that ABA swabbing treatments at relatively advance stage of fruit development had no effect on fruit length. Result showed that there were no significant differences in juice volume among all treatments; thus, there were a slight non-significant increase in juice volume observed in ABA treatments. Result showed that ABA swabbing treatments increased slightly fruit drop (Table 6.3). Only in 150 ABA treatment fruit drop was significantly little higher when compared to all other treatments. There were no significant differences among all ABA treatments in yield (Table 6.3). However, result shoed that ABA swabbing treatments slightly reduced yield. The highest yield was recorded in the control treatment and the lowest yield was recorded in 150 ppm ABA.

From the above conclusion, it can be suggested that ABA at the 100-150 ppm is benefaction to enhance fruit colour without adverse effects in other fruit quality parameters.

All NAA swabbing treatments had positive effect on Anthocyanin content (Table 7. 1). The highest anthocyanin content was observe in the 90 ppm NAA and the lowest anthocyanin content was recorded at the control treatment. NAA swabbing treatment exhibited increases in total phenol when compared to the control (Table 7.1). The highest phenol content was recorded in 90 ppm NAA treatment and the lowest phenol content was observed in the control treatment. Study result showed that NAA increased the pH values. The highest pH value was recorded in 90 NAA swabbing treatment and the lowest pH value was observed in the control treatment. Table 7.1 showed that there were no significant differences among 60 ppm NAA, 30 ppm NAA and the control treatments. However, 90 ppm treatment exhibited a slight significant increase in flavonoid content when compared to all other treatments. As shown in Table 7.1, all NAA treatments caused increase in K⁺ content among all NAA swabbing

treatments. Results in Table 7.2, indicated that there were significant increases in TSS content recorded in 90 ppm NAA and 60 NAA treatments when compared to 30 ppm NAA the control treatments. The highest TSS was recorded in 90 ppm treatment and the lowest TSS among all treatment was recorded in the control .Result in Table 7.2 showed that NAA swabbing treatment decreased the titratable acidity (TA). The lowest significant TA was recorded in 90 ppm NAA treatment and the highest TA recorded in the control treatment. There was no significant difference between 90 ppm NAA treatment and 60 NAA treatment in TA. The study result in Table 7.2 showed that all NAA treatments increased the inverted sugar. The highest inverted sugar was observed in 90 ppm NAA treatment and the lowest inverted sugar was recorded in the control .As showed in table 7.2, the NAA swabbing treatments increased the accumulation of fructose in wax apple fruit. The highest significant increase in fructose was obtained in 90 ppm NAA treatment and the lowest fructose was recorded in the control treatment. The SPAD values in Table 7.3 showed that there were no significant differences among all NAA treatments. It was clear that NAA swabbing treatments did not affect the chlorophyll content in the leaves of wax apple fruits and that appeared to be due the method of using swabbing fruit. All NAA swabbing treatments had a positive effect on fruit weight. As shown in Table 7.3, the highest significant fruit weight was observed in 90 ppm NAA treatment and the lowest weight was recorded in the control treatment From Table 7.3, the percentage of fruit juice per 100 g in 90 ppm NAA showed a significant increase in fruit percentage when compared to all other treatments. The highest juice was obtained by 90 ppm NAA treatment and the lowest fruit juice percentage was recorded in the control treatment. All NAA swabbing treatments had a positive effect in fruit length (Table 7.3). There were substantial significant increases in fruit length in all NAA swabbing treatments. The highest fruit length was observed in 90 ppm NAA treatment whereas the lowest fruit length was recorded in the control

treatment at 57.97±2.92 mm. Fruit width positively affected by NAA treatments (Table 7.3.). The fruit diameter increased significantly with all NAA treatments. The highest fruit width was observed in 90 ppm NAA treatment and the lowest width was recorded in the control treatment. NAA swabbing treatments had positive effect on bud drop (Table 7.3). The highest reduction in bud drop was observed in 90 ppm NAA treatment and the highest bud drop was recorded in the control treatment. The result showed that NAA had positive effect on fruit set in general (Table 7.4). The highest fruit set was obtained by 90 NAA treatment and the lowest fruit set percentage was recorded in the control treatment. NAA swabbing treatments reduced fruit drop in wax apple fruit (Table 7.4). The greatest reduction observed in 90 ppm NAA treatment and The highest fruit drop was observed in the control treatment. NAA swabbing treatments reduced fruit drop in wax apple fruit (Table 7.4). The greatest reduction observed in 90 ppm NAA treatment and The highest fruit drop was observed in the control treatment. NAA had a positive effect in wax apple yield. The greatest yield was obtained by 90 ppm NAA treatment and the lowest yield recorded in the control treatment.

From the previous conclusion, the study suggested the best NAA swabbing treatment for wax apple grown under field condition was 90 ppm NAA followed by 60 ppm NAA.

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Publications:

	Paper	Journal	Status	Date
1.	Fruit growth, pigmentation and quality of three cultivars of wax apple (<i>Syzygium samarangense</i> [Blume] Merrill & L.M. Perry). Authors: Abdullah I. Alebidi, K. M. Moneruzzaman, Kamaludin A. Rashid, ABM Sharif Hossain and Amru Nasrulhaq Boyce	African Journal of Agricultural Research (ISI)	Submitted	29-6-2012
2.	Effect of ABA treatments on fruit size, colour development and quality of wax apple (<i>Syzygium samarangense</i>) under field conditions. Authors: Abdullah I. Alebidi, Kamaludin A. Rashid, ABM Sharif Hossain and K.M.Moneruzzaman	African Journal of Biotechnology (ISI)	Submitted	21-6-2012
3.	Assessment of Genetic Diversity in Three Cultivars of <i>Syzygium</i> <i>samarangense</i> Grown in Malaysia by Using Morphological and Physiological Parameters. Authors: Moneruzzaman K. M., Alebidi A. I. and Al-Saif A. M.	Research Journal of Biotechnology (ISI)	Published 7(3):16-22	Aug.,2012
4.	An evaluation of the nutritional quality of three cultivars of <i>Syzygium samarangense</i> under Malaysian conditions. Authors: Moneruzzaman, K.M.; Boyce, A.N.; Normaniza, O., Hossain, ABM, A I., Alebidi, A.I., Al-Saif, A.M.	African Journal of Agricultural Research (ISI)	Published 6(3):545-552	Feb., 2011

Workshops:

- Literature Review: Reading, Critical Thinking, and Academic Writing Workshop. IGS Upskill Programme, 30 May 2013. University of Malaya.
- 2- Preparing For Viva Presentation Effectively, Workshop, IGS Upskill
 Programme, 18 April 2013. University of Malaya.
- Format in Thesis/Dissertation Writing (Science) Workshop on 26 January 2012,
 IGS Upskill Programme. University of Malaya
- 4- How to Defence Your Thesis Workshop, IGS Upskill Programme dated in 8/2012. University of Malaya.
- 5- Orchid Uses, Physiology and Biotechnology, University of Malaya, 19-20
 November, 2011. 2 days' Workshop. University of Malaya.
- 6- Briefing on Writing Unit Workshop, University of Malaya 2010.

Seminars:

- Presented my Finding Seminar on 11 July 2013, Auditorium, Institute of Graduate Studies, University of Malaya.
- 2- Presented my Candidature Defence Seminar on 17 January 2013, Auditorium, Institute of Graduate Studies, University of Malaya.
- 3- Attended PhD Candidature Defence Seminars, Institute of Biological Sciences, University of Malaya. 16 -24 April 2011.
- 4- Presentation Skills, Prof. Dr. Ng Seik Weng, on 18th March 2011, University of Malaya.

APPENDICES



Appendix 1 Map showes the natvie distribution of *Syzygium samarangense* (Orwa *et al.*, 2009).

Appendix 3 Food value of *Syzygium samarangense* (wax apple) per 100 g of edible portion.

Moisture	91.40-92.96 g
Protein	0.50 g
Sugar	6.56 g
Iron	0.001 g
Ash	0.21-0.27 g
Calcium	0.01 g
Phosphorus	0.03 g
Sulphuric Acid	0.17%
Citric Acid	0.15%

Source: Morton (1987)

Appendix 3 Climatology information of orchard located at Banting, Selangor (2011).

Average	Те	mperature	Celsius	Rainfall	Relative
	Min	Max	Average	(mm)	Humidity
Monthly	23	37	33	225	84%

Source: Malaysian Metrological Department

Place of Origin	Selangor, Malaysia
Brand Name	Tengfei
Model	sunshade net
Net width	2 m
Shade rate	50 and 70%
Colour	black
Material	HDPE PE
Туре	Warp knitted
Quality	ISO standard

Appendix 4 Shading net specification.

Mean square (MS)									
Variation source	df	Bud drop	Fruit Set	Fruit drop	Fruit length	Fruit length	Fruit width	Fruit Juice	Yield
Between Groups	2	506.69 [*]	271.27*	87.49*	379.41*	230.18*	278.41*	164.43 ^{ns}	0.551*
Within Groups	42	10.83	8.97	14.32	5.73	11.65	6.18	2.89	0.006
CV%	_	10.52	11.00	8.7	12.3	8.30	12.61	2.78	28.93

Appendix 5 Analysis of variance (ANOVA) for physiological characteristic of wax apple affected by shading.

Shading							
Variation source	df	Anthocyanin	Total phenols	рН	Total Flavonoid	K^+	SPAD
Between Groups	2	0.472*	183.20 ^{ns}	0.868*	48.12 [*]	33.48*	57.85 ^{ns}
Within Groups	42	0.117	148.49	0.093	1.33	0.68	52.66
CV%	_	14.87	3.43	7.64	15.94	11.47	12.29
*Significant differe	ence at	<i>p</i> <0.05	ns: not sig	gnificant.			

Appendix 6 Analysis of variance (ANOVA) for biochemical characteristic of wax apple affected by shading.

Shading		Mean square (MS)						
Variation source	df	TA	TSS	TSS/TA	Inverted sugar	Fructose		
Between Groups	2	0.011*	2.23*	10.82*	1.62*	1.67*		
Within Groups	42	0.002	0.106	0.571	0.18	0.144		
CV%	_	5.51	5.24	9.52	6.07	5.71		

Appendix 7 Analysis of variance (ANOVA) for biochemical characteristic of wax apple affected by shading.

*Significant difference at *p*<0.05

ns: not significant.

Thinning				Mean square (MS)				
Variation source	df	Fruit weight	Fruit Length	Fruit width	Fruit Juice	Yield		
Between Groups	3	2005.52*	353.87*	325.09 [*]	0.280 ^{ns}	0.253*		
Within Groups	56	11.82	7.06	4.84	3.739	0.005		
CV%	-	18.92	7.63	10.63	2.55	15.04		

Appendix 8 Analysis of variance (ANOVA) for physiological characteristic of wax apple thinning treatment.

*Significant difference at p < 0.05 ns: not significant.

Thinning Mean square (MS)										
Variation source	df	Anthocyanin	Total phenols	РН	Total Flavonoid	K^+	SPAD			
Between Groups	3	6.95*	4254.51*	0.337*	10.78*	7.93*	33.68 ^{ns}			
Within Groups	56	0.14	553.16	0.124	5.04	0.83	30.58			
CV%	-	20.20	7.49	7.68	15.21	6.56	8.83			

Appendix 9 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by thinning treatment.

Significant difference at p < 0.05ns: not significant.

Thinning						
Variation source	df	TA TSS		TSS/TA	Inverted sugar	Fructose
Between Groups	3	.007*	1.09*	7.17*	0.95*	0.85*
Within Groups	56	0.002	0.13	0.77	0.12	0.12
CV%	_	5.91	4.44	8.53	4.39	4.34

Appendix 10 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by thinning treatment.

*Significant difference at p < 0.05 ns: not significant.

GA ₃										
Mean square (MS)										
Variation source	df	Bud drop	Fruit set	Fruit drop	Fruit weight	Fruit length	Fruit width	Fruit juice	Yield	
Between Groups	3	289.13^{*}	723.53*	665.56*	1653.45 [*]	444.60^{*}	185.94^{*}	12.77 ^{ns}	3.35*	
Within Groups	56	8.13	12.53	9.65	14.11	9.57	6.37	3.28	0.02	
CV%	-	11.03	12.69	17.41	20.22	8.62	9.53	2.52	36.16	
*Significant differen	o<0.05	ns: not s	ignificant.							

Appendix 11 Analysis of variance (ANOVA) for physiological characteristics of wax apple affected by GA₃ plant growth regulators.

GA ₃ Mean square (MS)									
Variation source	df	Anthocyanin	Total phenols	РН	Total Flavonoid	K^+	SPAD		
Between Groups	3	9.30*	33551.71*	0.326*	112.41*	24.83*	31.86 ^{ns}		
Within Groups	56	0.17	796.85	0.098	6.62	0.82	27.69		
CV%	-	21.45	12.09	6.59	22.44	8.35	8.66		

Appendix 12 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by GA₃ plant growth regulators.

*Significant difference at p < 0.05 ns: not significant.

GA ₃	Mean square (MS)					
Variation source	df	TA	TSS	TSS/TA	Inverted sugar	Fructose
Between Groups	3	0.014*	1.41*	11.55*	1.35*	1.18*
Within Groups	56	0.002	0.09	0.56	0.09	0.095
CV%	_	6.20	4.10	8.43	4.16	4.21

Appendix 13 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by GA₃ plant growth regulators.

*Significant difference at p < 0.05 ns: not significant
ABA		Mean square (MS)						
Variation source	df	Fruit drop	Fruit weight	Fruit length	Fruit width	Fruit juice	Yield	
Between Groups	3	30.11*	1.71 ^{ns}	0.214 ^{ns}	0.314 ^{ns}	1.09 ^{ns}	0.005 ^{ns}	
Within Groups	56	11.5	12.80	17.62	14.97	1.23	0.004	
CV%	—	7.68	8.42	6.80	9.48	1.50	7.58	
*0: :0: 1:00		-0.05						

Appendix 14 Analysis of variance (ANOVA) for physiological characteristics of wax apple affected by ABA plant growth regulator.

ABA Mean square (MS)									
Variation source	df	Anthocyanin	Total phenols	РН	Total Flavonoid	K^+	SPAD		
Between Groups	3	2.75*	128.02 ^{ns}	0.184*	2.23 ^{ns}	1.99 ^{ns}	18.01 ^{ns}		
Within Groups	56	0.23	153.83	0.092	1.94	1.20	62.21		
CV%	_	18.17	3.51	6.36	10.35	7.44	12.69		
*Ciamificant diffor	maa at a	<0.05 mg	. mat significant						

Appendix 15 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by ABA plant growth regulator.

ABA Mean square (MS)							
Variation source	df	TA	TSS	TSS/TA	Inverted sugar	Fructose	
Between Groups	3	0.008^*	0.435*	4.97*	0.459*	0.121*	
Within Groups	56	0.001	0.112	0.56	0.126	0.126	
CV%	_	5.49	3.83	7.22	4.13	3.88	

Appendix 16 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by ABA plant growth regulator.

*Significant difference at p < 0.05

ns: not significant.

NAA Mean square (MS)									
Variation source	df	Bud drop	Fruit set	Fruit drop	Fruit weight	Fruit length	Fruit width	Yield	
Between Groups	3	294.17 [*]	662.57 [*]	600.14*	1095.78*	225.61*	216.88*	2.64*	
Within Groups	56	8.03	16.91	10.53	19.12	9.46	4.93	0.031	
CV%	_	11.41	13.27	16.98	17.30	7.21	9.80	15.55	

Appendix 17 Analysis of variance (ANOVA) for physiological characteristics of wax apple affected by NAA plant growth regulator.

NAA Mean square (MS)								
Variation source	df	Anthocyanin	Total phenol	РН	Total Flavonoid	K^+	SPAD	
Between Groups	3	6.92*	14624.19 [*]	0.369*	12.09*	21.22*	10.02 ^{ns}	
Within Groups	56	0.13	540.22	0.08	2.26	0.955	26.54	
CV%	_	19.02	9.42	6.30	11.39	9.06	8.30	

Appendix 18 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by NAA plant growth regulator.

NAA Mean square (MS)								
Variation source	df	ТА	TSS	TSS/TA Ratio	Inverted sugar	Fructose		
Between Groups	2	0.012*	1.61*	10.90*	1.50*	1.44*		
Within Groups	65	0.002	0.13	0.623	0.143	0.141		
CV%	-	6.20	4.63	8.58	4.78	4.68		

Appendix 19 Analysis of variance (ANOVA) for biochemical characteristics of wax apple affected by NAA plant growth regulator.