Chapter 1 : INTRODUCTION

1.1. INTRODUCTION

Wax apple (*Syzygium samarangense*) is a common fruit in Malaysia as well as other Asian countries. The fruit is widely cultivated and grown throughout Malaysia mainly as small scale gardener ranging from 1 to 5 ha with its hectare average estimated at 1500 ha in 2005 *(*Shu et al., 2006). The species presumptively originated in Malaysia and other South-East Asian countries. It is widely cultivated and grown throughout Malaysia and in neighboring countries such Thailand, Indonesia and Taiwan. Currently in Malaysia it is cultivated mainly as small areas ranging from 1 to 5 ha with its hectarage estimated at about 1500 ha in 2005 (Shu et al., 2006)

Water apple (wax apple) belongs to the Myrtaceae family, is botanically identified as *Syzygium samarangense* Merr.&Perry (Morton, 1987). Many species belonging to Myrtaceae family have been enhanced by some phytohormones to develop fruit growth and quality. *Syzygium* is a genus of flowering plants that belongs to the family Myrtaceae. The genus comprises about 1100 species (Little et al., 1989). *Syzygium* species are widely distributed, occurring in Africa, main-land Asia, Malaysia, New Zealand, the Western Pacific, and Australia (Hyland, 1983). High levels of diversity occur from Malaysia to Northeastern Australia, where many species are very poorly known and many more have not been described taxonomically (Morton, 1987).

Since antiquity, fruit development and ripening have been considered as the most important phenomena in agriculture and fruit production. Amelioration of fruit quality is being done in horticultural plant that has edible fruit. Idea to develop fruit growth was very old and increase of yield or weight using horticultural practices were reported by many researchers. One of the old used techniques was the pruning of trees to increase fruit growth and development (Savage and Cowart, 1942; Elfving and Forshey, 1976).

One of the major evolutionary stages in plant physiology is the discovery of plant hormones called phytohormones or plant bioregulators (PBRs). In 1935 and for the first time has isolated a component that stimulated growth when applied to rice root (Yabuta, 1935). After that Brian et al. (1954) have isolated the first phytohormone from *Gibberella fujikuroi* and named it Gibberellic Acid (GA_s).

Phytohormone is defined as a natural compound synthesized by plant cell at a very low concentration, then translocated to another plant tissue where it causes physiological responses (Romanov, 2002; Gaspar et al., 2003; Galston et al., 1980; Salisbury and Ross, 1992).

Phytohormones contribute in a large range of phenomena that occur during the growth, and the development of plants. There are five classes of phytohormones such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene (Taiz and Zeiger, 1998). Other compounds which affect plant growth and reproduction but are not generally classified as hormones include brassinosteroids, salicylates, jasmonates, and polyamines.

Many studies reported that the application of phytohormones enhance the plant growth and the crop yield (Hernandez, 1997; Ashraf et al., 1987, 1989). The use of the plant bioregulators is more frequent in tree fruit production rather than in the horticultural or agricultural application. It has been proven by many researchers that phytohormones can regulate fruit abscission. This regulation of abscission occurs at the beginning of fruit development then during the fruit ripening period. It was observed that auxins retarded leaf petiole abscission led to the finding at the end of 1930s by Gardner et al. (1939). They also reported that Naphthalene Acetic Acid (NAA) and naphthaleneacetamide (NAAm) brought down preharvest drop. Yuan and Carbaugh, (2007), have applied l-methylcyclopropene (1MCP) as a drop control plant bioregulators and reported which was released as a gas then binds irreversibly to

ethylene binding sites within the plant. It was first used in the mid 1990s to widen the postharvest life of ornamentals. It is now used to extend the storage life of apples and the extent of its use (Watkins, 2006). This compound which is normally administered to apples as a gas in an enclosed space has been formulated so that it can be sprayed on trees. Another effective bioregulator for both apples and peers is the Abscissic acid (ABA) that has been shown to be an effective thinner hormone (Greene, 2007; 2009). It has the added advantage of also being a naturally suitable plant hormone which should be useful in facilitating product registration and grower acceptance .

Appropriate regulation of vegetative growth is fundamental in some tree fruit production since there is an inverse relationship between growth and flowering. Excessive vegetative growth negatively impact fruit quality, postharvest life, and development of an efficient and fruitful tree structure. Batjer et al. (1964) reported that daminozide affected the inhibition of growth of apple trees. Paclobutrazol was used as a growth retardant in many countries, but its use has been limited due to long persistence in the tree, concerns about ground water contamination and a negative influence on fruit size in pome fruit (Miller, 1989).

Enhance of flower bud formation is a prime method that increases fruit crop. Harley et al. (1958) showed that NAA had the intrinsic ability to promote flower bud formation distinct from thinning. NAA and ethephon, despite their action as a thinners, they were also suggested as a potential advance fruit ripening (Cline, 2008).

Fruit size and taste have 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 cultural practices, such as application of plant growth hormones. Gibberellic Acid (GA_3) has been shown to increase fruit set and growth in apples, pears (Weaver, 1972). A spray of GA_3 at 50 mg/l using 5 weeks after full bloom (AFB)

reduced fruit dropped in 'Huaizhi' (Ji et al., 1992), and a spray of GA_3 at 50–100 mg/l at full bloom also enhanced fruit retention and fruit size in 'Early seedless' and 'Calcuttia' litchiin India (Singh and Lal, 1980). Onguso et al. (2004) reported that auxins spraying prevented the senescence of fruits presumably by maintaining the cell turgidity at the zone of abscission, which prevents the synthesis of hydrolytic enzymes, such as cellulase, which hydrolyze cell walls. The deep-red colored fruits are popular, factors influencing red color has become important for investigators. The red color in wax apple (water apple) is believed to be influenced by several factors such as; leaf: fruit ratio (Wang, 1991), sugars, position of fruits on the tree, fruit development stages, light and temperature (Shu et al., 2001).

Horticultural cultural practices such as, spray of plant growth hormone application (Guardiola, 1992), pruning and girdling techniques are applied to develop fruit growth and quality. These techniques are traditional methods and have been used for a long time. The spray of plant growth hormone or chemicals is considered as a traditional method. Nowadays, environmental scientists do not encourage the use of these techniques too because of their bad effect on the environment such as the air and water pollution, as well as human health (Miller, 2004; Tashkent, 1998). Dipping technique has been developed for the fruit growth and quality development instead of spray method due to not affecting environment and cost effective as it can control the liquid effluent much easier (Probert, 2009). Das et al. (2001), used dipping methods of 45 ppm GA_3 in grapes bunches at the full bloom stage, reported that the higher final fruit weight and total soluble solid (TSS) content was found in dipping methods rather than in spray method.

Asano et al. (2001) used dipping methods instead of spray and found better effects in grapes fruit.

Hewitt et al. (2009) reported that *spray* droplet size and drift were risks to nontarget organisms from *aerially* applied in controlling coca.

Attempts have been made to develop the fruit growth and quality using innovative technique of hormone application method of spray and dipping method application.

An innovative technique swabbing method has been developed because of using small quantity to get more output compared to spray and dipping methods. Swabbing method does not create any droplet and spray dirft which is caused by spray and dipping method. Hossain et al. (2007) developed swabbing technique and resulted in excessive flowering in peach plants. They also reported that swabbing method enhanced early flowering (blooming) by dwarfing plant growth while ABA (Abscissic Acid) was applied to the bark in peach plant.

Gibberellic Acids (GA_s) has been shown to increase fruit set and growth in clementine orange (Van Rensburg et al., 1996)**.** Choi et al. (2002) reported that spraying GA_3 increased the fruit size and firmness in cherry fruits**.** In addition, El-Sese (2005) worked on Balady mandarin trees reported that treatment with GA_3 increased the yield of fruits**.** GA_3 increased fruit firmness, total soluble solids and fruit weight (Basak et al., 1998). Every year a lot of wax apple (water apple) fruit is being dropped in Malaysia. That is also an issue to reduce the drop fruit.

Quantitative studies investigating the phenolic content and antioxidant potential of edible fruits are useful, since the role these factors played in health and disease chemoprevention have been widely reported and there is an upsurge of interest in phytochemicals as potential new sources of natural antioxidants. The leaves of *S. samarangense* have shown the presence of ellagitannins, proanthocyanidins (Nonaka et al., 1992), flavanones, flavonol glycosides, anthocyanidins (Kuo et al., 2004), triterpenoids, chalcones (Srivastava et al., 1995), and volatile terpenoids (Wong & Lai,

1996). Chalcones are a group of plant-derived polyphenolic compounds that are intermediate in the biosynthesis of flavonoids and are associated with several biological activities, including antiviral, antifungal, anti-inflammatory and antioxidant activities (Han et al., 2006). They have also been reported to display anticancer and cytotoxic activity (Goh et al., 2005).

Very little scientific information is available and known about the growth and development of wax apple (water apple) fruits. A search in the Thomson-Reuters and Scopus database revealed only a few articles reporting on its chemical constituents as cited above. In this project the growth and development as well as the pre and post harvest characteristics of the tree and fruits will be investigated and documented with the expectation it will lead to better quality fruits, which will benefit our local farmers.

1.2. OBJECTIVES OF THE PRESENT STUDY

Despite the importance of wax apple (water apple) as an edible fruit, the effects of phytohormone to increase quality characters of fruit and productivity in future, are still unknown. The use of growth regulators is becoming popular to enhance crop productivity and varieties of such substances are available in the markets which are used for crop production. Therefore, considering the importance of different growth regulators in increasing crop growth, ameliorating fruit characters studies were carried out to compare the effect of three hormones: Giberellic Acid (GA_3), auxin (NAA) and cytokinin CPPU on fruit yield and quality of wax apple (water apple).

The objectives of the present study were:

1. To investigate the effect of GA_3, NAA and CPPU on selected parameters of wax apple (water apple) fruit growth and development.

2. To investigate the effect of PGRs on the various physical and biochemical characteristics of the wax apple (water apple) fruit quality during development.

3. To study the effectiveness of the swabbing method for the application of PGRs instead of using the spray method.

Chapter 2: LITRATURE REVIEW

2.1. SPECIES DISTRIBUTION

2.1.1. In the world

The map below (**Figure 2.1.**)shows countries where the species has been planted. It does neither suggest that the species can be planted in every ecological zone within that country, nor that the species cannot be planted in other countries than those depicted. Since some tree species are invasive, biosafety procedures should be followed.

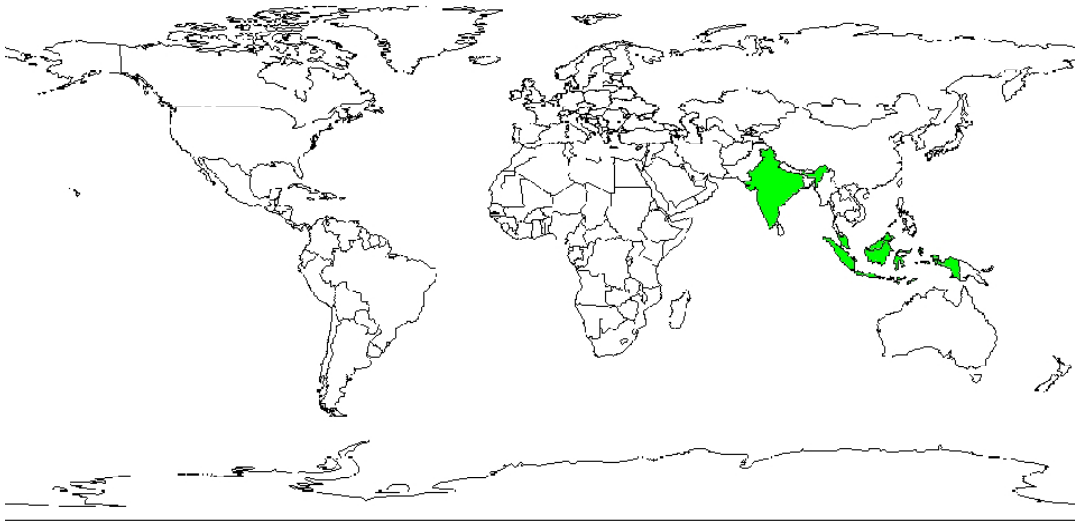


Figure 2.1. Map distribution of Wax apple

Fiji, India, Indonesia and Malaysia were showed as the native range (Green color). Exotic range shows countries where the species has been planted (Morton, 1987). Fruits of Warm Climates.

2.1.2. In Malaysia

Wax apple (Water apple) was widely cultivated and grown throughout Malaysia and in neighboring countries such Thailand, Indonesia and Taiwan. Currently in Malaysia it is cultivated mainly as smallholdings areas ranging from 1 to 5 ha with its hectarage estimated at about 2000 ha in 2005 (Shu et al., 2006).

Wax apple integrate *Syzygium* genus of flowering plants that belongs to the family Myrtaceae. This genus comprises about 1100 species (Little et al., 1989). High levels of diversity occur from Malaysia to northeastern 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* are planted throughout the tropics worldwide. In Malaysia, there are about three species which bear edible fruits, namely the water apple (*Syzygium aquem*), Malay apple (*Syzygium malaccense*) and wax jambu (*Syzygium samarangense*). The pink, red and green cultivars of wax apple are popular in Malaysia and other South East Asian countries. The fruit is rounder and more oblong in shape, also having a drier flesh.

2.1.3. Production in controlled environment

In 20th century, the use of the greenhouse, as it creates a favorable inside microclimate, opens a vast pat in plants and fruits production (Harmanto and Salkhe, 2006). Greenhouses protect plants and fruits from excessive heat or cold, shield plants from dust storms and blizzards, and help to keep out pests. Temperature and light control allows greenhouses to improve plant production control environments (Johannes et al., 2009).

Greenhouses are often used to increase growing flowers, vegetables and yield fruits as well as to reduce the frequency of pesticide application (Möller et al., 2004), and also to

decrease wind velocities and air exchange (Harmanto et al., 2006). Other types of physiological bees have been used, as well as artificial pollination.

Foliar spray by potassium and phosphate in greenhouse tomatoes incite early fruit ripening and increase fruit yield and quality (Chapagain and Wiesman, 2004). Percentage of firm fruit was increased, whereas and rotten fruits were decreased. By this technique, glucose content of tomatoes, dry matter after storage, magnesium, potassium and phosphorus fruit content were remarkably increased (Chapagain and Wiesman, 2004).

2.2. DESCRIPTION OF WAX APPLE (WATER APPLE)

2.2.1. Botanical classification

Kingdom: Plantae-Plants

Subkingdom: Tracheobionta-Vascular plants

Superdivision: 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. & Perry - Syzygium

(Morton, J. 1987. Fruits of Warm Climates).

Synonyms

Eugenia domestica Baillon

Eugenia malaccensis L.

Jambosa malaccensis (L.) DC.

Binomial name

Syzygium samarangense (Blume) Merrill & Perry

2.2.2. Common name

Syzygium samarangense (syn. *Eugenia javanica*) is a species in the Myrtaceae, native to Indonesia and Malaysia. Common names include wax apple, love apple, java apple, Chomphu (In Thai Language), Bellfruit (In Taiwan), Mận (in Vietnam), jambu air (in Indonesian), water apple, mountain apple, jambu air ("water guava" in Malay), wax jambu, Rose apple, bell fruit, makopa, tambis (Philippines), and chambekka in Malayalam.

It is known as jamalac in French, and zamalac in the French-based creole languages of Mauritius, Réunion, Seychelles and other Indian ocean islands. The wax apple tree also grows in the Caribbean. On Curaçao, Netherlands Antilles, the fruit is called kashu Sürnam in Papiamentu, which means ‘cashew from Surinam’, while in Surinam the fruit is called curaçaose appel (‘apple from Curaçao’ in Dutch), in Trinidad and Tobago it is known as pommerac, while in the Dominican Republic a small sub-species of the wax apple is known as cajuilito, or small cashew (Morton, 1987).

Some of the edible species of *Syzygium* are planted throughout the tropics worldwide. In Malaysia, there are about three species which contain edible fruits, called the water

apple (*Syzygium aquem*), Malay apple (*Syzygium malaccense*) and wax jambu (*Syzygium samarangense*). Regarding to fruit development, fruit from the Myrtaceae family, such as guava, follows a simple sigmoid curve with three phases of fruit growth: the first phase is of the cellular division‹ the second is an exponential growth phase of cellular elongation‹ and finally the ripening phase (Mercado-Silva et al., 1998; Nakasone and Paull, 1999). Fruit of Myrtaceae family exhibit great variability in their respiratory patterns. Fruit from *Eugenia* genus show a non-climacteric respiratory pattern‹ while fruit from the *Psidium* genus are climateric (Akamine and Goo, 1979). Araza´ is a climacteric fruit, as measured by Galvis and Hernandez (1993) using the dynamic technique to measure the fruit respiration rate (Kader, 2000), though its ethylene production is still unknown.

Among its various vernacular names are: wax apple, samarang rose apple, wax jambu and water apple. The waxy fruit is pearshaped, narrow at the base, very broad, flattened, indented and adorned with the four fleshy calyx lobes at the apex; 3.4–5 cm long, 4.5–5.4 cm wide. The skin is very thin, the flesh is white, spongy, dry to juicy, low acid and very bland in flavor. The color of the fruit is usually light-red, sometimes greenish-white or cream-colored (Morton, 1987). Almost unknown outside southeastern Asia, wax apple is an economically important fruit crop in Taiwan (Shu¨ et al., 1996; Wang, 1991). The fruit color of the most cultivar in Taiwan, is 'Pink', ranges from light-red to deep-red despite of its name. As more is paid for the deep-colored fruits, factors improving red color of 'Pink' are much interested. Red color of wax apples is influenced by such factors such as: leaf:fruit ratio (Wang, 1991), sugars (Liaw et al., 1999; Shu¨ et al., 2001), position of fruits on the tree (Shu, 1999a), fruit development stages (Chang et al., 2003), light and temperature (Shu¨ et al., 2001). According to the observation from the field, water apple fruits growing in winter and early spring, but fruits growing in warm seasons contain low pigmentation. Shu¨ et al. (2001) reported

water apple fruit discs cultured at 20°C have the best red color development. The effects of temperature shifting and day/night temperature regimes on quality attributes are still unknown.

2.2.3. Botanical description

It is a tropical tree growing to 5-20 m tall, with straight trunk, 20-45 cm diameter, often branched near the base and with broadly ovoid canopy. Leaves opposite, elliptic-oblong, 15-38 cm x 7-20 cm, thick-coriaceous, petiole 0.5-1.5 cm long, thick, red when young. Inflorescences exclusively on defoliate twig-parts, short and dense, 1-12-flowered; flowers 5-7 cm in diameter, red; calyx-tube ventricose towards apex, 1.5-2 cm long, with broad lobes 4-8 mm long; petals 4, oblong-ovate or orbicular-ovate, up to 2 cm long, dark red; stamens numerous, up to 3.5 cm long, with red filaments; style 3-4.5 cm long, red. Fruit is a bell-shaped edible berry, ellipsoid, 5-8 cm in diameter, crowned by the incurved non-fleshy calyx segments, dark red or purplish-yellow or yellow-white; flesh 0.5-2.5 cm thick, juicy, white, fragrant. Seed per fruit is one, globose, 2.5-3.5cm in diameter. When mature, the tree is considered a heavy bearer and can yield a crop of up to 700 fruits (Miami, 1987).

2.3. THE FRUIT

The ripened fruit varies in hue and can be light pink to a dark, almost purple, red. One of the most highly prized and sought after water apple in Taiwan are "black pearls," which are purplish-red. If it is ripe enough, the fruit will puff outwards, with the middle of the underside of the "bell shape" dented in a touch. Healthy wax apple have a light sheen to them. Despite its name, a ripe wax apple only resembles an apple on the outside in color. It does not taste like an apple, and it has neither the fragrance nor the density of an apple. Its flavor is similar to a snow pear, and the liquid to flesh ratio of

the water apple is comparable to a watermelon. Unlike either apple or watermelon, the wax apple 's flesh has a very loose weave. The very middle holds a seed that's situated in a sort of cotton-candy-like mesh. This mesh is edible but flavorless. The color of its juice depends on the cultivar of the fruit; it may be purple to entirely colorless. A number of cultivars with larger fruit have been selected. In general, the paler or the darker the color is, the sweeter the taste is. In South East Asia, the black ones are nicknamed "Black Pearl" or "Black Diamond," while the very pale greenish white ones are called "Pearl." They are among the highest priced ones in fruit markets . When choosing a good wax apple, look for ones with the bottom segments closed up because open holes signify worm eggs inside the fruit. Also, usually the reddest fruits are the sweetest. To eat, the core is removed and the fruit is served uncut, in order to preserve the unique bell shape presentation . Fruit skin discs of Wax apple (*Syzygium samarangense* Merr. & Perry) from different fruit development stages incubated with and without sucrose showed differential effects on diameter, weight, soluble solids (SSs) and skin color (anthocyanin concentration) (Chang et al., 2003).

Temperature has pronounced effects on quality attributes of water apple fruit discs (Hsia-hua and Zen-hong, 2007). Anthocyanin and total soluble solid (TSS) were greatest in the 20° C treated discs under constant temperatures. The concentration of soluble sugars (SS), starch, total phenolic compounds (TPC), free amino acids (FAA) and soluble protein (SP) all decreased with increasing temperature (Hsia-hua and Zen-hong, 2007).

The red color appears on the water apple fruit arising from the accumulation of anthocyanins (Chang et al., 2003). The synthesis of these pigments is affected by many factors, particularly light and temperature (Saure, 1990). The positive effect of low temperature on anthocyanin synthesis in apples has been noted previously (Creasy,

1968; Faragher, 1983; Proctor, 1974). However, the optimum temperature for maximum anthocyanin accumulation has varied. The optimum constant temperature for anthocyanin pigmentation for water apple fruits, although a tropical fruit is also 20°C (Shü et al., 2001).

2.4. BIOLOGICAL CYCLE

Shoot growth proceeds in flushes which are more or less synchronous‹ depending on the climate. The juvenile period lasts for 3-7 years. Bearing of clonal trees starts after 3-5 years. There are definite flowering seasons‹ often two, sometimes three in a year, but the timing varies from year to year. Water apple commonly flowers early or late in the dry season; the flowers appear to be self-compatible and the fruit ripens 30-40 days after anthesis (Morton, 1987).

2.5. ECOLOGY

The trees grow well in fairly moist tropical lowlands up to 1200 m elevation. Water apple grows best in areas with a fairly long dry season. This does not mean that this species is drought-resistant. The species require a reliable water supply and are often planted along streams or ponds. The trees prefer heavy soils and easy access to water instead of searching for water in light deep soils (Morton, 1987).

2.6. PLANT DEVELOPMENT AND PHYTOHORMONES

2.6.1. Concept of plant hormones and other techniques

Andrew et al. (2004) have studied the sensitivity of Chamelaucium, Myrtaceae genotypes to ethylene-induced flower abscission. In this family, fruit quality (Hernández et al., 2007) and postharvest quality of arazá (*Eugenia stipitata* Mc Vaugh) fruit during low temperature storage (Hernández et al., 2009) were reported. Marcelo

and Schaffer (2010) studied the photosynthetic and the growth responses of *Eugenia uniflora* to light intensity and obtained positive result.

Indeed, Lakso (1984) then Forshey and Elfving (1989) reported that excessive vegetative growth of tree reduced flower bud initiation, fruit set then fruit yield. Many other studies reported that pruning and bending trees improved a higher efficiency in tree yield and fruit quality (Tustin et al., 1988; Wünsche and Lakso, 2000; Robinson, 2003; Hampson et al., 2004a; Hampson et al., 2004b; Hossain et al., 2006).

In order to improve light distribution into the tree, thinning-out cuts were observed to increase fruit number, fruit quality and control best tree growth (Myers and Savelle, 1996; Jung and Choi, 2010). Recently, Wei-Hai Yang et al. (2009) reported a new method to ameliorate fruit quality of *Dimocarpus longan* (Lour). This method consists of fruit bagging with adhesive –bonded fabric bag that increase the size and the fruit retention rate. The application of this method reduces craking incidence and could be a very important practice for many species like cross-winter longan (*Dimocarpus longan*).

The concept of chemical messengers in plants is not new. For over two millennia, people have observed that one part of the plant can influence another. Duhamel du Monceau's experiments in 1758 declared that sap movement controlled the growth of plants. He showed that downward moving sap from the leaves was responsible for the roots healthiness (Du Monceau, 1758). Julius von Sachs who was known as the leader of plant physiology revised du Monceau's theory by presenting evidence that "organ-forming substances" were developed by the plant and transmitted to different parts of the plant where they controlled growth and development. He also suggested that these "organ-forming substances" were the response of the environmental stimuli (Von Sachs, 1880). Charles Darwin, is considered to be the scientist responsible for the beginning of

the modern research in plant growth substances considering his experiments on phototropism described in his book "The Power of Movement in Plants." (Darwin, 1880). In 1926 this compound was first isolated from plants by a graduate student in Holland named Fritz Went. It was the first plant hormone isolated and was later termed "auxin" (Greek auxein, "to increase") by Kogl and Haagen-Smit in 1931. Went's innovative work which greatly influenced researches on plant growth substances and much of our current knowledge regarding auxins are attributed to his work (Went, 1926). Few years later other attempts led to the discovery of another plant hormone such as gibberellins which were discovered in plant pathogenesis studies. In addition efforts to culture tissues led to cytokinins. After that attempts to control abscission and dormancy aimed to abscisic acid. Finally, the effects of illuminating gas and smoke brought us to ethylene. Other compounds contribute to plant growth but are not generally classified as hormones. They include brassinosteroids, salicylates, jasmonates, and polyamines .

One of the techniques that does not need chemicals, easy to practice and it gives wonderful result was the induction of phloem stress by partial ringing or dwarfing plant (Tukey, 1978, Hossain et al., 2006). The application of this method by Hossain and Boyce (2009) on fig tree promoted fruit growth and quality development. It has also been reported that ringing of the trees tends to increase the size and sugar content of the fruits and to cause them to mature a few days to a week earlier (Tukey, 1978). Furthermore, trunk growth above the girdling significantly increased whilst that below declined and that the increase in trunk girth above the girdling might be caused by an accumulation of carbohydrates (Arakawa et al., 1997; Onguso et al., 2004). They also reported that girdling in apple and peach significantly increased flowering the following spring (Hossain et al., 2007). It has been suggested that girdling can change the fruit quality (increased SSC and reduced acid concentration) by blocking the translocation of

sucrose from leaves to the root zone through phloem bundles. However, Onguso et al. (2004) reported that partial ringing of four-year-old peach trees reduced shoot growth and developing fruit quality. Jose (1997), working on mango trees, found lower vegetative growth in all the ringing (girdling) treatments in relation to control mango trees. The reason for the different responses among cultivars is still unknown.

2.6.2. Mechanism work of plant hormones

It is known that micromolar and smaller concentrations of hormones are necessary in order to observe a response to be observed. For that reason, three criteria are necessary to stimulate plant hormonal action (Salisbury and Ross, 1992).

These criteria are mentioned below;

a) The hormone must be presented in the correct quantity and in the correct location .

b) There must be a good recognition and a strong binding between the hormone and the responding molecules .

c) The receptor molecule must then trigger some other metabolic change which will trigger the amplification of the hormonal signal .

There are two generally accepted mechanisms by which hormones act. The first type deals with a steroid hormone. In this type the hormone can pass through the plasma membrane into the cytoplasm. Here it binds with its receptor molecule to form a hormone-receptor complex. From this point, the complex may dissociate (If there is not tight binding) or it may enter the nucleus and affect mRNA synthesis. The effect of the hormone on mRNA synthesis ultimately results in the physiological response (Arteca,1996; Wolfe, 1993). The second type, consists of a peptide hormone which binds to a receptor protein on the target cell. The receptor protein then undergoes a

conformational change leading to a cellular cascade ultimately resulting in modification of enzyme activity, altered metabolic processes, and different phenotypes (Arteca, 1996; Wolfe, 1993).

Plant hormones specifically control the gene expression. It is important to point out that the exact mechanisms by which hormones regulate gene expression are poorly understood. Gene expression is considered as part of a large amplification process. In this process the DNA transcription is repeated to give many copies of mRNA (1st amplification step); mRNA is processed and entered into the cytoplasm where it is translated many times by ribosomes into a gene product such as an enzyme (2nd amplification step); enzymes are modified in order to be functional and capable of high catalytic activity even at low concentrations. These enzymes catalyze the production of many copies of an important cellular product (3^{rd} amplification step).

It is common that gene regulation is affected by certain enzymes after initial hormone binding. Genes may be altered by secondary and tertiary messengers of a cellular cascade as well. Hormones may indirectly control gene expression, through these enzymes and messengers, at several control sites such as transcription, mRNA processing, mRNA stability, translation, and post-translation (Arteca, 1996; Salisbury and Ross, 1992).

2.6.3. The Auxins

The term auxin is derived from the Greek word ‘Auxein’ which means to grow. Compounds are generally considered as auxins if they can induce cell elongation in stems and otherwise resemble Indole Acetic Acid (the first auxin isolated) in physiological activity. Auxins usually affect other processes in addition to cell elongation of stem cells but this characteristic is considered critical of all auxins and

thus "helps" define the hormone (Arteca, 1996; Mauseth, 1991; Raven et al., 1992; Salisbury and Ross, 1992).

2.6.3.1. History of Auxins

Auxins were the first plant hormones discovered. Charles Darwin was among the first scientists to dabble in plant hormone research. In his book presented in 1880 and titled "The Power of Movement in Plants", he first described the effects of light on movement of canary grass (*Phalaris canariensis*) coleoptiles. The coleoptile was a specialized leaf originating from the first node which made sheaths the epicotyl in the plants seedling stage protecting it until it emerged from the ground. When unidirectional light shined on the coleoptile, it bends in the direction of the light. If the tip of the coleoptile covered with aluminum foil, no bending would occur towards the unidirectional light. However, if the tip of the coleoptile could leave uncovered, the portion just below the tip would cover and exposure to unidirectional light resulted in curvature toward the light. Darwin's experiment suggested that the tip of the coleoptile was the tissue, responsible for perceiving the light and producing some signal which was transported to the lower part of the coleoptile where the physiological response of bending occurred. He then cut off the tip of the coleoptile and exposed the rest to unidirectional light to see if curving occurred. Curvature did not occur confirming the results of his first experiment (Darwin, 1880).

Salkowski (1885) discovered indole-3-acetic acid (IAA) in fermentation media. The separation of the same product from plant tissues was not found in plant tissues for almost 50 years. IAA is the major auxin involved in many of the physiological processes in plants (Arteca, 1996). Fitting (1907) studied the effect of making incisions on either the light or dark side of the plant. His results aimed to understand if translocation of the signal occurred on a particular side of the plant but his results were

inconclusive because the signal was capable of crossing or going around the incision. Boysen-Jensen (1913) modified Fritting's experiment by inserting pieces of mica to block the transport of the signal and showed that transport of auxin toward the base took place in the dark side of the plant as opposed to the side exposed to the unidirectional light. Paal (1918) confirmed Boysen-Jensen's results by cutting off coleoptile tips in the dark, exposing only the tips to the light, replacing the coleoptile tips on the plant but off centered to one side or the other. Results showed that whichever side was exposed to the coleoptile, curvature occurred toward the other side (Paal, 1918). Soding was the next scientist to extend auxin research by extending on Paal's idea. He showed that if tips were cut off there was a reduction in growth but if they were cut off and then replaced growth continued to occur (Soding, 1925).

Went (1926) descrived that how he isolated a plant growth substance by placing agar blocks under coleoptile tips for a period of time. He also mentioned that they were removed and placed on decapitated Avena stems (Went, 1926). After placement of the agar, the stems resumed growth. Went (1928) developed a method of quantifying this plant growth substance. His results suggested that the curvatures of stems were proportional to the amount of growth substance in the agar. This test was called the avena curvature test. Much of our current knowledge of auxin was obtained from its applications. Went's work had a great influence in stimulating plant growth substance research. He was often credited with dubbing the term auxin but it was actually Kogl and Haagen-Smit (1931) who purified the compound auxentriolic acid (auxin A) from human urine. Later Kogl (1931) isolated other compounds from urine which were similar in structure and function to auxin A, one of which was indole-3 acetic acid (IAA) initially discovered by Salkowski (1985). A committee of plant physiologists (1954) was set up to characterize the group auxins. The term comes from the Greek auxein meaning "to grow." Compounds are generally considered auxins if they are

synthesized by the plant and are substances which share similar activity to IAA (the first auxin to be isolated from plants) (Arteca, 1996; Davies, 1995).

2.6.3.2. Biosynthesis and Metabolism of Auxin

IAA is chemically similar to the amino acid tryptophan which is generally accepted to be the molecule from which IAA is derived. Three mechanisms have been suggested to explain this conversion :

1- Tryptophan is converted to indolepyruvic acid through a transamination reaction. Indolepyruvic acid is then converted to indoleacetaldehyde by a decarboxylation reaction. The final step involves oxidation of indoleacetaldehyde resulting in indoleacetic acid .

2- Tryptophan undergoes decarboxylation resulting in tryptamine. Tryptamine is then oxidized and deaminated to produce indoleacetaldehyde. This molecule is further oxidized to produce indoleacetic acid .

3- As recently as 1991, this third mechanism has evolved. IAA can be produced via a tryptophan-independent mechanism. This mechanism is poorly understood, but has been proven using tryptophan mutants. Other experiments have shown that, in some plants, this mechanism is actually the preferred mechanism of IAA biosynthesis .

The enzymes responsible for the biosynthesis of IAA are most active in young tissues such as shoot apical meristems and growing leaves and fruits. The same tissues are the locations where the highest concentrations of IAA are found. One way plants can control the amount of IAA present in tissues at a particular time by controlling the biosynthesis of the hormone. Another control mechanism involves the production of conjugates which are, in simple terms, molecules resemble to the hormone but are inactive. The formation of conjugates may be a mechanism of storing and transporting

the active hormone. Conjugates can be formed from IAA via hydrolase enzymes. Conjugates can be rapidly activated by environmental stimuli signaling a quick hormonal response. Degradation of auxin is the final method of controlling auxin levels. This process also has two proposed mechanisms outlined below :

1- The oxidation of IAA by oxygen resulting in the loss of the carboxyl group and 3-methyleneoxindole as the major breakdown product. IAA oxidase is the enzyme which catalyzes this activity. Conjugates of IAA and synthetic auxins such as 2,4-D can not be destroyed by this activity .

2- C-2 of the heterocyclic ring may be oxidized resulting in oxindole-3-acetic acid. C-3 may be oxidized in addition to C-2 resulting in dioxindole-3-acetic acid .

The mechanisms by which biosynthesis and degradation of auxin molecules occur are important to future agricultural applications. Information regarding auxin metabolism would most likely lead to genetic and chemical manipulation of endogenous hormone levels resulting in desirable growth and differentiation of important crop species. Ultimately, the possibility exists to regulate plant growth without the use of hazardous herbicides and fertilizers (Davies, 1995; Salisbury and Ross, 1992).

2.6.3.3. Auxin roles

The most recognizable role of auxin is the phenomenon of apical dominance. Auxins synthesize in apex inhibit the activity of the lateral meristem (Cline, 1996; Leyser, 2002), whereas cytokinins, promote the growth of lateral meristems (Taiz and Zeiger, 1998), and thus auxins and cytokinins act as antagonists during lateral meristem development.

Auxin is also required for cell elongation, and has different effects depending on the organ in which it is present; it stimulates elongation in the shoot, but inhibits it in the

root (Taiz and Zeiger, 1998; Crozier et al., 2000). In addition to cell elongation, auxin is also involved in photo- and gravitropism, the processes whereby a plant grows toward light and gravity, respectively. Darwin demonstrated phototropism in 1880, while gravitropism was demonstrated later by Went (1926). Auxin also affects the differentiation of vascular tissue and vascular patterning in leaves (Naderi et al., 1997; Taiz and Zeiger, 1998). Recent research further suggests that auxin may be integral in regulating embryogenesis and plant totipotency (Ribnicly et al., 2002).

IAA, indole-3-acetic acid, considered as the major auxin, involved in many processes of growth and development in plants (Arteca, 1996). It represents the most abundant naturally occurring auxin in plants (Bartel, 2001). IAA promotes enlargement in leaves and increase photosynthetic activities and activates the translocation of carbohydrates during their synthesis (Awan et al., 1999; Ritenour et al., 1996).

NAA is frequently used for inducing fruit abscission and post-bloom thinning of 'Delicious' apple fruit. This method promotes better fruit size. This negative effect of NAA on fruit size was first reported by Greene (1943), and remains an important limitation in the use of NAA by the apple industry (Unrath, 1981). Brent et al. (1995) reported that application of NAA in adequate time and volume did not significantly reduce fruit size.

These NAA concentrations were similar to those reported by " the effect of spray volume and time of NAA application on fruit size and cropping of Red chief' Delicious' apple" . In this work, the recommended practice dose is 10 to 15 mg/l NAA (Brent et al., 1995).

Loquat trees (*Eriobotrya japonica* Lindl cv. Algerie) were treated with Naphthalene Acetic Acid at 25, 50 and 100 mg/l (NAA-25, NAA-50 and NAA-100) to fruit

develoment (Amorós et al., 2004). Bract longevity was found to be almost 10 days longer in NAA (50, 100 and 150 ppm) (Saifuddin et al., 2009).

NAA (0, 50, 100, 150 and 200 ppm) were sprayed on fruits of Barhee and Shahl date palm cultivars. The high doses applied were explicated by the use of spraying technique and not swabbing (Harhash and Al-Obeed, 2007).

Application of IAA on shoot of lentil (*Lens culinaris*, MEDIK) showed a decrease in length of shoot and number of internodes. The increase in the diameter, area and number of leaves was also observed (Naeem et al., 2004). The decrease in length of shoot with IAA for the same species was earlier reported by Pilot & Saugy (1985). Lee et al., (2000) working on *Zinnia* cultures reported that IAA causes increase in length.

Application on shoot of lentil (Lens culinaris, MEDIK), IAA induced branching with lush green colour of leaves. Komaratchi et al. (1981) reported that a minimum concentration of NAA was required to stimulate strawberry fruit growth. This was consistent with the higher equilibrium dissociation constant (lower affinity) for auxin binding to strawberry fruit membranes than to corn coleoptiles.

Synthetic auxins are well known plant growth regulators that can substitute for pollination and induce fruit setting and growth, development as well as quality (Kataoka et al., 2009). Synthetic auxins have been reported to be effective in enhancing fruit growth, when applied during the second stage of fruit development (Westwood, 1993). These auxins are known for their ability to increase cell enlargement (Davis, 2004), thus enhancing fruit growth in citrus (Agusti et al., 1995). More recently, it was observed that application of NAA before flowering, followed by three weeks after fruit setting significantly increased fruit length, diameter and fruit weight as well as yield in guava (Dutta and Banik, 2007). It was found that application of NAA reduced the fruit drop, increased yield, TSS, total sugar and vitamin-C contents in guava fruits (Iqbal et al.,

2009). They also reported that fruit quality improved with lower NAA concentrations and deteriorated at higher rates. Synthetic auxin has an increasing effects on total antioxidant capacity as well as the nutritional quality in transgenic silcora seedless grape (Elisa et al., 2007).

The following are resumed some of the responses that auxin is known to cause (Davies, 1995; Mauseth, 1991; Raven et al., 1992; Salisbury and Ross, 1992).

1- Stimulates cell elongation

2- Stimulates cell division in the cambium and, in combination with cytokinins in tissue culture

3- Stimulates differentiation of phloem and xylem

4- Stimulates root initiation on stem cuttings and lateral root development in tissue culture

5- Mediates the tropistic response of bending in response to gravity and light

6- The auxin supply from the apical bud suppresses growth of lateral buds

7- Delays leaf senescence

8- Inhibits or promote (via ethylene stimulation) leaf and fruit abscission

9- Induces fruit setting and growth in some plants

10- Involves in assimilate movement toward auxin possibly by an effect on phloem transport

11- Delays fruit ripening

12- Promotes flowering in Bromeliads

13- Stimulates growth of flower parts

14- Promotes (via ethylene production) femaleness in dioecious flowers

15- Stimulates the production of ethylene at high concentrations

2.6.4. The Gibberellins

Unlike auxins, which are classified on the basis of function, gibberellins are classified on the basis of structure as well as function. All gibberellins are derived from the ent-gibberellane skeleton. The structures of this skeleton derivative along with the structure of a few of the active gibberellins are shown above. All gibberellins are acidic compounds and are therefore also called gibberellic acids (GA) with a different subscript to distinguish between them. GA_3 has historically been called gibberellic acid but the term is also often used in describing all gibberellins. GAs are widespread and so far ubiquitous in flowering (angiosperms) and non-flowering (gymnosperms) plants as well as ferns. They have also been isolated from lower plants such as mosses and algae, at least two fungal species and most recently from two bacterial species. There have been over 90 GAs isolated, all of which are most likely not essential to the plant. Instead, these forms are probably inactive precursors or breakdown products of active gibberellins (Arteca, 1996; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

2.6.4.1. History of Gibberellins

Japanese farmers first observed the phenomenon of abnormal elongation in certain rice plants early in the season. These plants often became unhealthy and sterile. The agent of the disease bakanae was deduced as being a fungal pathogen of the genus *Fusarium* (Hori, 1898). Kurusawa (1926) discovered that the disease was caused by a substance

secreted by the fungal species *Gibberella fujikuroi* resulting to controversy over the true pathogen (Kurusawa, 1926). Wollenweber (1931) stated that the fungus *Fusarium moniliforme* Sheld., which was the asexual or imperfect stage of the ascomycete *Gibberella fujikuroi* (Saw.) Wr. was is the culprit for the disease bakanae (Wollenweber, 1931). Yabuta (1935) isolated the compound from *Gibberella fujikuroi* and called it gibberellin A. This compound was found to stimulate growth when applied to rice roots (Yabuta, 1935). Due to second world war (WWII), much of the work on gibberellins was put on hold and the Western civilizations did not have access to these findings (Arteca, 1996). A new compound from *G. fujikuroi* was discovered in Britain. This compound was named gibberellic acid (Brian et al., 1954). In 1955, a similar compound was also isolated by American scientists from *G. fujikuroi* and which they called gibberellin X (Stodola et al., 1955). Around the same period, Japanese scientists discovered that gibberellin was actually made up of three compounds which they called GA_1, GA_2, and GA_3. Gibberellin X, GA_3, and gibberellic acid are all the same compound. The latter two were accepted in describing the compound and are synonymous terms today (Takahashi et al., 1955). Radley (1956) described some compounds similar to gibberellic acid in plants (Salisbury and Ross, 1992). Takahashi (1957) isolated another compound from *G. fujikuroi* which he called GA_4. He showed that GA_1 was identical to what Stodola and his associates were calling gibberellin A (Takahashi, 1957). MacMillan and Suter (1958) isolated and identified GA_1 from plants. in the same year, West and Murashige also identified GA_1 in higher plants (Salisbury and Ross, 1992). MacMillan and Takahashi (1968) proposed that Gibberellins were assigned numbers in order to reduce confusion between the compounds (Takahashi et al., 1991). This idea proved to be a good one sionce the procedure is currently used and it is helpful in reducing confusion between the over 90 gibberellins known .

2.6.4.2. Gibberellin Biosynthesis and Metabolism

Gibberellins are synthesized from acetyl CoA via the mevalonic acid pathway. They all have either 19 or 20 carbon units grouped into four or five ring systems. The fifth ring is a lactone ring as shown in the structures above attached to ring A. Gibberellins are believed to be synthesized in the young tissues of the shoot and also in the developing seed. It is not confirmed yet that young root tissues also produce gibberellins. There is also some evidence that leaves may be the source of some biosynthesis (Sponsel, 1995; Salisbury and Ross, 1992). The pathway by which gibberellins are formed is outlined below .

1- 3-Acetyl CoA molecules are oxidized by 2 NADPH molecules to produce 3-CoA molecules as a side product and mevalonic acid .

2- Mevalonic acid is then Phosphorylated by ATP and decarboxylated to form isopentyl pyrophosphate .

3- Four of these molecules form geranylgeranyl pyrophosphate which serves as the donor for all GA carbon atoms .

4- This compound is then converted to copalylpyrophosphate which has 2 ring systems

5- Copalylpyrophosphate is then converted to kaurene which has 4 ring systems

6- Subsequent oxidations reveal kaurenol (alcohol form), kaurenal (aldehyde form), and kaurenoic acid respectively.

7- Kaurenoic acid is converted to the aldehyde form of GA_{12} by decarboxylation. GA_{12} is the first true gibberellane ring system with 20 carbons.

8- From the aldehyde form of GA_{12} arise both 20 and 19 carbon gibberellins but there are many mechanisms by which these other compounds arise.

Certain commercial chemicals which are used to inhibit growth apply the same method because they block the synthesis of gibberellins. Some of these chemicals are Phosphon D, Amo-1618, Cycocel (CCC), ancymidol, and paclobutrazol. During active growth, the plant will metabolize most gibberellins by hydroxylation to inactive conjugates quickly with the exception of GA_3. GA_3 is degraded much slower which helps to explain why the symptoms initially associated to the hormone in the disease bakanae are present. Inactive conjugates might be stored out or translocated via the phloem and xylem prior their release (activation) at the exact time and in the exact tissue (Arteca, 1996; Sponsel, 1995).

2.6.4.3. Functions of Gibberellins

The role of GA in plant development has been observed in a several plants such as barley, rice, pea, and *Arabidopsis thaliana* (Richards et al., 2001). Active gibberellins show many physiological effects, each depending on the type of gibberellin present as well as the species of plant. Application of GA_3 on lentil shoot (Lens culinaris, MEDIK) showed a marked elongation in the length of shoot and increase in the number of internodes and compound leaves (Naeem et al., 2004). Similar results were observed by Chaudhary (1997). The increase in length was accompanied by inhibition in the diameter. Furthermore, Chaudhry and Zahur (1992) worked on *Abelmoschus esculentus L.*, and Chaudhry and Khan (2000) worked on *Cicer arietnum* and reported similar effects. Increases in number of internodes were also observed in a number of crops (Hernadez, 1997; Bagatharia and Chanda, 1998). Applied exogenous GA_3 showed early flowering that was accompanied by more number of flower buds (Naeem et al., 2004). GA_3 had stimulatory effect on floral stem length and number of flowers in rice (Awan

et al., 1999) and Lilium (Lee et al., 1999). Strawberry foliar spray by GA_3 increased fruit set, whereas, production of malformed and button berries was reduced. Although individual berry weight was reduced slightly, but fruit number, total as well as marketable yield was increased (Sharma and Singh, 2009).

Applied dose of GA_3 was inspired from many other works. For example, in a study reported by Sharma and Singh (2009) on 'Chandler' strawberry, experiments were conducted to observe the effects of foliar application of gibberellic acid on vegetative growth, flowering, fruiting and various disorders in 'Chandler' strawberry. GA_3 was sprayed at a level of 75 g/l at fruit bud differentiation stage and pre-flowering stage.

Regarding the effect of Gibberellic Acid (GA_3) on the yield of the phenolics, chlorogenic acid and cynarin, both in leaves and in the edible part of the head of globe artichoke, Sharaf-Eldin et al. (2007) have applied GA_3 at 60 g/l either at 4, 6 or 8 weeks after transplanting date.

Iknur et al. (2008) reported that the most effective application time for enlargement of grape berries is when the size of small grape berries become 1 mm. All the applications done before or after this period make the grape berry smaller in size. The best effect was observed around 75-100 g/l dose. To enlarge bract size and increase longevity of *Bougainvillea spectabilis*, selected branches were applied with 100 and 150 g/l GA_3 (Saifuddin et al., 2009).

It has been well documented that the size and quality of the fruits can be affected by the application of plant growth hormones (Guardiola, 1992). Gibberellic Acid (GA_3) has been shown to increase fruit set and growth in clementine orange (Van Rensburg et al., 1996). Choi et al. (2002) reported that spraying GA_3 increased the fruit size and firmness in cherry fruits. In addition to this El-Sese (2005) working on Balady mandarin trees reported that treatment with GA_3 increased the yield of fruits. GA_3 increased fruit firmness, soluble solids and fruit weight (Basak et al., 1998). The application of

gibberellic acid (GA_3) to entire trees of 'Satsuma' mandarin (*Citrus unshiu Marc*.) retarded pigment changes in the fruit and prevented puffiness of the peel (Garcia et al., 1985). Peak responses for both effects were obtained at the onset of chlorophyll degradation in the peel, before the completion of fruit growth. This application prevented the late peel growth which takes place after the cessation of pulp growth and retarded the loss of juice from the ripe fruit, allowing on-tree storage of the fruit for more than 2 months after commercial ripening (Garcia et al., 1985). Early GA_3 application on seedless Clementine mandarin (*Citrus clementina* Hort. ex Tanaka) trees reduced peel thickness at maturation (Garcia et al., 1992).

Cultures of 'St. Julien A' (*Prunus instititia L*.) rootstock, treated with 12.5 mg l−1 gibberellic acid (GA_3), produced elongated shoots suitable for rooting (Reeves et al., 1985).

However, GA also influences a variety of other physiological processes such as seed germination and floral initiation (Langridge, 1957; Taiz and Zeiger, 1997; Richards et al., 2001). Many other roles in plant development were attributed to GA including barley, rice, pea, and *Arabidopsis thaliana* (Richards et al., 2001). Actually there were over 100 identified forms of gibberellin, but only a few are biologically active (Richards et al., 2001).

Some of the physiological processes stimulated by gibberellins are outlined below (Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

1- Stimulates stem elongation by stimulating cell division and elongation .

2- Stimulates bolting/flowering in response to long days .

3- Breaks seed dormancy in some plants which require stratification or light to induce germination .

4- Stimulates enzyme production (a-amylase) in germinating cereal grains for activation of seed reserves .

5- Induces maleness in dioecious flowers (sex expression) .

6- Causes parthenocarpic (seedless) fruit development .

7- Delays senescence in leaves and citrus fruits.

2.6.5. Cytokinins

Cytokinins are compounds with a structure that fits to adenine which promote cell division and have other similar functions to kinetin. Kinetin was the first cytokinin discovered and so named because of the compounds ability to support cytokinesis (cell division). Though it is a natural compound, It is not made in plants, and is therefore considered a "synthetic" cytokinin (meaning that the hormone is synthesized somewhere other than in a plant). The most common form of naturally occurring cytokinin in plants today is called zeatin which was isolated from corn (*Zea mays*). Cytokinins have been found in almost all higher plants as well as mosses, fungi, bacteria, and also in tRNA of many prokaryotes and eukaryotes. Today there are more than 200 natural and synthetic cytokinins combined. Cytokinin concentrations are highest in meristematic regions and areas of continuous growth potential such as roots, young leaves, developing fruits, and seeds (Arteca, 1996; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

2.6.5.1. History of Cytokinins

Haberlandt Gottlieb (1913) discovered that a compound found in phloem had the ability to stimulate cell division. Van Overbeek (1941) discovered that the milky endosperm from coconut had this ability. He also showed that various other plant species had

compounds which stimulated cell division (Van Overbeek, 1941). Jablonski and Skoog (1954) extended the work of Haberlandt showing that vascular tissues included compounds which promote cell division. The first compound isolated that induced plant cytokinesis, and named kinetin, was derived from autoclaved herring sperm (Miller et al., 1955). It promoted tobacco pith parenchyma differentiation in culture and stimulated totipotent plant cell growth (Sieberer et al., 2003).

However, the first naturally occurring cytokinin was isolated from corn (*Zea Mais*) in 1961 by Miller and called zeatin. Letham reported that zeatin acts as a factor inducing cell division and later described its chemical properties (Letham, 1963). Since that time, many others naturally occurring cytokinins have been isolated and today there are more than 200 natural and synthetic cytokinins combined (Arteca, 1996; Salisbury and Ross, 1992).

2.6.5.2. Biosynthesis and Metabolism of Cytokinins

Cytokinin is generally detected in higher concentrations in meristematic regions and growing tissues. They are believed to be synthesized in the roots and translocated via the xylem to shoots. Cytokinin biosynthesis happens through the biochemical modification of adenine. The process by which they are synthesized is as follows (McGaw, 1995; Salisbury and Ross, 1992) :

1- A product of the mevalonate pathway labeled isopentyl pyrophosphate is isomerized .

2- This isomer can then respond with adenosine monophosphate (AMP) with the aid of an enzyme called isopentenyl AMP synthase .

3- The result is isopentenyl adenosine-5'-phosphate (isopentenyl AMP).

4- This product can then be converted into isopentenyl adenosine by removal of the phosphate by a phosphatase and further converted to isopentenyl adenine by removal of the ribose group .

5- Isopentenyl adenine can be converted to the three major forms of naturally occurring cytokinins .

6- Other pathways or slight alterations of this one probably lead to the other forms .

Degradation of cytokinins occurs largely due to the enzyme cytokinin oxidase. This enzyme removes the side chain and releases adenine. Derivatives can also created but the pathways are more complex and poorly understood .

2.6.5.3. Cytokinin Functions

Cytokinins, like auxins are necessary for many plant developmental processes (Taiz and Zeiger, 1998). These compounds intensify branching (Wang and Below, 1996), retarded senescence (Richmond, 1957), and promoted chlorophyll biosynthesis (Kato et al., 2002). To study the effect of cytokinins on leaf senescence, Richmond incubated *Xanthium pennsylvanicum* leaves in a kinetin solution for 10 days and compared their senescence to leaves incubated in water. He noticed that the kinetin-incubated leaves remained green while the water-incubated leaves senesced. Further, Gan and Amasino (1995) were able to delay senescence by transforming tobacco with a senescence associated gene promoter (SAG12): Isopentenyl Transferase construct. The prolonged senescence was attributed to cytokinin biosynthesis occurring after the induction of the SAG12 supported by the senescence-signaling pathway. It was shown that the cytokinin, zeatin-O-glucoside (ZOG), thought to be a storage form of Z, promotes chlorophyll biosynthesis in the shoot of young *Cucurbita* maxima up to 100 times more effectively than either Z or zeatin riboside (ZR), (Kato et al., 2002). Cytokinins also

contribute to the growth and development of meristematic organs and enhance shoot formation (Johnston and Jeffcoat, 1977; Wang and Below, 1996). In the shoot, cytokinins act as positive regulators of SAM (shoot apical meristem) cell proliferation while acting as negative regulators in the root apical meristem (Werner et al., 2003).

Kinetin showed inhibition in length and in the number of internodes (Naeem et al., 2004). Cytokinins promote growth by swelling rather than elongation in soybean (Fatima and Bano, 1998). Zadoo (1986) confirmed that cytokinin induced expansion of growth in hypocotyl segments of morning glory and inhibited the extension growth. Applied cytokinin showed a conversion of protoplastid into chloroplast with grana, thus giving lush green colour to the leaves (Stetler and Laetsch, 1965).

Cruz et al., (1999) reported that the weight of 'Hayward' fruit increased by 20 g on average when the synthetic cytokinin CPPU was applied in combination with GA_3. In addition pineapple plantlets could be efficiently propagated by soaking defoliated stems in CPPU solution (Shinichi et al., 2004).

The applied of CPPU on shoot and fruit of apple enhanced fruit size and weight, though often inducing irregular elongation, a slight delay in coloring and a lower sugar content (Tartarini et al., 1993). Applied doses of CPPU were deduced from many other works like Bangerth and Schriider (1994) how they have sprayed fruit apple by CPPU at 20 g/l. Glozer (2006) applied a concentration of 10 to 15 mg/l to study fruit firmness and reduction of preharvest drop in *Prunus domestica L.* Doses of 5, 10 and 15 ppm of CPPU were applied during four weeks after fruit setting to study the yield, fruit weight and dimensions and chemical fruit quality of Le-conte pear (Faissal and Ahmed, 2007).

In Japanese persimmon, formation of a sunken fruit apex, which was observed in about 30% of fruits from untreated trees, was suppressed by application of CPPU. CPPU also delayed coloration of fruit (Sugiyama and Yamaki, 1995).

The application of CPPU on kiwifruit showed a significant increase in fruit size and was found to double the weight. Although a significant reduction in the concentrations of total soluble solids (TSS), titratable acids (TA) and ascorbic acid (AsA) in the CPPU-treated fruits was recorded. Quan (1999), reported that Parthenocarpy induced by CPPU prevents flower abortion in Chinese white-flowered gourd (*Lagenaria leucantha*). CPPU also increased fruit set and fruit growth of pollinated ovaries.

Some of the known physiological effects caused by cytokinins are listed below. The response would vary depending on the type of cytokinin and plant species (Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

1- Stimulates cell division .

2- Stimulates morphogenesis (shoot initiation/bud formation) in tissue culture .

3- Responses the growth of lateral buds-release of apical dominance .

4- Causes leaf expansion resulting from cell enlargement .

5- May enhance stomatal opening in some species .

6- Promotes the conversion of etioplasts into chloroplasts via stimulation of chlorophyll synthesis .

2.6.6. Ethylene

Ethylene was discovered by Neljubow (1901) and reported the defoliation effect of plants (Neljubow, 1901). Unlike the rest of the plant hormone, ethylene was a gaseous compound (Chang et al., 1993; Rodrigues-Pousada et al., 1999).

Of all the known plant growth substance, ethylene has the simplest structure. It is produced in all higher plants and is usually associated with fruit ripening and the tripple response (Arteca, 1996; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

2.6.6.1. Discovery of Ethylene in Plants

Ethylene has been used in practice since the ancient Egyptians, who would gas figs in order to stimulate ripening. The ancient Chinese would burn incense in closed rooms to enhance the ripening of pears. In 1864, leaks of gas from street lights showed stunting of growth, twisting of plants, and abnormal thickening of stems (the triple response)(Arteca, 1996; Salisbury and Ross, 1992). Neljubow (1901) showed that the active component was ethylene (Neljubow, 1901). After that Doubt discovered that ethylene stimulated abscission in 1917 (Doubt, 1917). Gane (1934) reported that plants synthesize ethylene. Crocker et al. (1935) proposed that ethylene was the plant hormone responsible for fruit ripening as well as inhibition of vegetative tissues.

2.6.6.2. Biosynthesis and Metabolism

Ethylene is produced in all higher plants and is made from methionine in essentially all tissues. The production of ethylene varies with the type of tissue, the plant species and also the stage of development. The mechanism by which ethylene is produced from methionine is a 3 step process (McKeon et al., 1995; Salisbury and Ross, 1992).

1- ATP is an essential component in the synthesis of ethylene from methionine. ATP and water are added to methionine resulting in loss of the three phosphates and S-adenosyl methionine .

2- 1-amino-cyclopropane-1-carboxylic acid synthase (ACC-synthase) facilitates the production of ACC from SAM .

3- Oxygen is then needed in order to oxidize ACC and produce ethylene. This reaction is catalyzed by an oxidative enzyme called ethylene forming enzyme .

The control of ethylene production has been significantly studied. Subsequently the study of ethylene has focused around the synthesis promoting effects of auxin, wounding, and drought as well as aspects of fruit-ripening. The ACC synthase is the rate limiting step for ethylene production and it is this enzyme that is manipulated in biotechnology to delay fruit ripening in the "flavor saver" tomatoes (Klee and Lanahan, 1995).

2.6.6.3. Functions of Ethylene

Ethylene has many physiological roles in leaf and flower abscission, fruit ripening, anaerobic stress response, flower senescence and the breaking of seed dormancy in cereals (Doubt, 1917; Chang et al., 1993; Taiz and Zeiger, 1998 ;Vogel et al., 1998).

Ethylene is known to affect the following plant processes (Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992):

1- Stimulates the release of dormancy .

2- Stimulates shoot and root growth and differentiation (triple response)

3- May have a role in adventitious root formation .

4- Responses leaf and fruit abscission .

5- Promotes Bromiliad flower induction .

6- Induction of femaleness in dioecious flowers .

7- Causes flower opening .

8- Stimulates flower and leaf senescence .

9- Causes fruit ripening .

2.6.7. Abscisic Acid

Abscisic acid is a single compound dissimilar the auxins, gibberellins, and cytokinins. It was first identified and characterized by Addicott (Ohkuma et al., 1963) and called "abscisin" because it was thought to take part in abscission of fruits (cotton) (Addicott et al., 1968). At about the same time another group named it as "dormin" because they thought it contributed in bud dormancy. The name abscisic acid (ABA) was created by a compromise between the two groups. Though ABA generally is thought to play mostly inhibitory roles, it has many promoting functions as well (Arteca, 1996; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

2.6.7.1. History of Abscisic Acid

Ohkuma (1963) reported that abscisic acid was the first set and distinguished from the other hormones. They studied the compounds responsible for the abscission of fruits (cotton). Two compounds were isolated and called abscisin I and abscisin II. Abscisin II is presently named abscisic acid (ABA)(Ohkuma, 1963). Two other groups at about the same time discovered the same compound (Addicot, 1968). One group was studying bud dormancy in woody plants and the other group was studying abscission of flowers

and fruits from lupine. Plant physiologists agreed to call the compound abscisic acid (Salisbury and Ross, 1992).

2.6.7.2. Biosynthesis and Metabolism

ABA is a naturally occurring compound in plants. It is a sesquiterpenoid (15-carbon) which is partially produced via the mevalonic pathway in chloroplasts and other plastids. Because it is synthesized partially in the chloroplasts, it makes sense that biosynthesis primarily occurs in the leaves. The production of ABA is accentuated by stresses such as water loss and freezing temperatures. It is believed that biosynthesis occurs indirectly through the production of carotenoids. Carotenoids are pigments produced by the chloroplast which have 40 carbons.The breakdown of these carotenoids occurs by the following mechanism :

1- Violaxanthin is a carotenoid which has forty carbons .

2- It is isomerized and then splitted via an isomerase reaction followed by an oxidation reaction .

3- One molecule of xanthonin is produced from one molecule of violaxanthonin and it is uncertain what happens to the remaining biproduct .

4- One molecule of xanthonin produced is unstable and spontaneously changed to ABA aldehyde .

5- Further oxidation results in ABA .

Activation of the molecule can occur by two methods. In the first method, an ABA-glucose ester can form by attachment of glucose to ABA. In the second method, oxidation of ABA can occur to form phaseic acid and dihyhdrophaseic acid .

The transport of ABA can occur in both xylem and phloem tissues. It can also be translocated through paranchyma cells. The movement of abscisic acid in plants does not exhibit polarity like auxins. ABA is capable of moving both up and down the stem (Walton and Li, 1995; Salisbury and Ross, 1992).

2.6.7.3. Functions of Abscisic Acid

Ohkuma (1963) stated that the two groups simultaneously discovered the compound now known as abscisic acid (ABA). One group named the molecule "abscisin II" (Ohkuma et al., 1963) for its putative role in leaf abscission, later disapproved, and the other group named the molecule "dormin" (Eagles et al., 1964) for its role in bud dormancy. Later, the name "abscisic acid" was given to this phytohormone, despite the fact that ABA has no role in leaf abscission (Addicott et al., 1968). ABA has roles in dormancy, freezing tolerance, drought tolerance, and water flux in the roots. Unlike auxins and cytokinins, abscisic acid is not an absolute requirement for plant growth and development (Koornneef et al., 1998). However, the loss of ABA sensitivity results in phenotypic *aberrations.* Dwarfing effects on peach tree were detected by the swabbing of abscisic acid (ABA), hinokitiol and tropolone (Sharif et al., 2007).

One of the most well characterised roles of ABA is the negative regulation of stomatal opening during periods of low water potential. Water flux in plants is perceived in the roots (Mantyla et al., 1995; Taiz and Zeiger, 1998). Specifically, the interruption in water uptake is sensed in lateral roots and root hairs and induces ABA transport through the xylem to the photosynthetically active leaves (Hetherington, 2001; Schroeder et al., 2001). The ABA concentration in the xylem sap increases from approximately 1-15 nM to 3 μM (Schurr et al., 1992) in *Helianthus annulus* plants when water uptake is interrupted. In leaves, ABA enters the guard cells and triggers a series of signal

cascades that lead to loss of turgor pressure and stomata closure (Schroeder et al., 2001).

Seed dormancy and desiccation tolerance are also influenced by ABA. Determination of the ABA content in seeds from a dormant ecotype of *Arabidopsis thaliana*, Cape Verde Islands, demonstrated that ABA content was highest in dormant seeds and subsequently decreased under seed-breaking conditions finally reaching a concentration similar to non-dormant seeds (Ali-Rachedi et al., 2004). Because its role and unlike auxins and cytokinins, abscisic acid is not an absolute requirement for plant growth and development (Koornneef et al., 1998).

The following are some of the physiological responses known to be associated with abscisic acid (Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

1. Stimulates the closure of stomata (water stress brings about an increase in ABA synthesis).

2. Inhibits shoot growth but will not have as much effect on roots or may even promote growth of roots .

3. Induces seeds to synthesize storage proteins .

4. Inhibits the effect of gibberellins on stimulating de novo synthesis of a-amylase .

5. Has some effect on induction and maintanance of dormancy .

6. Induces gene transcription especially for proteinase inhibitors in response to wounding which may explain an apparent role in pathogen defense .

2.6.8. Other Growth Regulating Compounds

2.6.8.1. Brassinosteroids

There are approximately 60 steroidal compounds known as brassinosteroids. They are named after the first one identified, brassinolide, which was found in mustard pollen. They appear to be widely distributed in the plant kingdom. Some of their effects include :

1- Stimulation of stem elongation .

2- Inhibition of root growth and development .

3- Promotion of ethylene biosynthesis and epinasty (Arteca, 1996).

2.6.8.2. Salicylates

Salicylates have been known to be present in willow bark for quite some time. They have only recently been recognized as potential growth regulators in plants. Salicylic acid is synthesized from the amino acid phenylalanine. It has numerous effects including :

1- Thermogenisis in arum flowers .

2- Plant pathogen resistance-stimulates plant pathogenesis protein production .

3- Enhance longevity of flower .

4- Inhibition of ethylene biosynthesis .

5- Inhibition of seed germination .

6- Blocking the wound response .

7- Reverse the effects of ABA (Arteca, 1996; Davies, 1995).

2.6.8.3. Jasmonates

Jasmonates are represented by Jasmonate and its methyl ester. They were first isolated from the jasmine plant in which the methyl ester is an important product in the perfume industry. Jasmonic acid is synthesized from linolenic acid which is an important fatty acid. Jasmonates have a number of effects such as :

1- Inhibition of many processes such as growth and germination .

2- Promotion of senescence, abscission, tuber formation, fruit ripening, pigment formation, and tendril coiling .

3- They appear to have important roles in plant defense by inducing proteinase synthesis (Arteca, 1996).

2.6.8.4. Polyamines

There is some controversy as to whether these compounds should be classified with hormones. They are widespread in all cells and exert regulatory control over growth and development at very low levels. Development is affected by plants having low levels of polyamines. Polyamines have a wide range of effects on plants. They appear to be essential in growth and cell division (Arteca, 1996; Davies, 1995; Salisbury and Ross, 1992).

2.6.9. Phytohormone Cross Interaction

Idea to combine different types of hormones gives sometimes unexpected responses. Normal plant growth and development requires phytohormones to interact to regulate the various processes. This interaction is termed “cross interaction”.

2.6.9.1 Auxin and Cytokinin

Skoog and Miller (1957) were the first to discover cross-talk when they observed that the ratio of auxin:cytokinin influenced organogenesis in plant tissue culture. An equal amount of auxin and cytokinin induced callus growth, while a higher auxin:cytokinin ratio induced root growth, and a lower auxin:cytokinin ratio stimulated shoot growth. Another plant response governed by the interaction of auxin and cytokinin is gravitropism. Prior to a graviresponse, cytokinins accumulate within stratocytes, resulting in decreased root elongation, while auxins, transported to the lateral roots by PIN3, stimulate root elongation (Friml et al., 2002; Aloni et al., 2004). The antagonism of the auxins and cytokinins in the roots results in differential growth. This differential growth rate produces root curvature. These data indicated that auxins and cytokinins were antagonists. Further evidence of auxin to cytokinin antagonism is seen in leaf primordia in *Arabidopsis thaliana*. Auxin-induced repression of KNOX (KNOTTED1-LIKE HOMEOBOX) expression in leaf primordia is necessary for correct leaf initial growth (Scanlon, 2003). The KNOX proteins may induce cytokinin biosynthesis (Ori et al., 1999; Hay et al., 2004).

Auxin to cytokinin antagonism includes each hormone's effects on the concentration of the other (Palni et al., 1988; Nordstrom et al., 2004). Although Palni et al. (1988) found that treating plants with α-NAA increased oxidative metabolism of zeatin riboside (ZR), it was eventually shown that cytokinins and auxins regulate each other by decreasing the rate of biosynthesis and transport rather than catabolism (Bangerth, 1994; Eklof et al., 1997). However, conversion of the active cytokinins, zeatin (Z) and zeatin riboside (ZR) in most plants to the inactive N-glycosylated forms is increased in the presence of auxin (Blagoeva et al., 2004).

Indeed, Naeem et al., (2004) showed that application of IAA decreased shoot and the number of internodes of lentil (*Lens culinaris*), however the mixed dose of IAA and kinetin promoted late flowering, increased number of floral buds and the expansion of leaves.

Catecholamines (CA) are additional small molecules that affect plant growth and that have also been found in plants. These molecules that stimulated by abscisic acid (Sweidrych et al., 2004) exhibited cytokinin (Christou and Barton, 1989; Kuklin and Conger, 1995) and indole-3-acetic acid oxidase antagonist activities (Protacio et al., 1992).

Auxin and cytokinin are absolute requirement for viability (Taiz and Zeiger, 1998). Auxin is also required for cell elongation; it promotes elongation in the shoot, but inhibits it in the root (Taiz and Zeiger, 1998; Crozier et al., 2000). The differentiation of vascular tissue and vascular patterning in leaves were also under control of Auxin (Naderi et al., 1997; Taiz and Zeiger, 1998).

The relationship between auxin content and cytokinin biosynthesis was examined in greater detail in *Arabidopsis thaliana* plants treated with α-NAA. In a dose dependant manner, the treatment caused a decrease in the amount of both ZR and its precursor by acting on the isopentenyladenosine-5'-monophosphate independent pathway (Nordstrom et al., 2004). Auxin perception by the AXR gene family mediated this effect on cytokinin biosynthesis.

Unlike the fast reduction in cytokinin amounts seen after auxin treatment (Bangerth, 1994), cytokinin repression of auxin occurred over a much longer period, requiring up to 48 h (Nordstrom et al., 2004). These researchers concluded that cytokinins indirectly influenced auxin content. Bangerth (1994) proposed that auxin to cytokinin cross-

interaction was a two-sided feedback loop involving auxin transport from the SAM (shoot apical meristem) and cytokinin transport from the root. Subsequent research by Bangerth (2004) and others (Eklof et al., 1997; Haver et al., 2003) indicated that feedback inhibition of auxin and cytokinin biosynthesis in the presence of high concentrations of the antagonist phytohormone was due to a decrease of IAA biosynthesis in the shoot apex and cytokinin biosynthesis in the root.

Auxin and cytokinin do not always act as antagonists. In young organs, they are thought to interact synergistically to control progression of the cell cycle. One of the earliest studies on the roles of auxin and cytokinin in the cell cycle examined the effect of the hormones on p34cdc2-like proteins; protein kinases activated when a cell is committed to division (Choi et al., 1991). In tobacco pith, auxin induces biosynthesis of a p34cdc2-like protein and cytokinin is required for activation of the protein (John et al., 1993). In alfalfa leaf protoplast-derived cells, the absence of cytokinin completely abolished cdc2MsA/B activity, preventing cell cycle progression from the G0-G1 phase to S phase and from the G_2 phase to mitosis. Further, in the absence of auxin, cyclin dependent kinases could not be isolated from the cells (Pasternak et al., 2000). Sieberer et al. (2003) obtained additional evidence for the interaction of auxin and cytokinin in cell cycle control. The prz1-1 (proporz) mutant was isolated from a screen for seedlings showing defective growth on auxin and cytokinin from a T-DNA-mutagenized population of *Arabidopsis thaliana.* When prz1-1 plants were grown in the presence of auxin and cytokinin uncontrolled cell proliferation increased dramatically. PRZ1 appears to be a gene involved in the switch from cell proliferation to cell differentiation. It is a putative transcriptional adaptor protein involved in the transcription of a cell cycle control protein (Sieberer et al., 2003).

Auxin and cytokinin also act synergistically to regulate cell differentiation. The highest concentrations of auxin and cytokinins are seen in young leaves (Nordstrom et al., 2004). The SAM (shoot apical meristem) was also found to contain high amounts of auxins and cytokinins, both of which were necessary for SAM cell division (Werner et al., 2001). Cytokinin rapidly induced expression of the *Arabidopsis thaliana* response regulator ARR4 (Yamada et al., 1998). In turn, ARR4 interacted with AtDBP1, a DNA binding protein (Alliotte et al., 1988). The interaction between ARR4 and AtDBP1 was induced by exogenous auxin, as part of an indirect, long-term auxin response (Yamada et al., 1998). This auxin- and cytokinin-inducible interaction and activation is required for phosphorelay activity in the cytokinin-responsive signaling pathway.

The mixed dose of GA_3 + IAA and GA_3 + kinetin showed increase in the number of leaves (Naeem et al, 2004). The area of first leaves showed average increase with applied of IAA (Tuominen et al., 1997).

2.6.9.2 Auxin and Abscisic Acid

Cross-interaction between auxin and ABA has not been studied as extensively as auxin and cytokinin cross-interaction. In 1990, Wilson et al. discovered that *Arabidopsis thaliana* axr2 mutant plants were resistant to auxin, ethylene and ABA, thus indicating an interaction among these phytohormones. Additionally, drought induced rhizogenesis, the formation of lateral roots that were short, tuberous, and lacking root hairs (Vartanian, 1981), was decreased in both ABA insensitive mutants and in the auxin mutant axr1-3 (Vartanian et al., 1994). A recent study examining the effect of drought on cross-interaction between auxin and ABA in two auxin mutants, axr1-3 and axr2-1, found that both of the mutants displayed decreased ABA signaling (Bianchi et al., 2002). All these results suggest an overlap in auxin and ABA signal perception.

The molecular mechanisms that mediate auxin and ABA signaling remain largely unknown. Research at the molecular level has shown that abscisic acid and auxin have antagonistic interactions. Auxin was shown to enhance the telomerase activity in synchronized tobacco cells (Tamura et al., 1999). ABA treatment, however, abolished the positive effect on telomerase activity induced by auxin and inhibited telomerase activity in untreated cells (Yang et al., 2002). Furthermore, ABA was shown to increase transcription of ICK1 (INHIBITORs/INTERACTORs OF CDK), a cyclin-dependent protein kinase (CDK) inhibitor, suggesting that ABA can inhibit cell cycle progression (Wang et al., 1997; Wang et al., 1998).

2.7. PHYTOHORMONES IN FRUIT PRODUCTION

2.7.1. Phytohormones uses in tree fruit production

Plant bioregulators (PBRs) are more frequently used in tree fruit production than in any other horticultural or agricultural commodity, and they are essential for effective and profitable production. Several commercial uses have been selected to prove the evolution of the involvement of PBRs (Greene, 2010) from infancy to the present and progress made in the fundamental understanding of how regulation by PBRs is achieved.

2.7.2. Fruit Abscission - Preharvest Drop

Fruit abscission is considered by many to be the most important physiological response that is regulated by PBRs (Greene, 2010). This regulation of abscission occurs at two very different times in the life and development of the fruit. The first occurs early in or at the begining of the fruit development and then refers to the flower abscission or chemical thinning period. Secondly during the fruit ripening period they turn to the development period until harvest.

It was observed that auxins delayed leaf petiole abscission led to the finding in the late 1930s by Gardner et al. (1939) that Naphthalene Acetic Acid (NAA) and naphthaleneacetamide (NAAm) reduced preharvest drop. Other auxins were tested including 2,4-D (2,4-dichlorophenoxyacetic acid) but most proved to be unsatisfactory. Fenoprop (2-[2,4,5-trichlorophenoxy]propionic acid) was discovered in the 1950s and proved to be very successful (Southwick et al., 1953) but it was dropped in the 1980s due to fear of contamination with the carcinogen dioxine. The preharvest drop control properties of daminozide (2,2-dimethylhydrazide) were recognized in the 1960s soon after its growth control properties were recognized (Edgerton and Hoffman, 1966). This compound was the dominant preharvest drop control compound for over 20 years, not only because of its effectiveness but also because it delayed ripening, increased red color, reduced ethylene production and enhanced flesh firmness. The use of daminozide in apples was withdrawn in 1989 because of health concerns. NAA alone remained viable drop control compound but the drop control properties were relatively short-lived, when two NAA applications were made or when the time between application and harvest was delayed, fruit softening and reduced storage life frequently occurred (Smock et al., 1954).

The ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG) was recognized as having stop drop capabilities (Bangerth, 1978) but it was not developed for this purpose because daminozide was a very acceptable compound, it possessed several additional assets and an economical way was not known to produce this product and competitively not cost effective. Following the loss of daminozide initiated drop control studies with AVG was used as a drop control compound on apples. It remains today as the prominent drop control PBR. The most recent candidate as a drop control PBR is l-methylcyclopropene (1MCP) (Yuan and Carbaugh, 2007). This is a compound that is released as a gas which then binds irreversibly to ethylene binding sites within the plant.

It was first used in the mid 1990s to extend the postharvest life of ornamentals. It is now used to extend the storage life of apples and the extent of its use, and the impact that it had on commercial postharvest handling of apples (Watkins, 2006). This compound which normally is applied to apples as a gas in an enclosed space has been formulated so that it can be sprayed on trees. It is the most effective drop control compound on apples and the sprayable form has been used as a drop control compound in several countries.

Some of the most exciting work related to the control of preharvest drop on apples is just now emerges from the lab of Yuan at Virginia Tech and other locations. Combination of NAA with AVG or 1-MCP more effectively control drop than when the individual drop control compounds are used. Further, these combinations could be recognized to more effectively suppress genes responsible for ethylene biosynthesis and cell wall degradation in the abscission zone. The recent and major progress being made in drop control has been achieved by combining molecular biology, good pomology and a better basic understanding of the physiology of abscission (Greene, 2010).

2.7.3. Flower Abscission - Chemical Thinning Period

The inherent characteristic of pome fruit to undergo biennial bearing has been recognized for centuries but practical and meaningful solutions emerge started in the 1930s. Two separate approaches have been taken in crop load reduction; one is use of hormonal sprays and the second is application of caustic sprays. Auchter and Roberts (1933) used tar oil distillates as caustic materials to remove crop by damaging some blossoms. The compound sodium dinitro-ortho cresylate (DNOC) evolved from this work and remained an important thinner of apples in arid regions until 1990 when it was discontinued. A flurry of activity followed the loss of DNOC that has been

continued. Further details and background on thinning of pome fruit with caustic materials can be found in this volume (Fallahi and Greene, 2010).

Abscission retardation was one of the early physiological responses identified with auxins. Gardener, Marth and Batjer (1939) reported that NAA and NAAm might retard preharvest drop in apples. Burkholder and McCown (I941) attempted to increase fruit set on shy-bearing' Starking Delicious' using these compounds but instead these PBRs caused abscission rather than prevent it. Batjer et al. (1964) refined the use of both NAA and NAAm as thinning agents and these compounds are used generally until today. Observations by Batjer and Westwood (I960) of reduced fruit set following the use of the newly introduced insecticide carbaryl (1-naphthyl methylcarbamate) led to general and widespread use of this compound as a fruit thinner that persists today. In some regions carbaryl is the favored thinner because it is mild, its response is not rate sensitive and over thinning is quite unlikely. Carbaryl is now under regulatory scrutiny, and in some areas including large portions of Europe, it may no longer be available for use.

BA (6-benzylaminopurine) emerged as a chemical thinner candidate in the late 1970s when it was found to be a very effective thinner on 'Winesap' apples, but the active ingredient was not packaged into a thinning product until the 1990s; and even then it appeared as an altered formulation of a previous product that also contained a small amount of GA (Greene, 2010). Although the amount of GA was presented a few and seemed unimportant, its presence altered the thinning activity of BA, making it an erratic product to usc. A thinning formulation that contained only BA was introduced several years later and this has proved to be very effective. When combined with carbaryl it was a potent chemical thinner (Greene and Autio, 1994).

ABA has appeared on the horizon as a new and potentially useful chemical thinner. It has been shown to be an effective thinner on both apples and pears (Greene, 2007; 2009). It has the advantage of naturally occurring plant hormone which should be useful in facilitating product for the grower acceptance. The mode of action has not been defined but undoubtedly, closing of stomata, thus restrict carbohydrate supply and prove to be a contributing factor.

An enormous number of field experiments have been done in an attempt to achieve consistent thinning results. Progress has been hampered because important pieces of the puzzle have been missing, but there is a reason to be optimistic. The missing links have been the lack of understanding of basic control points in the abscission process, the absence of a method to predict thinning responses, and sorting out the genes primarily responsible for abscission from the background noise of nonparticipatory genes. Byers et al. (1991) reported that there was a link of light, temperature and carbohydrates to the abscission process. Bangerth (2004) has described that auxins have linked with ABA and ethylene. The development of a computer model Lakso et al. (2008) has incorporated the important environmental signals that affect abscission into a model that quite accurately predicts thinner response and provides guidance in the selection of thinning programs prior to application. Fruit measurement systems have been developed that allowed prediction of thinner results in about 7 days (Greene et al., 2005). More recently Zhu et al. (2008) have identified specific genes involved in the abscission process and they have shown that activation can be linked to specific PBRs. Abscission is a complex process that undoubtedly involves several hormones and many enzymes. Hormonal signals upregulate and down-regulate genes to drive this process (Costa et al., 2006). Critical breakthroughs in understanding and regulating abscission would only occured increasing fundamental understanding components of the abscission process by specifically identifying genes that are regulated into action or inaction.

2.7.4. Vegetative Growth Control

Appropriate regulation of vegetative growth is an important in pome fruit production since there is an inverse relationship between growth and flowering and excessive vegetative growth negatively impacts fruit quality, postharvest life, and development of an efficient and productive tree structure. Batjer et al. (1964) reported that daminozide could effectively inhibit growth of apple trees. It was an important discovery. Since it could also reduce fruit size, affect fruit shape and increase fruit set, its use for growth control early in the season was generally limited to directed application to the tops of vigorous trees, use on young nonbearing trees or on bearing trees where the crop was partially or completely lost (Greene, 2010).

Ethephon was also identified as a very effective growth retardant in the 1960s but its use on bearing trees was limited because it was also a strong fruit thinner (Edgerton and Greenhalgh, 1969). It was used quite extensively in the 1970s and 1980s in combination with daminozide for growth control and increased flowering on nonbearing trees on semi-dwarfing rootstocks (Byers and Barden, 1976). Paclobutrazol and other triazole gibberellin biosynthesis inhibitors were extensively tested in the 1980s. Paclobutrazol was approved for the use as a growth retardant in several countries, but its use has been limited due to long persistence in the tree, concerns about ground water contamination and a negative influence on fruit size in pome fruit (Miller, 1989). There were no viable PBR options for growth control of bearing trees until the gibberellins biosynthesis inhibitor prohexadione-calcium (Pro-Ca) was identified and extensively tested in the early 1990s and eventually registered for use by BASF (chemical company : *Baden Aniline and Soda Factory*) as the proprietary products Apogee in the US and Canada and Regalis in Europe and elsewhere (Rademacher et al., 2004). ProCa degrades relatively rapidly in the tree necessitating repeat application for season long growth

control. This seeming short coming has a distinct advantage since it affords a high degree of growth control via metabolism and reapplication. ProCa must be applied quite early, as soon as sufficient leaf area has emerged for absorption, since it requires about 10 days on pome fruit to start to restrict vegetative growth.

2.7.5. Enhance Flower Bud Formation

Harley et al. (1958) showed that NAA had the intrinsic ability to promote flower bud formation distinct from effects related to thinning. Earlier the focus on NAA was to enhance flower bud formation by chemical thinning to reduce crop load. In the mid 1960s when daminozide came into general use, it was found that damiozide could enhance flowering when applied after bloom. High rates reduced fruit size so lower rates were used to reduce the impact on fruit size. Ethephon proved to be the most effective promoter of flower bud formation. However, its use on bearing trees was limited because ethephon also caused thinning (Byers, 2003). Many investigators concluded that a combination of damiozide plus ethephon was the appropriate combination to increase flowering. Because of the thinning response, most of this work focused on influencing flowering on young and nonbearing trees. Enhancement of flowering became a lower priority in the 1980s and 1990s because there was a shift to planting trees propagated on dwarfing rootstocks that tended to be much more precocious thus the need for increased flower formation was diminished.

A new need became very apparent starting in the 1990s when new, unique and better tasting apples were introduced and these were planted extensively. Many of these new varieties had much greater biennial bearing problems than previous standard varieties. 'Honeycrisp' is an excellent example of a new cultivar that is being afflicted by this problem. Many new high density orchards were planted that were highly dependent upon continuous and consistent production to be economically viable. Consequently,

the focus on flowering research is to find strategies to increase flowering that do not substantially affect either crop load or fruit maturity. The PBR options are NAA and ethephon, both of which are thinners and they have the potential to advance fruit ripening (Cline, 2008). The general approach at the present time is to use multiple applications of low rates of either NAA or ethephon starting near the end of June drop. Flowering in pome fruit undoubtedly is a very complex and interactive process. Lack of consistent flowering in high density plantings remains an important problem and it needs to be addressed in a more innovative way. Breakthroughs and ultimate regulation of flowering will only come after we have achieved a better understanding of the physiology and mechanisms of flower bud formation. With this knowledge we can then achieve success similar to those we are just now realizing in the understanding in fruit abscission process.

2.8. TREE MANAGEMENT

Tree spacing ranges from 8-10 m. The trees receive little attention after the first year or 2nd year. Manuring, weeding، mulching and watering ensure rapid increase of tree volume. Trees which bear well benefit from compound fertilizers applied after harvest and supplemented with a top dressing as soon as the inflorescences are being formed. There appears to be no experience with pruning or fruit thinning. The fruits have a thin skin and are delicate; they need to be picked by hand twice a week and handled with care. The fruit should be consumed or preserved within a few days from harvest. A five-year-old water apple may yield 700 fruit (Morton, 1987).

2.9. REPRODUCTIVE BIOLOGY

There are definite flowering seasons, often two, sometimes three in a year, but the timing varies from year to year. There seems to be no regular growth rhythm for Malay

water apple. Apparently the trees are triggered into bloom (by wet weather following a dry period) more readily than water apple (*S. aqueum*) and wax jambu (*S. samarangense*) trees; at any rate, Malay apple usually has the most crops per year. Malay apples ripen about 60 days after bloom (Morton, 1987).

2.10. HARVESTING

Better fruits can be produced by harvesting properly. The fruits have a thin skin and are delicate; they need to be picked by hand twice a week and handled with care. The fruit should be consumed or preserved within a few days from harvest. Wax apple yields of 20-85 kg/tree are reported (Morton, 1987).

2.11. USES

2.11.1. Food: The tree is grown for their fruits, which substitute for one another in the marketplace. It is not easy to distinguish between the various *S. aqueum* and *S. samarangense* fruits. The ripe fruit is sweet and is mainly eaten fresh. In Indonesia wax jambu is used in fruit salads ('rojak') and they are also preserved by pickling ('asinan') (Panggabean, 1992). Eighty per cent or more of the fruit is edible. The composition of the species per 100 g edible portion: water more than 90%, protein 0.3 g, fat none, carbohydrates 3.9 g, fibre 1 g, vitamin A 253 IU, vitamin B1 and B2 traces, vitamin C 0.1 mg, energy value 80 kJ/100 g (analysis for wax jambu in Thailand).

The pink fruits are juicier and more tasty and suitable for eating out-of-hand. In Malaysia, the greenish fruits are eaten raw with salt or may be cooked as a sauce. They are also stewed with true apples .(Morton, 1987).

2.11.2. Medicine: Various parts of the tree are used in traditional medicine, and some have in fact been shown to possess antibiotic activity. The flowers are astringent and used in Taiwan to treat fever and halt diarrhea. Investigators have found the flowers

principal constituent to be tannin. In scientific research the flowers have shown weak antibiotic action against *Staphylococcus aureus*, *Mycobacterium smegmatis*, and *Candida albicans.*

Leaves of *S. malaccense* have been used to treat a wide variety of inflammatory conditions in Western Samoa (Andersson et al., 1997). Previous phytochemical studies of the leaves of *S. samarangense* have shown the presence of ellagitannins (Nonaka et al., 1992), flavanones (Kuo et al., 2004), flavonol glycosides (Kuo et al., 2004; Nair et al., 1999), proanthocyanidins (Nonaka et al., 1992), anthocyanidins (Kuo et al., 2004; Nonaka et al., 1992), triterpenoids (Srivastava et al., 1995), chalcones (Resurreccion-Magno et al., 2005; Srivastava et al., 1995), and volatile terpenoids (Wong & Lai, 1996).

Several flavonoids, ellagitannins, and phenolic acids have been identified from the fruits of *S. samarangense* (Nair et al., 1999; Nonaka et al., 1992; Okuda et al., 1982; Srivastava et al., 1995).

2.12. LIMITATION OF THE PRESENT RESEARCH

The research has some limitations that the experiments done in the field are time consuming and laborious. However, if it can be overcome, swabbing technique would be a suitable method of hormone application rather than spray.

Chapter 3 : MATERIALS AND METHODS

3.1. EXPERIMENTAL SITE

This study was carried out during two successive seasons (2010 and 2011) in a private orchard located at a commercial farm in Banting, 2^0 30N, 112^0 30E and $1^0$28 N, 111^0 20 E at an elevation of about 45 m from sea level. The area under study has a hot and humid tropical climate. The soil in orchard is peat with a mean pH of 4.6 (Ismail et al., 1995). Banting is a leading town in affecting dominion appropriate to Kuala Langat, Selangor in Malaysia (**Figure 3.1.**). The latitude of Selangor is 2°35'-3°60'N and longitudes is 100°45'-102°00'E. Banting's climate typically consists of warm, sunny days, and cool nights all year round with occasional rain in the evenings. Temperatures range from 23°C to 33°C. Humidity usually exceeds 80 percent. Annual rainfall is 2,670 mm. December till February are the wettest months.

Figure 3.1. Experimental site located in Banting

3.2. PLANT MATERIALS

Twelve – year- old wax apple trees (water apple) were selected for the study. The trees were spaced at 20.25 m^2 (square pattern). It was meant that tree to tree distance was 4.5 m and row to row distance was 4.5 m (**Figure 3.2**). Twelve trees were used in the study. Three trees were used for each treatment. Five branches from each tree were used for each unit. All the insects and diseases infected branches were removed before the experiment launching. Sixty uniform branches of the same length, diameter, same number of leaves were maintained from the twelve trees for each experiment (**Figure 3.3.).**

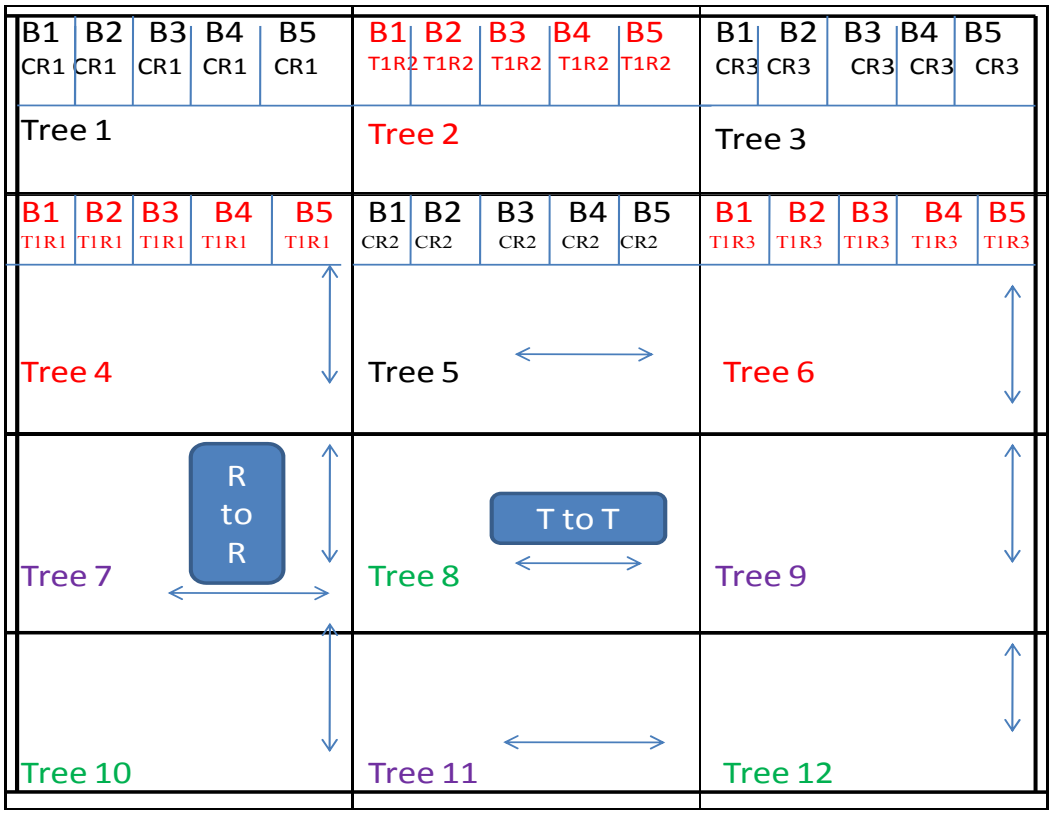


Figure 3.2. Experimental design: B = Branch, C = Control, T = Treatment, R = Replication, R to R = Row to Row distance, T to T = Tree to Tree distance. CRD = Completely Randomized Design.

A **B** **C**

Figure 3.3. Experimental trees in the field (A), choice of the uniformed experimental branches (B), labeling of the selected experimental branches (C)

3.3. TREE MANAGEMENT AND INTERCULTURAL OPERATION

The field was maintained properly and irrigation was done when necessary. Pesticides were applied once at growing season. Weeding was done at one month interval. Plant hormone was applied in the sunny day. Fertilizer was applied at the rate of 15-15-15% (N-P-K) yearly (Hossain et al., 2004).

3.4. TREATMENT APPLICATION AND DESIGN OF EXPERIMENTS

3.4.1. Experiment one: The effects of Gibberellic Acid (GA_3) at different concentrations on the growth and the development of wax apple fruit

The experiment was carried from September to December (2010).Twelve trees were used in this experiment. the experiment consists of 4 treatments including control (with fifteen replications). Five uniformed branches were taken as an experimental unit. The selected uniformed branches were swabbed with 30, 60 and 90 mg/l GA_3 and water (control) in three plants selecting five replications per tree, total of 60 branches. Fruits were selected in each branch to make swabbing instead of spray. Total number of fruits was 15×15=225 per treatments [n= (10×15) for fruit and n=15 for branch]. The design used in the experiment was Completely Rendomized Design (CRD). The swabbing method was applied to the branches once a week starting from bud formation stage to flower opening stage (blooming). It was stopped at the beginning of fruit set stage.

3.4.2. Experiment two : Influence of Naphthalene Acetic Acid (NAA) at different concentrations on the growth and quality development of wax apple fruit

The experiment was conducted from January to April (2011), starting from tree management to fruit harvesting in 2011. Twelve trees were selected for the second experiment. The experiment was conducted with four treatments namely, water control, 6, 12 and 18 mg/l of NAA. Different concentrations of NAA were swabbed starting from bud formation stage to flower opening stage (blooming) and then stopped. A total of sixty branches were used for 4 treatments. 15 branches in the 3 trees have been used for one treatment. The swabbing method for 4 treatments was followed as in experiment 1.

3.4.3. Experiment three : wax apple fruit growth and quality development as affected by N-(2-chloro-4-pyridyl)-N-phenylurea (CPPU)

The study was investigated from January to April (2011). Similar to the first and the second experiments, sixty uniformed branches from the twelve trees were selected for the third experiment. The Experiment was conducted with four treatments namely 10, 15 and 20 mg/l CPPU including control as a water treatment for the third experiment. Different concentrations of CPPU were swabbed starting from bud formation stage to flower opening stage (blooming). It was stopped at the beginning of fruit set stage. The swabbing method was same as experiment 1 and 2.

3.5. SWABBING TECHNIQUE

In this work a new technique called swabbing (**Figure 3.4.**) was used. This method consists of swabbing PBRs with wetting cotton and forceps without any contamination to the fruits. This method was applied successfully followed the method of Hossain et

al. (2007), where aqueous solutions of growth regulators were applied by swabbing two-to-three times with cotton wool held with forceps. The swabbing technique was applied on the bud at the same time for all treatments in the same experiment. It was done in the sunny day.

(A) Bud stage

(B) Initial flower stage **(C) Final flower stage**

Figure 3.4. Swabbing, by cotton applied, at bud flower and flower blooming stage of wax apple, by three Plant Bioregulators: GA_3, NAA and CPPU.

3.6. MEASUREMENT OF PHYSIOLOGICAL PARAMETERS

3.6.1. Total number of buds

The total number of buds was determined when bud size was 0.8-1.0 mm. the numbers of buds grown in 60 cm selected branch were counted before the opening of the flower bud.

3.6.2. Bud drop (%)

The percentage of bud drop was calculated by dividing the total number of buds before anthesis minus the number of buds at anthesis with the total number of buds before anthesis.

3.6.3. Initiation of flower

Flower initiation was reported at the beginning of the experimental and counted the flower initiation at 60 cm of the selected branches.

3.6.4. Blooming percentage

Blooming percentage were calculated by the bloomed bud divided by total number of buds then multiply the result by hundred.

3.6.5. Fruit set (%)

The Percentage of fruit set was calculated from tagged branches of the experimental trees immediatly after anthesis. The number of flower buds and total number of fruit set were counted before and after anthesis. Fruit set percentages were calculated using the following formula;

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

3.6.6. Fruit drop (%)

Fruit drop percentage was determined from tagged branches on the experimental trees by counting the number of initial fruits and the total number of fruits immediately after anthesis. Drop percentage was calculated 35 days of anthesis fruit, using the following formula;

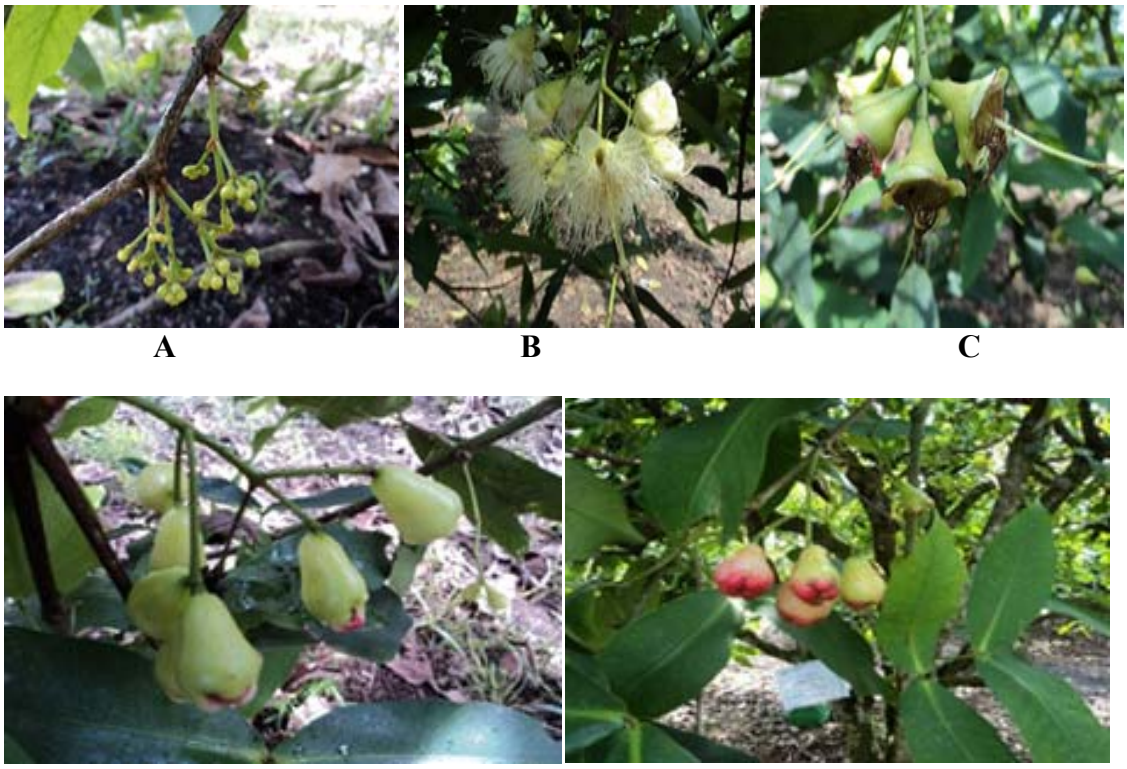
Fruit drop (%) = Number of fruits at final harvest/ Total number of initial fruit x100

3.6.7. Fruit length, diameter and fruit growth

Fruit length, diameter, and growth was measured weekly with digital caliper (Japan, Model). For fruit growth measurement of 15 fruits in selected branches were tagged after anthesis until the fruits were harvested. Final length and diameter were measured immediately after harvest (**Figure 3.5.).**

3.6.8. Fruit maturity (Observing color development)

The surface color of each tagged fruit was determined at three different points of the fruit using a standard color chart (Minolta, Osaka, Japan) and expressed as the percentage of maturity (peal color) (**Figure 3.5.**).



A **B** **C**

D **E**

Figure 3.5. Different stages of fruit development of wax apple. Where A is Bud satge, B is Flower initiation, C is Flower setting, D is Fruit setting, E is after Fruit setting.

3.6.9. Chlorophyll content (Represented by SPAD unit)

Chlorophyll content in leaves in the treated branches was determined using a Minolta SPAD meter 502, Japan model and measured usually after 1.5 month of treatment application. SPAD value of the leaves was expressed as the chlorophyll content.

3.6.10. Fruit harvesting

Fruits were harvested at different periods (1st experiment, December- 2010; 2nd experiment, Aprill-2011; 3rd experiment, April-2011). After two months of treatment application, fruits were harvested at the same day from all trees and packed in the plastic bag then fruits were taken to the laboratory for measurement.

3.6.11. Fruit volume

Fruits were kept in the scaled glass water for 2 minutes, after that volume was measured by this visual observation of water level in the scaled glass (**Figure 3.6**). This is laboratory traditional method of fruit volume measurement.

Volume= Initial level of water – Final level of water

3.6.12. Juice volume (ml)

After fruit harvest, it was taken to the laboratory for measurement. Volume was measured by this visual observation of juice level in the scaled glass by measuring cylinder in the laboratory.

3.6.13. Fruit yield

Yield per treatment was recorded by weighing the total number of fruits per treatment after harvesting at the same days for doing statistically uniformity.

3.7. MEASUREMENT OF BIOCHEMICAL PARAMETERS

3.7.1 Fruit grinding (Collection of fruit juice)

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

3.7.2. Total soluble solid (TSS) content

Total soluble solids (TSS) content in the fruits were evaluated at 25ºC with abbe Refractometer. TSS were expressed with % Brix. A hand-held refractometer (Atago ATC-1, 32-10 Honcho, Itabashi-ku, Tokyo 173- 001, Japan) was used from 2010 and a digital refractometer (Atago PR-101) was used from 2011 for TSS determinations. A few drops of juice were kept on the refractometer prism surface (**Figure 3.6**.) and reading was collected from skin pad.

Figure 3.6. Measurement of biochemical parameters

(C: control, T1 and T2: Treatements)

3.7.3. Fructose and inverted sugar

Centrifuged clear juices of fresh harvested water apple/wax apple were used for fructose and inverted sugar determination. Fructose and inverted sugar were evaluated at 25°C with Atago 8469 digital handled fructose and inverted sugar refractometer (Atago Co. LTD., Tokyo, Japan) and expressed as percentage. 2-3 drops of juice samples were put on the sensor of refractometer and reading was displayed in percentage.

3.7.4. pH of fruit juice: Sample Preparation and Reference Method

Immediately after harvest, fruits were clean, washed and dried of surface water with a fan. The fruits were then blended and fruit juices were kept in glass bottles. All fruit juice samples were first allowed to equilibrate to room temperature (25°C) before pH determination. pH was measured using a Microprocessor pH meter (Hanna Instrument). Prior to the measurement of pH, the Microprocessor pH meter was calibrated properly.

3.7.5. Potassium (K^+) content

Fruit juice was taken for K^+ determination from each treatment. Then 3 to 5 drops of the supernatant liquid of centrifuged juice (4000 rpm for 10 min) were dropped onto the calibrated sensor pad (Cardy Potassium Meter, Model-2400, USA), on a sampling paper placed on the sensor. The reading in ppm was taken from the display pad after it stabilized (30 to 43 sec).

3.7.6. Total phenols

The total phenolic content of wax apple fruits were determined by using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Folin-Ciocalteau (FC) colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The intensity of light absorption at that wavelength is proportional to the concentration of phenols.

1ml of fruit juice, gallic acid calibration standards, folin-Ciocalteau (FC) reagent stored in the dark and discarded if reagent becomes visibly green, Sodium carbonate solution, 100-ml were used in volumetric flask.

Spectrophotometer was set to 765 nm, with 1-cm, 2-ml plastic or glass cuvettes. 1ml of fruit extract was added to 25 ml of volumetric flax, containing 9 ml of distilled water. A reagent blank was also prepared. 1 ml of Folin –Ciacalteu's phenol reagent was also added to the mixture. The solution was diluated with distilled water and mixed Thoroughly incubation at room temperature at room temperature. The absorbance against reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer Lambda 5 and expressed as mg gallic acid equivalent GAE/ 100g fresh weight.

3.7.7. Total flavonoids

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

Catechin equivalents (CE) / 100 g fresh mass. Samples were analyzed in triplicates.

3.7.8. Total anthocyanin content

The total anthocyanin contents of the hydrophilic extracts were measured by the pH-differential method described by Rodriguez-Saona et al. (2001).

The matured wax apples were harvested and the crude extract was prepared in the following method. The wax apple was washed thoroughly with distilled water at room temperature (27 ± 2 °C). The outer layer of wax apple (skin, containing color) was

removed manually with the help of peeler. The pigment from peels was extracted with water using food processor (Singer, FP-450). The pigment extract was filtered to remove the fibrous particles and then it was centrifuged at 10,000 rpm for 5–10 min to remove the tiny suspended solid particles. The color extract was then stored at 4–5 °C in the refrigerator and used for the experiments.

The anthocyanin content was determined from the pH-differential method using the following equation (Ronald et al., 1982 and Rodriguez-Saona et al., 2001). The development and the process optimization of water apple concentration extract as potential natural red colorant,

$$\textbf{Anthocyanin content (mg/100g)} = \frac{\textbf{A}\times \textbf{Mw}\times\textbf{DF}\times 10^{3}\times 100}{\varepsilon\times \textbf{L}}$$

Where, $A = A_{510}$ (pH 1.0) – A_{510} (pH 4.5), Mw is the molecular weight of anthocyanin (433.2 g mol^{-1}), DF is the dilution factor, ε is the extinction coefficient (31,600 L cm^{-1} mol^{-1}) and L is the path length (1 cm).

It was employed by coupling reaction of 2, 4-dinitrophenyl hydrazine dye with vitamin C and followed by spectrophotometric determination.

3.8. STATISTICAL ANALYSIS

The data from the seasons (2010 and 2011) were plotted and analyzed using MSTAT statistical software. One way ANOVA was applied to evaluate the significant difference in the parameters studied in the different treatments. Least significant difference

(Fisher's protected LSD) was calculated, following significant F-test (p=0.05). Standard error (SE) was measured by Excel.

Chapter 4 : RESULTS AND DISCUSSION

4.1. EXPERIMENT ONE : THE EFFECTS OF GIBBERELLIC ACID (GA_3) AT DIFFERENT CONCENTRATIONS ON THE GROWTH AND DEVELOPMENT OF WAX APPLE FRUIT

4.1.1. The effect of Gibberellic Acid (GA_3) on number of bud, fruit set and drop

GA_3 which was applied at different concentrations has affected the bud and fruit drop as well as fruit set over the control process. The fruit set percent was observed to be 30% in water control, while it was 70% and 68% in 60 ppm and 90 ppm GA_3, respectively. In the case of fruit drop, the percent of fruit drop per branch was 38% and 37% in water control and in 30 ppm GA_3, respectively. The most significant difference was observed when the branch was treated with the 60 ppm GA_3 and 90 ppm GA_3. In this case, the fruit dropping was higher in each concentration (**Table 4.1.1.**).

4.1.2. The effect of Gibberellic Acid (GA_3) on fruit size and weight

GA_3 (30, 60 and 90 ppm) has increased the average fruit length and diameter. The plant in which GA_3 (60 ppm) was applied, produced maximum sized fruit (43.37 mm diameter) with maximum total (70.30 mm) fruit length (**Figure 4.1.1.**). Although plants, in which GA_3 (90 ppm) was applied produced fruit of the second largest size (41.85 mm diameter) with the total fruit length (64.3 mm/fruit) (**Figure 4.1.2**.). Whereas, water swabbing produced the lowest sized fruit (39.04 mm) with minimum fruit length (60.39 mm).

The fruit weight is the most important parameter in fruit quality, on which fruit value (price) is dependent. As it is shown in **Table 4.1.2**., Gibberellic Acid (GA_3) treated fruits were larger than untreated fruits (in fruit weight), but the differences were more significant in 60 ppm than other concentrations. The most important benefits of

GA_3 are a reliable increase of fruit size of about 10%. Increased firmness is a more consistent response to GA_3 and there are always change in fruit yield parameters.

4.1.3. The effect of Gibberellic Acid (GA_3) on fruit juice

Significant differences among the treatments in fruit juice were observed. Fruit yield (weight and volume) were significantly affected by the treatment with 60 ppm GA_3. The fruits treated with 30 ppm GA_3, were a little bit lower in weight and volume than 60 and 90 ppm GA_3. In general treated fruits contained more juice content than untreated fruits. The highest juice content was 72.3 ml/100g of fruit in 60 ppm GA_3 treated fruits (**Table 4.1.2.**).

4.1.4. The effect of Gibberellic Acid (GA_3) on total soluble solids (TSS), pH in fruit juice

Results shown in **Figure 4.1.3** illustrated that the total soluble solids (TSS) in fruit juice of wax apple was significantly increased by GA_3 treatments. GA_3 at 30 and 90 ppm concentrations decreased TSS in fruit juice as compared to the control. The pH also significantly affected by treatments. The lowest fruit pH value in fruit juice was recorded in control compared to all GA_3 concentrations. Percentages of fructose and inverted sugars in fruit juice decreased significantly by the higher concentration (90 ppm) of GA_3 compared to the water control and low concentration of GA_3 in present experiment (**Figure 4.1.4.** and **Figure 4.1.5**). In addition, the highest contents of fructose and inverted sugars in fruit juice were exhibited in 60 ppm of GA_3.

4.1.5. The effect of Gibberellic Acid (GA_3) on fruits maturity (color), flavonoid content and total phenol

The influence of treatments on maturity development was observed throughout the experiments. All concentrations were able to enhance the color associated component with respect to experimental periods. The most effective concentration to earlier maturity of wax apple fruit was 60 ppm of GA_3. In the case of flavonoid, lower content was observed in control, 30 and 90 ppm GA_3 than 60 ppm GA_3 concentration. However, the most effective concentration for flavonoid content was in the 60 ppm GA_3. In contrast, the maximum anthocyanin content was observed in 60 ppm GA_3 and the minimum was observed in water control (**Table 4.1.3**.). The results showed that color, total flavonoid, total phenolic and anthocyanin compounds were significantly increased by GA_3 treatment (**Figure 4.1.6. and Figure 4.1.7.**). It was found that total phenolic content also followed the same trend as total flavonoid and anthocyanin content in all treatments. It was clear that 60 ppm GA_3 had a positive effect on anthocyanin and maturity colour improvement compared to 30 and 90 ppm GA_3.

4.1.6. The effect of Gibberellic Acid (GA_3) on fruit K^+ content and chlorophyll content

Fruit K^+ content was significantly increased by GA_3 treatments, especially at 60 ppm (**Figure 4.1.8.**). However, no significant differences were found between K^+ content at 30 and 90 ppm treatment. Thus, there were differences in sensitivity of fruits to GA_3 at various fruit development stages. The photosynthetic pigment, chlorophyll (SPAD) showed a significant difference with respect to the applied hormone treatments. The accumulation of chlorophyll was significantly higher in plants which underwent in GA_3 application than control (**Figure 4.1.9**). The lowest amount of chlorophyll was

observed in the control treatment. Hence, it was visualized that 60 ppm GA_3 was the optimum rate for wax apple leaves to maintain the highest chlorophyll content.

4.1.7. Discussion

The discussions on some physiological aspects of hormonal application of GA_3 on wax apple fruits are presented. The researches done on wax apple fruit were focused only on physiological aspects, mainly on yield, fruit set and drop studies. These researches were focused on offering a wide view about the physiological parameter as well as the chemical analysis and the effects related to plant hormone. A new hormone application method (Swabbing technique) has been introduced under natural sunlight growing condition.

The transition from vegetative to reproductive growth is a critical event in the life cycle of plants. Plant hormones play an integral role in controlling the growth, development, metabolism and morphogenesis of higher plants (Claus, 2008). Auxins, gibberellins, cytokinins, ethylene and abscisic acid are well known plant hormones. However, growth hormones especially GA_3 differ from others considerably in their mode of actions (Goro et al., 2001; Andrea et al., 2004). Fruit set is a phenomenon induced by pollination and fertilization or chemical treatment which culminates in the initiation of growth in fruit tissues. Fruit development begins with the initiation of growth and continues through maturity. During wax apple bud to fruits setting, GA_3 was found to be essential for the development of fruits and in the mobilization of nutrients to the developing organs of bud (Claus, 2008, Saifuddin et al., 2009; Moneruzzaman et al., 2011). High concentration of GA_3 showed a positive role on flower formation in olive during the induction and the initiation period. In addition, the application of gibberellic acid (GA_3) has the potential to control growth and flowering and induce earliness of

meristem. Enhancement of synthesis of chlorophyll pigment by GA_3 concentration had previously been reported and it has been suggested that the enhanced synthesis was attributed to the increased cytokinin activity in rose and bougainvillea plants (Angeles et al., 2008; Saifuddin et al., 2009). Thus finding of the present study agrees with the reports on the enhancement of the photosynthetic pigments by GA_3 hormones (Moneruzzaman et al., 2011). In strawberry, GA_3 application increased petiole length and leaf area. It reduced the time needed for inflorescence emergence, accelerated flowering and therefore, increased the number of flower buds and open flowers in most growing conditions (Khan and Chaudhry, 2006; Sharma and Singh, 2009). In the case of the tropical reason, high temperatures and humidity inhibit pollen development and result in failure of fruit set and growth (Sato et al., 2000, 2002). Plant hormone, GA_3, is well known plant growth regulators that can substitute for pollination and induce fruit setting and growth, and these are used for stabilizing fruit production in commercial growing. However, under high temperature and humidity conditions may not be supportive to induce a sufficient fruit set (Sasaki et al., 2005). Fruit set and development are two developmental processes at the reproductive phase of a plant which are controlled by internal hormonal balance. Consequently both fruit set and development could be regulated by external application of plant growth substance similar as flowering and sex expression. The role of endogenous GA_3 in seed and fruit development was reported also in tomato fruits (Oded and Uzi, 2003). More resent work has indicated that final fruit size, weight and length of treated GA_3 in wax apple is higher than that of pollinated controls which is similar to this research findings (**Figure 4.1.10**).

The significant increase of TSS and pH content of wax apple fruits was observed in this study. In many reports, it was generalized that fruits were shown to

have higher levels of soluble solids and sugar, but lower level of acid compared with the non treated fruits (Gelmesa et al., 2010). As was discussed earlier in this discussion, successful induction of fruit set in response to application of exogenous growth regulators varies among different fruits. Many researchers reported that plant hormones exert their effect on fruit set by controlling the direction of transport of nutrients such as TSS, fructose and sugar content rather than by direct control of fruit set process. According to the authors, the rate of assimilate export from the leaves; rate of import by fruits, and the fruit carbon metabolism are factors that finally influence the TSS, fructose and sugar of wax apple fruit. The role of GA_3 in increasing TSS of various fruit was reported by many authors. For instance, Graham and Ballesteros (2006) reported that GA_3 increased proteins, soluble carbohydrates, ascorbic acid, starch and carotene in the tomato. Higher sugar content in this and previous study (wax apple and tomato fruits) were obtained from plants treated with 50 ppm GA_3 (Kataoka et al., 2009,). Hossain and Fusao (2008) explanted a clear understand on the relationship in TSS and acid content in peach fruit having same experiment for six years. They reported that obviously when TSS is increased the acid value exponentially decreased. In general, TSS has been of major interest to the food processing industries that manufacture concentrated fruit products (Ram, 2005) and for fresh market consumption (Ho, 1998). It is believed that increased TSS content of fruits could give more finished product per ton of raw tomato fruit and thus, require less energy to produce a certain quantity of concentrated product. Hence, the use of GA_3 application for fruit production is one option to improve TSS content of various fruit. Though fruits pH is dependent on several factors, including cultivar, maturity stage, cultural practices as well as growing location and seasonal variations (Gould, 1992) but achievement of low fruit pH and high TSS value by swabbing 60 ppm GA_3 in our study could be a useful investigation. Comparable to the present result, significant increase of fructose and inverted sugar

content in fruits due to application of PGRs was reported due to increased formation of fructose and sugar in the tissues (Graham and Ballesteros, 2006). Thakur et al. (1996) indicated that acidity of tomato fruits was reduced when the whole plant was sprayed with GA_3. On the other hand, increased pH value of fruit is a desirable quality and essential factor accounting for flavor. Thus processors typically add more sugar and fructose to juice to ensure high pH values. Thus, relationship between increased pH and increased sugar content is the desirable fruit quality parameter (Erdal et al., 2007; Fontes et al., 2000) to reduce the risk of microbial spoilage and requires moderate conditions for processing and enzyme inactivation.

Flavonoids are the most important plant pigments for flower and fruit coloration producing yellow or red/blue pigmentation in petals and fruits skin. An important role of flavonoids is to serve as visual signals for animals in attracting pollinators in flowers, and later for animals eating the fruits and thereby helping in seed dispersal. In fruits, flavonoids may contribute in a number of ways to fruit quality, for instance to traits such as color, flavor, and bitterness or texture (Amiot et al., 1997). The composition of flavonoids in different fruit species varies greatly. Anthocyanins are pigments that give most fruits their red, violet and blue color. In addition, environmental factors such as nutrients, temperature and light conditions can have an effect on flavonoid composition and on the final hue of the fruit. In addition, phenolic component, as well as other molecules, such as purines, has the ability to function as co-pigments. Also, the temperature and pH of the vacuolar solution may affect the final color (Brouillard and Dangles 1994). The change in color in these cultivar mutants might be due to mutations in structural or regulatory genes involved in anthocyanin biosynthesis (**Figure 4.1.11**).

Potassium (K^+) is important as it's an activator of many enzymes and a regulator of the osmotic potential in cell (Bussakorn et al., 2003). Known as a 'quality element', K^+ could increase fruit development of apple by enhancing synthesis and translocation of carbohydrates in plants (Han et al., 1995), citrus (Chen et al., 2000). Generally, application of plant hormones could increase both fruit setting rate and content of the soluble solids, sugars (Zhang et al., 1998; Huang et al., 2000; Gao et al., 2001).

In this experiment following GA_3 application, it was found that fruit K^+ was significantly increased. Niu et al. (2008) reported that at early stage, it is required for sufficiently large molecular substrates like carbohydrates and fruit tissues such as peel and seeds to develop its normal cell division and cell enlargement. As fruit species with high demand for K^+, nutrient contents in grape fruits have an important effect on their quality. Niu et al. (2008) reported that the application of GA_3 to grape fruits enhanced K^+ and fruit growth as well as the endogenous hormones (IAA) during fruit growth and development. This result was also attributed to growth acceleration by the GA hormone (Chen et al., 2000; Ma and Liu, 1998; Huang et al., 2002), which enhanced both the enlargement of grape fruits and sink capacity of grape fluster to absorb water or nutrients, such as K^+.

Finally, it is believed that fructose, inverted sugar and K^+ were related to the fruit weight and size which were considered as effective on the fruit set and the high growth rate as well as the color content. It was determined that swabbing of GA_3 had a tendency to increase juice content per fruit by 15–17%. Higher concentration of GA_3 might enhance sugar translocation from fruits into other parts of plant. The decreased fructose, inverted sugar and K^+ accumulation resulted in decreasing both fruit weight and size. Fruit set and development in wax apple fruit could be regulated by the external application of GA_3. Gibberellin acid (60 ppm GA_3) improved the quality of wax apple

fruit by increasing length, and yield of the fruits as well as by increasing the content of total sugar inside the fruits.

Table 4.1.1. Effects of GA_3 treatments on physiological characteristics of wax apple: growth parameters, bud and fruit set. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatments (mg/L)	Number of bud	Bud drop (%)	Fruit set (%)	Fruit drop (%)
Control	57.3 ± 0.33d	31 ± 0.57c	30 ± 0.57d	38.6 ± 0.33c
GA_3 30	64 ± 0.57b	27.3 ± 0.33cd	33.6 ± 0.33c	37.3 ± 0.33cd
GA_3 60	67.3 ± 0.33a	41 ± 0.57a	70 ± 0.57a	49 ± 0.57a
GA_3 90	61± 0.57bc	37.6 ± 0.66b	68 ± 0.57ab	46 ± 0.57b

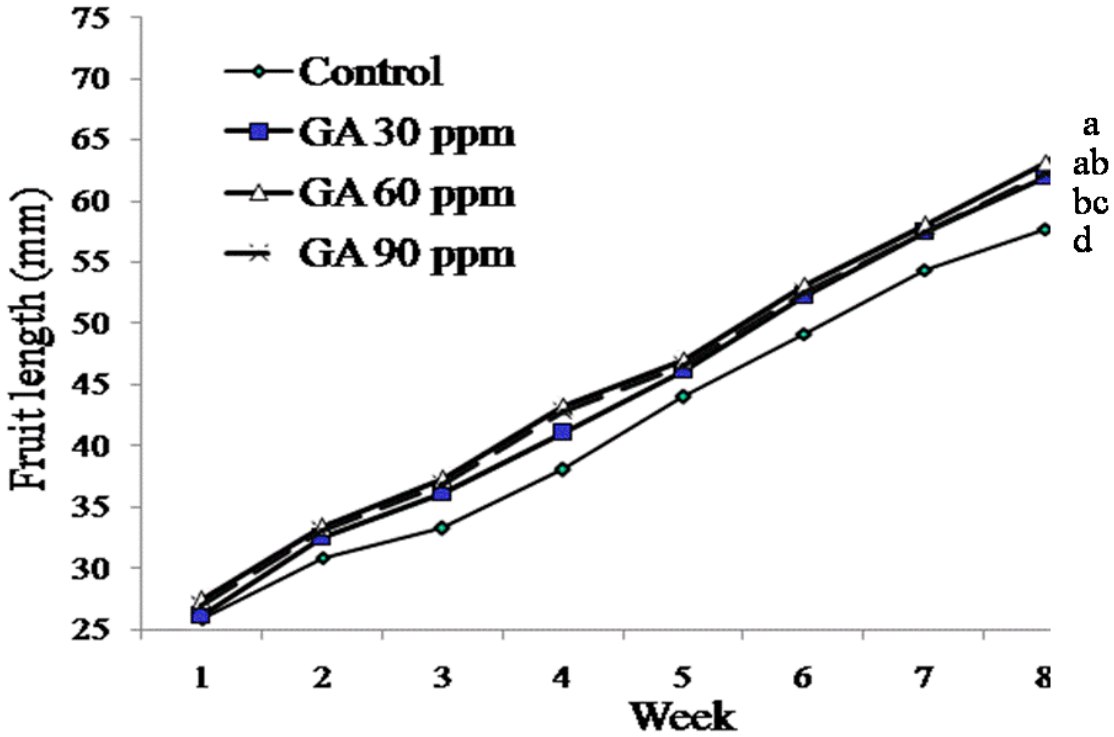


Figure 4.1.1. Fruit growth (Length /week) as influenced by different concentration of GA_3 (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

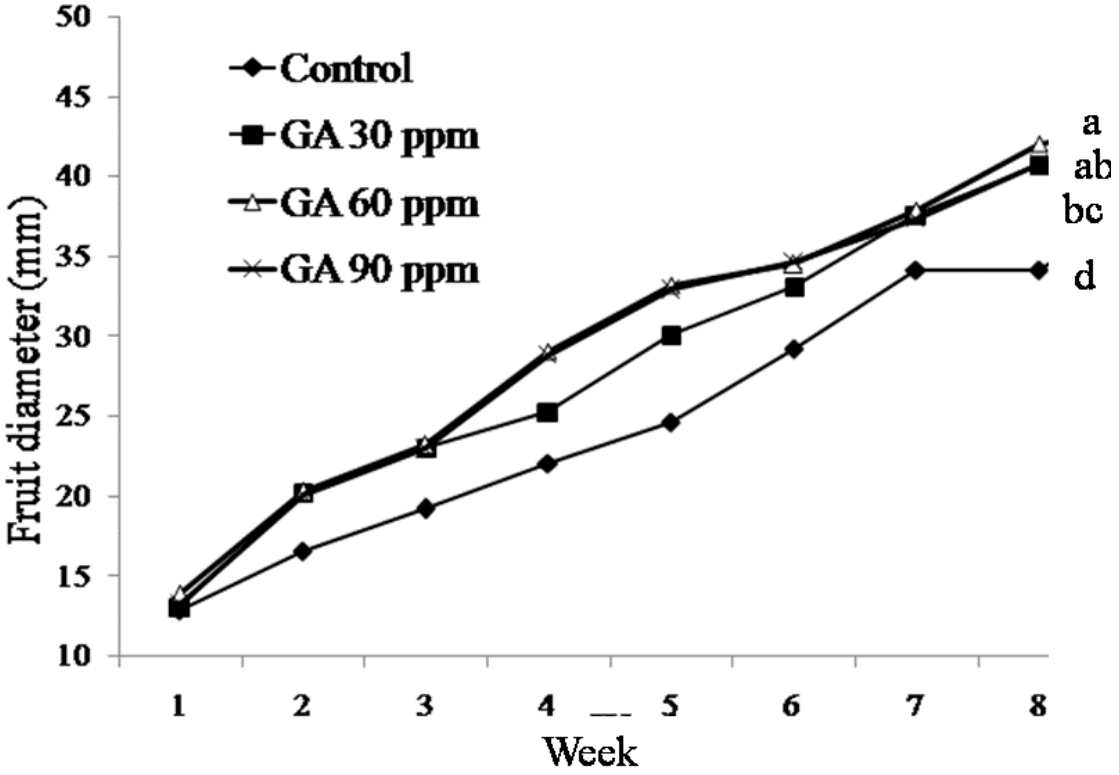


Figure 4.1.2. Fruit growth (diameter/week) as influenced by different concentration of GA_3 (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

Table 4.1.2. The effect of GA_3 treatments on yield contributing characteristics and fruit juice in wax apple fruit. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatments (ppm)	Yield/branch (g/branch)	Fruit weight (g/fruit)	Fruit volume (ml/fruit)	Juice content (ml/100g fruit)
Control	510.3 ± 1.4d	48 ± 0.57d	49 ± 0.57cd	63 ± 0.57d
GA_3 30	529 ± 2c	54 ± 0.57c	52 ± 0.57c	66 ± 0.57bc
GA_3 60	808 ± 6.1a	69 ± 0.57a	70.3 ± 0.88a	72.3 ± 0.88a
GA_3 90	781 ± 3.7b	61 ± 0.57b	62 ± 1.15b	66.6 ± 2.84b

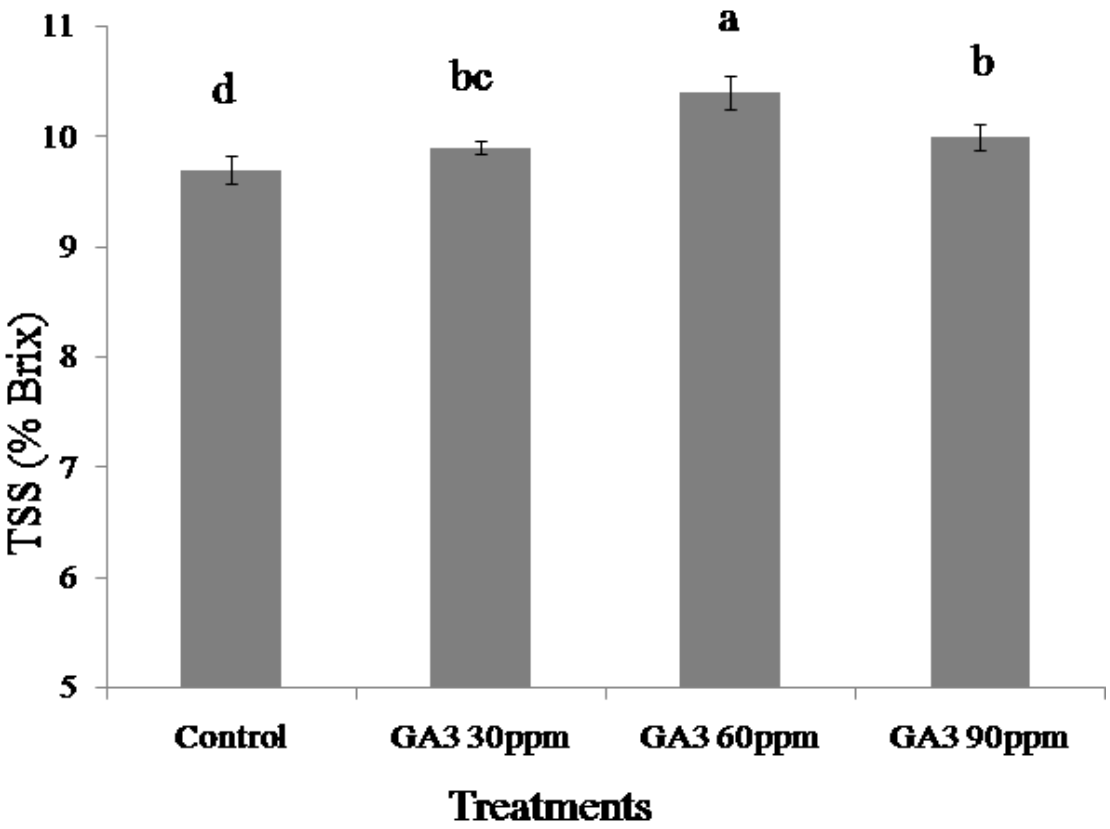


Figure 4.1.3. Effect of GA_3 treatments on total soluble solids (TSS) content of wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

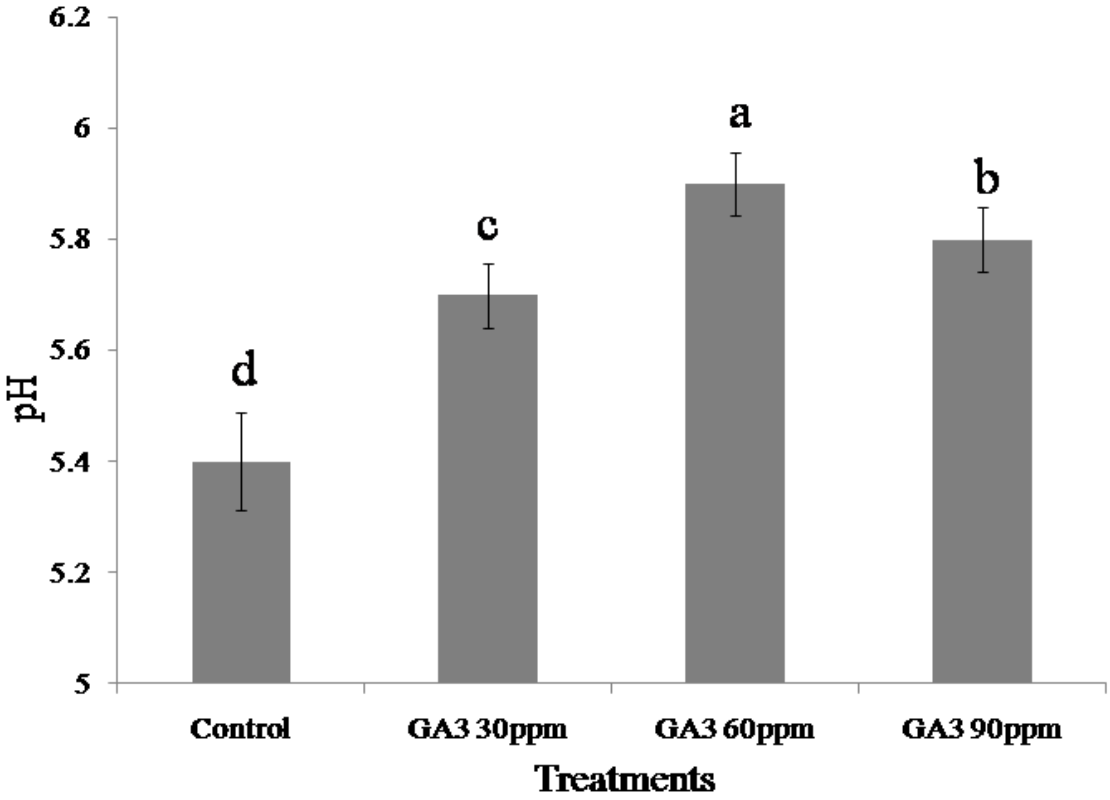


Figure 4.1.4. Effect of GA_3 treatments on pH of wax apple fruit juice (Different alphabets mark significant differences, $P < 0.05$ by LSD). S.E. ± (n=5).

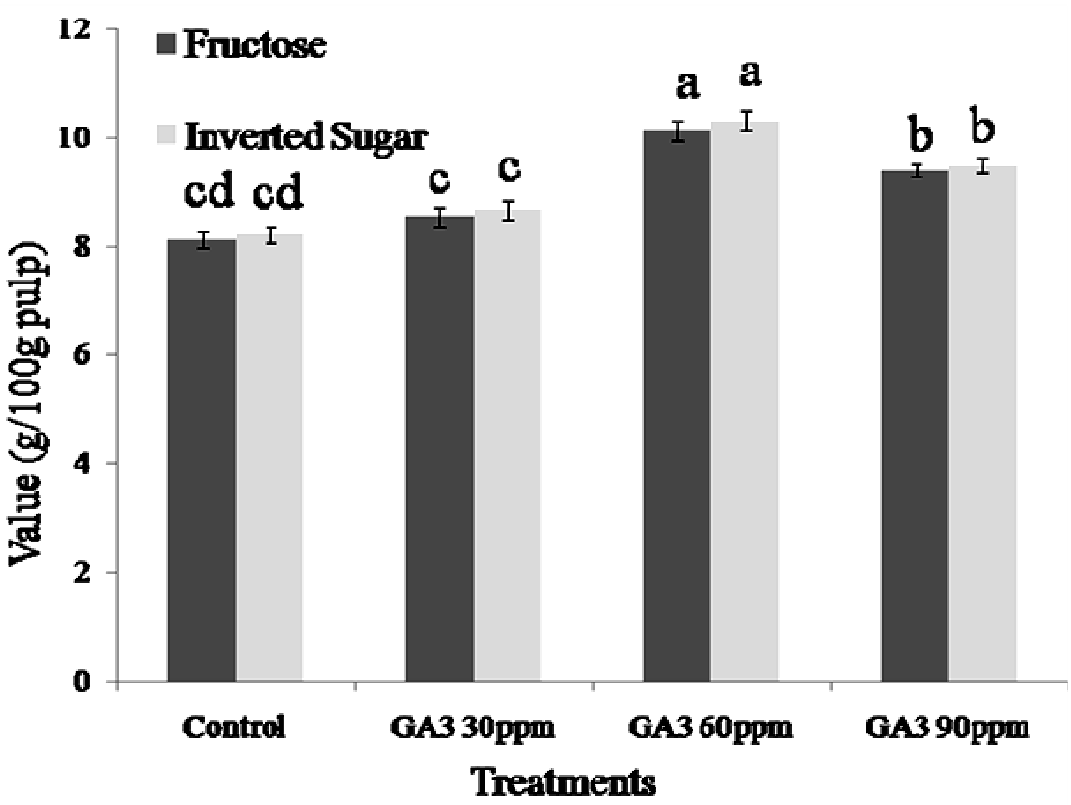


Figure 4.1.5. Effect of GA_3 treatments on fructose and inverted sugar content of wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

Table 4.1.3. The effect of fruits GA_3 treatments on peel color (%) development of wax apple fruit under field conditions. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatment (ppm)	Maturity development peel color (%)
Control	88.3 ± 0.04d
GA_3 30	94.3 ± 0.03b
GA_3 60	98 ± 0.09a
GA_3 90	93 ± 0.01bc

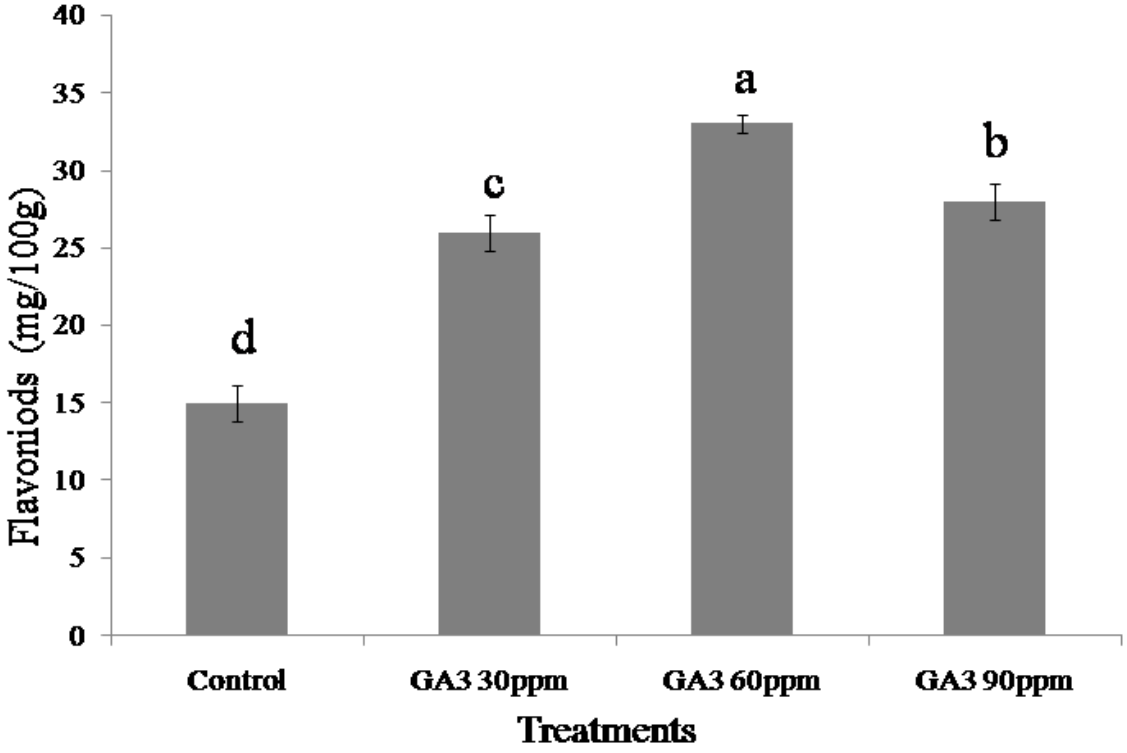


Figure 4.1.6. Flavonoid content as affected by different treatments of GA_3 applied to wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

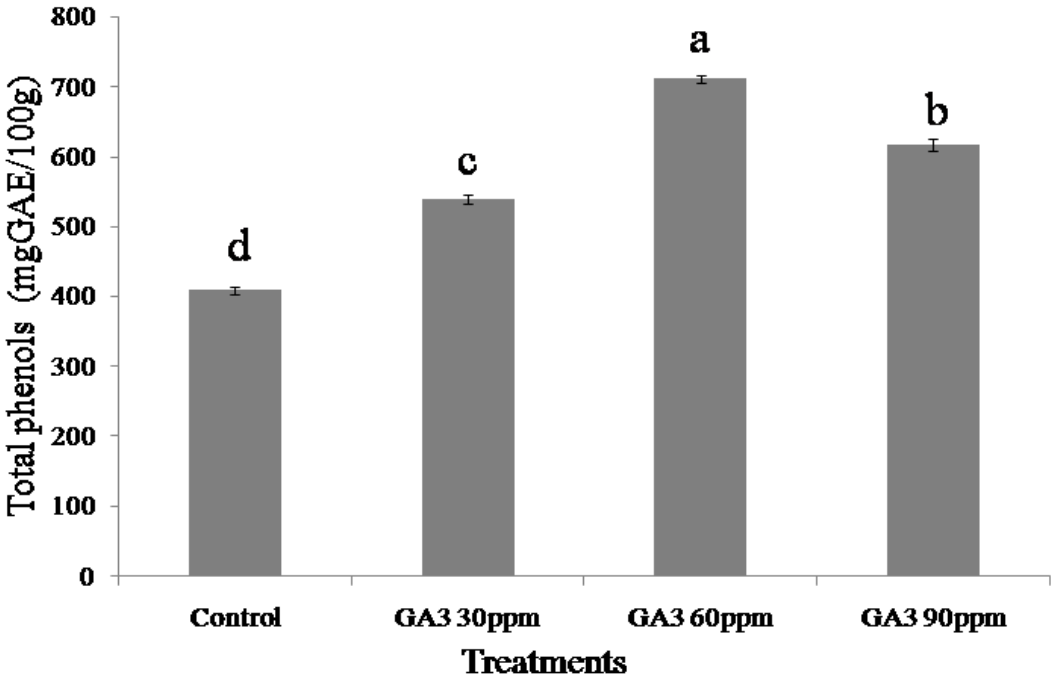


Figure 4.1.7. Total phenol content in wax apple fruit as affected by different treatments of GA_3 (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

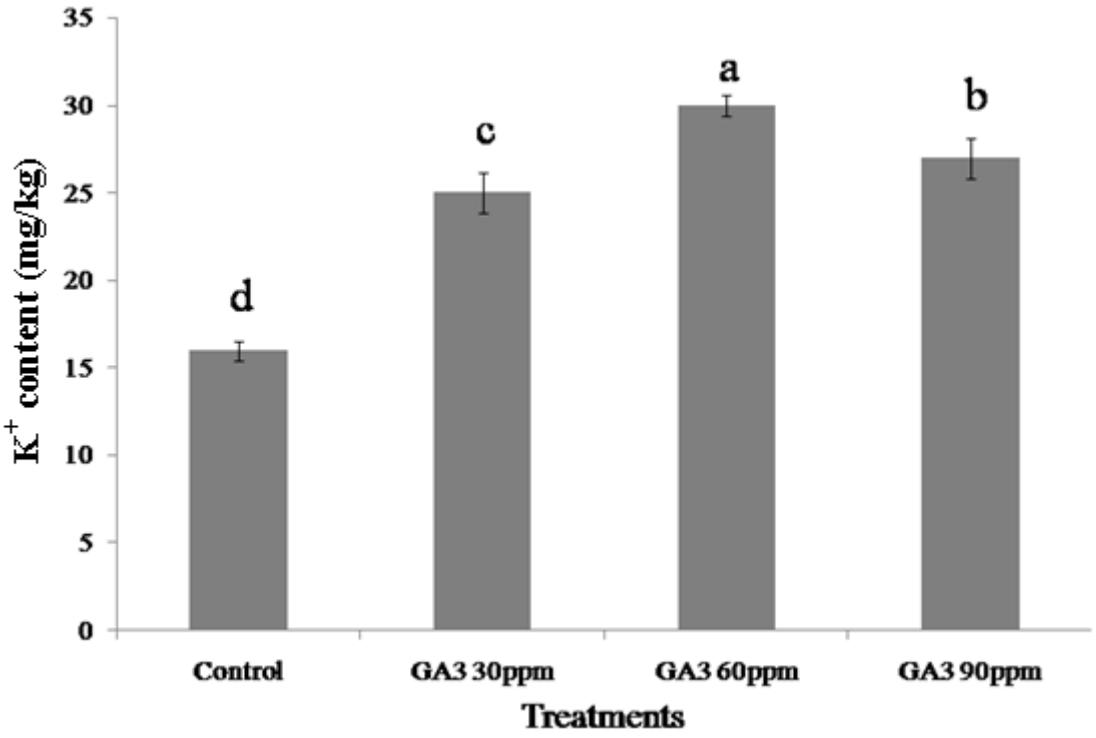


Figure 4.1.8. K^+ content of wax apple fruit as affected by different treatments of GA_3 (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

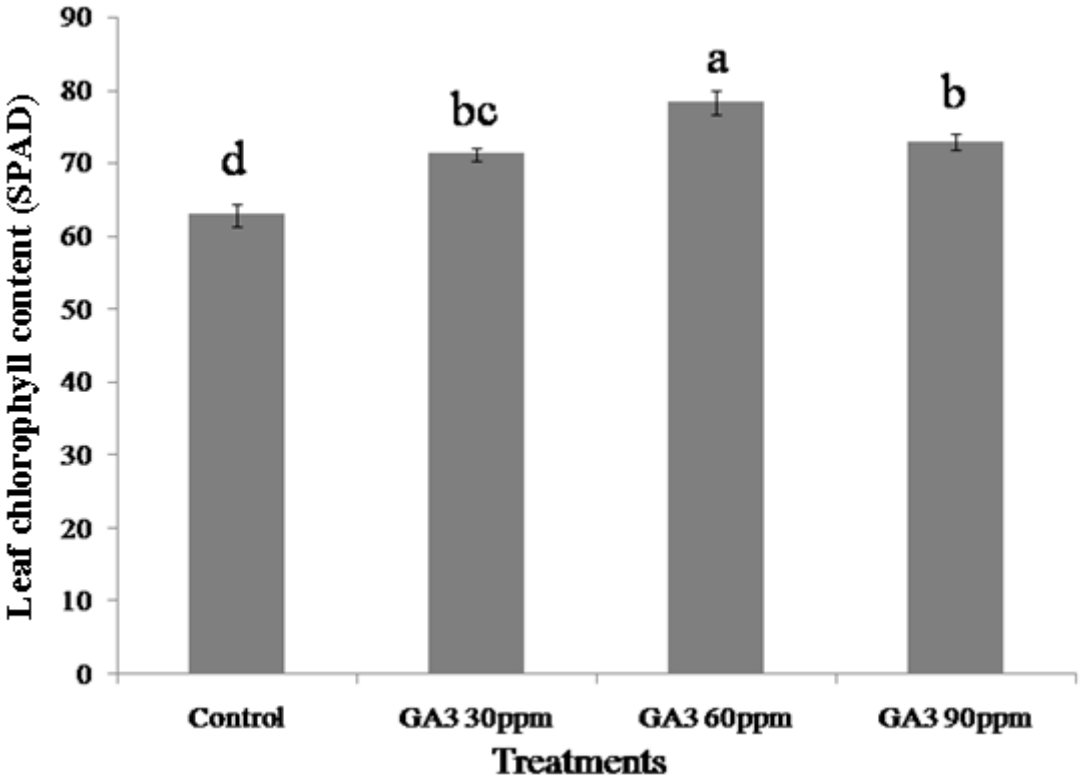


Figure 4.1.9. The leaf chlorophyll content of leaves in different treated-branches of wax apple (Different alphabets mark significant differences, $P < 0.05$ by LSD). S.E. ± (n=5).

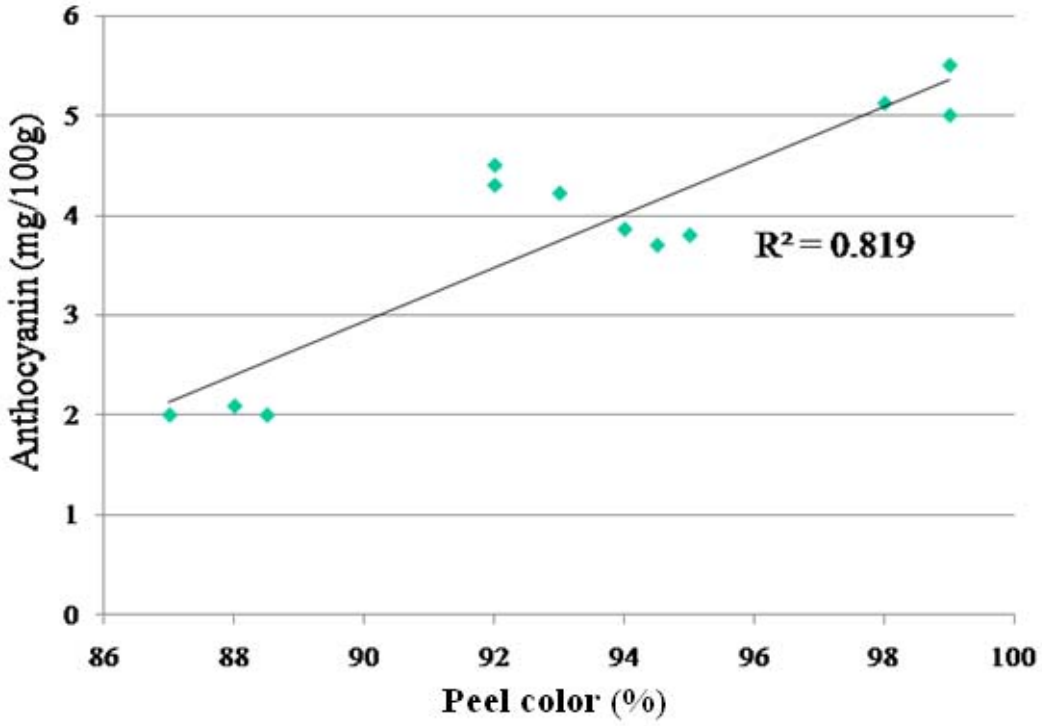


Figure 4.1.10. Correlation between peel color (%) and anthocyanin content of wax apple.

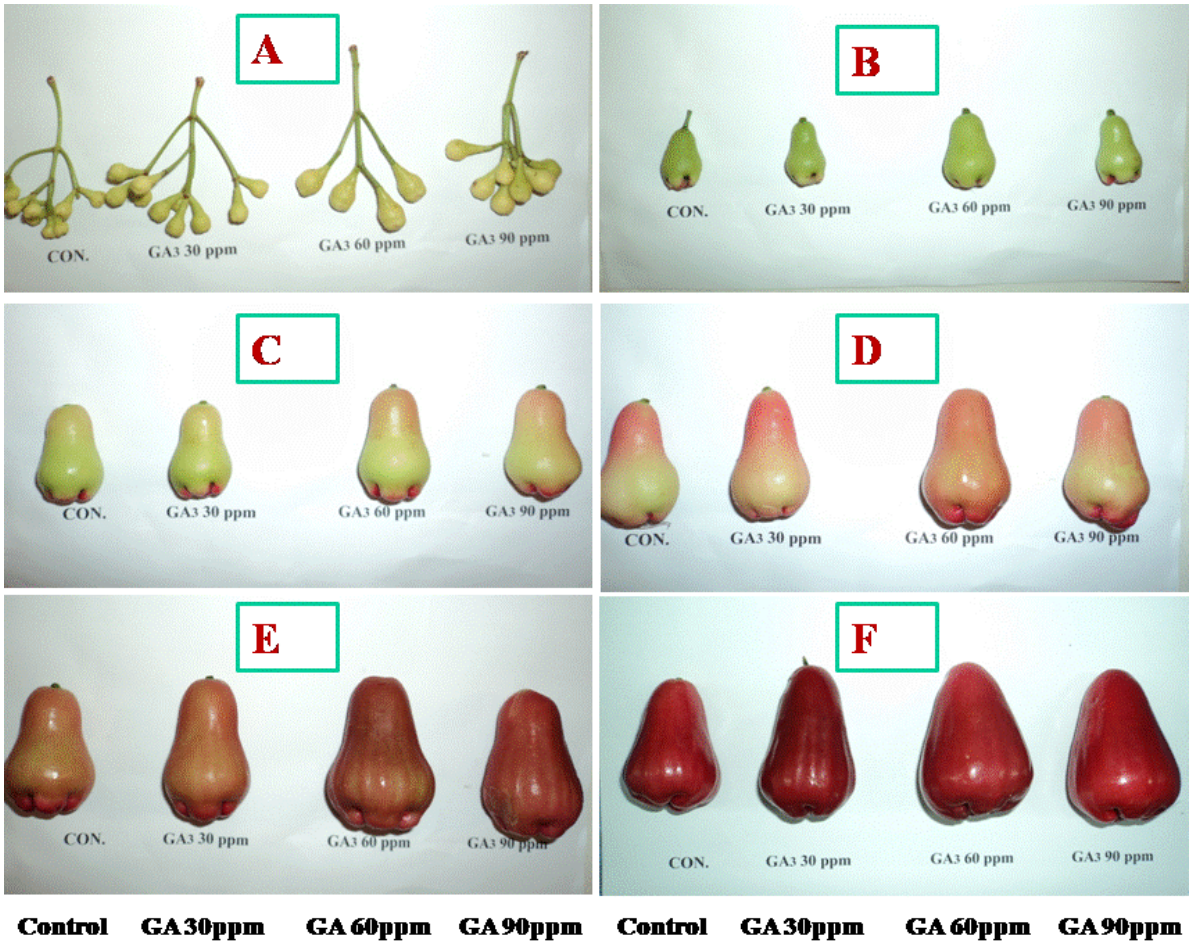


Figure 4.1.11. Photograph shows the effect of different concentrations of GA_3 on wax apple fruits: (A) Initial budding, (B) Green stage- 0-7 days, (C) light Green stage- 4-7 days, (D) Light red 14-28 days, (E) Red 28-35 days and (F) Deep red or harvesting stage 35-45 days.

4.2. EXPERIMENT TWO : INFLUENCE OF NAPHTHALENE ACETIC ACID (NAA) AT DIFFERENT CONCENTRATIONS ON THE GROWTH AND DEVELOPMENT OF WAX APPLE FRUIT

4.2.1. The effect of Naphthalene Acetic Acid (NAA) on bud and fruit drop

The effects of NAA on bud number and drop of wax apple fruits are shown in **Table 4.2.1.** Number of bud has been increased with increase the NAA concentration. Bud number for control branches (pollinated fruit) reached 54, whereas, bud number for 12 ppm NAA treated brunches showed maximum compared to others NAA treated brunches. Bud drop has been increased with the increase of the NAA concentration too.

4.2.2. The effect of Naphthalene Acetic Acid (NAA) on fruit set (%), growth (length and diameter) and drop (%).

The fruit development started from the beginning of fruit set or initial fruit growth to until maturity stage. Assessments of fruit development are based on the measurement of fruit size and weight from its initial growth to maturity stage. It is well documented that plant hormone, NAA has a distinct characteristics to control fruit set and fruit development. **Table 4.2.2.** showed that the effects of NAA at different concentrations on the induction of fruit set and final fruit size. Fruit set was extended by applying NAA (6, 12 and 18 ppm) at initial developmental stage. Application of 12 ppm NAA showed the highest fruit set compared to the other treatments. However, fruit drop was increased as a result of applying NAA (12 and 18 ppm) by 16% and 17%, respectively, compared to the control. Fruit length and diameter of wax apple were greatly enhanced as a result of the activity of NAA. In the present study, it was observed that the best result was exhibited by 12 ppm NAA among the different concentrations of NAA.

4.2.3. The effect of Naphthalene Acetic Acid (NAA) on fruit yield, fruit weight, fruit volume and juice volume

Considering all data in the experiments, yield per branch of wax apple was 455 g in control, whereas, yield per branch of wax apple was significantly higher with, 489, 517 and 493 g in treated branches with NAA (**Table 4.2.3.**). However, fruit weight was significantly increased in the case of 12 ppm NAA per branch. As a result, fruit volume and juice content also was increased by the application of 12 ppm NAA.

NAA can enhance fruit set and it can be applied early in the growing phase to prevent abscission of flower buds. The effect of the NAA treatments on the increase of fruit maturity was evaluated by measuring fruit length and diameter from first to eight harvest weeks. Thus, in the first week, the fruit length and diameter were not significant and subsequently, fruit length and diameter showed difference at different weeks and significant difference was observed especially in the 8^{th} week (**Figure 4.2.1. and Figure 4.2.2.**).

4.2.4. The effect of Naphthalene Acetic Acid (NAA) on chlorophyll content

The chlorophyll content (SPAD value) showed significant difference among the applied hormone treatments (NAA) and control leaves. The highest amount of chlorophyll content was observed in the 12 ppm NAA treated branches leaves. The accumulation of chlorophyll was lower in plants which was undertaken as control (**Figure 4.2.3.**). It was observed that higher concentration of NAA (18 ppm) had the lower chlorophyll content in wax apple leaves.

4.2.5. The effect of Naphthalene Acetic Acid (NAA) on potassium (K^+) content

Potassium (K^+) content was higher in NAA treated branches fruits than in control and potassium (K^+) content was reduced by high concentration of NAA. This was the effective mechanism for increasing potassium content in fruits. The potassium (K^+) content was higher in 12 ppm NAA treated fruits than other concentrations (**Figure 4.2.4.**).

4.2.6. The effect of Naphthalene Acetic Acid (NAA) on total flavonoid

Total flavonoid content of fruits was measured at the end of the experiment, where the content was 50% higher in treated fruits than in untreated fruit. The maximal total flavonoid content was obtained in 12 ppm NAA treated fruits (**Figure 4.2.5.**).

4.2.7. The effect of Naphthalene Acetic Acid (NAA) on total soluble solids (TSS) fructose content and inverted sugar

Total soluble solids (TSS) content was affected significantly by the application of different concentrations of NAA (**Figure 4.2.6.**). The highest TSS was observed by 12 ppm NAA concentration, through affecting the metabolism of high physiological process which led to increase sugar content in fruits. The 6 and 18 ppm NAA concentrations resulted significant reduction of solids content in fruits. Hence, it was observed that 12 ppm NAA was the optimal concentration for wax apple fruits to maintain the highest soluble solids content.

In addition, inverted sugar and fructose content were improved significantly by all NAA treatments. The highest increase in inverted sugar was recorded by 12 ppm NAA followed by 18 ppm NAA, whereas, the lowest content was recorded in 6 ppm NAA .

Both of inverted sugar and fructose were reduced by higher concentration of NAA (18 ppm). There was a similar increasing trend witch were observed by the same concentration in the case of both inverted sugar and fructose (**Figure 4.2.7.** and **Figure 4.2.8.**).

4.2.8. The effect of Naphthalene Acetic Acid (NAA) on color development

Furthermore, it was noted that there was almost a similar difference in physiological and biochemical observation. The remarkable effect in different NAA concentrations on fruit color that was assessed by measuring anthocyanin content of untreated fruit was almost same as untreated fruits. It was observed that the biochemical (anthocyanin) content was showed same trend as harvest color level (maturity) and the effects decreased as the NAA concentration was increased (**Figure 4.2.9.**). Consequently, color and maturity were earlier in NAA-treated fruit than in NAA-untreated fruits.

4.2.9. The effect of Naphthalene Acetic Acid (NAA) on pH of fruit juice

NAA treatment produced significant effect on pH of fruit juice **(Figure 4.2.11.**). The highest pH value was recorded with 12 ppm NAA treated-fruit, followed by 6 and 18 ppm NAA. This differences were statistically significant among the treatments. The lowest pH was recorded in control fruit.

4.2.10. The effect of Naphthalene Acetic Acid (NAA) on total phenol

The application of different concentrations of NAA had a significant effect on the total phenolic content of wax apple fruits. Fruits from 12 ppm NAA treated branches exhibited the highest amount of phenols followed by 6 and 18 ppm treated fruits. Control fruits showed the lowest (311mg GAE/100g) phenol content (**Figure 4.2.12**.)

4.2.11. Discussion

Naphthalene Acetic Acid (NAA) is an organic synthetic compound which is a plant hormone of the auxin family. The impacts of NAA hormone on a plant often depend on the stage of the plant's development and the concentration. NAA has been reported to raise flowers and fruits drop off trees before maturation (Chang and Chen, 2001). Similar results have been observed in this study where initial bud and fruits drop increased by 60% in NAA than in control. Brent et al. (1995) also reported that when NAA was sprayed on young buds or fruits such as apple and olive, some of the buds and fruits dropped off so that the remaining fruits could grow larger. Consequently, fruits drop were stopped until the maturity stage. The effects of this hormone on a plant often showed better activity than any other plant hormone in case of bud and fruit drop. Many researchers have recommended that maximum fruits were sensitive to ethylene in the young stages of development or middle maturity stages (Yoko et al., 2006). For that reason, a lot of fruits were dropped at the young stage before maturity. Elgar et al. (2003) reported that NAA might reduce abscission more successfully, as well as ethylene production might be neutralized by NAA in the young stage. Chang and Chen (2001) also showed that NAA delayed flower abscission. In this study, therefore fruit set was significantly increased by NAA.

Application of 12 ppm NAA resulted in significantly higher fruit length and diameter than pollinated control. It was observed that growth of NAA treated fruits closely related to changing carbohydrate level. However, the carbohydrate content among the treated fruits varied with the enzymatic activity. This is resembled to the work done by Agusti et al (2002) on citrus fruits. They described that NAA hormone stimulated cell elongation by stimulating naturally produced hormone, cytokinin which has an ability to increase the cell dimension. Application of the NAA increased final fruit quality such as size, color and juice content without causing any fruit damage. The effectiveness of

NAA depended on the concentration (12 ppm) applied in this study and many researchers mentioned that it also depended on seasons and periods of application (Issam, 2010). When NAA was applied at initial fruit stage, final fruit diameter and distribution of fruit color showed a significantly increase in fruits size. Therefore, fruit maturation and harvest time were earlier in treated branches than in untreated branches. In general, it was showed that NAA induced fruit set and development in varieties of fruit crops, but very few research has been carried on wax apple in this concern. Spraying of NAA successfully induced fruit development from initial fruit set to maturity stage.

In the present study, it was observed that the treatments of NAA significantly improved fruit size, weight, and biochemical content of wax apple (**Figure 4.2.13.**). The improvement of fruit quality and fruit management could possibly be resulted of enzymatic action of NAA (Chang and Chen, 2001). The variable difference in juice content, TSS and maturity was observed among the different concentrations. That was probably due to the various activity levels of different concentrations of NAA (Saifuddin et al., 2009). It could be changed in cell wall of fruits and leaves in different ways. The action of NAA might have been increased soluble carbohydrates in the fruits than the other parts of plant. As a result, the fruit volume and juice content of treated fruit was increased at the time of fruit set and reached a maximal level in fruit maturity stage.

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

and the simultaneous extra uptake of water would enhance the cellular turgor pressure, which might effect in greater fruits expansion.

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

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

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

Actually flavonoids considerate a large group of phenolic compounds that are synthesized various enzymatic steps. Flavonoids might work as protecting the fruits from excess light, defense against pathogens, and attracting for pollinator. Flavonoids also may contribute to maintain the wax apple quality such as its taste, color and

bitterness or texture like other fruits (Amiot et al. 1997). Flavonoid biosynthesis in tissue may be accumulated extensively in presences of phenolic compounds. Color and pH are the most important harvest parameter in fruit harvest index because of adjusting juice pH, it is dependent extensively on the fruits color (Tehrani et al., 2011, Moneruzzaman et al., 2008).

According to the pH content anthocyanin pigments that may appear as a red, purple, or blue tint. They belong to a parent class of molecules called flavonoids, synthesized via the phenylpropanoid pathway (Raghvendra et al., 2011). Anthocyanins found in all tissues of higher plants, including leaves and maximal was found in flowers and fruits. Hydrocarbons what can be produced via photosynthesis and laterally might be converted into flavonoids and their derivatives compound is anthocyanins. Generally fruits contain many compounds such as anthocyanins, chlorophyll, carotenoids, and flavonols which can be combined together to make its color formation. The most important composite for the red coloration in wax apple are the anthocyanins, situated specially in fruit skin. The anthocyanin can rise more than 4 times during the ripening stage. Many biochemical process led to anthocyanin production. The most important steps in this biochemical process were the increase of the availability of sugars and the activity of the enzyme in presence of K^+ content. These impacts combined with physiological and environmental factors speed up cell activity to raise the net color as well (Brouillard and Dangles, 1994).

Therefore, the commercial value of wax apple is depended on fruit size and color. The application of 12 ppm NAA at the onset of fruit set has been found to be effective in wax apple fruit, increasing fruit diameter by 10%, and yield by 20% per treatment compared with other NAA concentration. However, it was observed that each concentration of NAA significantly increased yield and fruit size (**Figure 4.2.13.**)

compared with control fruit. The aforementioned effects could be due to an increase of cell enlargement (Ohmiya, 2000). According to other findings, NAA and other auxins, such as 3,5,6-TPA, have been begun to have a similar effect on fruit growth (Ortola et al., 1991). This might function via encouragement of leaf chlorophyll allowing more photosynthesis and carbohydrate accumulation.

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

Table 4.2.1: Effects of treatments of NAA on bud number and bud drop of wax apple fruit. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatment (ppm)	Bud number	Bud drop (%)
Control	54.0 ± 0.57d	29.0 ± 0.57d
NAA 6	57.3 ± 0.33c	41.3 ± 0.88c
NAA 12	60.6 ± 0.33a	51.3 ± 0.33b
NAA 18	59.6 ± 0.33ab	55.3 ± 0.33a

Table 4.2.2: The effects of NAA treatment on fruit set, drop and final fruit size (length and diameter) of wax apple. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

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

Table 4.2.3: Effects of treatments of NAA on fruit yield, fruit weight, fruit volume and juice volume of wax apple. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

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

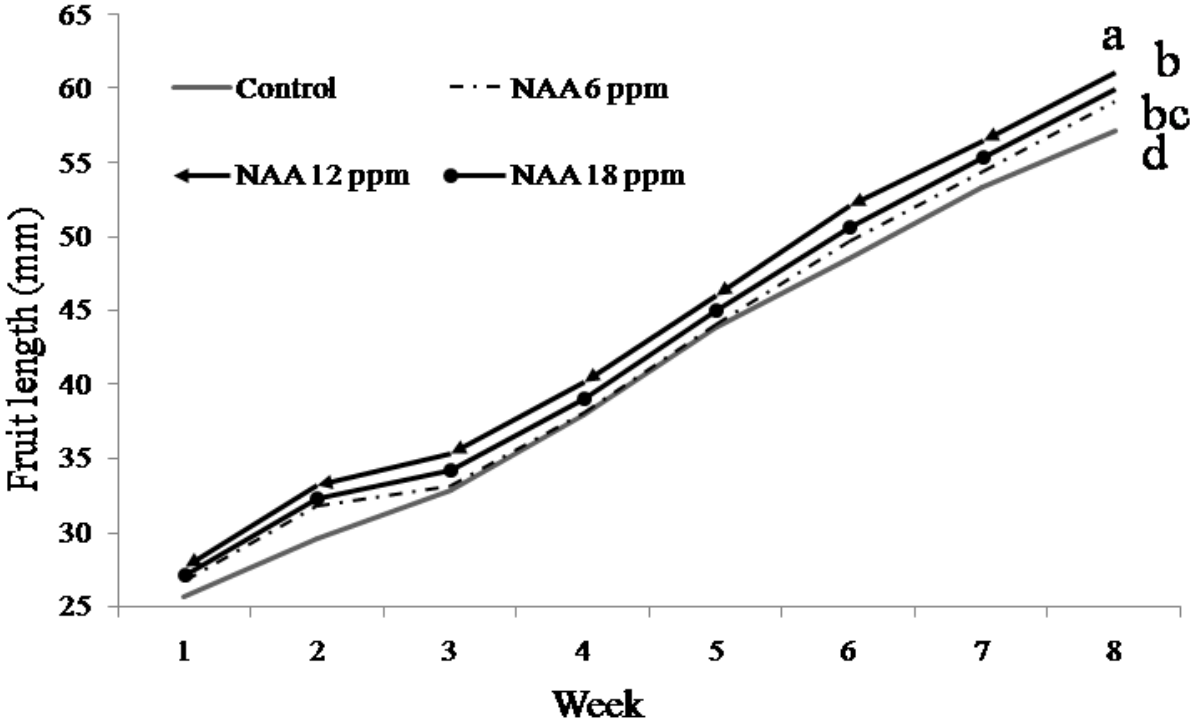


Figure 4.2.1. Fruit growth (length/week) as influenced by different concentrations of NAA. (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

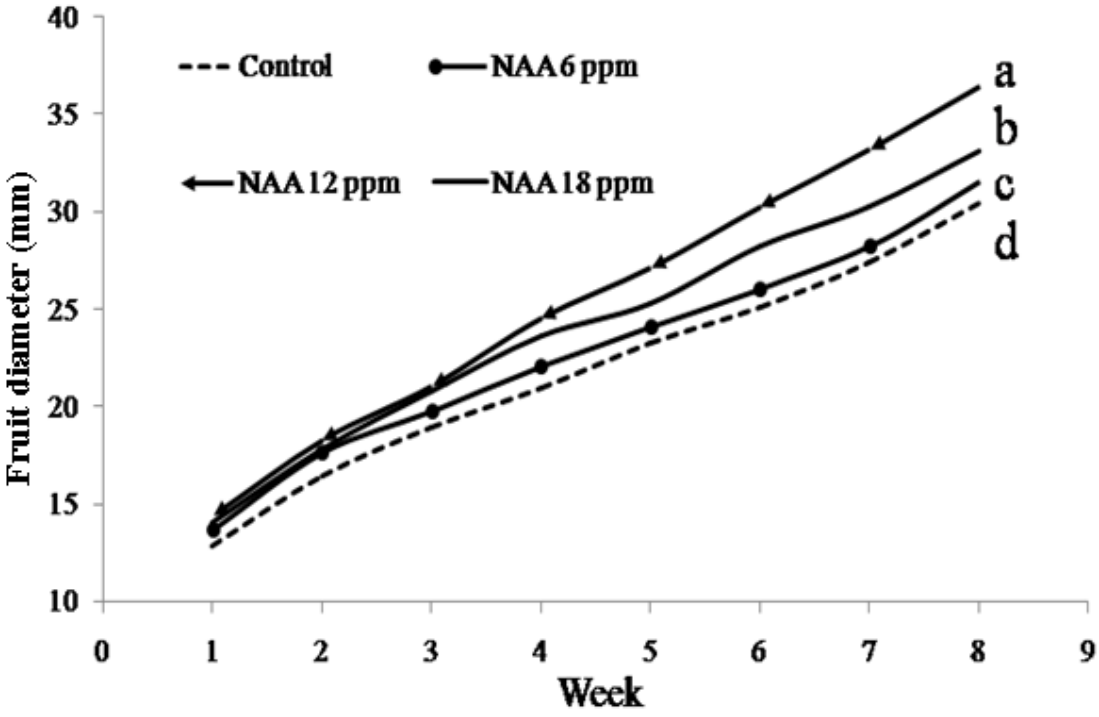


Figure 4.2.2. Fruit diameter per week as influenced by different concentrations of NAA (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

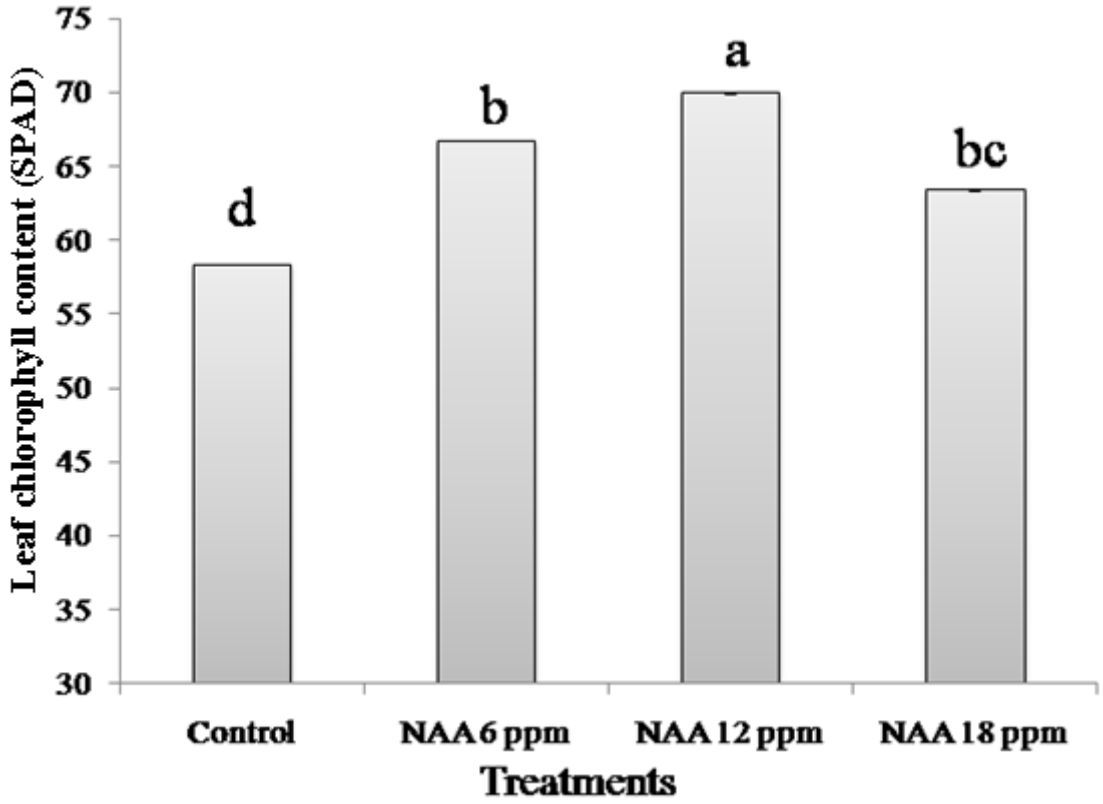


Figure 4.2.3. Effect of NAA treatments on leaf chlorophyll content (SPAD) of wax apple (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

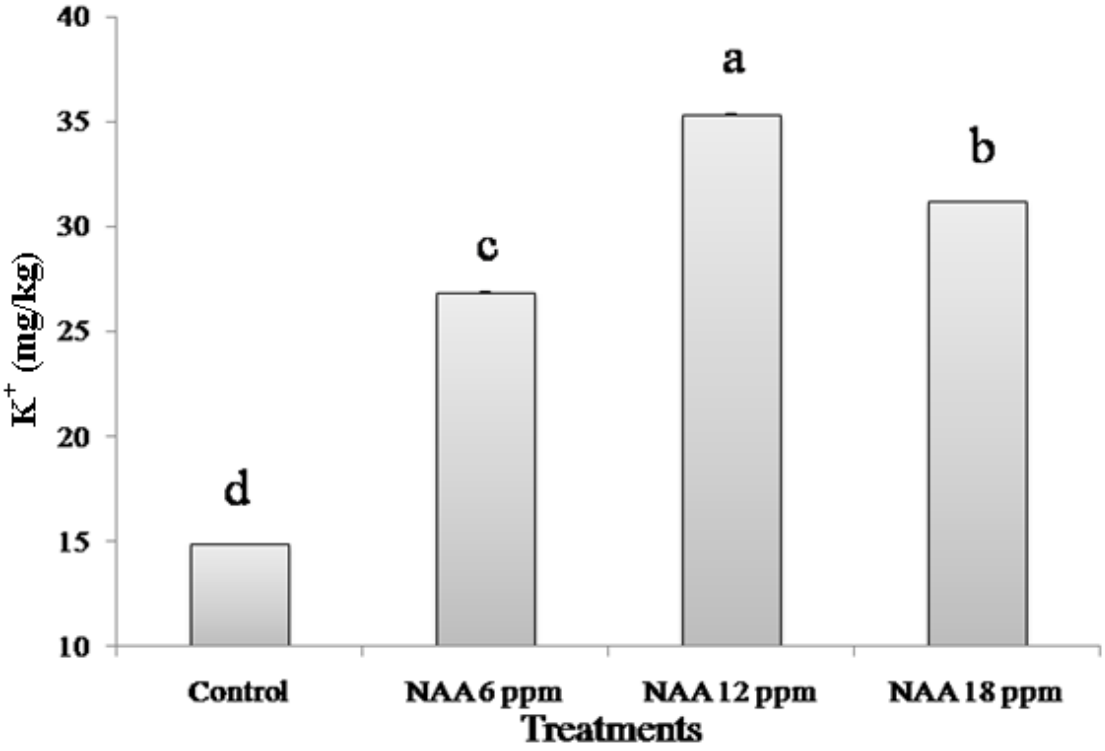


Figure 4.2.4. Effect of NAA treatments on potassium (K^+) content of wax apple fruit juice (Different alphabets mark significant differences, $P < 0.05$ by LSD). S.E. ± (n=5).

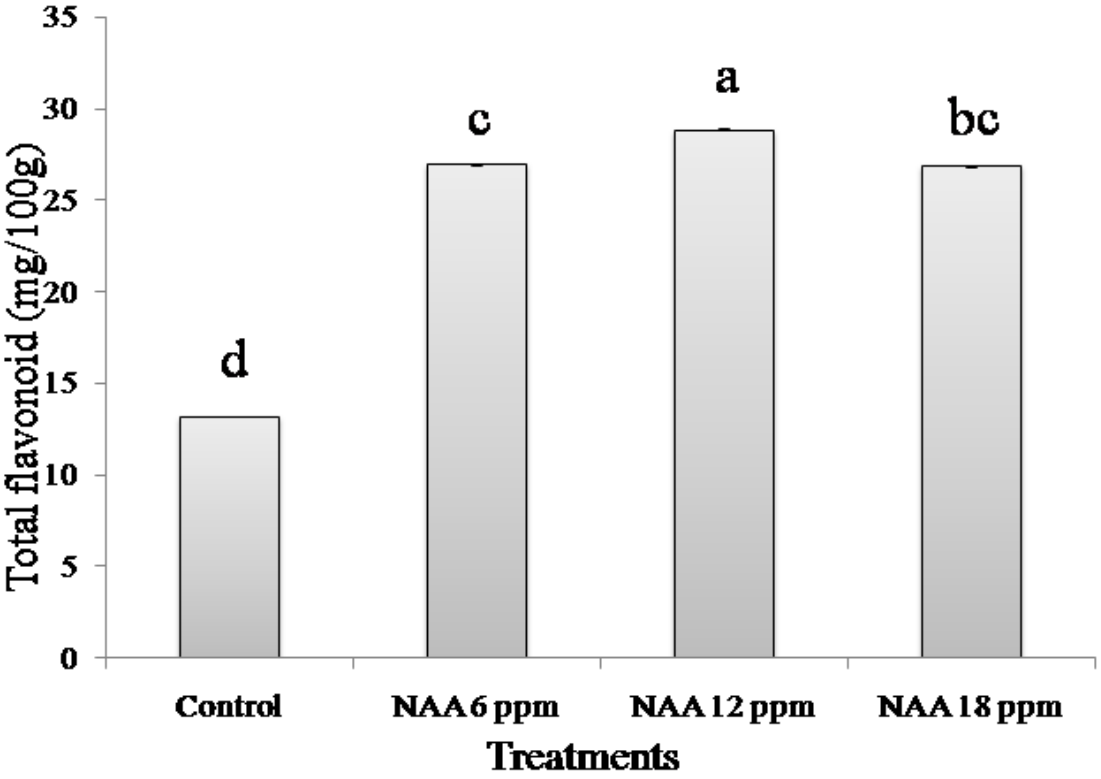


Figure 4.2.5. Total flavonoid content of wax apple as affected by treatments of NAA (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

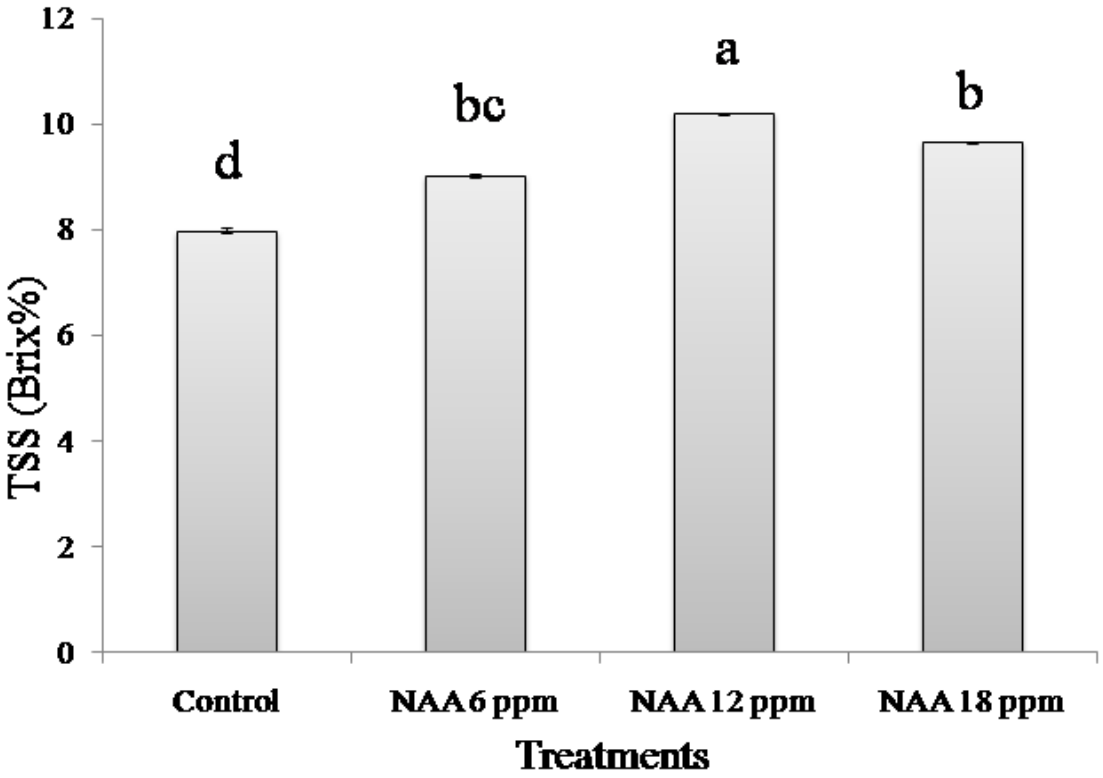


Figure 4.2.6. Effect of treatments of NAA on total soluble solids (TSS) content (°Brix) of wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

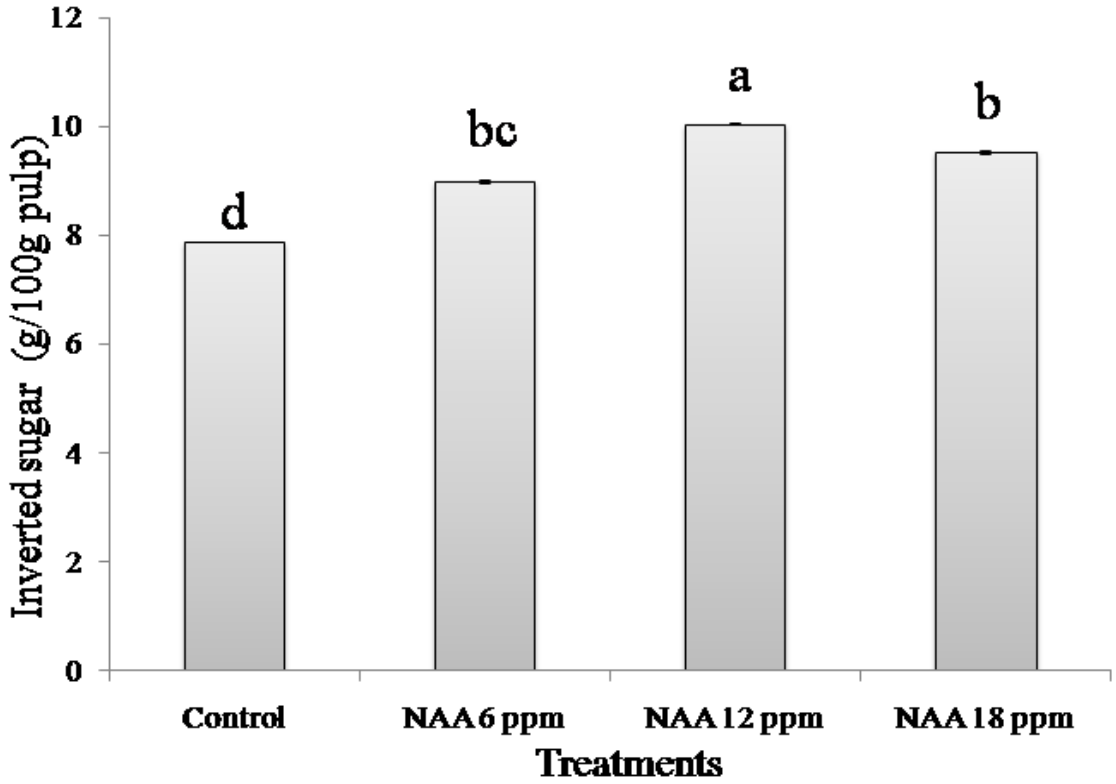


Figure 4.2.7. Effect of NAA treatments on inverted sugar content of wax apple (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

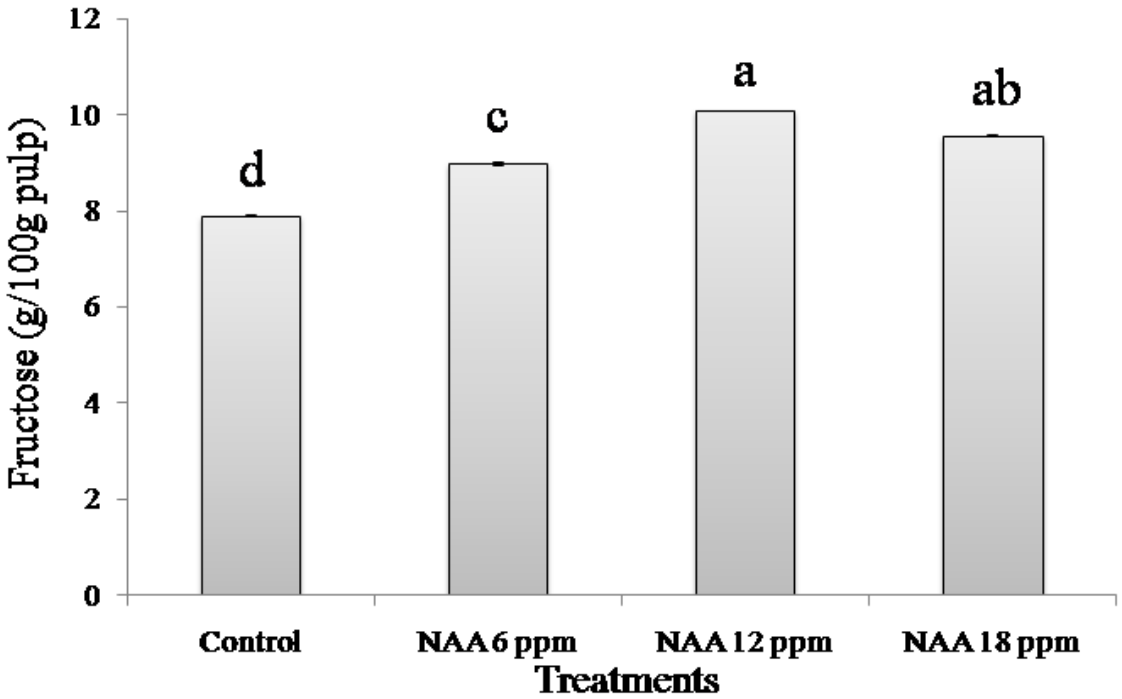


Figure 4.2.8. Effect of NAA treatments on fructose content of wax apple (Differentalphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

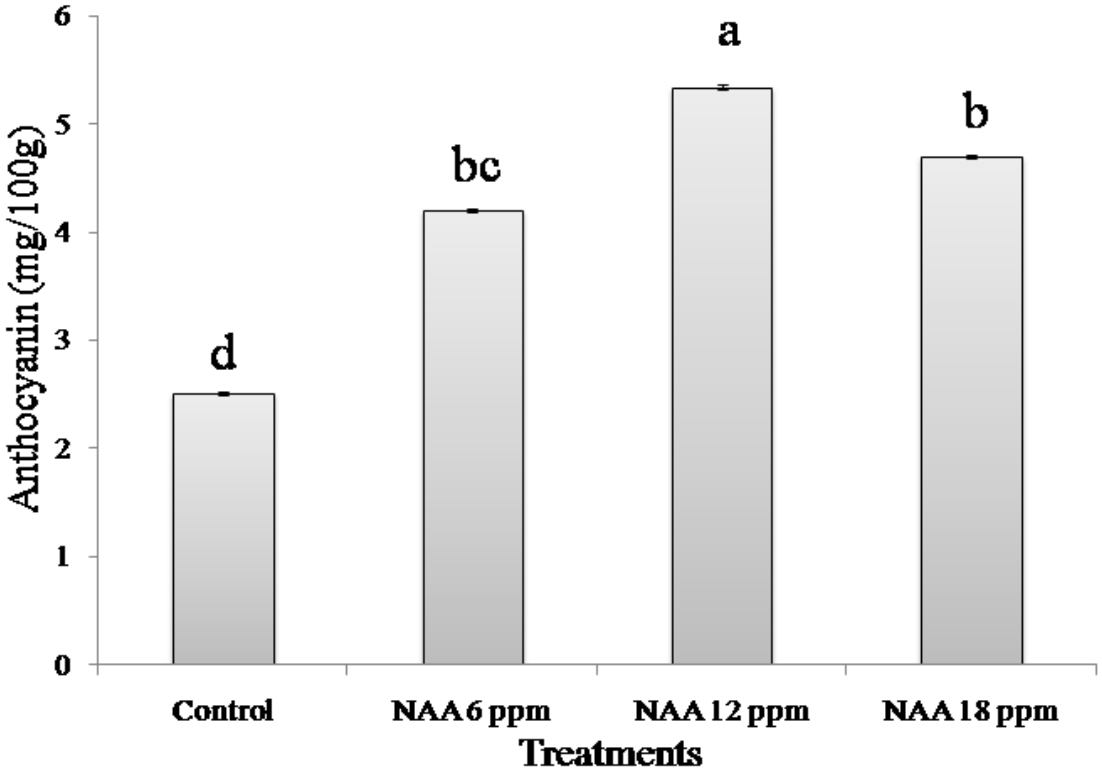


Figure 4.2.9. Effect of NAA treatments on anthocyanin (mg/100 mg) content of wax apple (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

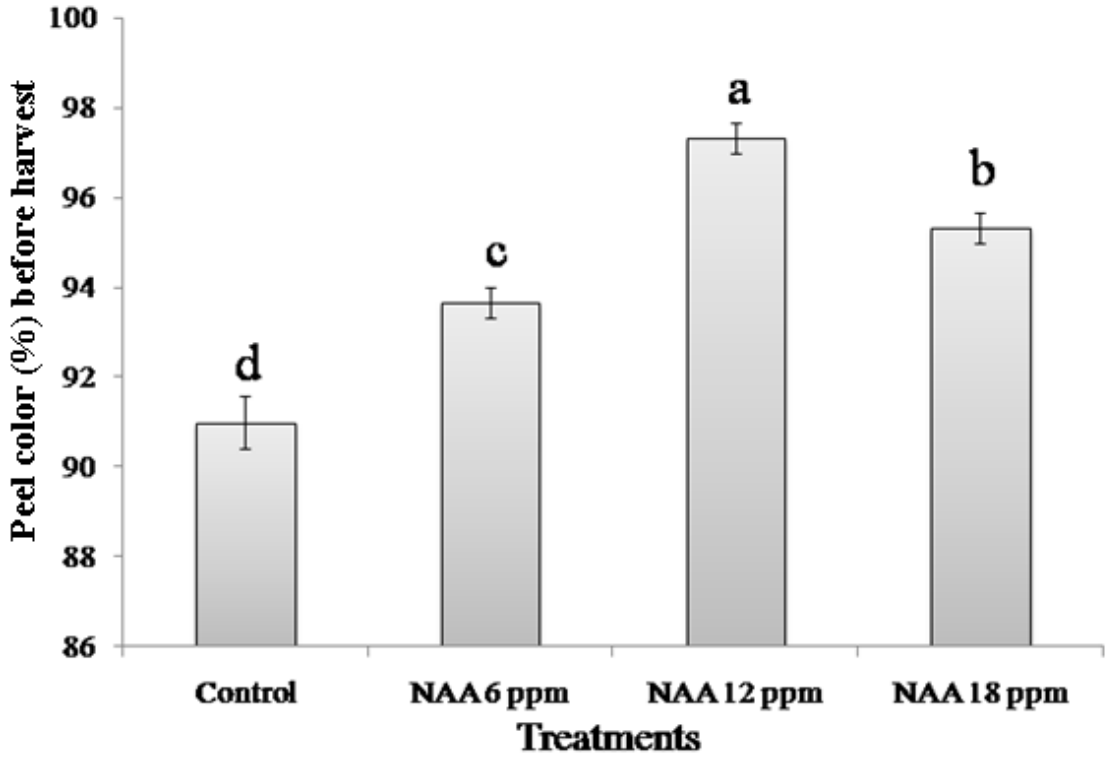


Figure 4.2.10. Peel color (%) before harvest as affected by treatments of NAA applied to wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

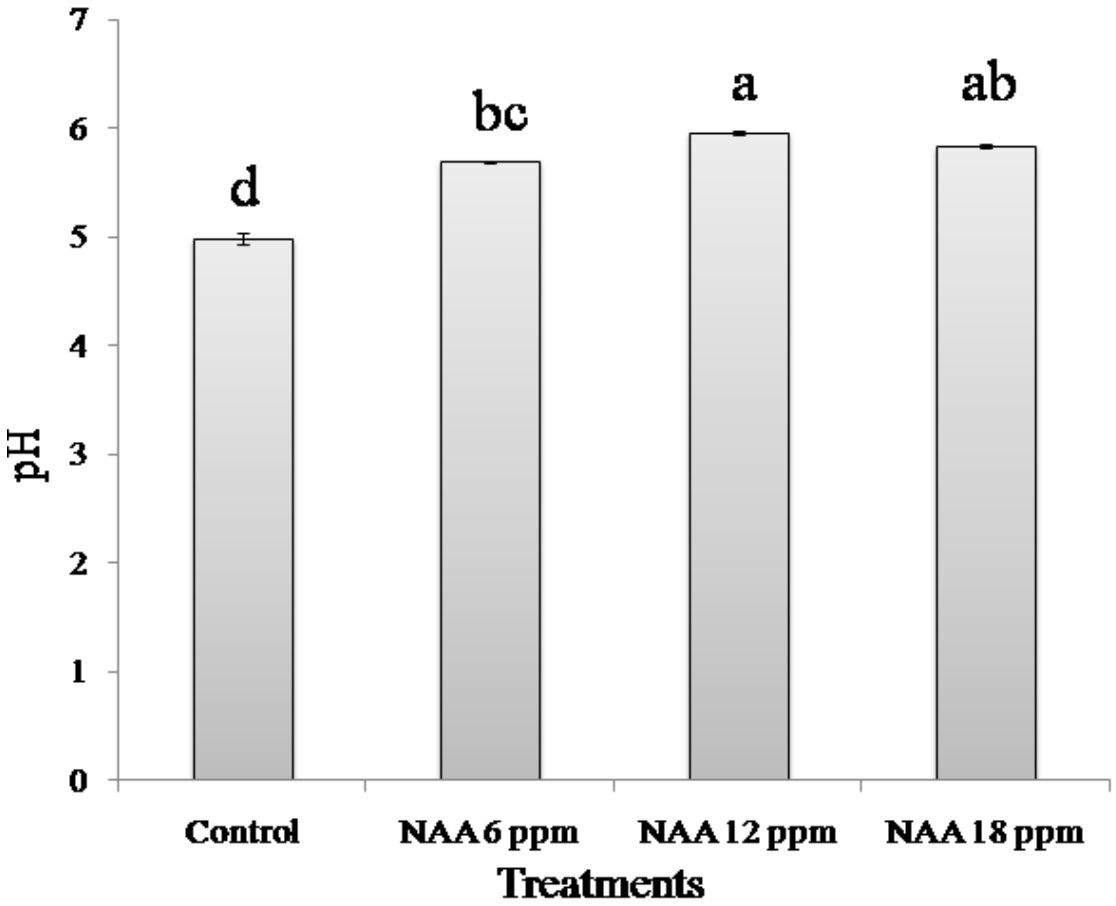


Figure 4.2.11. Effect of treatments of NAA on pH of wax apple fruit juice (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

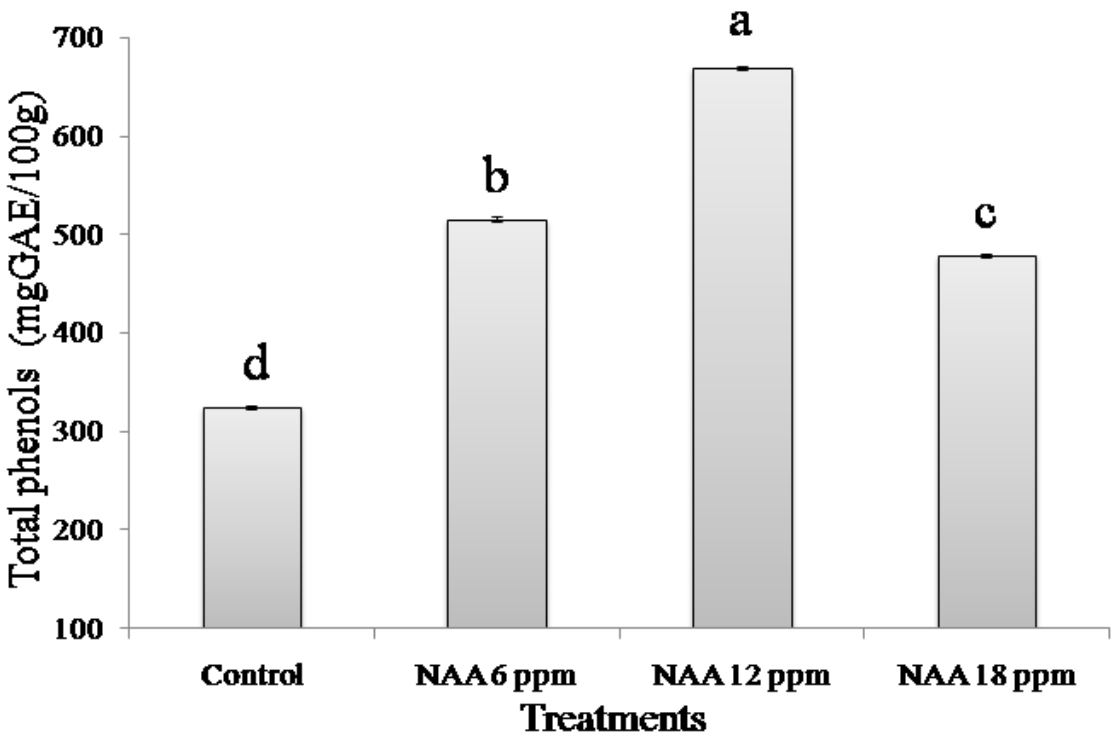


Figure 4.2.12. Total phenolic content of wax apple as affected by treatments of NAA under field conditions (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

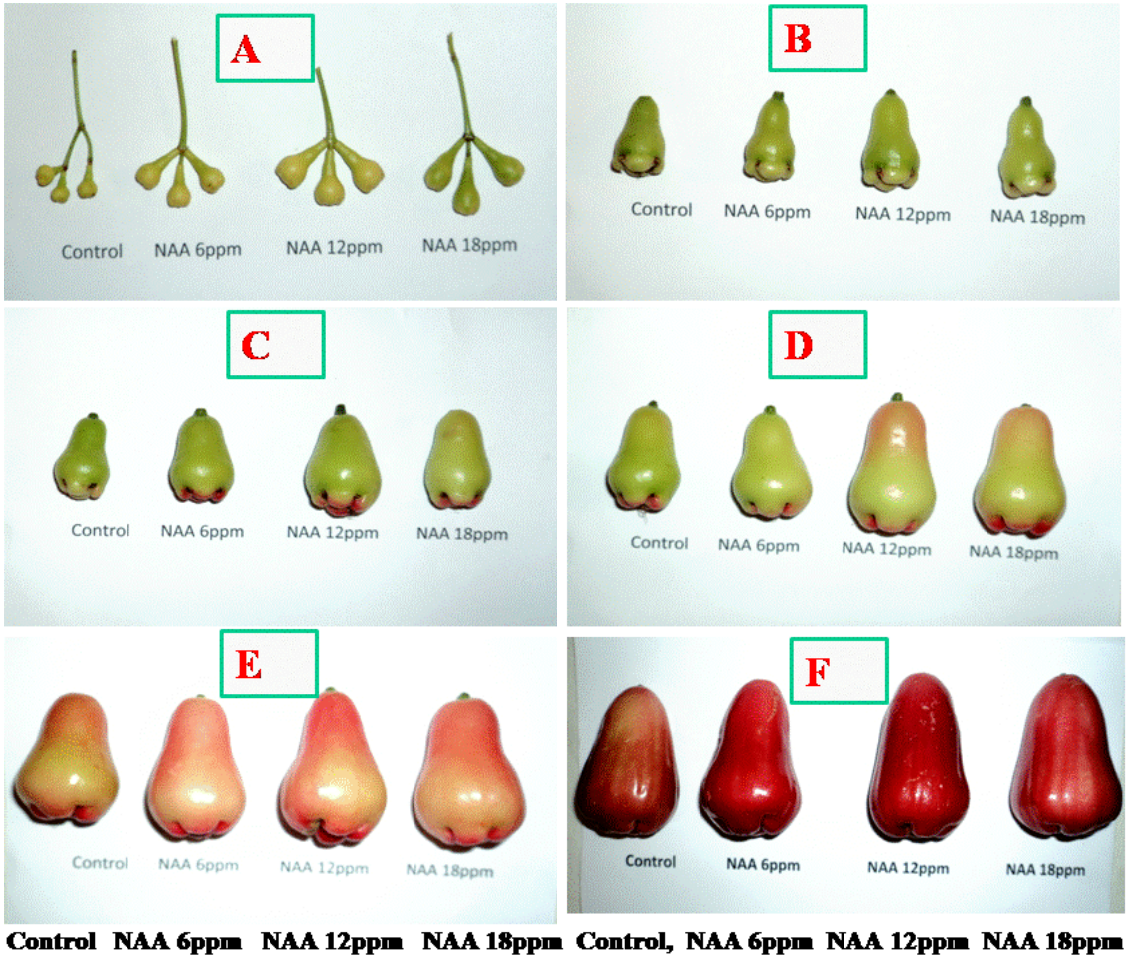


Figure 4.2. 13. Photgraph shows the effect of different concentrations of NAA on wax apple fruits, (A): Initial budding, (B): Green stage, (C): light Green stage, (D): Light red, (E): Red and (F): Deep red or harvesting stage.

4.3. EXPERIMENT 3 : WAX APPLE FRUIT GROWTH AND QUALITY DEVELOPMENT AS AFFECTED BY N-2-CHLORO-4-PYRIDYL-N-PHENYLUREA (CPPU)

4.3.1. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on bud and fruit drop

The impacts of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on bud number and drop of wax apple fruits are shown in **Table 4.3.1**. The number of bud has been increased at 15 ppm CPPU concentration. In contrast, the maximum bud drop was observed in 15 ppm CPPU concentration and the minimum was observed in control.

4.3.2. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on fruit growth parameters: fruits set, fruit drop, fruit length and diameter

In **Table 4.3.2.**, parameters of fruits growth and development are presented (fruit length, fruit set, fruit drop and fruit diameter). The see parameters were investigated to monitor the quality of wax apple fruit. All concentrations showed better initiation of fruit set than control in the experimental period. The results showed that fruit length and fruit diameter were significantly increased by CPPU compared to control. The highest fruit length was observed in 15 ppm CPPU as compared with control. Fruit diameter was almost similar in 15 and 20 ppm CPPU concentrations. It was clear that CPPU had a positive effect on fruit development compared to control.

4.3.3. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on fruit yield, fruit weight, fruit volume and juice volume

The influence of CPPU on fruit yield, weight and fruit volume was observed throughout the experiments. The most effective result was found to increase fruit yield and fruit weight by 15 ppm CPPU treatment (**Table 4.3.3.**). In the case of fruit volume, higher value was observed in 15 ppm CPPU treatment than other treatments. Higher juice volume was also observed in 15 ppm CPPU than other treatments. It was observed in **Figure 4.3.1**. and **Figure 4.3.2.** that fruit growth (length and diameter) per week was greatly influenced by different concentrations of CPPU.

4.3.4. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on total soluble solids (TSS), inverted sugar and fructose content

One of the most important quality of fresh fruit is attributed to TSS content. The influence of different CPPU concentrations on TSS content of mature fruits was measured at the end of the experiments. All CPPU concentrations were able to enhance the TSS content in mature fruit. The highest increase of TSS content was recorded in 15 ppm CPPU treated fruit. Lower TSS content was noticed in the fruit treated with 20 ppm CPPU than 10 and 15 ppm CPPU treatments (**Figure 4.3.3.**).

4.3.5. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on fruit pH

The fruits acidity level represented by pH value was significantly affected by the application of different concentrations of CPPU (**Figure 4.3.4.**). The highest pH value was observed in 15 ppm CPPU concentrations. The 10 and 20 ppm CPPU concentrations resulted in significant reduction of pH value in fruit compared to other concentrations. Hence, it was observed that 15 ppm CPPU was the optimum for wax apple fruits to maintain the lowest acidity.

4.3.6. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on chlorophyll content

This work shows, that chlorophyll content (photosynthetic tint represented by SPAD unit), was higher in CPPU treated leaves than the control leaves. This was the effective technique for increasing chlorophyll content in leaves. The chlorophyll content was highest in 15 ppm CPPU treated leaves than other CPPU concentrations (**Figure 4.3.5**.). The accumulation of chlorophyll pigment was lowest in leaves in case of high CPPU concentration. However, intensive photosynthesis, high carbohydrates accumulation leads to good fruit quality.

4.3.7. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on fruit color

The maturation of wax apple fruit was associated with a series of the physiological and biochemical changes, but the most important is the color changes. It is well proved that, fruit color is refereed to be one of the important external factors in determining fruit quality, since the fruit appearance greatly influence the customers. Fruits color was measured after harvest. The influence of different concentrations of CPPU on the color was clearly observed in **Figure 4.3.6.** and represented also in **Figure 4.3.14.** The results showed that 15 ppm CPPU had greatly influenced the treated fruits by increasing color.

4.3.8. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on Potassium (K^+) content

The potassium (K^+) content of mature fruit was measured at the end of the experiment. As can be seen in **Figure 4.3.7.**, the potassium (K^+) content was 50% higher in all CPPU treated branches fruits than in control. The maximum potassium (K^+) content

was obtained in 15 ppm CPPU treated fruits. This result suggest that CPPU treatment increased K^+ contents in fruits.

4.3.9. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on fruit pigment and flavonoid content

The result showed in **Figure 4.3.8** were illustrated that the fruit pigment and flavonoid content of wax apple were significantly increased by the application of CPPU compared to control. The highest flavonoid content of fruit was recorded in all of the CPPU concentrations as compared to control. The 10 and 20 ppm concentrations of CPPU decreased flavonoid content in fruit as compared to the 15 ppm concentration of CPPU.

4.3.10. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on total phenol and fructose contents

The results showed that the total phenolic and fructose contents in fruit varied considerably among the treatments (**Figure 4.3.9.** and **Figure 4.3.10.**). The 15 ppm CPPU concentrations showed highest phenolic and fructose content than other concentrations of CPPU. The highest concentrations resulted in decreasing trend of nutrient content that might be attributed to less stimulation of phenolic and fructose content in fruit. The lowest phenolic and fructose content were obtained in 15 ppm CPPU treated fruit among all CPPU concentrations.

4.3.11. The effect of N-2-chloro-4-pyridyl-N- phenylurea (CPPU) on inverted sugar

The concentration of inverted sugar is an important indicator to characterize the stage of fruit ripeness, and also to determine fruits quality. The inverted sugar concentration did not show the significant difference between 10 and 20 ppm CPPU concentrations. Only

15 ppm CPPU treated fruit held more inverted sugar than other treatments, probably by indicating an advanced ripening stage (**Figure 4.3.11.**).

4.3.12. Discussion

Fruits yield is the result of many morpho-physiological and biochemical processes of plant which is depended on many factors including natural and endogenous environmental applications. This is why many applications and mechanisms were executed by the researchers on regulating fruit growth and quality development. Historically, hormones have been viewed as a controlling reason in maintaining the fruits development. CPPU (N-(2-chloro-4-pyridyl)-N-phenylurea) is a cytokinin group plant hormone that has shown activity in promoting fruit growth. Now-a-days plant growth regulator, CPPU, has been focused by many researches on various fruits to increase fruit physical quality and biochemical contents.

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

Assessments of fruit quality are actually based on fruit developmental measurement such as, fruit growth, fruit set and fruit nutrients. In this present work, the number of fruit per branch was higher in 20 ppm CPPU than in control branches. This might be due to the deposition of satisfactory nutrients at treated brunches. Consequently, a sound fruit development was started from the onset of bud initiation and continued until maturity stage. Similar findings have been reported in other fruits by many researchers. Yang-Gyu and Woolley (2006) stated that endogenous hormones and its balance in plants played a vital role in mobilization of produced nutrients into fruits. **Table 4.3.2.** showed that the physical improvement of fruits by different concentration of CPPU

application. When fruit size (length and diameter) was plotted as function of CPPU concentration, the 15 ppm CPPU treated fruit was larger than the control fruit and other CPPU concentrations also increased yield (**Figure 4.3.1.** and **Figure 4.3.2**.). It was assumed that upgrading of water balance in fruits might enhance the juice content and overall fruit size. Therefore, the dynamic changes of internal fruits water levels during the period of fruit growth have been investigated by assessing the fruit length and diameter or biochemical content. Woolley et al. (1992) assumed that application of CPPU might regulate the plant physiological processes specially growth and development, organ formation and so on, through the cell division and increasing cell volume, which attributed to comprehensive effects of others plant hormones. Huang et al. (1994) also hypothesized that fruit growth depended on the endogenous of all hormones in stimulating the growth of flesh tissues. Therefore, treatment of CPPU hormones played an imperative part in the growth and development of wax apple fruits. The increase of fruit size percentage, observed in this study and proved in previous studies (Manabu et al., 2008). The results presented in the present work and supported by the previous results (Antognozzi et al., 1996) that fruit set and fruit growth in wax apple depended on the presence of tolerable levels of endogenous CPPU. Caixi et al. (2007) found that the CPPU, a synthetic cytokinin, was effective in enhancing Japanese pear fruit enlargement by stimulating cell division and/or cell expansion and also involved in improving fruit set.

The photosynthetic pigment (chlorophyll) of treated leaf was substantially increased by CPPU. This enhancement might result in more photosynthesis taken place in treated leaf and fruit enlargement is mostly dependent on the input of excess water, minerals and assimilates from other parts of plants into fruits. According to Johnson et al. (1992), most of the essential substance on which fruit growth depends on the translocation from

the leaf and stem in the fruit through the xylem and phloem. Lewis et al. (1996) presented that CPPU applications accelerated fruit ripening showing higher SPAD value (chlorophyll content) of treated leaf than the control leaves in kiwifruit. In addition, fruit juice content, which was related to fruit size, was increased by CPPU application. Therefore, the different treatments produced significant differences in potassium (K^+) content in fruit juice. Results showed that the potassium (K^+) content of fruit juice was higher in 15 ppm CPPU treated fruit whereas control fruit produced the lowest amount of potassium.

As mentioned above fruit quality depended on the level of total soluble solids (TSS) contents what could generally improve with increasing fruit maturity and color. TSS included the sucrose, glucose and fructose as well as many organic acids and soluble substances. In this study, the increase of TSS in wax apple possibly due to the hydrolysis of starch to soluble sugars such as glucose, sucrose and fructose (Soltani et al., 2010). Kader et al. (2002) stated that fruit consumer preferred the TSS contents in mature stage. The effect of CPPU on TSS content of fruit has been positively addressed in a number of studies (Lewis et al., 1996; Antognozzi, et al., 1996).

Flavonoids are the most essential plant pigments for fruit coloration (maturity development) producing, green, yellow or red/blue tint in fruits skin and commonly known for its antioxidant activity. Therefore, in fruits, flavonoids may contribute as fruit quality regulator not only color but also flavor, bitterness or texture (Lin and Tang, 2007). In this study, the composition of flavonoids in different fruit varies greatly due to the different CPPU concentrations (**Figure 4.3.12.**). Similar results were described by Winkel-Shirley (2001) and it was refereed that the few enzymes were involved in flavonoid's metabolism and this flavonoid's pathway are regulated by plant hormones. Woolley et al. (1992) found that CPPU stimulated both cell expansion and cell division in the fruit tissue. In addition, anthocyanins are also commonly contributed to the

pigmentation of fruits that give its red and yellow color (**Figure 4.3.13.**). The flavonoids biosynthetic pathway has been described by Weisshaar and Jenkins (1998). In wax apple fruits, anthocyanins levels have been differed from different concentrations of CPPU application. Factors affecting fruit color are primarily endogenous hormonal activity resolution.

The relative sweetness or sourness of wax apple fruits was evaluated by measuring the TSS and pH value (**Table 4.3.4.**). Most of the TSS of fruit is considered as sugars. It can be denoted as a maturity index and used to determine the level of maturity between treated fruits. The ratio of TSS and acid value gives relative measure of fruit maturity (Mohsen, 2010). The fruit would be sweeter if it contains more sugars in relation to acid. Fruit with higher TSS/acidity ratio, would not be very tasty. Therefore, the most dependent point to maturity is titratable acidity or the ratio of total soluble solids (TSS, °Brix) to pH. The association between color and maturity level in many fruits have been widely well accepted. As can be seen in **Figure 4.3.13.** and **Figure 4.3.14**., the improvement of the wax apple peel color with maturity was the result of a massive accumulation of anthocyanin content. Zhang et al. (2008) described that this was also becouse of chlorophyll degradation during the maturing period. Additionally, CPPU application has been exposed to increase the ability of the fruit to attract carbohydrates in wax apple. In this study faster growth has been showed in treated fruits compared to control fruit. The promotion on physical characters as well as the early fruit maturity could be considered as a result of increasing total soluble solids (°Brix) and inverted sugars. This promising treatment had great effects on fruit quality of wax apple.

Finally it can be seen that The application of CPPU contributed in improve of fruit quality and yield in wax apple. In this study, different concentrations of CPPU and control treated fruit were examined concerning bud and fruit set and their stimulatory

activities especially on fruit physiological and biochemical altering. The biochemical compounds and nutrient content in fruit were associated with treated conditions. The composition of these compounds may vary greatly on CPPU concentrations. It can be assumed that deep-colored fruits are anthocyanin-rich, especially flavonoid-rich. It was also observed that total phenolic and TSS contents in the wax apple fruit varied considerably when treated with different CPPU concentrations. Maximum fruits weight, length, biochemical content such as total inverted sugar and fructose were observed in the 15 ppm CPPU treated fruits.

The results suggested that the application of CPPU could increase the fruit set and fruit development via stimulating or synthesizing and translocation of carbohydrates such as TSS and fructose in fruits part. The best results with regard to yield and fruit quality of wax apple were obtained when CPPU was swabbed at 15 ppm after bud initiation. These findings exhibited the effectiveness of CPPU in wax apple and showed that it highly contribute to development of yield and fruit quality without any depressing features.

Table 4.3.1. Effects of different treatments of CPPU on bud number and bud drop of wax apple fruit. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatment (ppm)	Bud number	Bud drop
Control	57.33 ± 0.33d	31.0 ± 0.57d
CPPU 10 ppm	60.3 ± 0.33bc	41.6 ± 0.33c
CPPU 15 ppm	63.6 ± 0.33a	45.0 ± 0.57a
CPPU 20 ppm	61.0 ± 0.57b	43.33 ± 0.33b

Table 4.3.2. Effects of different concentrations of CPPU on fruit set, drop and size (length and diameter) of wax apple. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatments (ppm)	Fruit set (%)	Fruit drop (%)	Fruit length (mm)	Fruit diameter (mm)
Control	31.0 ± 0.33d	38.0 ± 0.57ab	59.32 ± 0.25d	34.2 ± 0.03cd
CPPU 10	43.0 ± 0.33c	36.6 ± 0.57c	60.69 ± 0.25c	35.5 ± 0.04c
CPPU 15	44.0 ± 0.33a	38.0 ± 0.57a	65.01 ± 0.04a	40.2 ± 0.01a
CPPU 20	42.0 ± 0.57b	35.6 ± 0.33cd	63.76 ± 0.03b	40.0 ± 0.01ab

Table 4.3.3. Effects of different treatments of CPPU on fruit yield, average weight, volume and juice volume of wax apple. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatment (ppm)	Fruit yield (g/branch)	Fruit weight (g/fruit)	Fruit volume (ml/fruit)	Juice volume (ml/100g)
Control	455 ± 3.3d	58.7 ± 0.19d	60.0 ± 0.1d	67.2 ± 0.18d
CPPU 10	489 ± 2.3c	60.9 ± 0.36c	61.7 ± 0.39bc	72.6 ± 1.38c
CPPU 15	610 ± 2.8a	64.2 ± 0.09a	64.4 ± 0.13a	78.9 ± 0.38a
CPPU 20	584 ± 7.6b	62.6 ± 0.03b	62.8 ± 0.02b	75.2 ± 0.10b

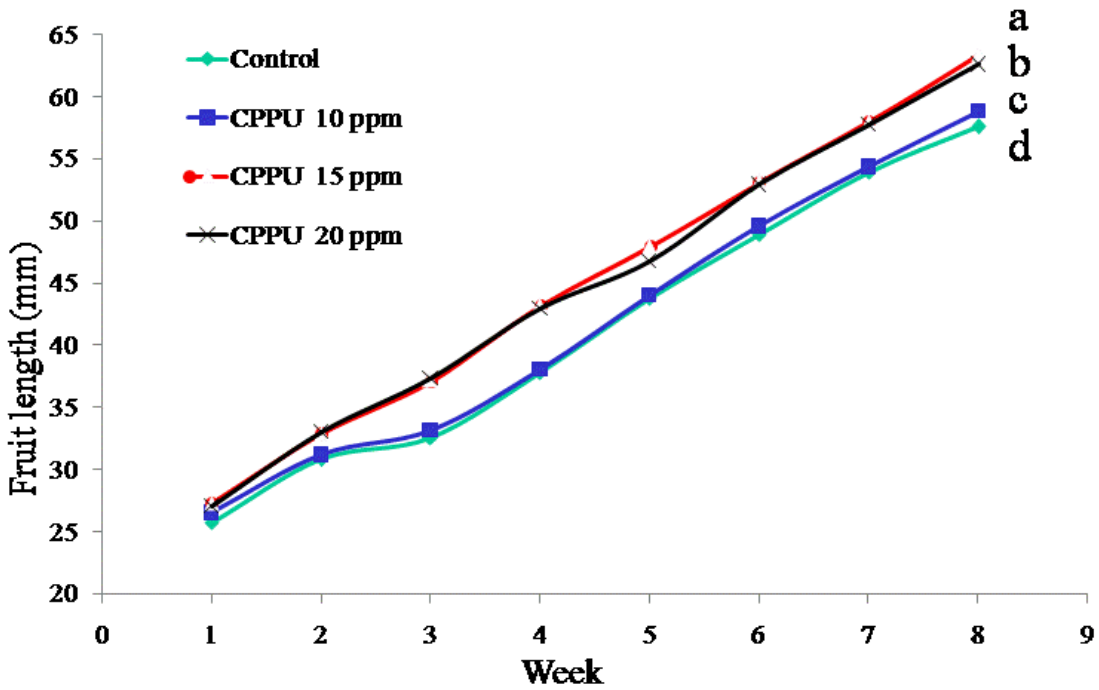


Figure 4.3.1. Fruit growth (length/week) as influenced by concentrations of CPPU (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

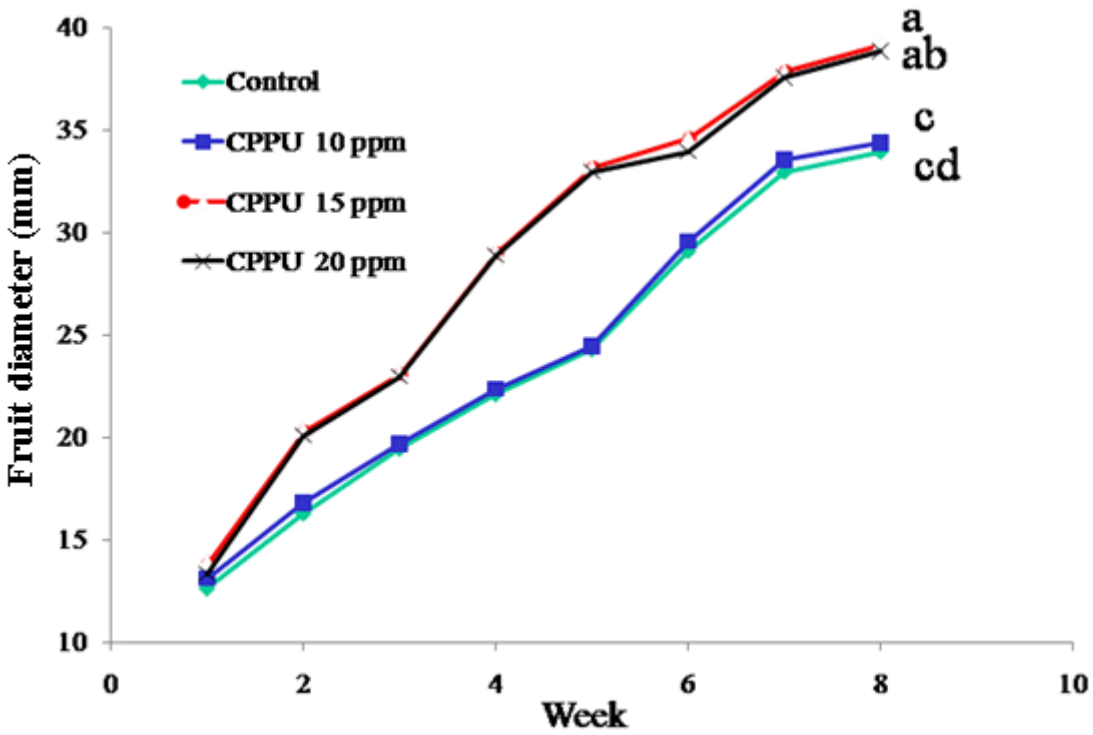


Figure 4.3.2. Fruit diameter per week of wax apple as influenced by different concentration of CPPU (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

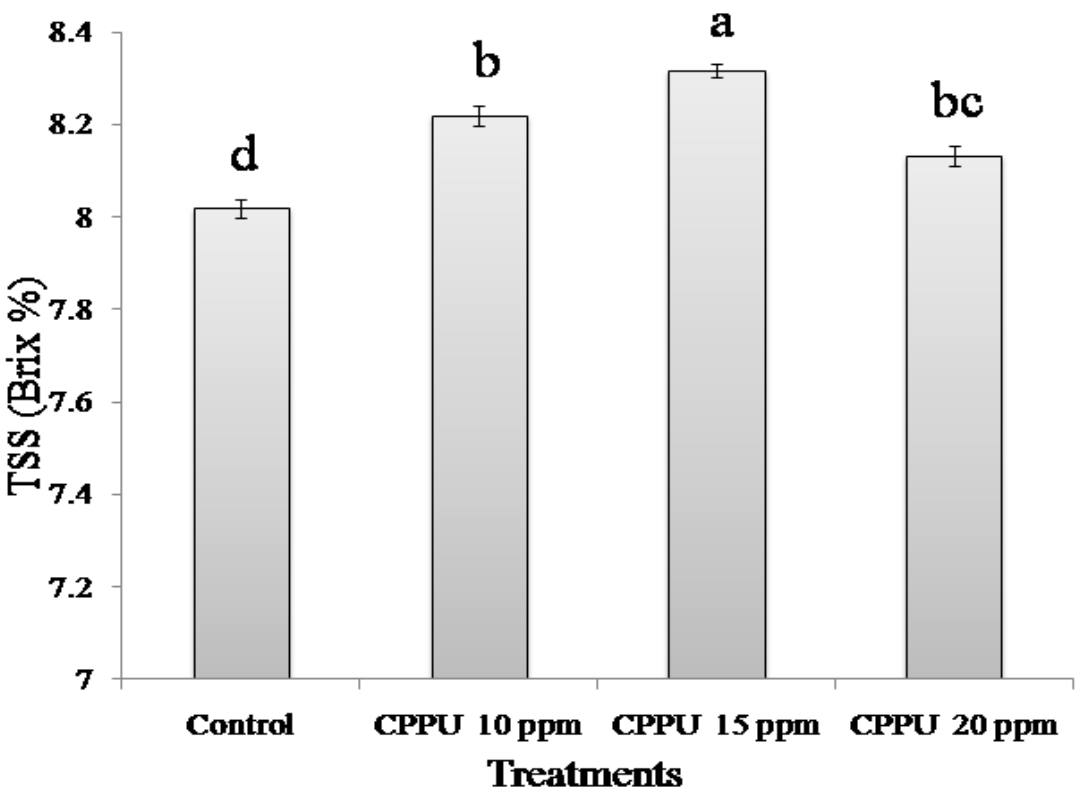


Figure 4.3.3. Effect of treatments of CPPU on total soluble solids (TSS) content of wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

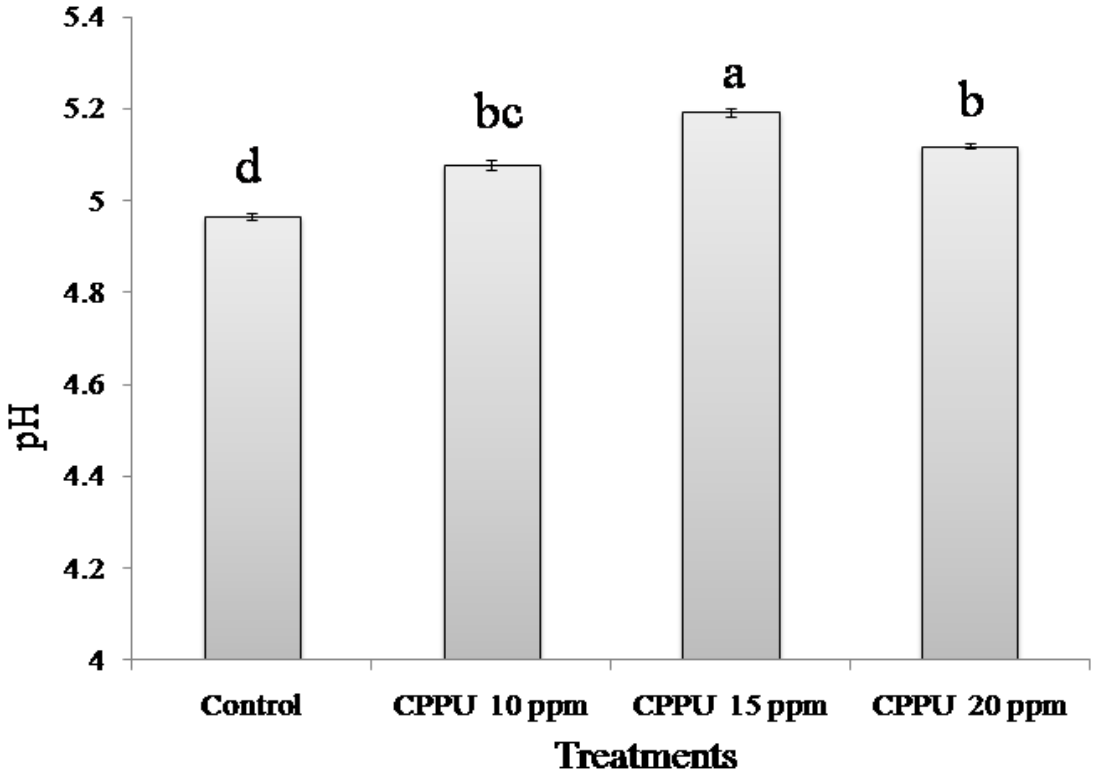


Figure 4.3.4. Effect of treatments of CPPU on pH of wax apple fruit juice (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

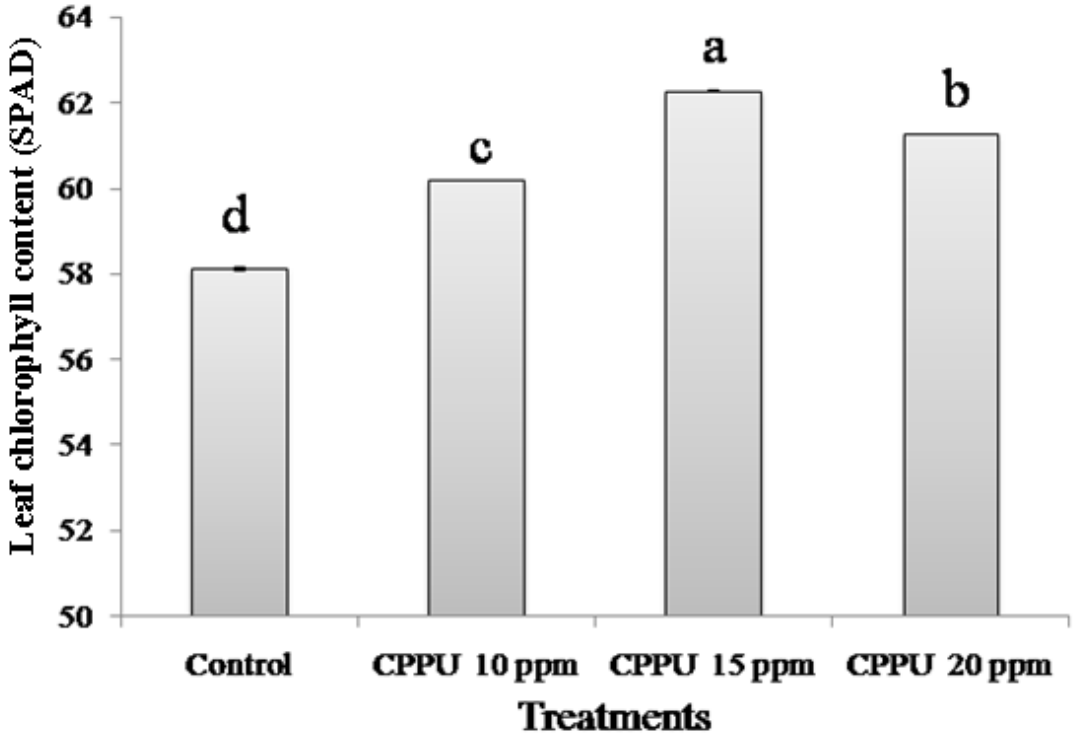


Figure 4.3.5. Effect of CPPU treatments on leaf chlorophyll content (SPAD) of wax apple (Different alphabets mark significant differences, $P < 0.05$ by LSD). S.E. ± (n=5).

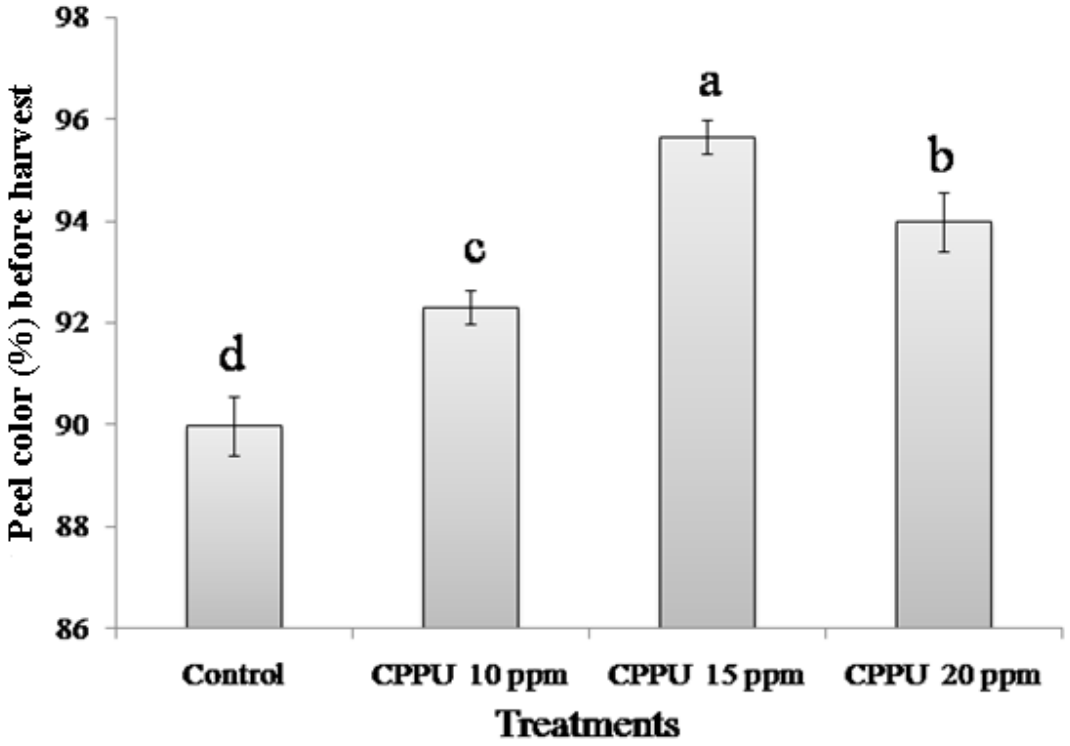


Figure 4.3.6. Peel color (%) before harvest as affected by treatments of CPPU applied to wax apple fruit (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

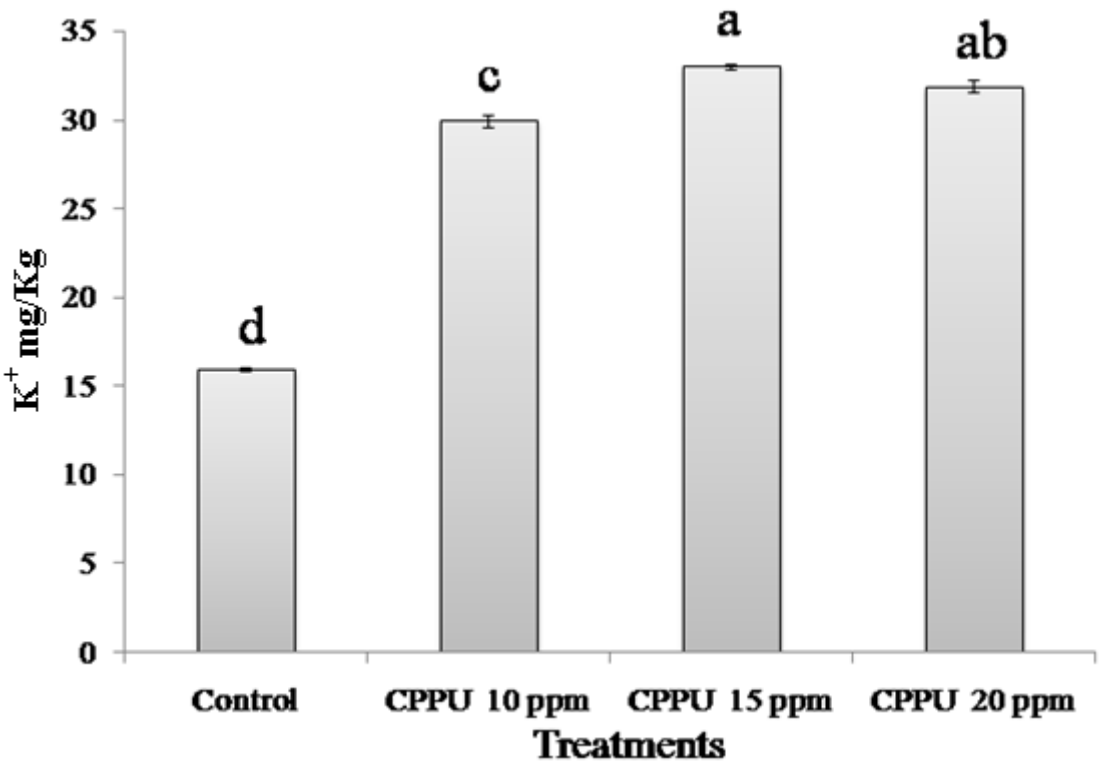


Figure 4.3.7. Effect of CPPU treatments on potassium (K^+) content of wax apple fruit juice (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

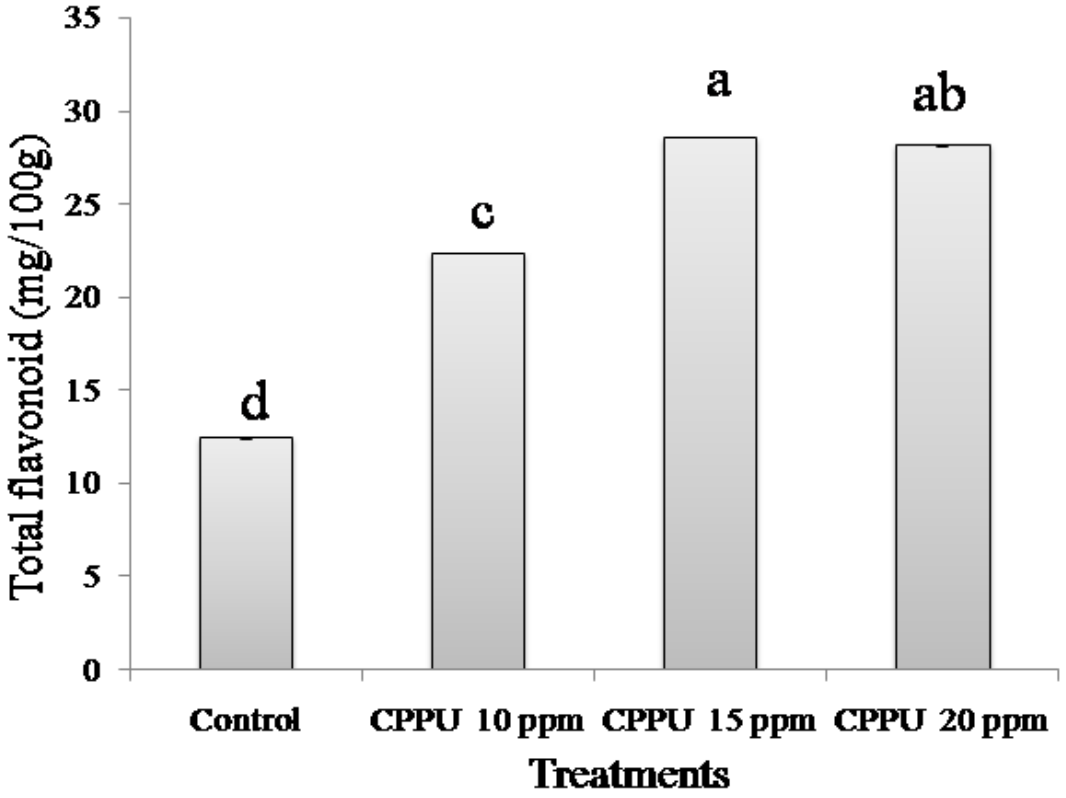


Figure 4.3.8. Effect of CPPU treatments on flavonoid content of wax apple (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

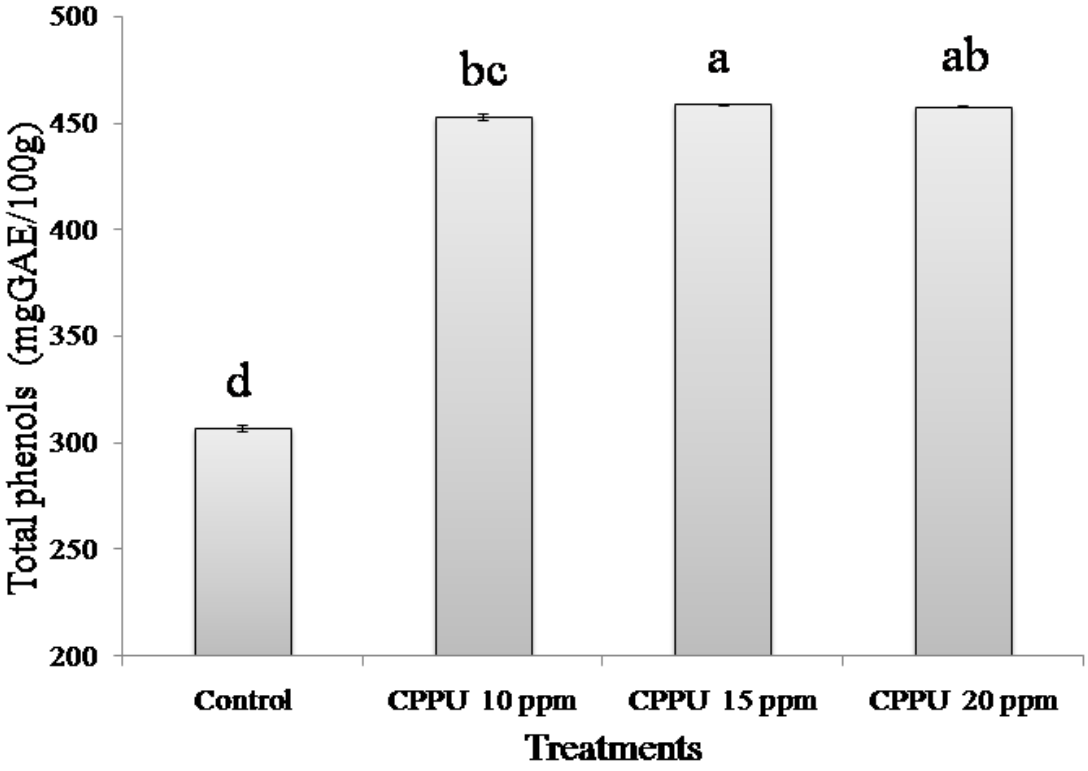


Figure 4.3.9. Total phenols content as affected by treatments of CPPU applied to wax apple fruit (Different alphabets mark significant differences P < 0.05 by LSD). S.E. ± (n=5).

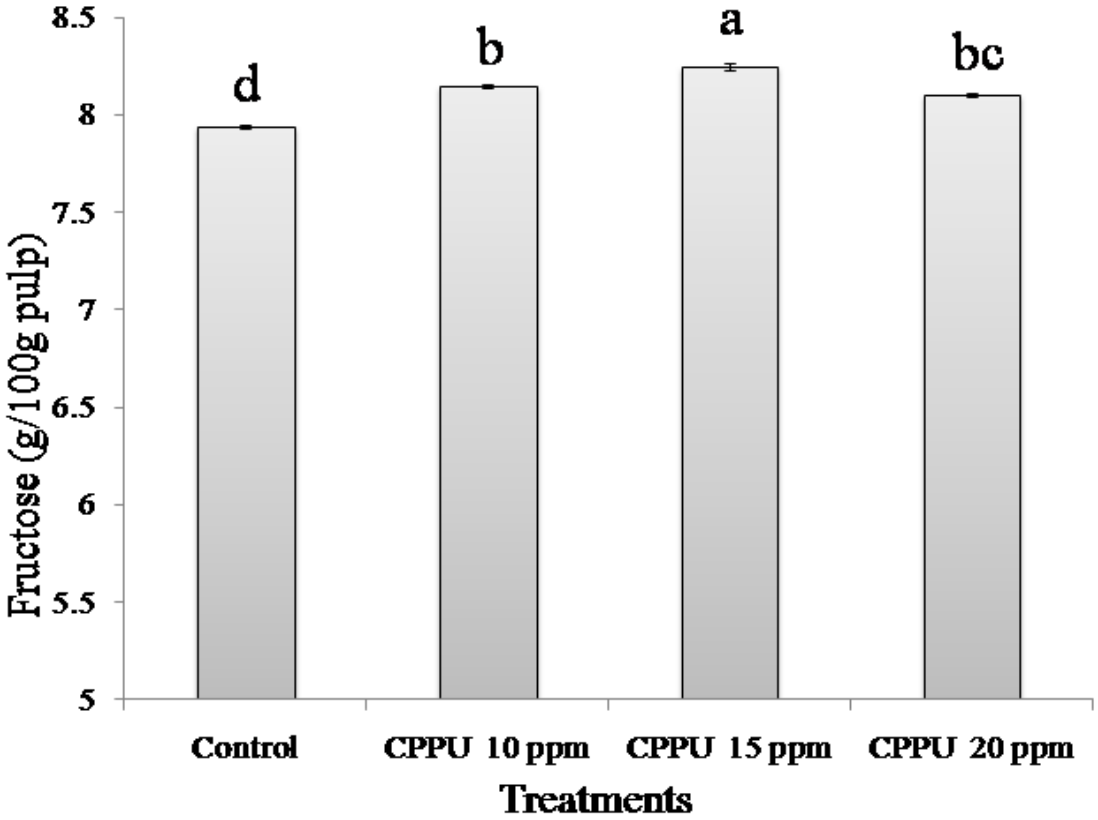


Figure 4.3.10. Effect of CPPU treatments on fructose content of wax apple (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

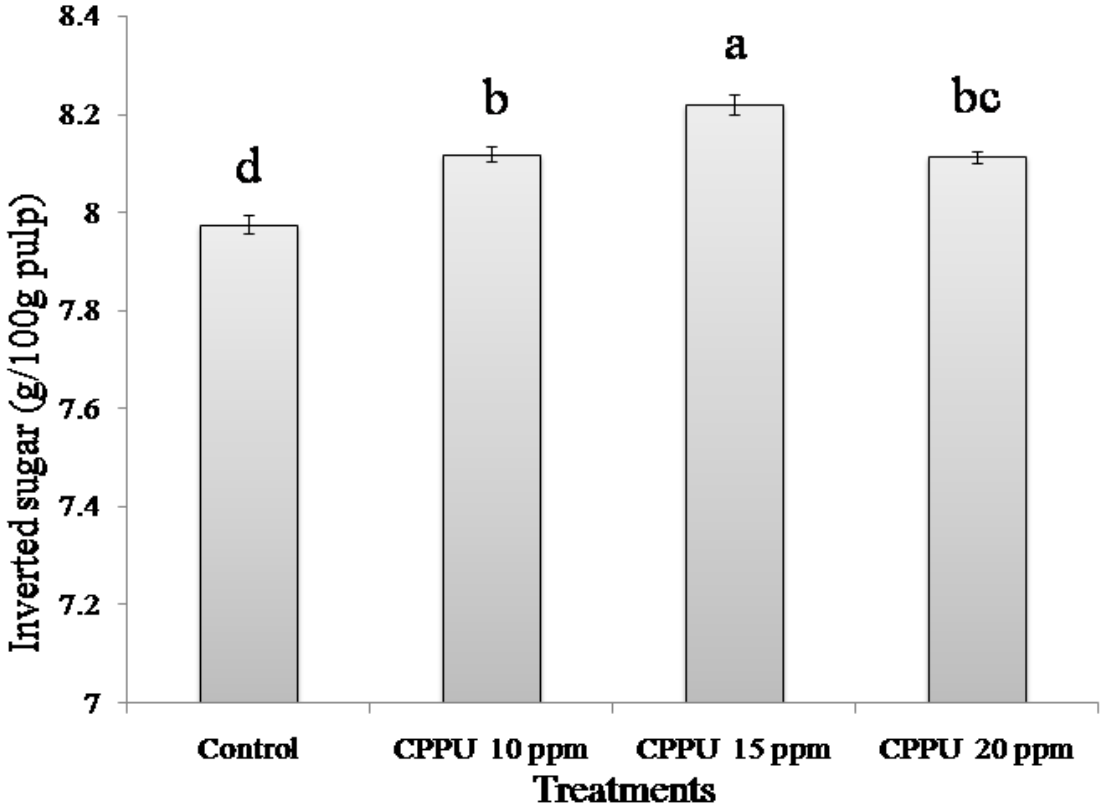


Figure 4.3.11. Effect of CPPU treatments on inverted sugar content of wax apple (Different alphabets mark significant differences, P < 0.05 by LSD). S.E. ± (n=5).

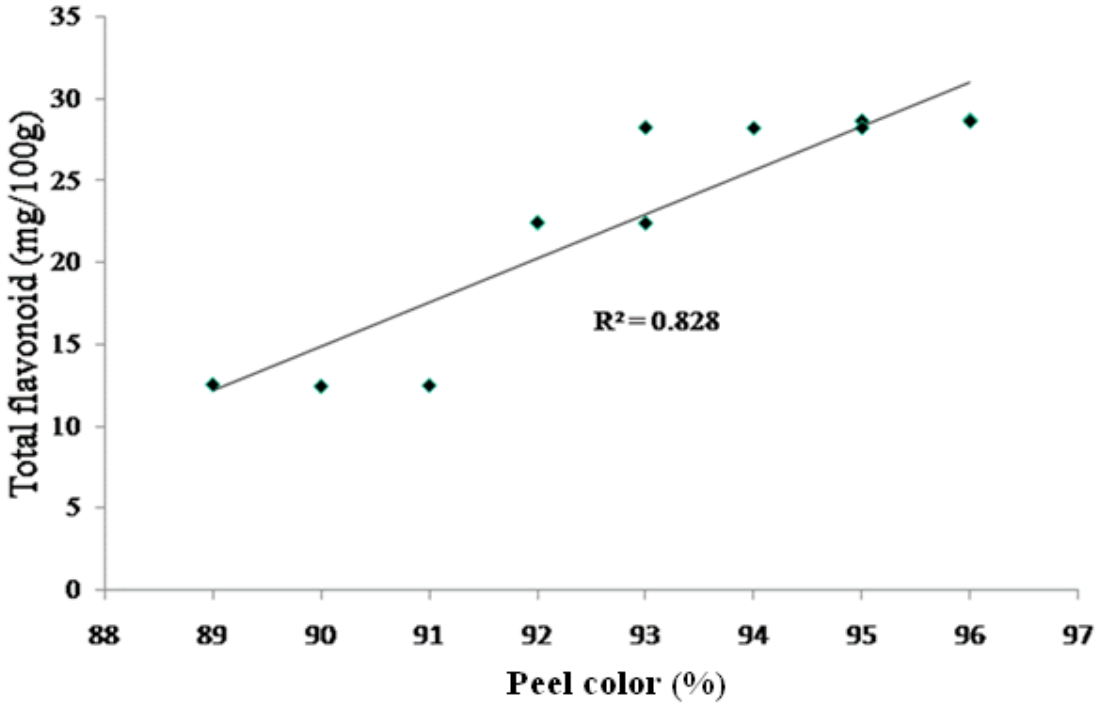


Figure 4.3.12. Correlation between peel color (%) and total flavonoid content of wax apple.

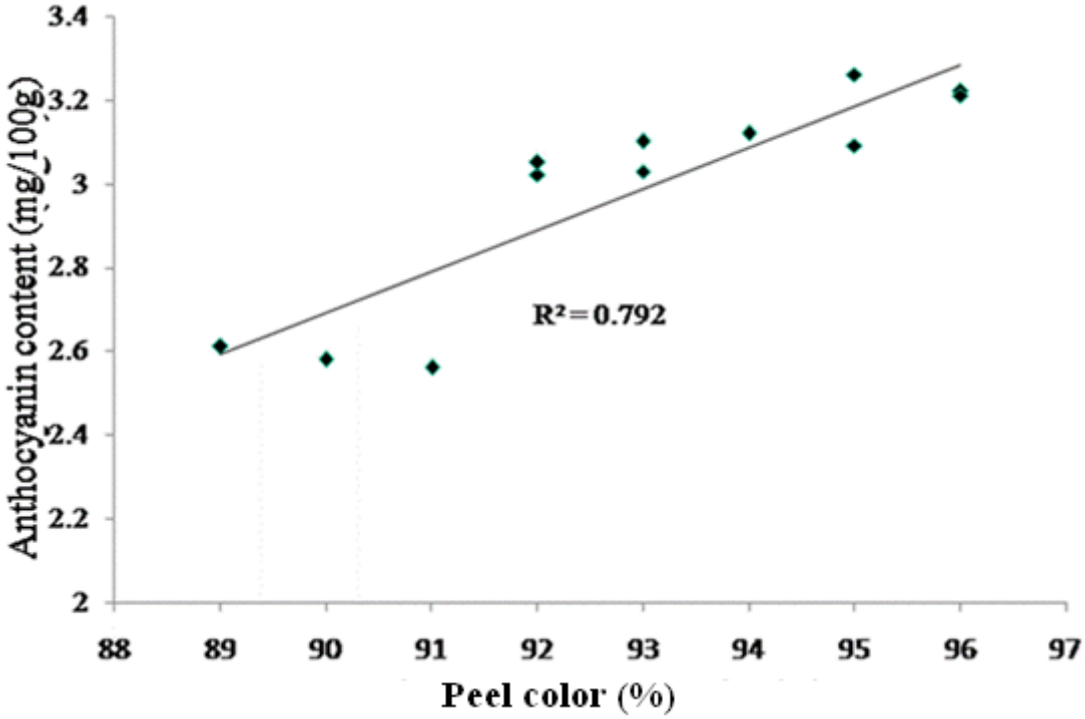


Figure 4.3.13. Correlation between peel color (%) and anthocyanin content of wax apple.

Table 4.3.4. Effects of different concentration of CPPU on TSS and pH ratio. Values are means S.E. ± (n=5). (Different alphabets mark significant differences, P < 0.05 by LSD).

Treatment (ppm)	TSS:pH ratio
Control	1.61
CPPU 10	1.61
CPPU 15	1.60
CPPU 20	1.58

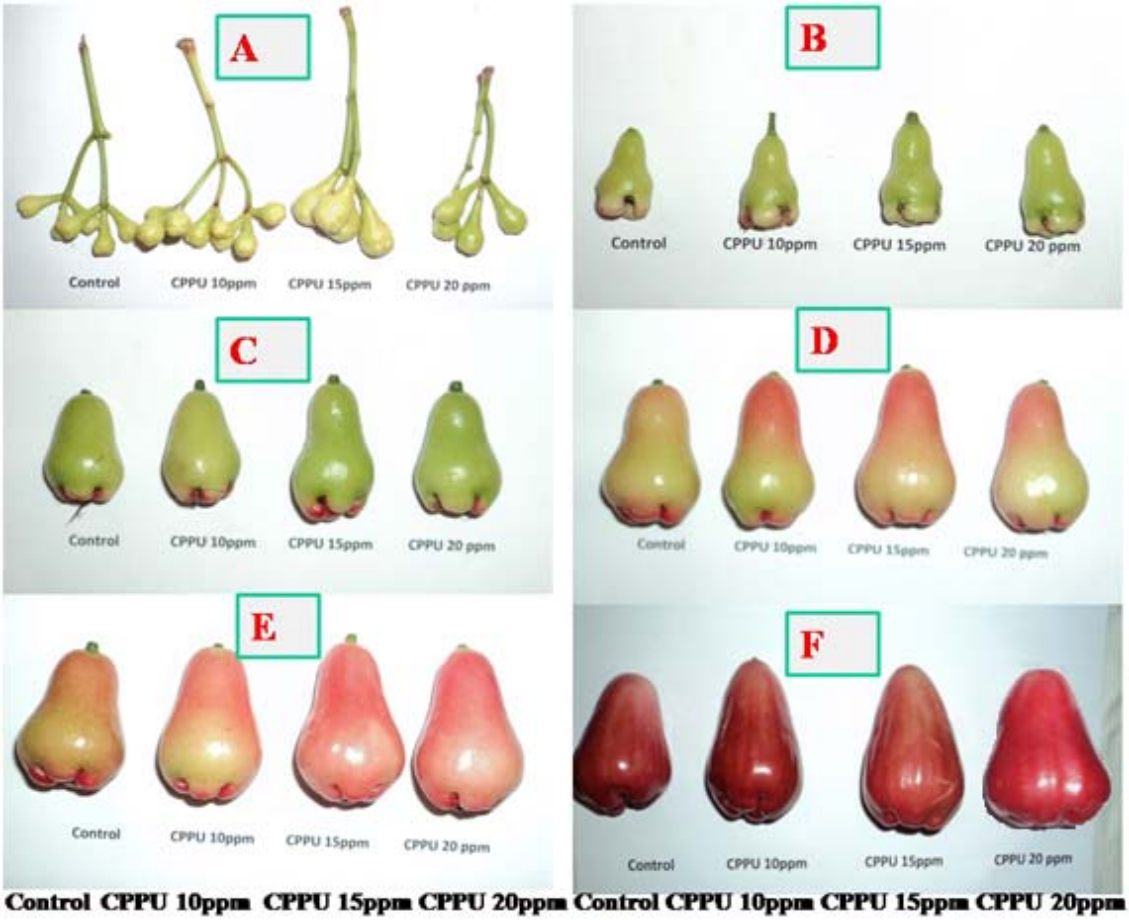


Figure 4.3.14. Photograph shows the effect of different concentrations of CPPU on wax apple fruits, (A): Initial budding, (B): Green stage, (C): light Green stage, (D): Light red, (E): Red and (F): Deep red or harvesting stage.

Chapter 5 :

CONCLUSION AND RECOMMENDATIONS

5.1. EXPERIMENT 1 (THE EFFECT OF GA_3)

This work shows that fruit set yield was observed as two folds higher in 60 ppm GA_3 treatment than in water control. The maximal fruit drop percentage per branch was in 60 ppm GA_3 and it was low in water control. In general, fruit drop was higher in each concentration than in control. GA_3 (30, 60 and 90 ppm) increased the fruit length and diameter. GA_3 60 ppm produced maximum fruit size with maximum total fruit length. The swabbing water method produced lowest fruit size with minimum fruit length. Fruit yield (weight and volume) were significantly affected by the concentration with 60 ppm GA_3. The highest juice content was recorded in 60 ppm GA_3 treated fruits. GA_3 at 60 ppm concentration showed higher TSS than other concentrations and control .

The percentages of fructose and inverted sugars in fruit juice decreased significantly by the high concentration (90 ppm) of GA_3 compared to the water control and the low concentration of GA_3. The highest contents of fructose and inverted sugars in fruit juice were exhibited in 60 ppm of GA_3. The maturity development (represented by color) was observed and lead to the conclusion that all the concentrations enhanced the fruit color. The most effective concentration which made earlier maturity of wax apple fruit was 60 ppm of GA_3. Higher flavonoid content was observed in 60 ppm GA_3 than control, 30 and 90 ppm GA_3. The maximum anthocyanin content was observed in 60 ppm GA_3 and the minimum was observed in water control. Total phenolic content was significantly increased by different concentrations of GA_3. The fruit K^+ content highly increased by different GA_3 concentrations. The highest K^+ content was found in 60 ppm GA_3 treatment and the highest chlorophyll content (represented by SPAD unit) was marked in 60 ppm GA_3. These experiments lead to the conclusion that 60 ppm GA_3 was the optimum concentration for wax apple growth and development.

5.2. EXPERIMENT 2 (THE EFFECT OF NAA)

Bud number for 12 ppm NAA treated branch showed the maximum values compared to others NAA treated branch and control. Bud drop has been increased with the increase of the NAA concentration. The NAA had a distinct characteristic to control fruit set and fruit development. The application of 12 ppm NAA showed the highest fruit set compared to the other treatments. However, fruit drop was increased by applying NAA (12 and 18 ppm). Fruit length and diameter of wax apple were greatly enhanced as a result of the activity of NAA. In this research, the best result was exhibited by 12 ppm NAA among the different concentrations of NAA.

The yield per branch of wax apple was significantly higher in treated branches with NAA than in control. However, fruit weight was significantly increased in case of 12 ppm NAA per branch. The maximum fruit set was initiated by the concentration of 12 ppm NAA. Fruit length and diameter showed variability at different weeks. Significant difference was observed especially in the 8^{th} week of observation. The highest amount of chlorophyll was observed in the 12 ppm NAA treated branches leaves. The potassium (K^+) content was higher in 12 ppm NAA treated fruits than in other concentrations. The maximal total flavonoid content was obtained in 12 ppm NAA treated fruits.

The total soluble solids (TSS) content was affected significantly by the application of different concentrations of NAA. The highest inverted sugar content was observed by 12 ppm NAA concentration. Both inverted sugar and fructose were increased by higer concentration of NAA (12 and 18 ppm). It was observed that anthocyanin content showed the same trend as harvest color level (maturity) and the effects decreased as the NAA concentration was increased. Consequently, color and maturity were earlier

treated fruit than in the untreated ones. 12 ppm NAA concentration showed a highest pH value which was significantly different from others treatment .

5.3. EXPERIMENT 3 (THE EFFECTS OF CPPU)

The maximum bud drop rate was observed in 15 ppm CPPU concentration while the minimum rate was observed in control. All the concentrations showed better initiation of fruit set than control. The highest fruit length was observed in 15 ppm CPPU compared to control. The highest fruit yield and fruit weight were found in 15 ppm CPPU treatment. Higher juice volume was also noticed in 15 ppm CPPU rather than in any other treatments. The highest increase of TSS content was recorded in 15 ppm CPPU treated fruit .

Fruits acidity level represented by pH value was significantly affected by the application of different concentrations of CPPU. The highest pH value was observed in 15 ppm CPPU concentrations. The chlorophyll content was higher in 15 ppm CPPU treated leaves than in any other concentrations of CPPU. The result showed that 15 ppm CPPU had a great influence on the treated fruits especially on color development. The maximum potassium (K^+) content was obtained in 15 ppm CPPU treated fruit and the minimum was in control fruit. The highest flavonoid content of fruit was recorded in all of the 15 ppm CPPU concentrations as compared to control. The total phenolic and fructose contents in fruit varied considerably in the treatments. The 15 ppm CPPU concentrations showed higer phenolic and fructose content than in other concentrations of CPPU. The higher concentrations resulted in decreasing trend of nutrient content. Inverted sugar was higher in 15 ppm CPPU than in other treatments .

Finally it can be concluded that 60 ppm GA_3, 12 ppm NAA and 15 ppm CPPU are the best concentrations in respect to the size, color (maturity), sugar, anthocyanin, nutrient

and phenolic content development in wax apple fruit using innovative swabbing technique of application methods of plant growth regulators. These concentrations of GA_3, NAA and CPPU may be used in the commercial orchard.

Swabbing method of hormone applications might be used in the orchard commercially for the growers who are interested in developing the quality of wax apple fruit. This method can reduce the excessive use of chemicals as well as the cost of production. It also prevents hazarding the environment compared to the spray method of chemical application. This swabbing technique can be applied on all fruit tree varieties.

5.4. RECOMMENDATIONS

This innovative swabbing technique can be used in the area of fruit growth and development. Still it is under research and it has a scientific value. Since it takes longer time rather than spray, it may not be recommended for commercial fruit grower. Further research should be conducted regarding this to invent the suitable method of application which would be cost effective, less time consuming and environmentally friendly.