

Chapter One

1. Introduction

The genus *Citrus* belongs to the Rutaceae family which is believed to have originated in the parts of Southeast Asia bordered by Northeastern India. In this genus *Citrus* *C. paradisi* is one of the most important species; originated in the West Indies as an accidental hybrid of pummelo and sweet orange (Samson, 1986). It is the refined descendant of the bigger and rougher fruit called pummelo or shaddock (Davidson, 1999), and is a relatively new comer to the citrus industry. It is a popular fruit throughout the tropics and subtropics. Its commercially propagated by eye-budding.

Citrus species have been propagated in an ever-widening area since ancient times. The best-known species are grapefruits, oranges, limes, mandarins and lemons. Citrus production comes second to grapes in world production and has high economic and nutritive values. The fruits are consumed fresh or processed (Purseglove, 1984).

Grapefruit cultivation in Sudan is receiving much attention from private and governmental sectors. There has been an increasing awareness of the potential and importance of grapefruit as a fruit crop over the last few years and the area under cultivation is increasing steadily due to an expanding consumer demand.

Grapefruit is customarily a breakfast fruit, chilled, eaten fresh or made into desserts. Its juice is marketed as a common beverage; fresh, canned or dehydrated as powder, or concentrated and frozen and is used as a dietary supplement supplier and is commonly believed to reduce cholesterol in the blood stream. The oil of grapefruit seeds can be refined for use in culinary

purposes and the fruit peel oil is mainly used to flavor soft drinks and other beverages.

The United States is the world leading grapefruit producer, China ranks second. Other grapefruit producers include Spain, Italy, Palestine, India and Argentina. The Arab world grapefruit market is dominated by Sudan, Tunisia and Lebanon with an approximate production of 154, 72 and 15.3 thousand metric tons, respectively (AOAD, 2010).

Grapefruit is a major cash crop and a common component of diet rich in vitamins (Sidahmed and Geneif, 1984). It is one of the most important citrus fruits in Sudan and can be successfully grown throughout the country where there are suitable soils and sufficient water to sustain vigorous tree growth. Sudanese grapefruit is well known for its large size, excellent quality and good coloration (Khalil, 1985). Several grapefruit cultivars have been introduced into Sudan and were evaluated for their growth, yield, and fruit quality (Dinar and Osman, 1984; Hamid *et al.* 1999). Grapefruit is an important fruit crop in Sudan, and there is potential for exports of Sudan's main cultivars to Gulf States and the European markets. The introduced cultivar, "Redblush", proved to be a high yielder with excellent fruit qualities and vigorous growth habits. It is mainly cultivated in the Northern, River Nile, Kassala and Kordofan States. The fruits are consumed locally as fresh fruit and juice, with negligible tonages exported to the Gulf States and Europe.

The most noteworthy grapefruit cultivars in Sudan are "Foster" and "Duncan". More recently "Redblush" was introduced into the country from the United States of America and trials showed its superiority to other tested cultivars, producing excellent quality fruits and high yield.

Problem Statement:

Superior citrus cultivars are typically propagated asexually by eye-budding on open-pollinated suitable rootstock seedlings (Platt and Opitz, 1973). One of the main obstacles to large scale production of citrus nursery clonal seedlings is the erratic ability of scion buds to take and to establish themselves (Maxwell and Lyons, 1979). The magnitude to-take varies with season of the year and age and physiological state of the scion bud.

Propagation of citrus by eye-budding is rather troublesome, requiring special conditions and technical know-how and has limitations because it can be accomplished only during a short period in the spring. The physiological condition of the mother plant from which bud wood sticks are taken can affect bud take and a sufficiently long growing season is needed for the rootstock before budding and for the scion bud to take and grow after budding. Additionally, the method is too slow for producing many plants in a short time and is often tedious and impractical when carried out on a large scale. Demand is great for clonal citrus nursery trees to replace alternate and shy bearer trees and for establishing new citrus groves. Though citrus appears to be amenable to tissue culture propagation (Al-Khayri and Al-Bahrany, 2001; Al-Bahrany, 2002; Ali *et al.*, 2004; Usman *et al.*, 2005; Almeida *et al.*, 2006), the techniques of tissue culture for propagation are economically prohibitive for many commercial citrus growers. Uniform, vigorous and normally appearing growth of budded scion buds in citrus propagation is essential for efficient production of high quality nursery citrus trees.

The perennial evergreen *Citrus* grows in flushes (Schneider, 1973) that are synchronous under Sudan conditions. The main flushes arise in February and September, which are periods of increased atmospheric humidity. Uniform and timely growth of inserted is essential for efficient production of high quality citrus nursery trees. Experience in Sudan has shown that successful bud-take can be obtained only during February through April. Citrus buds inserted at most of the other months of the year are often slow to start growth, even under greenhouse conditions. More or less similar observations were reported elsewhere (Skene, 1980; Wainwright and Price, 1984) that bud-break is seasonal. Scion buds of citrus require chilling to release from dormancy. This requirement is localized primarily in the bud. Poor bud-break and consequently low bud-take percent and reduced and delayed scion shoot development and/or cessation of early growth, are attributed to inadequate chilling for release from dormancy (Erez, 1987).

Scion bud-break in citrus nurseries is commonly hastened after plants have entered dormancy by a number of cultural practices such as manual removal of the terminal portion of the rootstock seedling above the inserted scion bud (topping) or by reducing apical dominance of the intact rootstock seedling by bending (looping) or half-ringing (notching) 3-10 cm above the inserted scion bud (Rouse, 1988; Williamson and Castle, 1989). Defoliation and with-holding irrigation (Edwards, 1987), pruning of mother plant (Krajewski and Rabe, 1995), defoliation of the bud-stick before its excision from mother plant (Popenoe and Barritt, 1988), scion bud position (Unrath and Shaltout, 1985), age (Krajewski and Rabe, 1995) or use of chemical growth regulators (Erez, 1987) are some of the factors that have been reported to influence bud-break. Generally nursery trees grow better when rootstocks remain

intact after bud-take than when they are topped, bent or notched (Rouse, 1988; Williamson and Maust, 1994; 1995; Williamson *et al.* 1992).

The downwards translocation of nutrients and growth regulators from the terminal portion of the rootstock to the scions and roots of young actively growing budded citrus nursery trees has been documented (Williamson *et al.* 1992; Williamson and Maust, 1994; 1995). Moreover, the commonly used physical cultural practices of bud forcing are time consuming, laborious and perhaps uneconomical and result in tissue loss.

During the recent decade several growth regulators, including cytokinins, have been shown to be effective in forcing bud growth in many plant species (Rubinsten and Nagao, 1976; Cody *et al.* 1985). Benzyl adenine (BA), a synthetic cytokinin, has been found to induce bud break of insufficiently chilled fruit trees substituting for the chilling requirements (Young and Werner, 1986). It has been identified as one of the most effective dormancy-breaking agent for several ornamental plants (Larson, 1985) and a variety of fruit trees (Boswell *et al.*, 1981; Nauer and Boswell, 1981, Wainwright and Price, 1984; Cody *et al.*, 1985; Pritts *et al.*, 1986; Popenoe and Barritt, 1988, Shaheen and Said, 1988; Abedrabo and Said, 2012). The optimum concentration and time of application of the chemical for stimulation of lateral shoot growth is not the same for all plant species; each species appears to have a growth period when it is most sensitive to cytokinin treatment. Reliable and rapid bud-break and scion shoot elongation are desirable for the production of citrus nursery trees.

Inducing growth of the scion buds before excision and insertion onto a suitable rootstock may enhance bud-take and subsequent scion shoot growth. A number of chemical compounds have been found to be effective in

releasing bud dormancy (Hosoki *et al* 1985). There are few published reports (Nauer *et al* 1979; Nauer and Boswell, 1981) on the use of growth regulators to artificially release scion buds of citrus from apical dormancy.

It has been specifically verified that systematic pesticides such as insecticides, herbicides, fungicides and acaricides exhibit growth regulators-like activity and influence plant growth and morphogenesis. The motivation for using these chemicals for enhancement of growth and development in plants comes from the scarcity and lack of availability and virtually prohibitive cost of the natural and synthetic growth regulators, (i.e. auxins and cytokinins), that have been used extensively in plants to elicit specific morphogenic responses. The effects of sub-lethal levels of some of these chemicals suggest that they have growth-regulating properties (Neuman, 1959; Welker, 1976; Jansson and Svensson, 1980; Olofinboba and Kozlowski, 1982; Scora *et al.* 1984; Idris *et al* 2010; El-Khair, 2013).

Glyphosate (Roundup), {N-(phosphono-methyl) glycine}, (Gly), a nonselective, very broad spectrum, foliar applied, systematic post-emergence herbicide which is effective in controlling deep-rooted perennial, annual and biennial species of grasses, sedges and broad-leafed weeds. In low rates can stimulate plant growth (Velini *et al.*, 2010). The effects of sub-lethal concentrations of glyphosate on plant growth suggest that it has growth regulating properties. Some of these effects include increasing branch formation in intact plants (Coupland and Casely, 1975; Welker, 1976; Fernandez and Bayer 1977; Lee, 1984) as well as marked increase in growth and development of *in vitro* cultured plant tissues and organs (Scora *et al.* 1984; Gowda and Prakas, 1998). The positive effects of glyphosate has

been attributed to its ability to overcome apical dominance and release of quiescent buds enhancing the formation of shoots and branching in a similar manner to BA releasing quiescent buds and promote branching in intact plants (Abedrabo and Said, 2012).

Furadan, (Carbofuran), is an important chemical pesticide. It has been found to exhibit growth regulator-like effect at sub-lethal concentrations under *in vitro* conditions (Idris *et al.*, 2010; Hussein, 2012; Mohamed, 2014)

Sevin (carbaryl: 1-naphyl-N-methyl carbamate), a pesticide, has been shown to exhibit growth-regulator like effects on intact plants (Stebbins, 1962; Byers *et al.*, 1982; Rogers and Thompson, 1983). El-Khair, (2013) evaluated the influence of sevin, BA, furadan and stroby on scion graft-take percentage in mango. Sevin, BA and furadan produced positive scion graft growth responses of equivalent magnitude. Stroby significantly enhanced percentage of graft take and subsequent growth and development of scion shoots over all other treatments. The effects of BA were more or less equal to those of stroby in most parameters measured (El-Khair, 2013). On the other hand, Hussein, (2012), used *in vitro* tissue techniques to test the efficacy of confidor, sevin, furadan and stroby on shoot proliferation in ginger *in vitro* cultured tissues. Sevin proved to be the most effective in increasing shoot formation at the low concentration .

Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives, and pesticides (Balandrin and Klocke, 1988). In search for alternative sources of growth promoting substances, attention has been shifted to plants as a sustainable source of natural products that might be of

use in increasing yield potential of plants. Production of bioactive plant metabolites from plants that could result in better or more efficient growth and development of plants, as alternatives to synthetic and natural growth regulators, has received much attention in the last few years (Ramachandra Rao and Ravishankar, 2002).

The use of juices of fruits or extracts of certain plants as biostimulants (sometimes referred to as botanical activators or botanicals) was used successfully by several investigators for improvement of plant growth (Helmy, 1992; Chandrasekaran *et al.*, 2000; DongZhi *et al.*, 2004; Abdalla, 2013).

Inducing growth of the scion buds before excision and insertion onto an appropriate rootstock may enhance bud-take and subsequent scion shoot growth and development . As yet, it seems that only very scanty information on induction of buds of mature citrus species in Sudan (Abedrabo and Said, 2012) is available. Hence it seems reasonable to find out if growth regulators , pesticides and plant extract would be effective in increasing bud-break and subsequent bud-take and shoot elongation of budded scion buds of mature citrus trees.

Eye-budding is frequently the only economical method of propagating citrus, although success is often low and variable and the process is seasonal. Routine production of large numbers of nursery citrus trees has not yet been accomplished . A continuing challenge for citrus growers interested in expanding in citrus planting has been the inaccessibility of propagules of desired varieties. The potential demand for planting material for replanting and expansion is enormous. There is a pressing and undeniable need for the rapid build-up of techniques so that clonal citrus nursery trees of desired

cultivars to be available in sufficient quantities to keep pace with the rising demand for planting material. Therefore, the objectives of this research were:

1. To search for new suitable procedures for improving the propagation of grapefruit by bud grafting.
2. To develop a quick, easy and cost-effective system that would allow for the production of nursery grapefruit seedlings ready for field planting on a year-round basis.
3. To maximize the percent bud-take and enhance the sustainability of scion shoot growth.

Chapter Two

2. Literature Review

2-1 Origin:

Citrus belongs to the large family Rutaceae which includes 130 genera and more than 160 species.(Purseglove, 1984) Grapefruit is the only citrus fruit that originated in the West Indies and is considered a natural hybrid of pummelo and sweet orange as is evidenced by the morphological characteristics of the vegetative and reproductive organs (Samson, 1986). It was first grown commercially in the United States.

The first records on grapefruit were written in 1750 by Griffith Hughes who reported on a small Shaddock that is called the “forbidden fruit” in Barbados (Purseglove, 1984). The grapefruit name is derived from the fact that fruits are commonly borne in clusters rather than singly resembling grapes.

Two groups of grapefruit cultivars are known: the white and the pigmented cultivars. In both groups seedy and seedless cultivars occur .In general seedy cultivars mature earlier than seedless ones. Under favorable conditions it takes about eight months for fruits to mature, but over a year if there is insufficient heat (Purseglove, 1984; Samson, 1986).

The common grapefruit is increasingly referred to in the trade as the white grapefruit to distinguish it from the pigmented varieties. Of the common grapefruit varieties known, only "Marsh" and "Duncan" are currently being planted on large commercial scale. The "Duncan" variety is one of the oldest grapefruit known and has remained the standard of grapefruit excellence. It is very seedy (Samson, 1986)."March" variety was also known as “Thompson”. Soon afterwards a sport of "Thompson" was found with much

deeper colors of flesh and rind and was given the name of "Redblush". It is seedless and otherwise similar to "Thompson" but display redder color. The first pigmented grapefruit "Foster" became undesirable because it is very seedy although, it has a good flavor and early maturity (Samson, 1986).

2-2: Botany

The grapefruit tree is a large shrub or small tree which reaches 4.5 to 6 m or even 13.7 m with age, with spreading growth habit and rounded top; the trunk may exceed 15 cm in diameter; that of a very old tree actually attained nearly 2.4 m in circumference. The twigs are spiny; the evergreen leaves are ovate, 7.5-15 cm long and 4.5-7.5 cm wide, alternately arranged; dark green above, lighter beneath, with minute rounded teeth on the margins, and dotted with tiny oil glands; the petiole has broad, oblanceolate or ovulate wings. The white, 5-petals flowers are axillary borne singly or in clusters, and are 4.5-5 cm across. The fruit is large and nearly rounded or oblate to slightly pear-shaped 8 to 25 cm wide with smooth finely dotted peel, up to 1 cm thick, pale-lemon, sometimes blushed with pink, and aromatic outwardly; with spongy and bitter inside. The center may be solid or semi-hollow. The pale-yellow, nearly whitish, or pink, or even deep-red pulp is in 11-14 segments with thin, membrane, somewhat bitter walls; very juicy, acid to sweet-acid in flavor when fully ripe. While some fruits are seedless or nearly so, there may be up to 90 white, elliptical, pointed seeds about 1.25 cm in length and are polyembryonic. The number of fruits in a cluster varies greatly; a dozen is unusual but there have been as many as 20.

The root system of grapefruit trees is composed of an integrated network of woody lateral roots from which arise bunches of fibrous roots. A prominent tap root may not always be present in mature citrus trees. Major pioneer

roots radiate out in all directions from the tree trunk, forming the framework of lateral roots which are primarily responsible for the root system. The studies of citrus root distribution showed that depth of rooting is influenced by tree age, rootstock, and soil and drainage characteristics.

2:3: Cultural practices

Cultural practices for established citrus trees are designed to maintain good growth and vigor of plants and to maximize the production of quality fruits. The common components of all cultural practices are irrigation, fertilization and weed control. Pruning is rarely necessary. Pest control, harvest and postharvest may be necessary to produce bright, clean fruit and occasionally to maintain health and vigor of leaves.

2-3-1: Planting

The field where planting out is to take place must be well cleared, prepared and weeded. The planting density usually varies from 200 to 400 trees/ ha but much closer planting is sometimes used. This induces high yields for a number of years, but when trees become crowded yield start to decline. A system of close planting for the early years, followed by thinning out is a recent development in citrus industry. However, growers usually wait too long to eliminate the unwanted trees. The age of citrus tree may reach 40 years. Citrus trees seldom reach that age in the tropics.

The planting system can be square, rectangular, or triangular. A spacing of 5 X 6 m is frequently used for orange and mandarin, and 7 X 7 m for grapefruit. However, 5 X 7 m and 6 X 8 m leave more space between the rows. The size of the holes is 30 X 30 X 30 cm if drainage is good, otherwise it should be 50 X 50 X 50 cm. In the bottom of the hole, 1 kg of

rock phosphate or basic slag is applied before planting. Citrus tree should be planted on a mound, 10 cm high, after the soil has settled. They must be a little higher than they were in the nursery to avoid infection by foot rot disease. The best time for planting is the beginning of a rainy season. The soil around the plant must be thoroughly wetted and irrigation should be used if some dry days follow. The direction of the rows is important for high yields. In the tropics a north-south direction ensures maximal sunlight (Samson, 1986).

Grafted citrus seedlings are usually transplanted to the field with a ball of earth in plastic bags or wrapped in a piece of jute sacks (balled) or bare-rooted when they are planned to be planted in their permanent place in nearby fields immediately after transplanting. Bare-rooted transplants are easier to handle and transports and are amenable to inspection for diseases, abnormal root growth, damage or infection by soil-borne pests. On the other hand, balled transplants allows for long distances transport without great losses to desiccation and it can be stored in a shaded wet area for a number of days prior to planting. However, the method is expensive, laborious, but it may be useful in drier areas.

Wind-breaks are necessary for protection from hot dry winds which often scorch trees by drying young leaves. Nevertheless high wind velocities will also scar fruits and cause flower and fruit drop, but continuous shade should be avoided (Rice *et al.*, 1987).

Intercropping of citrus fields is a common practice in several citrus growing areas where vegetables or legume fodder crops are grown in the inter-spaces between the trees. These crops (cover crops) improve soil chemical and physical properties and control weeds. Usually these crops are mowed at the

time of drought conditions to lessen the intensity of competition for water (Samson, 1986).

2-3-2.: Fertilization

In most citrus growing areas nitrogenous fertilizers are the most usually applied at the rate of 0.6 kg / tree / year to young non- bearing trees and 3 kg / tree / year for adult trees; often in two doses: the first at the beginning of the rainy season and the second dose 3 to 5 months after flowering. Time of application, type and amount of fertilizer used varies with locality and depend on soil type and on availability (Samson, 1986; Rice *et al.*, 1987).

Citrus trees in general benefit from nitrogen, phosphorus, potassium, iron, manganese, magnesium, zinc and copper fertilization. Of these, nitrogen and, perhaps potassium are the nutrient most likely to be needed routinely by adult citrus trees. Potassium affect fruit quality and 2 kg / tree/year potassium sulphate may be applied routinely to adult trees. Zinc and magnesium deficiencies are quite common in citrus and may be controlled by foliar sprays, often together with copper and manganese. Iron deficiency is corrected using chelated iron as soil drench or foliar spray. Organic manures are important and are beneficial and are recommended where available (Samson, 1986).

Nutrition experiments with citrus have shown that excessive nitrogen results in malformed fruits with coarser texture and less juice. Lack of certain minor essential nutrient elements is evident in symptoms often mistaken for diseases. The condition called exanthema is caused by copper deficiency and mottle leaf results from zinc deficiency.

2-3-3: Irrigation

Citrus trees require irrigation throughout the year to maintain good vigorous growth and subsequently profitable fruit yield. Ideal rainfall for citrus is 914 to 1117 mm rather evenly distributed the year around .Water requirements however, vary according to climate, locality, and soil type (Rice *et al.*, 1987).

Citrus trees withstand a drought period of up to four months if planted in deep soil with good water holding capacity. The amount of irrigation water needed to be applied depends on rootstock, soil type, rainfall and the prevailing temperature. The soil should be kept moist but not wet to a depth of at least one meter. Too much water is just as harmful as too little. The even distribution of irrigation water over the tree root zone area is vital. Irrigation following a period of at least six weeks of drought has been found to induce flowering of adult citrus trees.

2-3-4: Pruning

Citrus trees have small reserves of photosynthate unlike temperate fruit trees. So pruning is usually done for shaping seedlings in the nurseries before out-field planting and budding. Inward growing suckers in juvenile trees, dead branches, nests of birds, ants and termites, bee-hives and infected plant parts are usually removed manually. Heavy pruning in adult citrus trees should be avoided since it delays flowering and thus reduce yield. Very little pruning is done in the tropics and sometimes heavy pruning for rejuvenation purposes done to very old citrus trees that have desirable horticultural characteristics (Samson, 1986).

2-3-5: Pests and diseases

Citruses are subject to most of the pests and diseases that attack other fruit trees. A number of insect pests including mealy bugs, fruit flies, mites and aphids have been known to cause damage to citrus trees. Also several scale insects prey on citrus trees. The most harmful enemy is citrus snow scale infesting the woody portions of the tree. Purple scale and glover scale suck sap from the branches, twigs, leaves and fruit. Florida red scale and yellow scale induce shedding of fruit and foliage. Chaff scale may be found on the fruit, foliage and bark and produces green spots on the fruit. Cottony cushion scale often infests young trees. Maintaining populations of the Vedalia lady beetle, a predator, in nurseries and groves is a fairly effective means of controlling this scale. Parasitic wasps (*Aphytis* spp.) are able to control citrus snow scale, purple scale and Florida red scale. Control could be achieved biologically by the use of predators. The whitefly in its immature stage congregates on the lower side of the leaves, sucking the sap, and also excreting honeydew leading to sooty mold. Immature whiteflies are preyed upon by the parasitic fungi *Aschersonia* spp. and *Aegerita* sp., which are frequently mistaken for harmful pests. Aphids (plant lice) cause leaves to curl and become crinkled. The brown citrus aphid, *Toxoptera citricidus*, is the main vector of the tristeza virus. These pests damage the trees in summer and autumn.

Over 15 species of nematodes attack citrus, the most serious of which are the burrowing nematodes (*Radolphus similis* Cobb.). Control of nematodes is difficult. Preventive measures are usually followed by careful inspection of orchard site for freedom from nematodes prior to planting or use of resistant rootstocks such as Swingle citrumelo.

Citrus are susceptible to many virus and virus like-diseases including crinkly virus, psorosis, tristeza, xyloporosis and infectious variegation. Fungal and bacterial diseases that attack the citrus tree include leaf spot, tar rot, gummosis, heart rot, dieback, charcoal root rot, foot rot, damping off (Rice *et al.* 1987). Gummosis or root rot is a serious problem in Sudan soils (Dafalla, 2004) which are poorly drained. It has been a traditionally adopted practice to grow citrus trees on mounds to prevent the direct contact of the stem with irrigation water. Control of this disease is possible by surgical removal of infected areas, growing citrus trees in well drained areas or by use of resistant rootstock such as sour orange, Swingle citumelo or trifoliolate orange.

2-3-6: Weed control

Weeds should be controlled for rapid establishment and attainment of vigorous growth of citrus trees in an area of 2 m in diameter around the base of each tree. This can be accomplished through cultivation or chemically with herbicides (Rice, *et al.* 1987).

2-3-7: Harvesting, post harvesting and processing

Citrus fruits stay on the tree until they are fully mature. Fruit color is not considered as an indicator of maturity and subsequent harvesting in the tropics. Color changes do not develop unless the temperature has remained below 13⁰C for several hours during maturity. Many citrus fruits retain a green color when fully mature in Sudan. Only lemons and limes are usually picked according to size. Citruses are harvested by climbing the trees or using picking hooks which frequently damage the fruit. Fruits on low branches are picked by hand from the ground; higher fruits are usually

harvested by workers, on ladders who snap the stem, or clip the fruits near the calyx with special clipper. Fruits should be picked carefully to avoid bruises and blemishes. Mature green fruits can be de-greened by exposure to ethylene gas (Samson, 1986).

The first sign of breakdown is dehydration and collapse of the stem end. Fruits for marketing are washed and waxed after harvest. When kept in prolonged storage the grapefruit is subject to chilling injury at temperature below 10⁰C.

Most citrus fruits can be stored in the pantry or the refrigerator. However, more fragile citrus fruits such as tangerines should be refrigerated. Refrigerated citrus fruits will keep up to 3 weeks without damage. Fruits stored at room temperature will keep for only 4-5 days. Grapefruit is remarkable for its durability and keeps well at 18⁰C or higher for a week or more in the compartment of home refrigerator. Lemons and limes should stay fresh for about a week at room temperature if kept out of bright sunlight. For ideal storage, place lemons and limes in the crisper drawer of the refrigerator. They should keep for up to a month. Oranges and pummelos stay fresh at room temperature for about a week, and can be stored in the refrigerator crisper for up to 2 weeks. Ideally, tangerines should be eaten soon after purchase, but they will keep in the refrigerator for 1 or 2 weeks. The control of pests and diseases of citrus through its postharvest and storage stage is beneficial to the citrus industry. Many pests can attack citrus fruit within shelf life such as fruit fly, mealy bug and pine aphid. On the other hand there are many diseases which cause a huge reduction in quality of citrus fruit thus the decay control must be standard by early fungicides applications as well as for arresting moulds. Also bacteria (citrus canker) cause unsightly lesions on all parts of the plant, affecting tree vitality and

early drop of fruit. While not harmful to human consumption, the fruit becomes too unsightly to be sold, and entire orchards are often destroyed to protect the outbreak from spreading (Gottwald *et al.* 2002).

Citrus industry was faced by many problems in processing citrus fruits such as monitoring feed rates to each citrus extractor, adjustment as needed for efficient fruit processing and technology to maintain sanitation levels throughout the entire citrus processing system. About a third of citrus fruit production goes for processing: more than 80% of this is for orange juice production. Demand for fresh and processed oranges continues to rise in excess of production, especially in developed countries.

2-3-8. Propagation:

Citrus species are commonly propagated sexually by seeds or asexually by vegetative methods. Sexual propagation is usually used for breeding purposes, production of rootstocks and principally for the propagation of citrus species that are difficult to propagate by vegetative means such as limes. It is easy, cheap and fast and does not need much expertise and technical know-how. It results in the production of complex heterogeneous seedlings with considerable variation in genetic make-up. The unique characteristics for many fruit trees are immediately lost if propagated by seeds. Seedling fruit trees tend to be rather more vigorous, thornier and are slower to come into bearing. Sexual propagation is used as a method of obtaining disease free-plants, production of new cultivars and as a tool in breeding programs (Mathew and karikari, 1990). On the other hand, vegetative (asexual) propagation is used for the clonal multiplication of known desired varieties. Genetic variation is eliminated unless a sport or mutation occurs. It results in the production of uniform plants that are

genetically homogeneous and true-to-type. In addition to that, fruit trees propagated by vegetative means come into bearing earlier compared to their seed propagated counterparts.

2-3-8-1. Seed propagation:

In many citrus growing countries, citrus species have been grown from seeds (Hartmann *et al.* 2002). Seeds of many citrus species and cultivars are polyembryonic. A seed may contain sexual tissues resulting from a fertilized egg cell and up to 9 or more asexual embryos, which develop from the somatic cells of the nucellar tissues.

Seedling trees from the sexual embryos do not breed true-to-type and usually result in the production of inferior fruit and low yield while seedling trees from nucellar embryos will be of the same genetic make-up as the female parent. Nucellar seedlings are thus used both for direct planting in the field as well as for raising uniform rootstock (Platt and Opitz, 1973).

The degrees of polyembryony vary among citrus species and varieties (Motial, 1983; Prasad and Ravishanker, 1983). Usually limes, lemons and mandarins show a high degree of nucellar embryony (80-100%), while pummelo and citrons are mono-embryonic. Citrus species with relatively high degree of polyembryonic seeds give remarkably true-to-type seedlings, while pummelo and citron seeds, being mono-embryonic, produce genetically variable seedlings. However, propagation of citrus by seed is used where no other method is available. The propagation of desired citrus varieties has not been practiced by using seeds on large-scale for the establishment of commercial orchards even in varieties that are known to produce high proportions of nucellar asexual seedlings such as mandarins, oranges and grapefruit.

The presence of a zygotic embryo makes the use of seeds for the propagation of poly-embryonic citrus species difficult. The identification of the single zygotic seedling from nucellar seedlings is still a difficult task. Several attempts including the use of modification of rootstock colour-reaction test gas chromatography (Pieringer *et al.*, 1964), infrared spectroscopy (Pieringer and Edward, 1965), isozyme analysis (Soost *et al.*, 1980; Anderson *et al.* 1991), ISSR (Fang *et al.* 1997) or morphological characteristics of seedlings and isozyme analysis (Ashari *et al.* 1988) failed to offer a basis for accurately distinguishing zygotic seedling in progenies. It is important to be able to accurately distinguish between nucellar and zygotic seedlings at an early stage of growth.

Vegetative propagation techniques are used to ensure the clonal status of desired commercially known varieties and cultivars of citrus trees.

2-3-8-2. Vegetative propagation:

Often the term vegetative propagation is used more or less synonymously with asexual propagation, which means in its broad sense taking a part or a multi-cellular mass of tissues removed from or attached to the parent plant and making it to grow and develop directly into a new separate individual.

The most obvious advantage of vegetative propagation as a horticultural practice is that all off-springs propagated by vegetative means are clonal. Vegetative propagation also results in the production of plants that come into bearing earlier compared to their seed produced counterparts. The most important advantage of vegetative propagation in citrus however, is that it makes possible the propagation of some citrus varieties that have lost their

capacity for seed reproduction even though they have flowers, such as Navel orange, Bearess lime and Satsuma mandarin. The most obvious disadvantage of vegetative propagation is that it plays an important role in the spread of pests and diseases especially virus diseases. In addition , it needs technical know-how.

Any part of a plant whether an organ, tissues, or even a single cell may be used for vegetative propagation of plants. They all have the ability (totipotency) to form whole individual plants.

The classification of the technique of vegetative propagation in vascular plants is based on the plant part used (the propagules) for propagation such as a stem, root, or leaf-cutting, rhizome, corm bulb, offshoot, sucker, organ, tissue or single cell culture (Hartmann *et al.* 2002)

Two methods of vegetative propagation techniques have been developed for the propagation of citrus. The first one is propagation of plants on their own roots. This method includes rooting of cuttings and layering. Plants produced are called “own-rooted” citrus trees. The other method of vegetative propagation is propagation on a rootstock as in budding and grafting. Plants produced are called budded or grafted citrus trees. Budding and grafting are essentially just other modified methods of propagation by cuttings or layering. The cutting, instead of being rooted are budded or grafted to another plant of the same species or genus but generally of a different variety.

2-3-8-2-1. Own-rooted trees:

This form of vegetative propagation has been used successfully for citrus propagation in many citrus growing countries. It is the most commonly propagation method used in the Mediterranean countries and California.

Layering, however, is most used in tropical and subtropical Asia, Florida and South Africa (Sutton, 1954).

Wide variation in rooting ability of stem cuttings among citrus species and varieties has been observed. While lime, lemon and citron cuttings root most readily, sweet orange, grapefruit and sour orange are intermediate and mandarin proved to be difficult to root. Age and physiological status of the mother plants, type of wood, time of planting and media composition determine the extent of successful rooting of citrus cuttings (Bajwa *et al.* 1977). Leaf-bud cutting have been used as a propagule for citrus propagation in the late thirties. It has been used for the propagation of larger number of citrus species where the parent material is in limited supply (Solomon, *et al.* 1965; Yelenosky, 1987) A leaf-bud cutting contain only a single bud similar in that respect to the budding technique, whereas the standard stem cutting may contain from five to nine buds. Stem cuttings may be hard, semi-hard or soft according to the type of wood used. Stem cuttings have been used for the vegetative propagation of several fruit trees including citrus (Halma, 1931; Gabricidze, 1970; Platt and Opitz, 1973; Grewal and Singh, 1975; Debnat, *et al.* 1986; Sabbah *et al.* 1991; Swelih and Said, 2009) and kiwifruit (Caldwell *et al.*, 1988).

Layering is the principal method of propagation of citrus in South Africa. Air-layering is much used in the humid regions of tropical and subtropical Asia. A branch is made to root while still attached to its mother plant it is then cut from its mother and planted as a separate individual. The method, however, does not allow for the production of large number of trees. It has been used with success for the propagation of Beares lime in the Southern part of Florida (Platt and Opitz, 1973). Nonetheless the use of cutting for propagation of citrus is still quite limited (Swelih and Said, 2009).

Tissue culture has been used for citrus propagation (Perez-Molphe-Balch and Ochoa-Alejo, 1997; Al-Khayri and Al-Bahrany 2001; Ali *et al.* 2004; Rashad *et al.* 2005; Saini *et al.* 2010; Idris *et al.* 2014).The technique is however, economically prohibitive for many citrus growers.

2-3-8-2-2. Propagation on a rootstock:

In most citrus growing countries citrus is propagated by budding the scion bud of the desired variety onto a suitable rootstock (Platt and Opitz, 1973). The absence of a dormant season in tropical regions allows budding to be done at any time of the year provided that irrigation water in the dry season is available (Samson, 1986) On the other hand, Hartmann *et al.* (2002) advocated that budding should be done when the vascular cambium is actively dividing. In Sudan, budding is done during the months of February to April. Few published research on vegetative propagation of grapefruit (Ali *et al.* 2004, Salih and Said 2012) exist. The time and type of budding vary with locality. Great emphasis should be placed on selecting and using scions obtained from healthy virus-free mother trees known to be true-to-type and high yielders in areas where they are planted (Hartmann *et al.* 2002; Platt and Opitz, 1973).

The most important budding methods for woody ornamentals and fruit trees are the chip and T-budding methods. Budding may result in a strong union, particularly for the first few years, than is obtained by some other grafting methods. Budding makes more economical use of propagation wood than grafting. Budding height varies. Generally it is done higher on dwarfing rootstock for fruit crops to prevent scion rooting and allow deep planting. Higher budding also increases the dwarfing effect of the rootstock (Hartmann *et al.* 2002).

Rootstocks are commonly used in citrus production to provide resistance or tolerance to various biotic and abiotic production stresses. On the other hand, a rootstock should have desired characteristics of vigor, proper growth habit, and resistance to soil-borne diseases, as well as being easily multiplied. When producing a citrus tree, the scion cultivar is budded onto the rootstock up to 30 cm above the soil line. Some of this portion of the rootstock, (stem shank), may be buried when the tree is planted in the orchard.

A rootstock may be considered a rooted cutting, a seedling or a micro-propagated plant. The length of time before budding depends on rootstock vigor, length of growing season, and the prevailing climatic conditions. As little as six months to one year growth in the nursery row is needed to produce a rootstock plant large enough to be budded (Hartmann *et al.* 2002). Sour orange seedling grow slowly in their first growth cycles, both in seedling beds and in nursery line-out beds leading to the use of 1-year old transplant for the budding process.

The shield or 'eye-', T- or inverted T- methods are practiced in many citrus growing countries including Sudan. Using a sharp knife, an eye or a bud with a shield-shaped piece of bark, 2 -3 cm in length is sliced from a bud-wood stick. A vertical cut through the bark to the wood of the seedling rootstock is made. This is followed by a horizontal cut at the upper end of the vertical cut to form a "T" or at the lower end to form an inverted T. This opens the bark so that the bud may be inserted at the junction of the two cuts in such a way the bud faces upwards. During the time when the vascular cambium of the seedling rootstock is actively dividing the bark will slip freely. For successful take' the bud shield must be in contact with the exposed surface of the seedling rootstock. The inserted bud is then tied or

wrapped with a wrapping material starting at the bottom to ensure a firm contact between the shield bud and the rootstock (Platt and Opitz, 1973). No water should penetrate to avoid rotting and failure of "bud-take". The wrapping material should also cover the shield bud to avoid drying but the eye of the shield bud must be left free (Samson, 1986).

After one month to six weeks from budding, the seedling rootstock is inspected, and if the shield bud is still green, union has probably been affected (Nauer and Goodale, 1964). Buds that failed to 'take' turn brown in colour. The tape is then partially unwrapped. The rootstock may be used again by re-budding (Nauer and Goodale, 1964) with a new shield bud. Under cases where bud growth is weak or slow after take' forcing is practice by either cutting of the top of the seedling rootstock just above the inserted green buds, (topping), or bending (lopping) or half ringing the seedling rootstock, (notching), 3-10 cm above the inserted green bud (Nauer and Goodale, 1964). Topping effectively overcome apical dominance while lopping breaks apical dominance selectively on the upper side only, meanwhile, allowing continued transport of food and growth regulators on the lower side to the roots (Samson and Bink, 1975).

Exogenously applied cytokinins have been shown to promote the outgrowth of dormant axillary buds of such diverse fruit tree species including apple (Williams and Stahly 1968), avocado (McCarty *et al.* 1971); orange (Nauer *et al.* 1979); macadamia (Boswell, *et al.* 1981); tangerines (Nauer and Boswell, 1981); and apple and peach (Young and Werner 1986), to mention but some. These chemicals have been found to be more effective when used in combination with proper cultural practice and at the proper time of the year (Diaz *et al.*, 1987)

Chapter three

3. Materials and Methods

The experiments were carried out at the plastic greenhouse of the Date palm Company of the Agricultural Research Corporation, Shambat Research Station, Khartoum North. All experiments were conducted using scion buds obtained from a single 10-year-old "Redblush" grapefruit tree grown in the open fields of the Department of Horticulture, Ministry of Agriculture and Forestry, Al-Mogran (Latitude 15 35 N; Longitude 32 33 E), selected for uniformity of fruiting and vigorous growth habit.

Non-flowering, intact, and actively growing branches were cut and the leaves were acropetally removed and bud-wood, 10-15cm in were tied in bundles and wrapped in paper with moist saw dust and taken to the greenhouse for chemical treatment.

In all experiments the technique of T-budding was used. Sour orange (*Citrus aurantium* L.) seedlings, 9-months-old, 60 cm in height were used as rootstock. The seedlings were grown from open-pollinated seeds in a soil mix of 2:1 sand: clay in 15 cm diameter black plastic bags (one seedling per bag), watered daily with tap water and no fertilizer was applied. All budding operations were carried out under greenhouse conditions. The inserted scion buds were wrapped with polyethylene stripes and the bud-union area was covered by a narrow ice cream polyethylene bag. Experience has shown that, slipping a polyethylene bag around the graft union area, promotes bud-take, probably through maintenance of relatively high humidity and were watered once every other day with tap water and no fertilizer was applied. Budded seedlings were incubated under greenhouse conditions, watered once every other day with tap water and rootstock suckers were removed as soon as they appeared.

The chemical substances and the concentrations were chosen on the basis of preliminary experiments, (not reported here), in which various quantities were previously tested in laboratory for morphogenic response using a diverse number of plant species.

Benzyl-amino-purine (BAP) powder was weighed using a precision balance in amounts of 100, 200, 400, and 800 mg. Each weighed amount was dissolved in 1N HCl and made to 1000 ml volume with distilled de-ionized water containing 0.05 % surfactant Tween-20, (polyoxyethylene sorbitan monolaurate), to increase solubility of the chemical (Garren, 1969). Distilled de-ionized water containing an equivalent volume of each of 1N HCl and 0.05% Tween-20 was used a control. The soaking duration for all experimental chemicals was two hours.

Experimentations:

In the first experiment the potential efficacy of benzyl-amino-purine, (BAP) was tested at concentrations of 0, 100, 200, 400 and 800 mg/l.

Experiment 2 was performed to examine the effect of different concentrations of Sangral, with the concentration of benzyl-amino-purine. (BAP), held constant at 400 mg/l, on scion bud-take and growth of scion shoot. The following concentrations of Sangral in (mg/l): 0, 50, 100, 150, 200, 250, or 300 were tested with 400 mg/l BAP for comparison.

The third experiment was conducted to study the influence of topping of the rootstock and dipping of the scion bud-woods in 200 mg/l of Sangral on bud-take and scion shoot growth and development. The 200 mg/l Sangral was chosen because it was found to be the most effective concentration in increasing scion bud-take in experiment 2 above. Treatments include a

control (no treatment), soaking of bud-wood in 200 mg/l Sangral plus topping, soaking in 200 mg/l Sangral only without topping and topping only without soaking in 200 mg/l Sangral.

In experiment 4 three types of pesticides were tested for influence on grapefruit bud-take and subsequent scion shoot growth and development . Concentrations of 50 mg/l of each of glyphosate furadan, and sevin were used. Bud-wood were soaked in one of each of these chemicals for 2-h prior to grafting onto sour orange seedling rootstocks.

Experiment 5 evaluated the influence of stroby at concentrations 0.0, 50, 100, 150, 200, 250 or 300 mg/l on scion bud-take and subsequent growth and development of scion shoots.

A sixth experiment was performed to study the effect of water extract of vegetative parts of rocket, spinach and garlic on bud break and scion shoot growth and development. Green leaves of rocket and spinach and cloves of garlic were weighed, each separately, using a precision balance 5; 10; or 20 g. Each weighed amount was blended in an electric blender and diluted to 100 ml with distilled deionized water.

Experiments were terminated 6 weeks from budding. Parameters measured included percentage of scion take, number of scion leaves, and scion shoot length . Only inserted buds that break and form a branch were considered to have taken. Inserted buds that swollen and remain green without forming a visible branch were not included in the total count. Treatments were arranged in a randomized complete design replicated 4 times. Each treatment consisted of 10 budded rootstock seedlings and each treated budded rootstock is a replicate. All observations were based on 10 grafted rootstocks per treatments. The percentages refer to the proportion of buds produced scion shoots. Data on the percentage of scion buds that took were

subjected to transformation and then converted back to original values for inclusion in tables. Data were analyzed using the analysis of variance procedures on Excel computer programme and Duncan Multiple Range Test was used to separate treatment means.

Chapter Four

4. Results and Discussion

This study was undertaken to incorporate certain aspects of the work of Abedrabo and Said, (2012), Mohamed, (2014) and Saadalla. (2015). Bud break was the principal morphogenic response desired in this study.

BAP concentrations.

All BAP concentrations promoted bud-take at varying degrees. The magnitude of the response of scion buds to take and scion shoot to grow and develop varied with the BAP concentrations (Table 1). Soaking of bud-wood in 400 mg/l BAP resulted in significant increases in number of scion bud-take and number of leaves compared to the untreated plants (control) or plants treated with lower or higher concentrations of BAP. Bud-break and leaf number were highest at 400 mg/l, decreased at 100 and 200 mg/l and were greatly decreased at 800 mg/l BAP. On the other hand, scion shoot elongation was not affected by any of the BAP concentrations. This was in accordance with the findings of other investigators (Miller, 1982; Young, 1987; Malik and Archbold, 1992; Abedrabo and Said, 2012) that BA alone has no effect on elongation of newly formed scion shoots.

A significant increase in bud-take in BAP-treated scion buds would appear to be a result of a loss of apical dominance. All BAP concentrations tested affected bud-break and increased the number of leaves of the scions that took. BAP has to be absorbed by the quiescent buds to be effective (Carpenter 1975; Little 1985). Direct contact of the axillary scion buds with the right concentration of BAP seems to be important for bud-break and

scion shoot elongation. The soaking application method allows for the direct contact of the right BAP concentration to all quiescent axillary buds present on the soaked bud-wood at the time of treatment. BAP permits the development of shoots from budded scion buds which normally would remain dormant. These results are parallel to those of Nauer *et al.* (1979) on navel orange, where effective BAP test solutions were applied directly to individual budded scions with cotton swabs.

Differences in response to BAP application may be due to the result of differences in plant genotype, the concentration of BAP, bud developmental stage and environmental conditions in the location of the experiments. Variations within species in BAP concentrations optimal for bud-break and shoot elongation in citrus have been documented (Nauer *et al.* 1979). The effectiveness of BA soaking probably caused most of the dormant buds present at time of treatment to start active growth during the time following the excision and insertion. The failure of some of inserted and wrapped treated buds to take could be attributed to variation in the age of scion buds and/or scion bud position along the bud-wood stick in (Unrath and Shaltout, 1985; Krajewski and Rabe, 1995).

Sangral concentrations:

Sangral, a proprietary commercially available chemical formulation, that is widely used, but at a single concentration, for rooting plant cuttings under nursery conditions. The composition of this chemical is confidential to the manufacturer.

Table 2 summarizes the results of an experiment conducted with the objective of evaluating the influence of different concentrations of Sangral on scion bud-take, scion shoot length and number of leaves of grapefruit.

Bud-wood sticks were soaked in different concentrations of Sangral solution for 2 hours prior to bud excision and insertion. Significant differences in leaf number and scion bud-take were found among the four Sangral levels examined (table 2). Not only was percentage of scion bud-take greater with 200 mg/l Sangral, but all other growth attributes measured were likewise better and greater (Fig.1 and Fig.2 and Fig. 3). Scion bud-take percentage and leaf formation were significantly promoted by soaking in 200 mg/l Sangral with vigorous and dark green leaves compared to the other treatments. Scion bud-take was increased significantly by Sangral but this chemical had no effect on scion shoot length (table 2). This is an unexpected result since Sangral is marketed as a rooting “hormone” not as a promoter of scion bud-break and growth.

The significant increase in scion bud-take in Sangral treated scion buds reported herein would appear to be the result of a loss in apical dominance. The concentration of 200 mg Sangral/l effectively increased scion bud take response as well as leaf formation but had no effect on scion shoot elongation. There were no significant differences between the 200 mg/l Sangral and the 400 mg/l BAP treatments in all growth variables measured however, both treatments significantly increased all growth variables measured compared to all other treatments.

At present, no explanation can be offered for the effect of Sangral concentrations on scion shoot elongation. It can be speculate on scion buds competition between the scion bud and the scion shoot for assimilate (source/sink relationship). Competition for the available nutrients between the newly formed shoots may inhibit their elongation. Most food reserves and growth substances in the rootstock tissues might have been diverted for bud forcing and bud-take. Initiated scion shoots cannot compete with the

newly forced buds and the subsequent bud-take. It is also possible that Sangral resulted in an alteration in the balance between endogenous growth regulators that are involved in controlling apical dominance in plants. It is to be noted that the 200 mg/l Sangral and the 400 mg/l BAP, which was used as a control in the current study, produced positive responses of equivalent magnitude. This could be considered as an indication that Sangral has a BAP-like effect.

Combination of soaking and topping

The results of the experiment conducted to evaluate the effect of the treatment combinations of topping and the concentration of 200 mg/l Sangral for maximum percentage of scion bud-take, scion shoot length and production of leaves are depicted in Table 3. The most effective was the soaking and topping combination treatment. Growth differences between treatments were evident for all parameters measured. The combination treatment of soaking and topping had the greatest effect on the various growth variables measured. The highest bud-take percentage (100), the greatest number of leaves (44) and the longest shoots (43.0 cm) were obtained with the soaking and topping combination treatment (table 3).

Topping without soaking was the least effective in promoting bud-take and subsequent scion shoot growth and development (Figs.4 and Fig.5). Consistent with these results were those reported by others (Carpenter 1975; Nauer *et al.*1979; Nauer and Boswell 1981) who obtained successful bud-break by chemicals treatments only when the chemical treatment is combined with pruning or topping. However, other investigators (Parups 1971; Ohkawa 1979; Rouse 1988; Abedrabo and Said 2012) enhanced chemical bud-take and branching in intact plants without topping or pruning. The importance of current photosynthates from the rootstocks for nursery trees growth and development and the reduction of growth and development of nursery trees forced to take by topping, bending or

notching has been realized (Rouse, 1988; Williamson and Maust, 1994; 1995). The discrepancies in results are primarily due to differences in plant genotype, type of chemical and concentration, method of application and environmental condition at the location of the experiment.

The current results showed that all measured responses in grapefruit were significantly higher with the combination treatment than with soaking alone or topping alone. Neither soaking alone nor topping alone was able to optimize percent scion bud-take and subsequent growth and development of scion shoots as was obtained with the combination treatment. Both, soaking and topping, are needed to enhance scion bud-take and to maximize growth variables of inserted scion buds.

This would appear to be the result of a synergistic positive effect between soaking and topping on scion bud-take and subsequent growth of scion shoot. An alteration in the endogenous balance between growth regulators and a shift in assimilates in the rootstock shoot to levels inducing scion bud-take and subsequent scion shoot growth and development might be involved. This conclusion is supported by the speculations of several investigators (Sachs and Thiman 1967; Shindy and Weaver 1967; Phillips 1975) that exogenous application of growth regulators to intact plants resulted in changes in the balance between growth regulators controlling apical dominance and the concentrations of stored nutrients in the plant tissue.

Stroby concentration:

The results of the effects of stroby on growth attributes of grapefruit scion budding are depicted in Table 4. All growth parameters measured were

affected by all stroby concentrations tested. The magnitude of response, however, varies with concentration. The most effective concentration on all parameters was 200 mg/l stroby giving significantly higher bud-take and leaf formation values compared to other treatments; with a 50% increase in percent scion take over the control. However, scion shoot elongation was not influenced by all stroby concentrations. A sharp decline in percent bud-take and leaf formation was obtained by increasing the concentration of stroby above 200 mg/l. The percentage of scion bud take ranged from 25% to 75%. The highest values for percent scion shoot take, and leaf number were recorded with the 200 mg/l Stroby concentration. However, stroby concentrations higher or lower than 200 mg/l, reduced growth variables significantly compared to the control. No phytotoxicity was noted from the stroby treatments. The leaves appeared to be larger and darker green in colour than those of the control treatment.

These results are consistent with those reported by El-Khair (2013) which indicate that stroby application significantly enhanced percentage of graft take and subsequent growth and development of scion shoots of mango over all other treatments. The positive effect obtained with stroby on plant growth and development reported herein corroborated the findings of a number of researchers using tissue culture (e. g. Hussein 2012, Mohamed, 2014, Saadalla, 2015) who obtained higher growth responses with medium amended with optimum concentrations of stroby than the control.

The enhancement of scion graft take obtained in the current study would primarily be attributed to loss of apical dominance; a phenomenon that is associated with cytokinins. It appears that stroby exhibited a cytokinin-like effect on scion bud-take and subsequent scion shoot growth and development. Stroby had little effect on scion shoot elongation but did

enhance bud-take percentage. Scion shoots were largely unresponsive to stroby treatments. Similar results were obtained and similar conclusions were reached by El-Khair, (2013) in mango scion shoot grafting and the effects of stroby were more or less equal to those of BA in most parameters measured.

Stroby exhibits considerable potential as an agent for increasing bud-take in grapefruit. The results attested to its potency as a chemical compound with cytokinin-like effects. Although a precise explanation for the effects of Stroby on bud-take and subsequent scion shoot growth and development is lacking, the results of this study demonstrate that presoaking of scion buds in a concentration of 200 mg/l stroby significantly increased bud-take percentage and leaf formation.

Effect of Carbofuran (furan), Glyphosate and Sevin:

Based on literature and our professional experience, these three chemical compounds were selected for experimentations because of their considerable potential as agents for increasing bud-break and shoot proliferation at sub-lethal concentrations. The research did not attempt to explain the difference in effectiveness between these chemicals when used for bud release from dormancy.

Table 5 illustrates the result of the effects of furadan, glyphosate and sevin at 50 mg/l each on bud-take and subsequent scion shoot growth and development of budded grapefruit scion buds. Furadan and sevin treatments, effectively increased scion bud-take percentage as well as formation of leaves with significant difference between the two treatments and the control. Shoot length was unresponsive to both treatments. Glyphosate, on

the other hand, resulted in the death of all inserted scion buds within the first week of culture.

The results concur with those reported by El-Khair (2013) who found that sevin and furadan and benzyl adenine (BA) produced positive scion graft growth responses of equivalent magnitude on scion grafting of mango. The current results indicate that sevin and furadan effects on grapefruit scion bud-take are cytokinin-like effects a conclusion that is supported by findings of others (Hussein, 2012, Mohamed, 2014; Saadalla. 2015) that inclusion of low concentrations of sevin or furadan in the culture media results in the proliferation of shoots as a result of loss in apical dominance and release of quiescent buds of in vitro cultured shoot tips of plant species. A similar finding was also reported by Abedrabo and Said (2012) that cytokinins release quiescent buds and promote bud-break in intact plants.

It is worth mentioning that Lee (1977) attributed the promotion of growth in pea stem segments by furadan to its ability to preserve an optimum level of auxins required for growth and development.

Soaking of scion buds in 50 mg/l glyphosate resulted in the browning of the inserted buds and the surrounding rootstock tissues. It is most probable that the concentration of 50 mg/l glyphosate used in the study was lethal.

The current results disclosed that the single concentration of furadan tested (50 mg/l) significantly increased scion bud-take and leaf formation relative to the control. The results were comparable to those reported by (Idris, *et al.* 2010; Hussein 2012) working with ginger where furadan at low concentrations increased shoots formation in ginger. The results of this study supported the view of Idri, *et al.* (2010) that furadan exhibited cytokinin-like effects.

This study also showed that seven has a pronounced positive effect on bud-take and leaf formation. Shoot elongation was unresponsive to the 50 mg/l seven treatment. The results parallel those of Hussein (2012) working with ginger and Mohamed (2014) working with strawberry tissue culture who found that low concentrations of seven in the culture medium significantly enhanced shoot proliferation indicating that seven has cytokinin-like effects.

Plant botanicals (biostimulants, botanical activators).

Table 6 illustrates the results of the effects of leaf extract of rocket on scion bud-take and growth of scion shoot of grapefruit. There was a significant difference between the rocket leaf extract treatments and the control. Differences in all measured growth variables among treatment concentrations were also evident. Rocket leaf extract had a significant influence on all growth responses over the control, but differences were not significant among treatments. However, the 10% rocket leaf extract concentration gave non-significantly higher values of all measured growth responses than the other rocket leaf extract concentrations.

The data portrayed in Table 7 summarize the influence of spinach leaf extract on scion bud-take and scion shoot growth. Significant difference in percent scion bud-take, leaf formation and scion shoot length among treatments (spinach leaf extract concentrations) were noted. All concentrations of spinach leaf extract resulted in a prompt increase in number of leaves, scion shoot elongation and scion bud-take over the control. The lowest concentration of spinach leaf extract (5%) significantly increased the values of all measured growth responses relative to the control.

The values of all measured growth responses however, progressively declined with increasing spinach leaf extract concentration above 5%.

It was apparent from the data displayed in Table 8 that the values of all growth responses increased with all concentrations of garlic clove extract and growth differences among treatments were evident for all parameters measured. Neither leaf formation nor scion shoot elongation differed for garlic clove extract concentration levels. However, both growth responses were non-significantly higher at the lowest concentration (5%) of garlic clove extract. However, the 5% and 10% garlic clove extract concentrations significantly increased percent scion bud-take over other concentrations tested with no significant difference among them. The 20% garlic clove extract concentration reduced scion bud-take percentage compared to the other two garlic extract concentrations but it was still significantly higher than the control.

The results of the effects of soaking scion buds prior to budding in aqueous solutions of leaf extract of rocket and spinach, each tested separately, revealed that rocket and spinach leaf extracts enhanced scion bud-take and subsequent scion shoot growth. The magnitude of response varied with concentration and source of plant extract used. The results are consistent with those reported by others (Chandrasekaran *et al.* 2000; DongZhi *et al.* 2004.; Hanafy *et al.* 2012; Abdalla 2013; Saadalla 2015) who indicate that aqueous leaf extract of some plants promotable growth and development of intact plants or plants parts under *in vitro* conditions. The enhancement of grapefruit scion bud-take and subsequent growth and development of scion shoot by soaking in aqueous extract of leaves of rocket and spinach is thought to be of the presence in these extracts of macro-and micro-nutrients

and/or hormones that are necessary for growth and development of plants. This view is supported by the conclusion of Hosoki, *et al.* (1986) that the positive effects of rocket leaf extract on release of dormancy of corms is primarily related to the presence of high sulfur-containing compounds and that of DongZhi *et al.* (2004) who advocated considering leaf extracts as natural growth regulators.

The significant increase in percent scion bud-take of scion buds soaked in garlic clove extract concentrations appeared to be a result of a loss of apical dominance, since leaf formation and scion elongation were significantly inhibited while scion buds take in intact rootstock shoot, apparently a direct response of lateral buds to the release of apical dominance. The results agreed with previous studies (Hosoki *et al.* 1983; 1984; 1985; Kubota *et al.* 1983; Helmy 1992; Hanafy *et al.* 2012; Abdalla 2013) that garlic promotes sprouting of dormant buds in a diverse number of plant species, The enhancement of bud-take by garlic could be due to its content of chemical compounds that are essential for promotion of bud-take and subsequent growth of scion shoots. This speculation is parallel to the findings of others (Kojima 1982; Hosoki, *et al.* 1985; 1986) that garlic contains many sulfides including the volatile methyl disulfide which was found to be the best dormancy breaking chemical since it was effective without causing injury to plants over a wide spectrum of plant species (Hosoki *et al.* 1986; Hosoki and Kubara 1989; Kubota *et al.* 1999).

A comparison between the potential of the extract of plant tissues of these vegetable species as chemical compounds with growth regulators-like effect is beyond the scope of this study.

Chapter Five

5. References

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Table 1. Effect of different concentrations of benzylamino-purine (BAP) on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding.

BAP conc. (mg /l)	No. of leaves	Shoot length (cm)	bud-take (%)
0.00	9.00 c	11.12 a	10 c
100.00	12.00 bc	13.25 a	48 b
200.00	14.30 b	14.00 a	46 b
400.00	18.00 a	13.67 a	91 a
800.00	12.50 b	11.37 a	44 b

*Means in a column followed by same letter(s) are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Table 2. Effect of different concentrations of Sangral on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding.

Sangral conc.			
(mg /l)	No. of leaves	Shoot length	bud-take (%)
		(cm)	
400 mg/l BA	15.00c	17.00b	85a
0.00	13.6c	13.9c	50b
100.00	14.2c	14.5c	50b
200.00	26.2a	17.1b	75a
400.00	18.2b	20.5a	50b
800.00	6.4d	6.4d	25c

*Means in a column followed by same letter are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Table 3: Effect of combinations of 200 mg/l Sangral with or without topping on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding.

Treatment	number of leaves	shoot length (cm)	bud take %
Control	0.0c	0.0c	0.0c
Soaking without topping	35.4b	33.0b	80b
Soaking and topping	44.0a	43.0a	100a
Topping without soaking	30.6b	38.4b	50c

*Means in a column followed by same letter are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Table 4. Effect of different concentrations of stroby on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding.

Stroby			
conc. (mg /l)	No. of leaves	Shoot length(cm)	bud take(%)
0.00	-	-	-
50.00	10.00c	5.50c	25c
100.00	13.00b	8.00b	25c
150.00	15.00b	9.00a	50b
200.00	23.00a	10.40a	75a
250.00	8.00c	4.00c	25c
300.00	7.00c	4.50c	25c

*Means in a column followed by same letter are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Table 5: Effect of furadan, glyphosate and seven on the percentage of bud take , shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding.

Treatment (Conc. 50 mg/l)	number of leaves	shoot length (cm)	bud take %
0.0	4b	5c	10b
Furadan	7a	5c	30a
Glyphosate	0c	0c	0c
Seven	8a	5c	30a

*Means in a column followed by same letter are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Table 6: Effect of aqueous leaf extract of rocket on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding.

Conc. of Leaf extract of rocket (%)	number of leaves	shoot length (cm)	bud take %
0.0	4b	5b	10b
5	8a	8a	30a
10	10a	10a	50a
20	8a	8a	40a

*Means in a column followed by same letter are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Table 7. Effect of aqueous leaf extract of spinach on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding.

Conc. of leaf extract of spinach (%)	number of leaves	shoot length (cm)	bud take %
0.0	4b	5c	20b
5	10a	14a	50a
10	8aa	10ab	30ab
20	6ab	8b	30ab

*Means in a column followed by same letter(s) are not significantly different at P=0.05, according to Duncan Multiple Range Test.

Table 8. Effect of aqueous extract of garlic clove on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding

Conc. of garlic clove extract (%)	number of leaves	shoot length (cm)	bud take (%)
0.0	4c	5bc	10c
5	7a	8a	50a
10	6b	7ab	50a
20	5c	6b	20b

*Means in a column followed by same letter are not significantly different at P=0.05, according to Duncan Multiple Range Test.

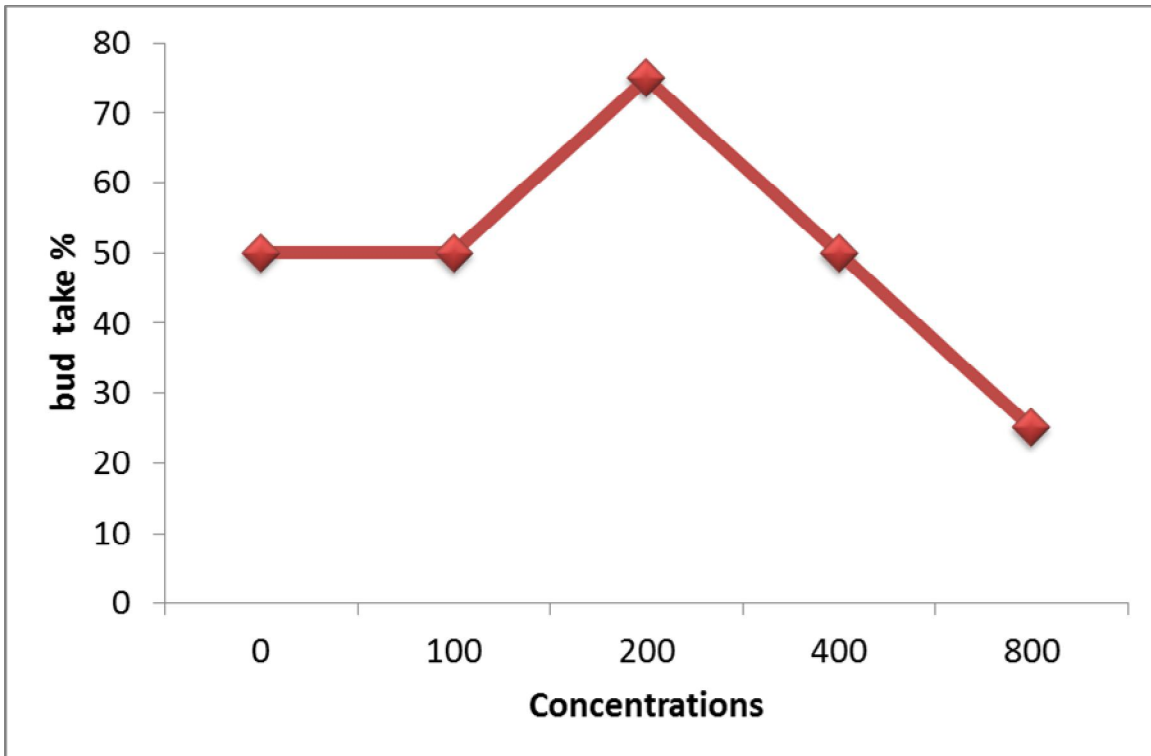


Fig.1. Effect of different concentrations of Sangral on scion bud-take of grapefruit. Data taken after 6 weeks from budding.

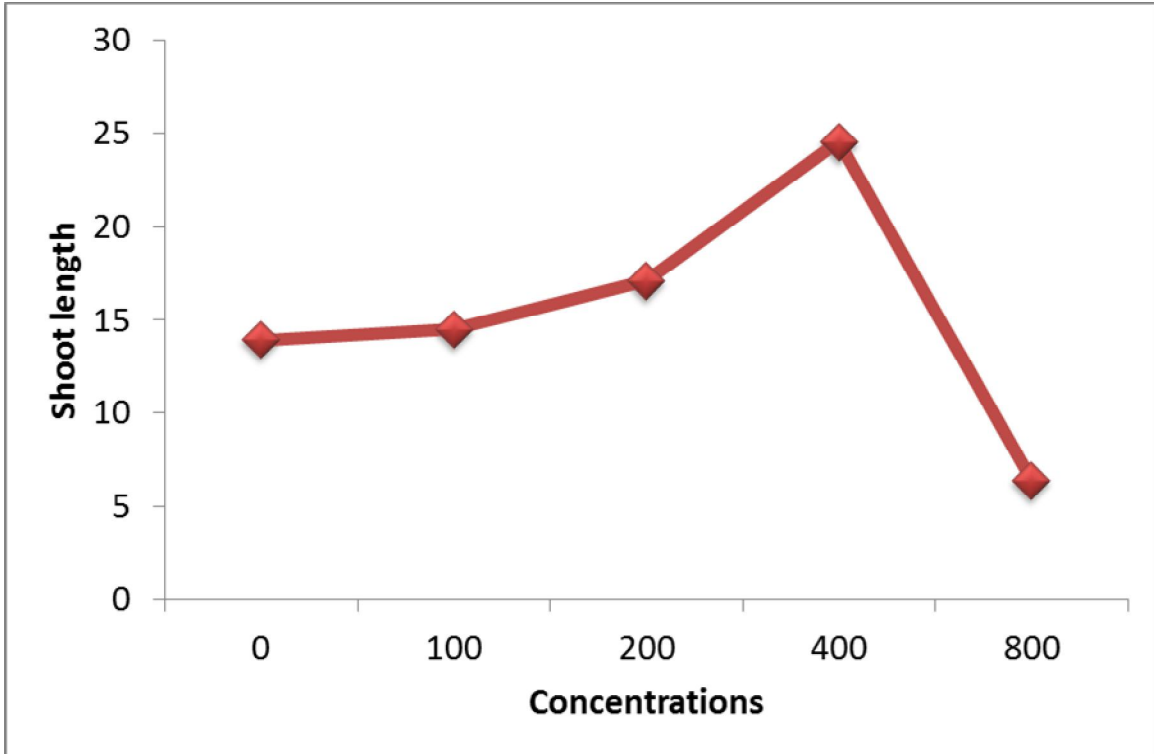


Fig.3. Effect of different concentrations of Sangral on scion shoot elongation. Data were taken after 6 weeks from budding.

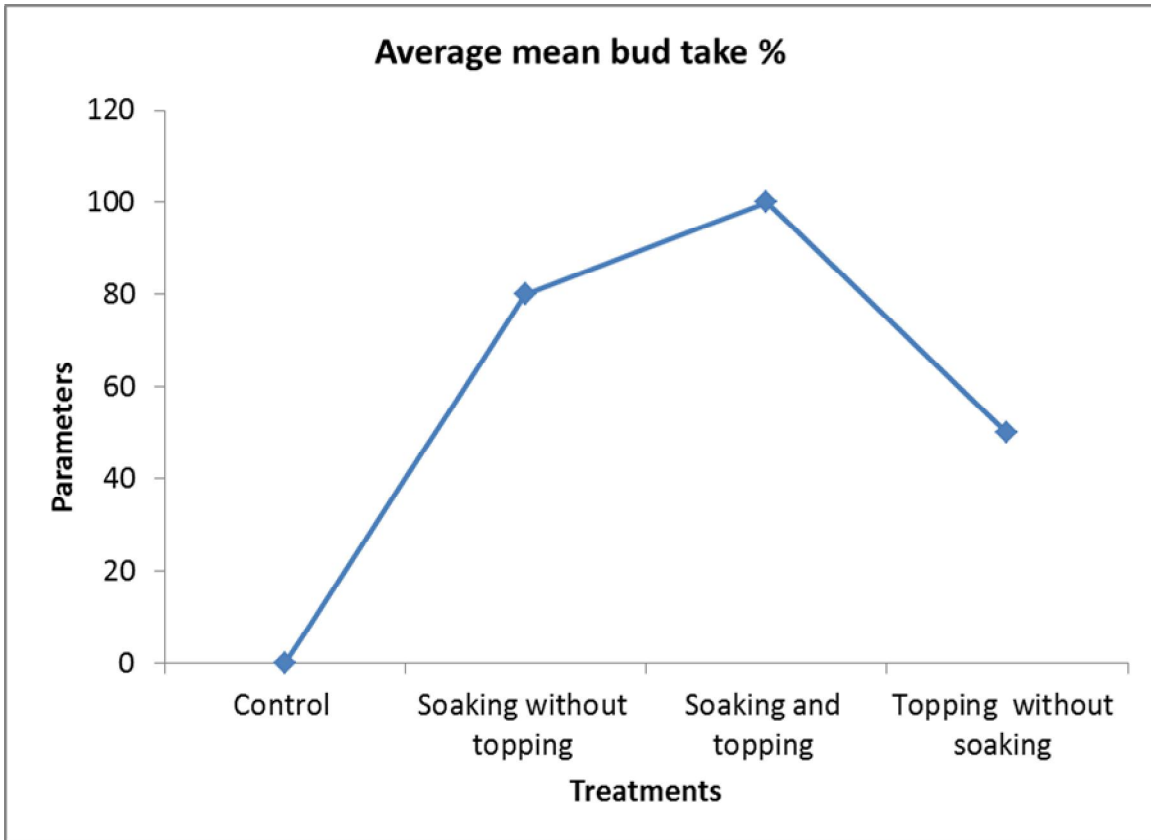


Fig.4 Effect of soaking/topping treatments on scion bud-take percentage of grapefruit. Data taken after weeks from budding.

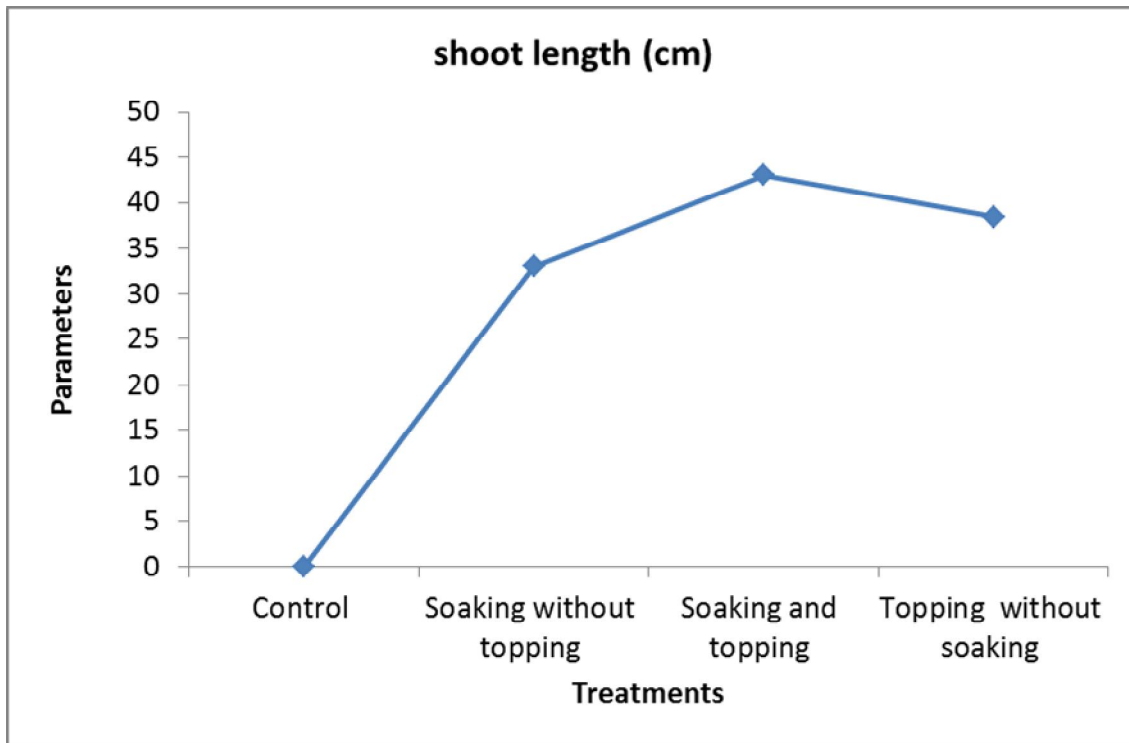
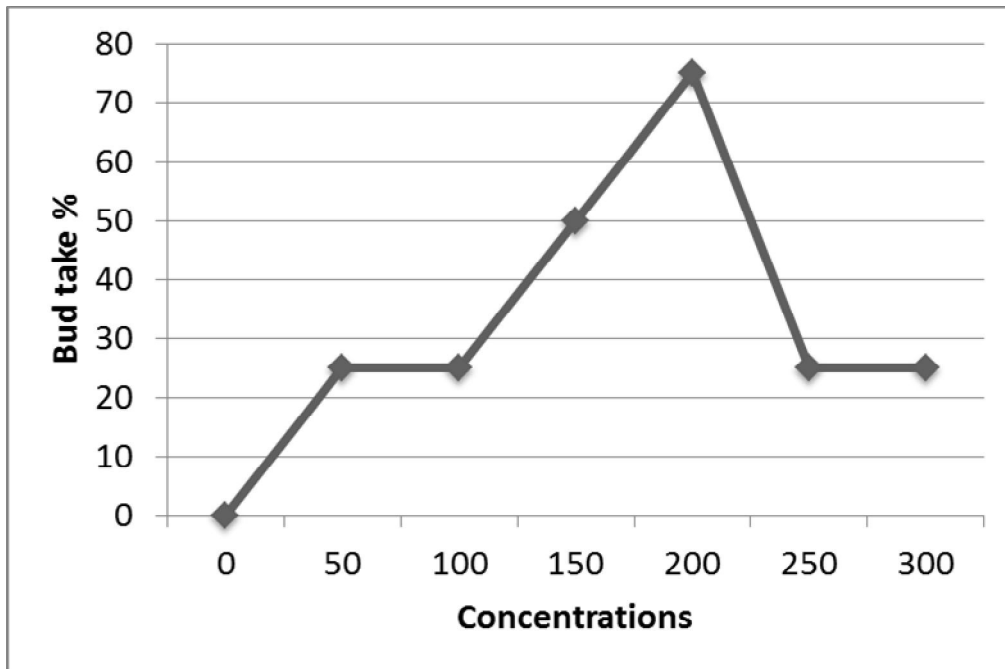


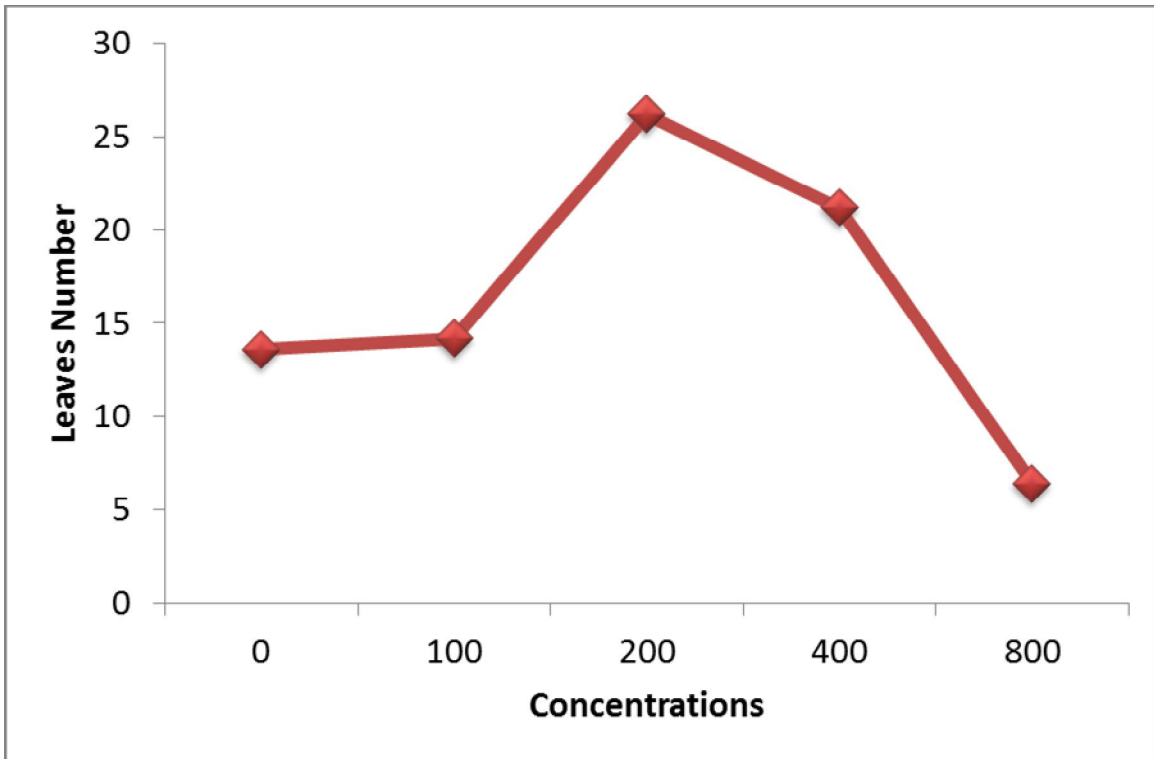
Fig. 5. Effect of soaking/topping treatments on elongation of scion shoot of grapefruit. Data taken after weeks from budding.

Chapter 6

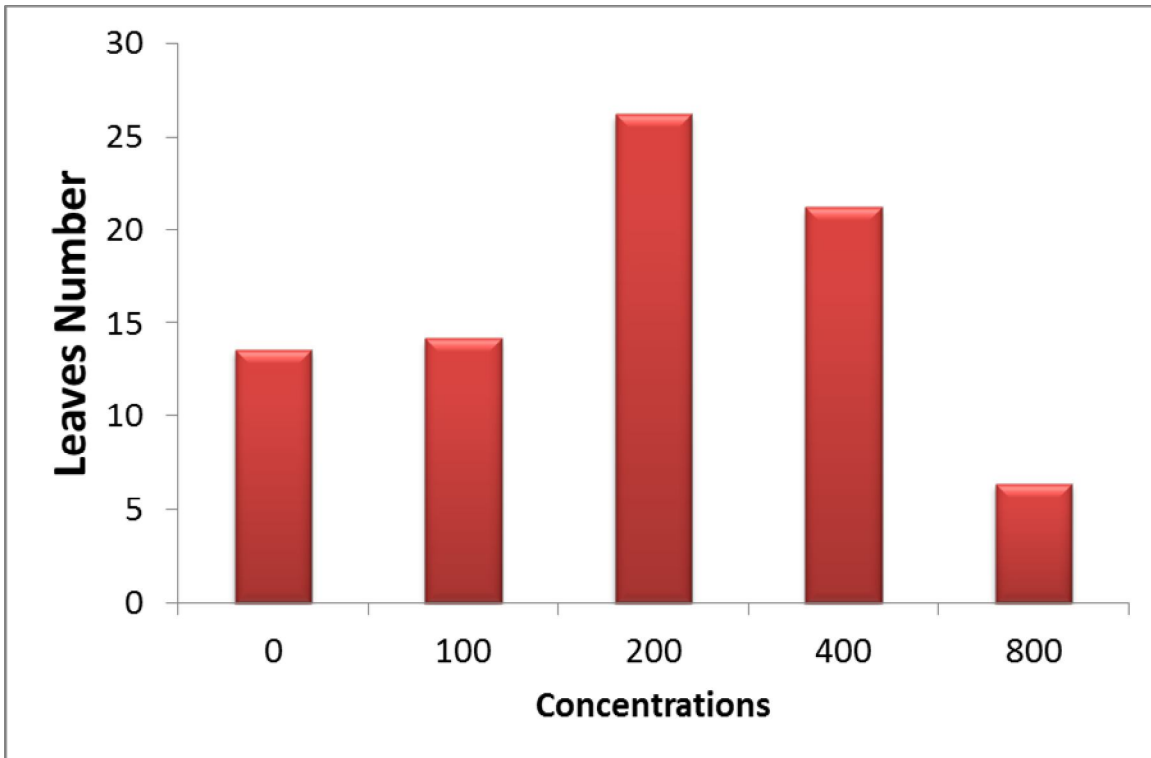
Appendices



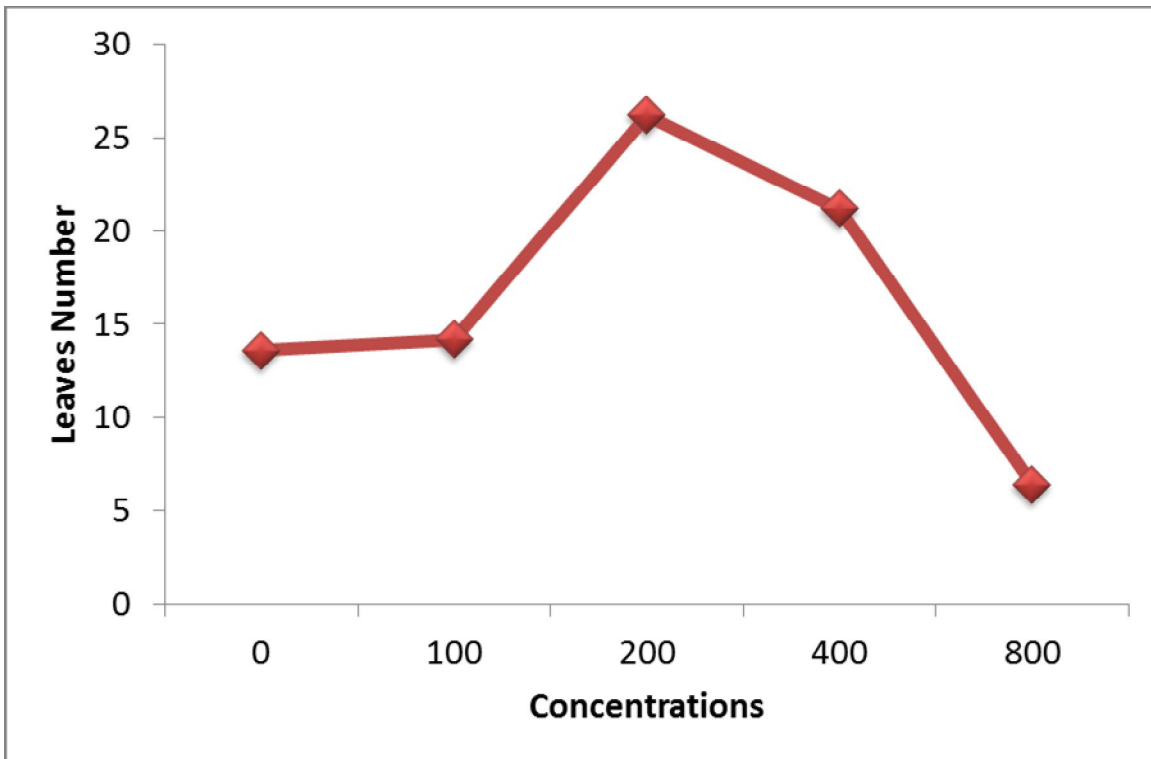
Appendix 1. Effect of different concentrations of stroby on percent scion bud-take. Data were taken after 6 weeks from budding.



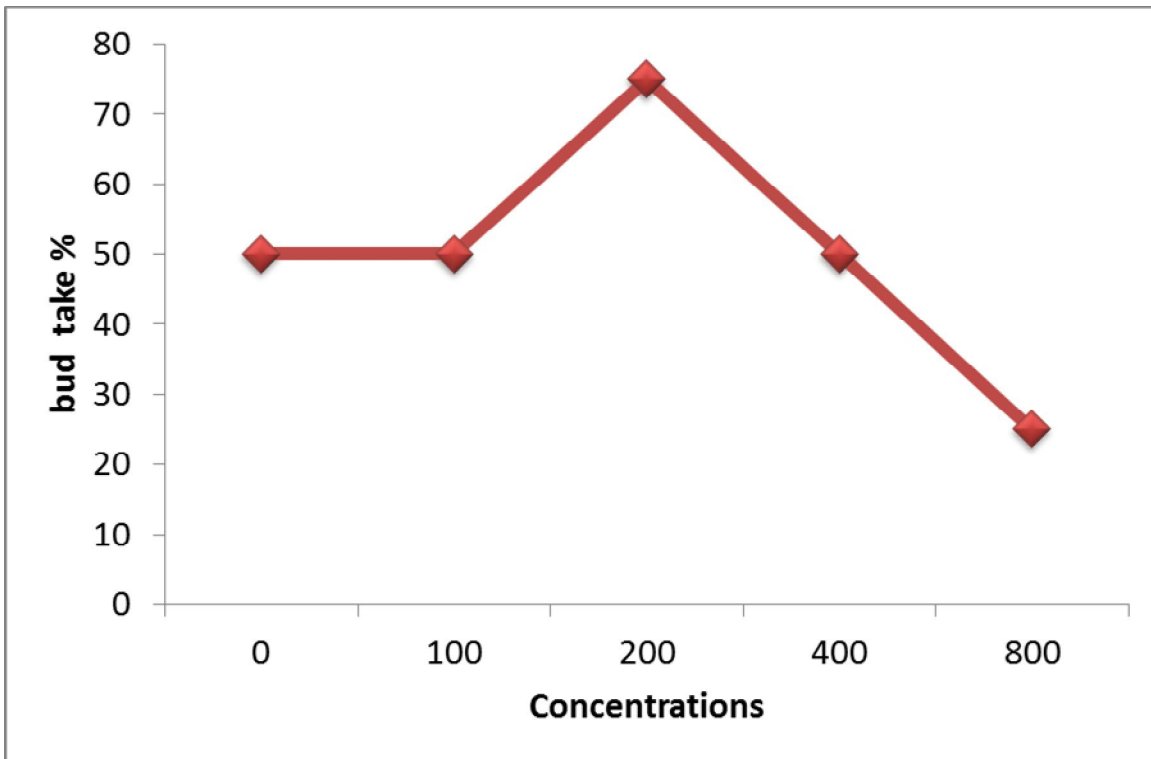
Appendix 2. Effect of different concentrations of Sangral on leaf number of grapefruit scion buds. Data taken were after 6 weeks from budding.



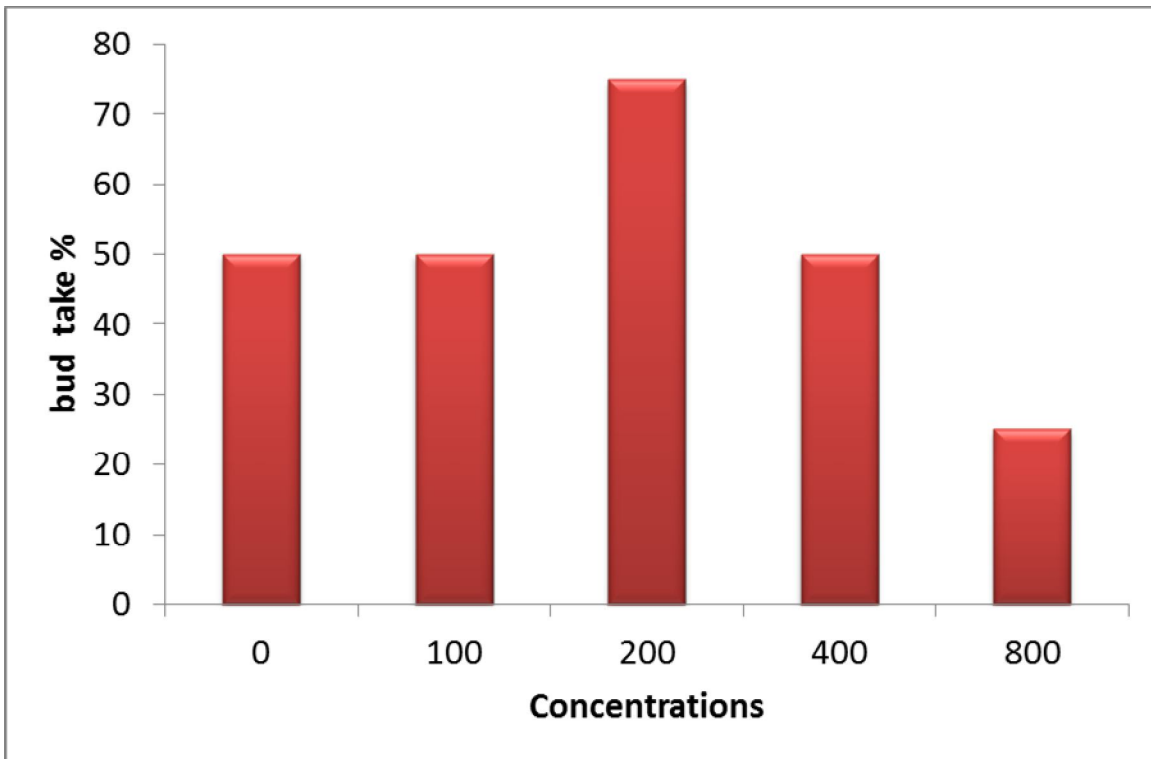
Appendix 3. Effect of different concentrations of Sangral on leaf number of scion shoot of grapefruit. Data taken after 6 weeks from budding.



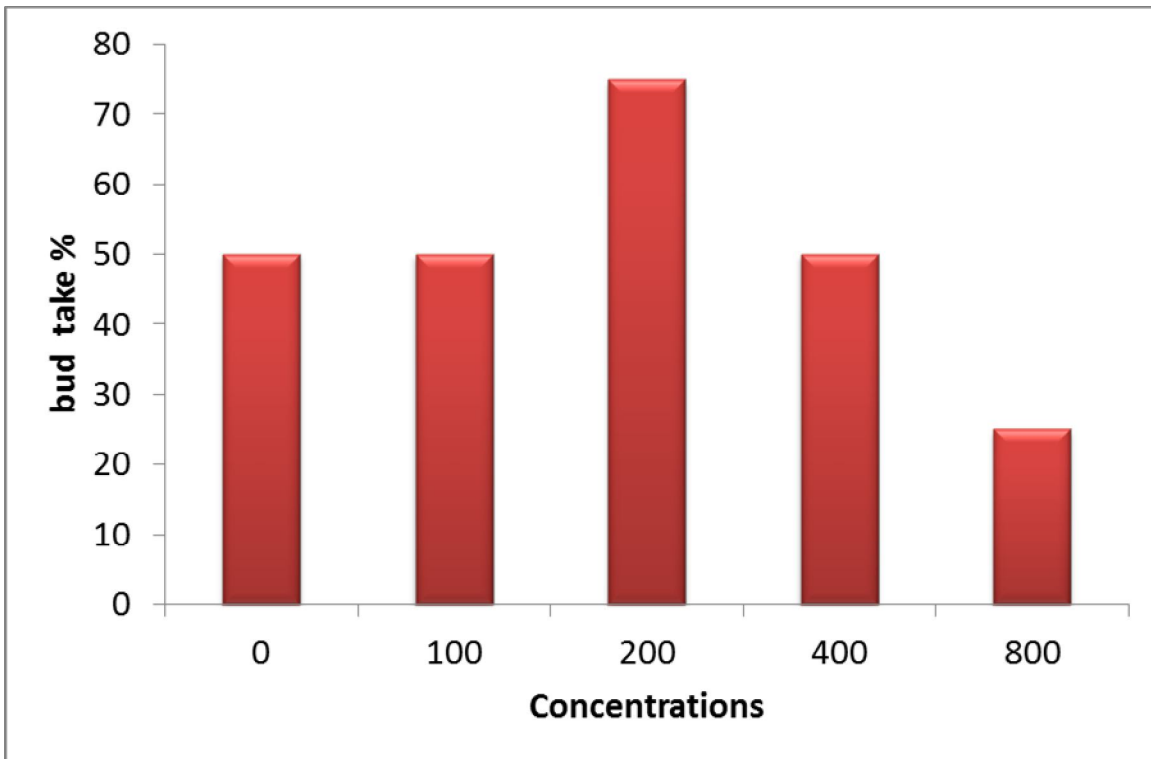
Appendix.4. Effect of different concentrations of Sangral on leaf number of leaves of grapefruit scion shoot. Data taken after weeks from budding.



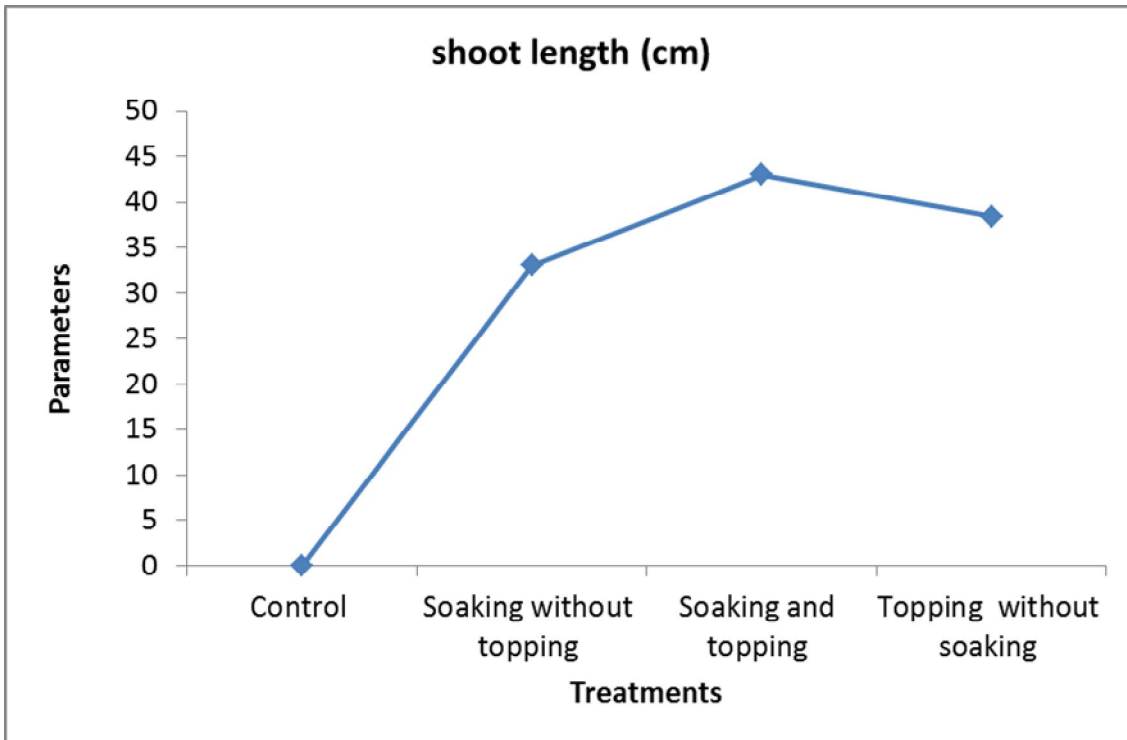
Appendix 5. Effect of different concentrations of Sangral on scion bud-take of grapefruit. Data were taken after 6 weeks from budding.



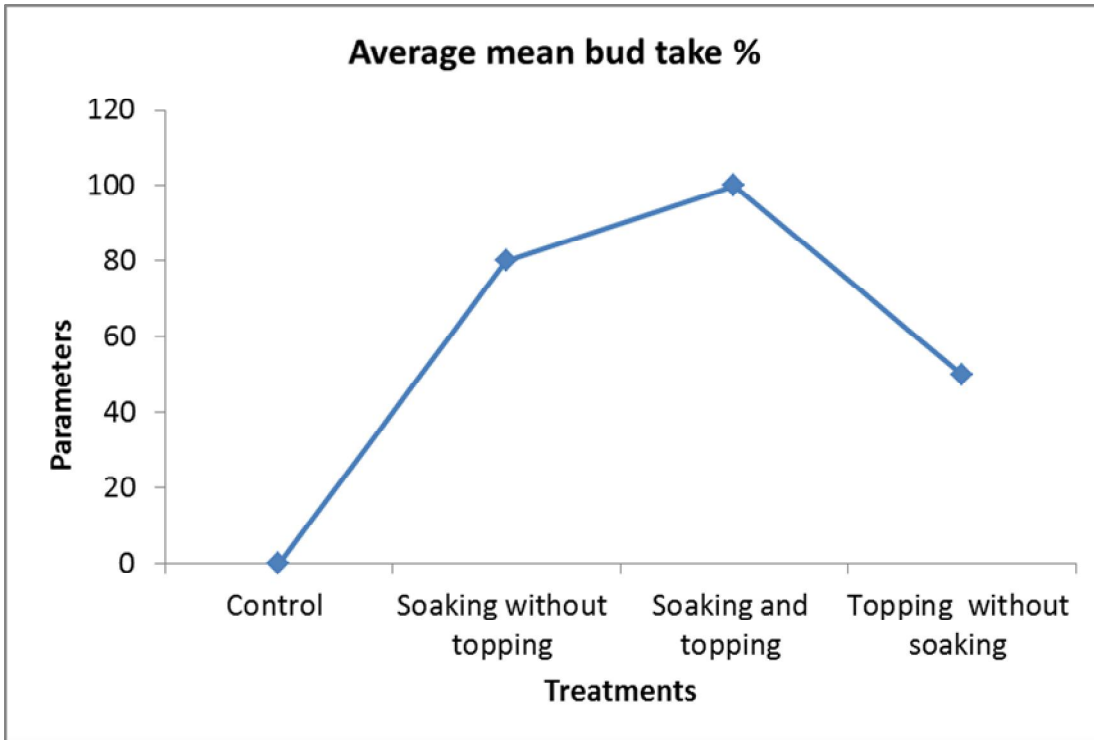
Appendix 6. Effect of different concentrations of Sangral on scion bud-take of grapefruit. Data were taken after 6 weeks from budding.



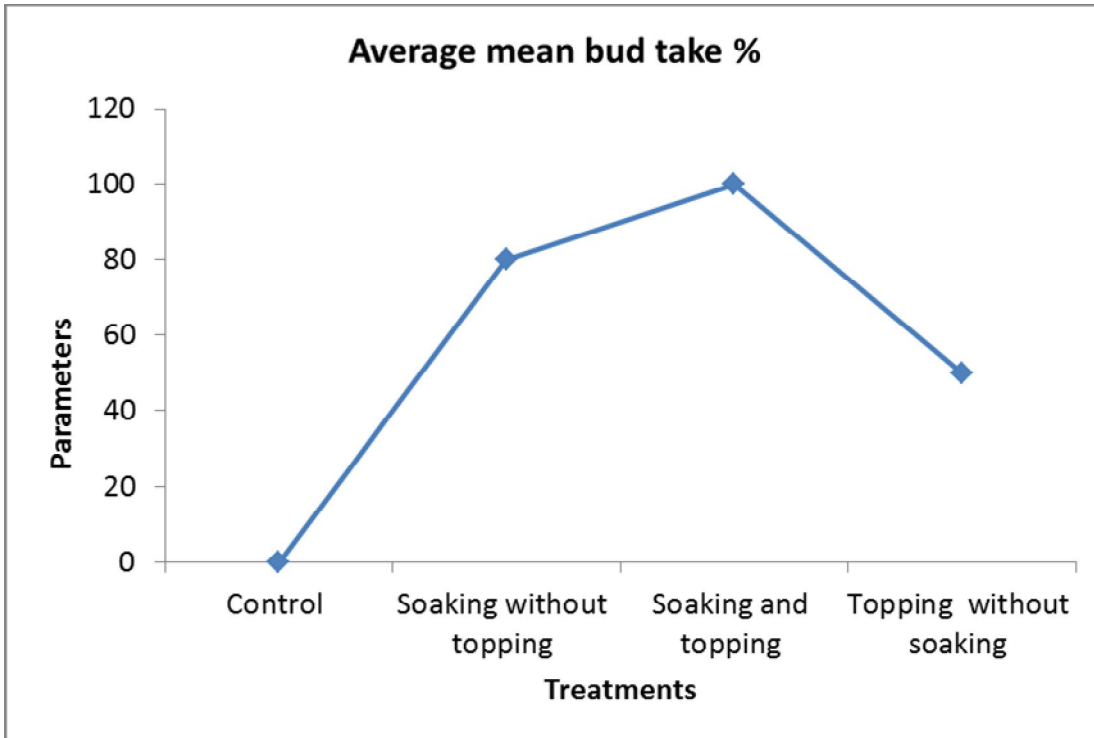
Appendix 7.. Effect of different concentrations of Sangral on scion bud-take of grapefruit. Data taken after weeks from budding.



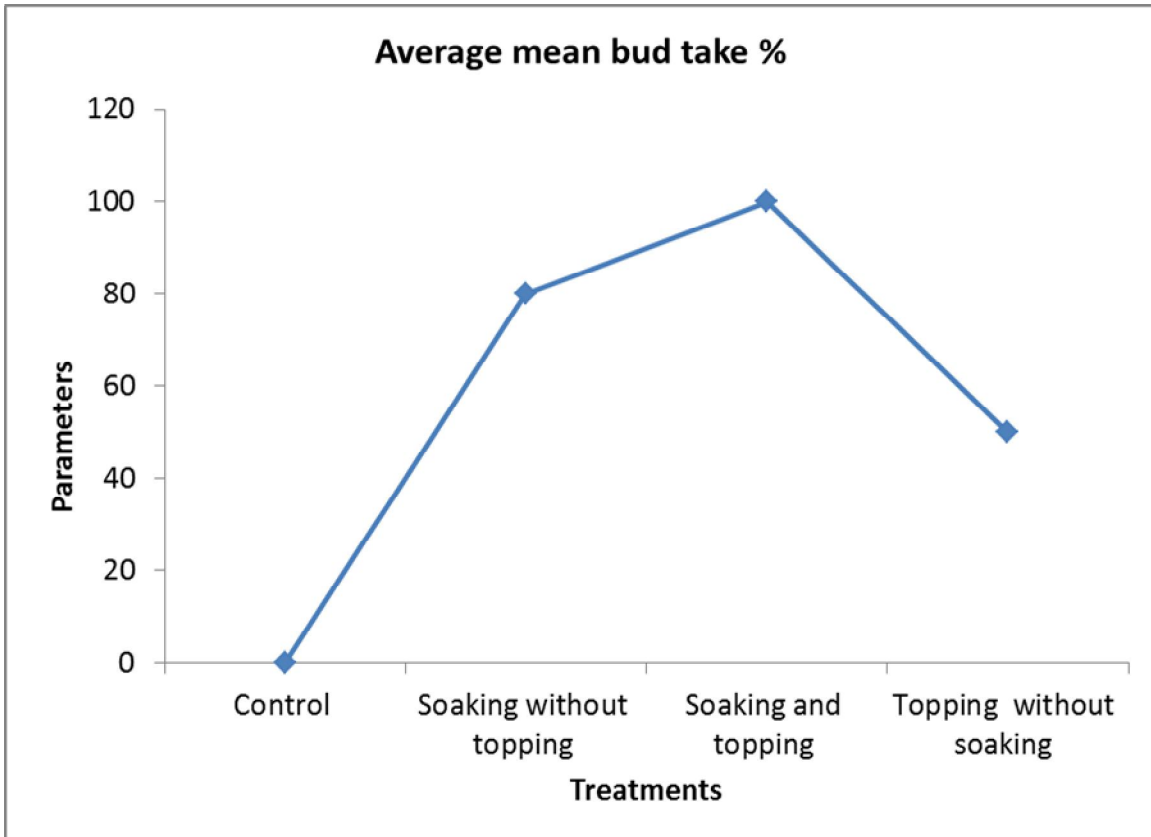
Appendix 8. Effect of soaking/topping treatments on scion shoot elongation of grapefruit. Data were taken after 6 weeks from budding.



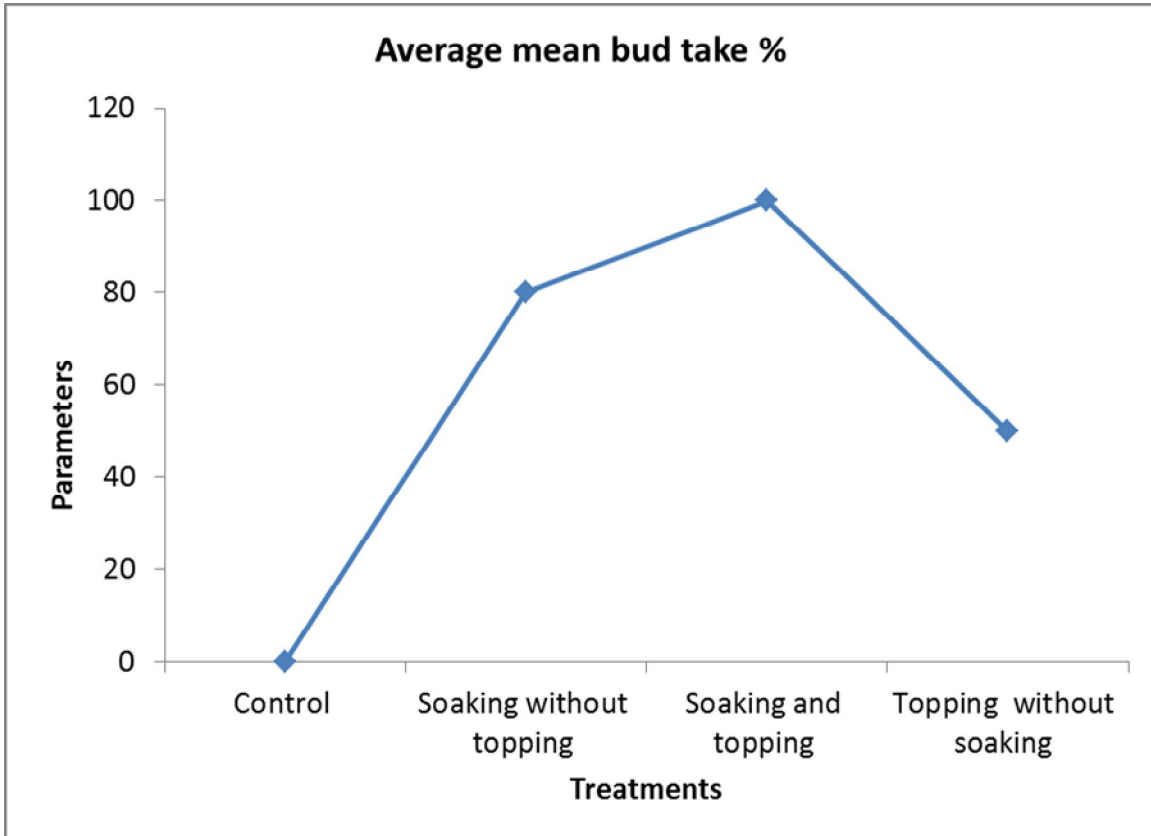
Appendix 9. Effect of soaking/topping treatments on scion bud-take percentage of grapefruit. Data were taken after 6 weeks from budding.



Appendix 10. Effect of soaking/topping treatments on scion bud-take. Data taken 6 weeks after budding.

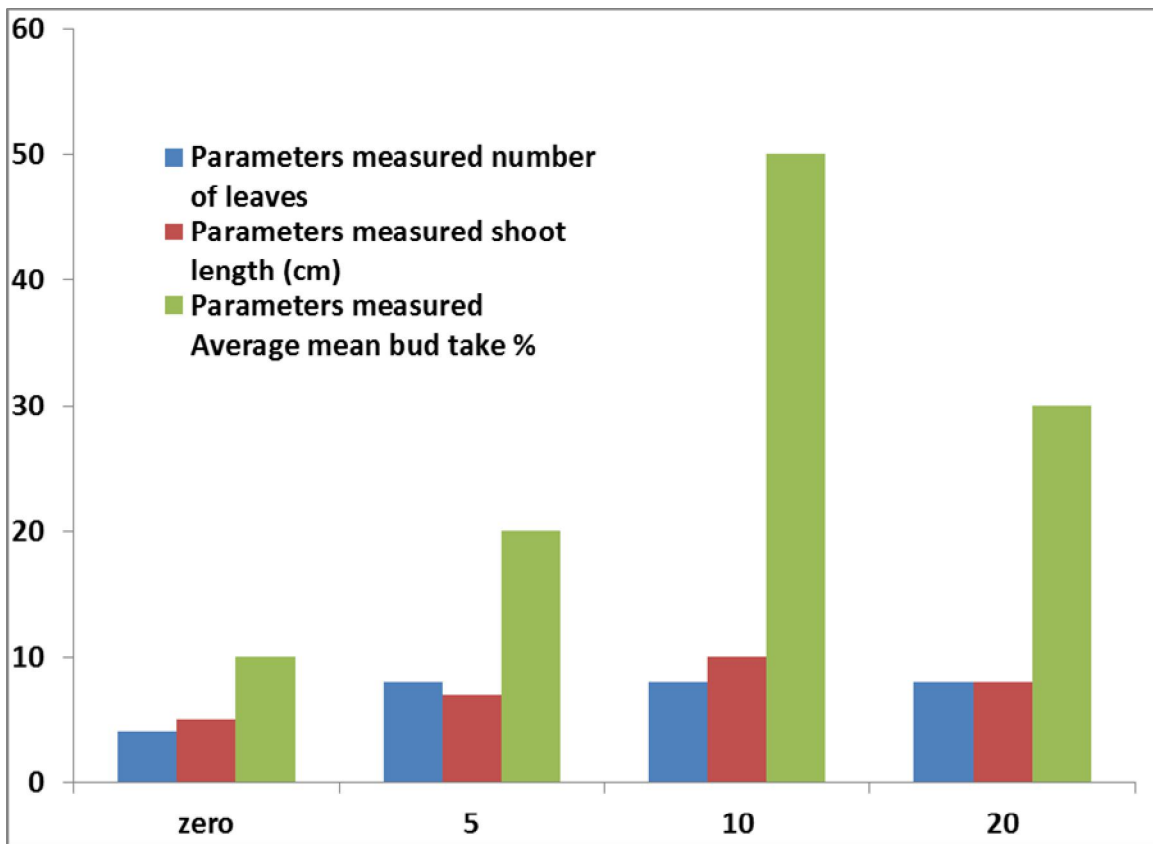


Appendix 11. Effect of soaking/topping treatments on scion bud-take of grapefruit. Data were taken after 6 weeks from budding.

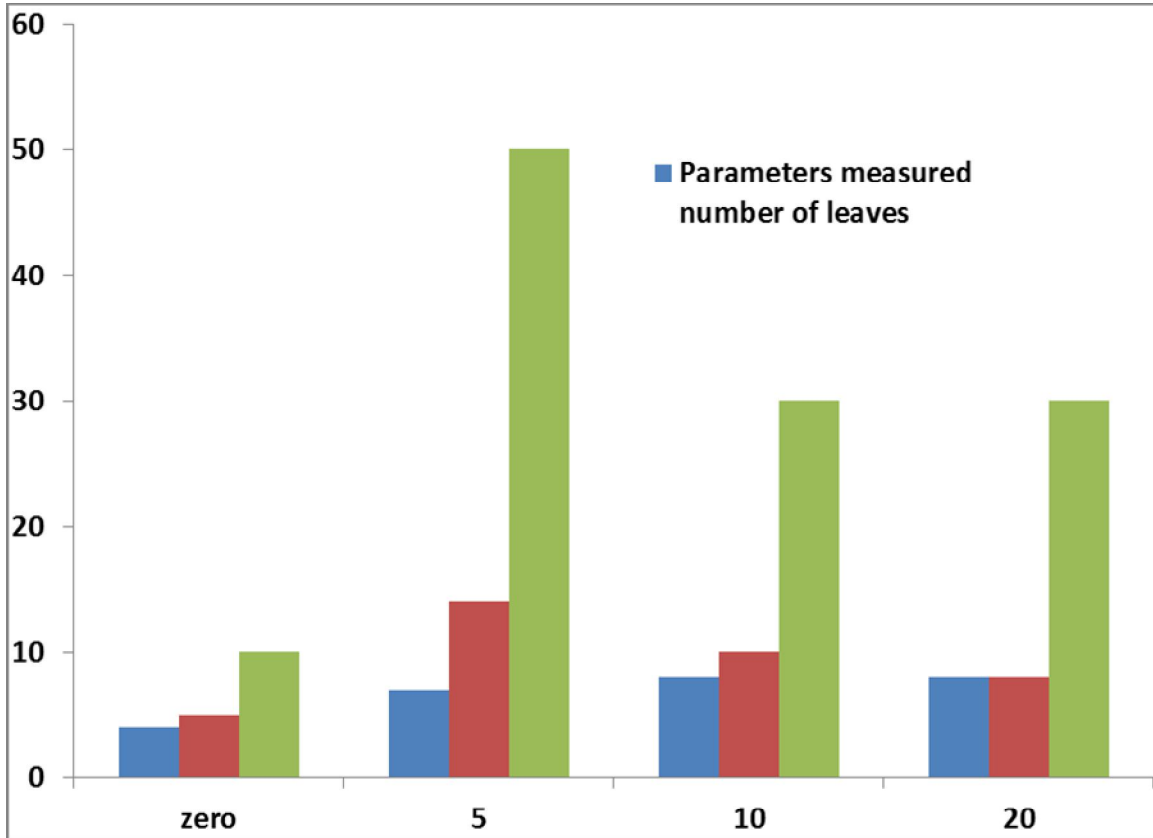


Appendix 12. Effect of soaking/topping treatments on scion bud-take of grapefruit. Data taken after 6 weeks from budding.

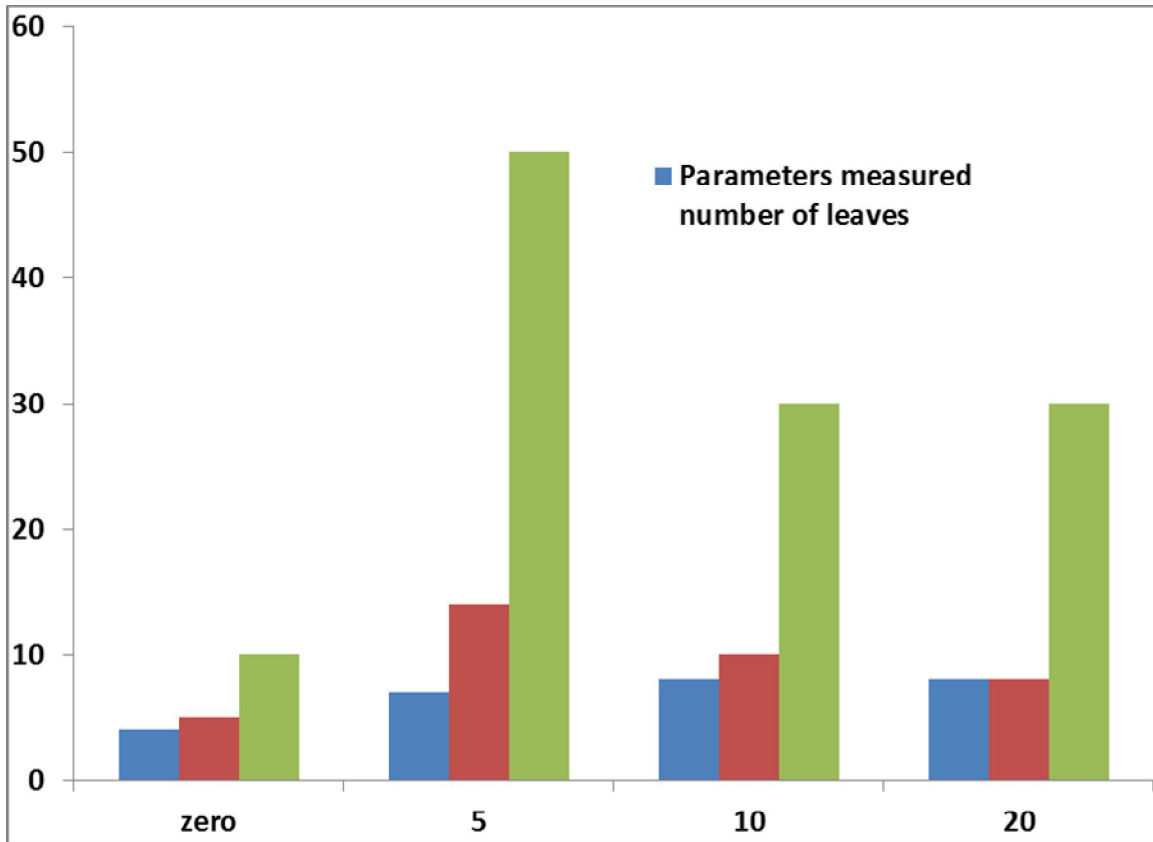
Effect of aqueous leaf extract of rocket on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding



Effect of aqueous leaf extract of spinach on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding



Effect of aqueous extract of garlic clove on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding



: Effect of furadan, glyphosate and seven on the percentage of bud take, shoot elongation and number of leaves of grafted grapefruit scions, 6 weeks from budding

