A REVIEW ON SWEET ORANGE (*CITRUS SINENSIS* L Osbeck): HEALTH, DISEASES AND MANAGEMENT

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Abstract

Citrus sinensis (L. Osbeck) or sweet orange originated from south East Asia, but is consumed all over the world as an excellent source of vitamin C, a powerful natural antioxidant that builds the body immune system. Important phytochemicals like liminoids, synephrine, hesperidin flavonoid, polyphenols, pectin, and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium are also present. These biologically active compounds prevent arteriosclerosis, cancer, kidney stones, stomach ulcers and reduction in cholesterol level and high blood which promote human health. However, the impact of diverse diseases caused fungi (sweet orange scab, citrus black spot, powdery mildew) bacteria (pierce's disease, citrus variegated chlorosis ctrus greening or huanglgbin, citrus canker), viruses (citrus tristeza, citrus ringspot, etc) and a complex of nematodes limits sweet orange production, nutritional value and market qualities. These diseases can be controlled through chemical treatment of fruits, use of biological control agents, proper packaging and storage facilities and other disease management practices to reduce postharvest damages. Considering its health benefits, there is need for public awareness on the importance of sweet orange, especially in the rural areas as the fruit is relatively cheap and common almost all year round.

{Citation: E. Etebu, A. B. Nwauzoma. A review on sweet orange (*Citrus Sinensis* Osbeck): health, diseases, and management. American Journal of Research Communication, 2014, 2(2): 33-70} www.usa-journals.com, ISSN: 2325-4076.

1. Introduction

The sub-genus *Citrus* (Swingle), family Rutaceae and subfamily *Aurantioideae* is of three types: *Citrus, Fortunella (Kumquat)* and *Poncirus Trifoliata*. There are three genera and eighteen defined species, but other natural mutations exist resulting to numerous hybrids which are widely spread throughout the world (Guo and Deng, 2001). Citrus is widely grown in Nigeria and many other tropical and subtropical regions (Piccinelli *et al*, 2008). In terms of volume in production, citrus ranks after banana as the world second fruit crop with more than 108 million tons (FAO Statistics 2006). Sweet orange (*Citrus sinensis* L. Osbeck) commonly called orange is a member of this family and a major source of vitamins, especially vitamin C, sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium (Angew, 2007). Economically, oranges are important fruit crops, with an estimated 60 million metric tonnes produced worldwide as at 2005 for a total value of 9 billion dollars. Of this total, half came from Brazil and the United States of America (Goudeau et al, 2008; Bernardi et al 2010). The global citrus acreage according to FAO statistics in 2009 was nine million hectares with production put at 122.3 million tons, ranking sweet oranges first among all the fruit crops (Xu et al, 2013**)**.

Oranges probably originated from south East Asia, and were cultivated in China by 2500 BC (Nicolosi *et al*, 2008), where it was referred to as ‘’Chinese’’ apple (Ehler, 2011). Today, it is grown almost all over the world as a source of food for humans because of its high nutritional values, source of vitamins and other uses. Propagation through seed is associated with problems like poor pollen production, self-incompatibility and muscular embryo (Mortton, 1987). Therefore budding onto appropriate rootstocks is the common means of assuring the reproduction of cultures of known quality. Arsingrin (2011), posited the use of tissue culture to produce good quality plantlets, but are highly susceptible to root diseases, and this limits production (Katzer *et al*, 1999). This paper reviews the importance of

oranges to human health, the impact of diseases on its production and control measures that should be adopted to realise its maximum benefits in the promotion of human health.

2. Botany

Sweet orange (*Citrus sinensis* L. Osbeck) (to distinguish it from closely related species like sour orange, *C. aurantium C. reticulata* and mandarin orange), is a small evergreen tree 7.5 m high and in some cases up to 15 m. It originated from southern China where it has been cultivated for many years, but is today grown commercially worldwide in tropical, semi-tropical and some warm temperate regions to become the most widely planted fruit tree in the world (Nicolosi *et al.*, 2000; Ehler, 2011). Orange produces leathery and evergreen leaves of different shapes, ranging from elliptical to oblong to oval, 6.5-15 cm long and 2.5-9.5 cm wide, often bearing narrow wings on the petioles. It bears fragrant white flowers either singly or in whorls of 6, about 5 cm wide, with 5 petals and 20-25 yellow stamens. The small, white or purple scented hermaphroditic flowers produce nectar for pollination by insects. The fruit, which may be globose to oval is 6.5 to 9.5 cm wide, and ripens to orange or yellow. Anatomically, the fruit consists of two distinct regions (Fig. 1): the pericarp also called the peel, skin or rind, and the endocarp, or pulp and juice sacs. The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell. The quantity of wax is dependent on the variety, climatic conditions and growth rate. A plethora of microflora consisting mainly of fungus and bacteria are present on the skin and more copious in damp climates. This justifies the need for appropriate washing of the fruit before eating or proceeding to extract juice and essential oils. The pericarp consists of the outer flavedo, or epicarp largely made of parenchymatous cells and cuticle. Embedded oil glands create terpenoid aromatic compounds such as valencene, limonene, and alpha/beta sinsenal (Goudeau et al, 2008; Sharon-Asa, et al, 2003). Beneath

the epidermis is the flavedo, with its characteric yellow, green or orange colour. The flavedo is very fine and fragile containing oliferous vesicles on the inside which can be collected by scraping on the flavedo layer. The flavedo is a generally colorless, spongy inner layer of mesophyll that changes character and thickness throughout fruit development, properties that determine ease of peeling (Webber, 1989). The albedo, or mesocarp lying beneath the flavedo consists of tubular-like cells joined together to constitute the tissue mass compressed into the intercellular area. The albedo is rich in flavonoids, which if transferred to the juice imparts a bitter taste.

The flesh or pulp of the fruit is typically juicy and sweet, divided into 10 to 14 segments (although there are seedless varieties) and ranges in color from yellow to orange to red. The ripe fruit is classified as a hesperidium which is a type of berry with multiple seeds and is fleshy. Fleshy juice sacs accumulate sugars, organic acids and large amount of water, causing difficulties in the extraction of nucleic acids and proteins. The endocarp and the *carpels* in which the juice containing vesicles are found and which from a synthetic biology point of view should be considered as the liquid released by the cytoplasm and by the vacuoles in the vesicles' internal cells. A spongy tissue similar to that of the albedo constitutes the the greater part of the fruit.

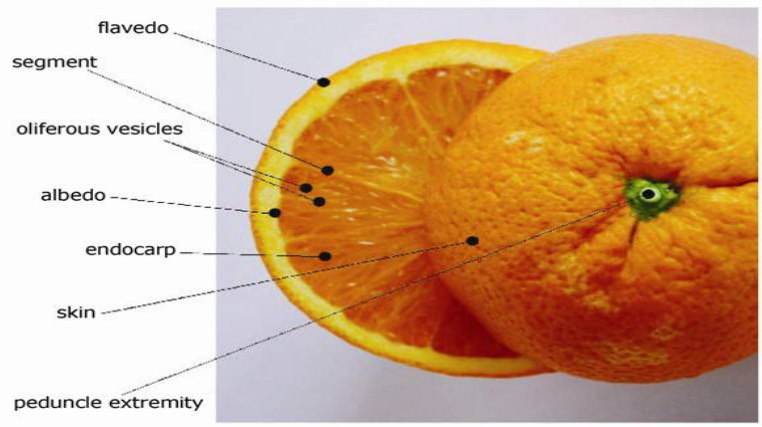

Fig. 1: Structure of Citrus fruit. (Goudeau *et al*, 2008).

The centre of origin of most citrus cultivars is perhaps unknown, but the ancient relatives of citrus are native to China, the Southeast Asia, the Malay Archipelago, New Caledonia and Australia (Atta et al, 2012). The genetic origin of sweet orange is not clear, although it is believed to be derived from the interspecific hybridization of some primitive citrus species (Xu et al, 2013). Hence, its many cultivars are grouped into four major categories or varieties according to origin (Mediterranean and Spanish oranges) and taste. The navel oranges named because of its shape, (segmented skin looks like a human navel) originated as a single mutation in a Brazilian monastery in 1820 (Ehsani *et al.* 2007). Every single navel orange originates from it because they do not produce seeds and propagation is through graft cuttings. The popularity of these oranges continued to grow resulting to its success across the world. The Valencia orange was named by William Wolfkill, an agronomist after the Spanish city of Valencia known for its orange production (Ehsani *et al.*, 2007). Blood oranges named from their crimson flesh range from sweet to tart depending on the fruit. There are three types of blood oranges: moro, tarocco and sanguinello, each differing in taste, size and origin. Blood oranges are used to make sorbets, marmalade and beverages.

The Seville orange is a type of bitter orange, primarily grown around the Mediterranean. Many countries have claimed origin to the Seville orange. There are reports of this fruit in ancient Fuji, Guam and Sicily. However, it is not known it was first cultivated in Seville, Spain. Spaniards brought this orange over to St. Augustine, Florida,\ where settlers cultivated it and sent it to England. It is used to make marmalade, liquor and wine (Ehsani *et al.*, 2007).

The Chinotto bitter orange is mainly cultivated in Italy as one of the main ingredients in many Italian wines and soft drinks. They possess unusual leaves compared to others, because they are from myrtle-leafed orange trees (Ehsani *et al.*, 2007). Peel quality is

of particular economic importance in orange production because factors such as shape, color, aroma, texture, and ease of peeling influence consumer demand. For example, the Clementine Mandarin has replaced navel varieties largely due to convenient size and ease of peeling (U.S. Citrus Genomics Steering Committee, 2003).

3. Oranges, human health and nutrition

The human diet contains important micronutrients namely vitamins C and E, carotenoids and flavonoids, essential for maintenance of human health. Multiple dietary sources of these compounds are present virtually in all plant material (Di Majo et al, 2005). The nutritional importance of foods is due to the presence of these functional food ingredients and antioxidant nutraceuticals or phytochemicals. Phytochemicals are present in edible fruits and vegetables and when eaten potentially modulate human metabolism in a favourable manner, thereby prevent chronic and degenerative diseases (Tripoli et al, 2007). Increase in fruits and vegetables consumption protect against degenerative pathologies such as cancer and therosclerosis (Keys, 1995); as epidemiological surveys had shown an inverse relationship between dietary flavonoid intake from citrus and cardiovascular diseases (Hertog *et al*., 1993; Di Majo et al, 2005). Citrus fruits are the main source of important phytochemical nutrients and for long have been valued for their wholesome nutritious and antioxidant properties. It is scientifically proven that oranges being rich in vitamins and minerals have many health benefits. Moreover, it is now appreciated that other biologically active, non-nutrient compounds found in citrus fruits such as phytochemical antioxidants, soluble and insoluble dietary fibres are known to be helpful in reducing the risk for cancers, many chronic diseases like arthritis, obesity and coronary heart diseases (Crowell, 1999).

3.1 **As antioxidant**: A high quality orange is one that is mature with good color intensity uniformly distributed over the surface. Such oranges must be firm with a fairly smooth texture and shape that is characteristic of the variety, free from decay, defects and other blemishes. The biological activity and the healthy effects of citrus flavonoids as antioxidants have been reported (Tripoli et al, 2007). These group of pigments as found in plants and together with anthocyanin play a role in flower and fruit colouration. Also, they are present in dietary fruits and vegetables (Macheix et al., 1990), and exercise their antioxidant activity in several ways, including the activities of metal chelation (Bombardelli and Morazzoni, 1993). Studies indicate that flavonoids are excellent radical-scavengers of the hydroxyl radical (Cillard and Cillard, 1988; Darmon et al., 1990), due to their to ability to inhibit the hydroxyl radical and donate hydrogen atom (Di Majo et al., 2005, Tripoli et al. 2007). Oranges as excellent source of vitamin C, contain powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases (Ejaz et al., 2006). Consumption of foods rich in vitamin C improves body immunity against infectious agents and scavenging harmful, pro-inflammatory free radicals from the blood. Sweet orange contains a variety of phytochemicals like hesperetin and narigenin. Naringenin has a bioactive effect on human health as antioxidant, free radical scavenger, anti-inflammatory, and immune system modulator.

3.2 **Anti-inflamation**: Citrus flavonoids contain compounds with anti-inflammatory activity due to the presence of regulatory enzymes (protein kinase C, phosphodiesterase, phospholipase, lipoxygenase, and cyclooxygenase) that control the formation of the biological mediators, responsible for the activation of endothelial cells and specialized cells involved in inflammation. Flavonoid inhibition of the immune and inflammation responses can be associated with their inhibition of these enzymes (Tripoli et al 2007). Indeed, citrus

flavonoids are able to inhibit the kinases and phosphodiesterases essential for cellular signal transduction and activation. They also affect the activation of a number of cells involved in the immune response, including T and B lymphocytes (Manthey et al., 2001). Citrus flavonoids also prevent atherosclerosis, inhibiting the formation of atheroma (Hertog et al., 1993). Tripoli et al, (2007) reported that hesperidin obtained from citrus cultures may have a potential therapeutical use as a mild anti-inflammatory agent, being also useful as a precursor of new flavonoids endowed with this activity (Da Silva et al., 1994). Studies using mouse macrophage cells also show that hesperidin has an inhibitory effect on lipopolysaccharide (LPS)-induced over expression of cyclooxygenase-2, inducible nitric oxide synthase (iNOS), over-production of prostaglandin E2 and nitric oxide (NO) (Sakata et al., 2003).

3.3 **Anti-Cancer and anti-Arteriosclerosis**: Citrus flavonoids can prevent câncer through selective cytotoxicity, antiproliferative actions and apoptosis (Elangovan et al, 1994; Hirano et al., 1994). Flavonoids are antimutagenic, thus protects the DNA from damage by their ability to absorb ultraviolet light (Stapleton and Walbot, 1994). They neutralize free radicals that promote mutations when they are generated near DNA. This hás been shown in mice body irradiated with c-ray (Shimoi et al., 1994). Flavonoids can also protect the DNA by interacting directly with the tumoral agents, as in the induced chromosomal aberrations by bleomycin (Heo et al., 1994). The inhibitory effect of citrus flavonoids on tumoral development and cell proliferation by rat malignant cells, in cardiac and hepatic tissue of syngenetic rats have been reported (Bracke et al., 1989). The ability to function as such by citrus flavonoids are based on cell mobility inhibition (Bracke et al., 1989, 1991). Oranges are also rich in iron, chlorine, manganese, zinc, sodium, phosphorous, iodine, calcium, folic acid, potassium, pectin, beta-carotene and amino acids and fibre. A single orange is said to have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory,

blood clot inhibiting and antioxidant properties. All these properties help to promote overall health (Cha et al. 2001).

3.4 **Anti-Obesity**: Sweet oranges contain low calories and no saturated fats or cholesterol, but is rich in dietary fibre, pectin which is very effective in persons with obesity. Pectin as bulk laxative protects the mucous membrane from exposure to toxic substances, as well as by binding to cancer causing chemicals in the colon. Pectin has also been shown to reduce blood cholesterol levels by decreasing its re-absorption in the colon by binding to bile acids in the colon (Walton *et al.* 1945). Orange peels contain the alkaloid synephrine, which reduces the production of cholesterol in the liver. The antioxidant elements in oranges combat oxidative stress that oxidizes the LDL (low-density lipoprotein) in the blood.

3.5 **Wholesome health**: Oranges also contain very good amount of vitamin A, and other flavonoid antioxidants such as alpha and beta carotenes, beta-cryptoxanthin, zeaxanthin and lutein, compounds that have antioxidant properties. Vitamin A is rnecessary for maintaining healthy mucus membranes, skin and essential for vision. It is also a very good source of B-complex vitamins such as thiamin, pyridoxine and folates. These vitamins are essential in the sense that body requires them from external sources to replenish. Orange fruit also contains a very good amount of minerals like potassium and calcium. Potassium in an important component of cell and body fluids helps control heart rate and blood pressure. Vitamin A also required for maintaining healthy mucus membranes and skin and is also essential for vision. Consumption of natural fruits rich in flavonoids helps body to protect from lung and oral cervical cancers. Orange fruit also contains a very good amount of minerals like potassium and calcium (Table 1). Potassium is an important component of cell and body fluids and helps to control heart rate and blood pressure. The alkaline properties in the orange stimulate

the digestive juices, thus, reliving constipation. Regular intake of orange juice reduces the chances in the formation calcium oxalate which causes kidney stones. Polyphenols present in oranges prevents viral infections. Oranges protect the skin from damage caused by free radicals, thereby helping you look young and keeps the skin fresh and glowing (Tsuda *et al*, 2004).

Oranges can be processed into juice, which can be consumed directly or further processed into concentrate, both used in numerous soda and cocktail drinks, punches, orangeades, and liqueurs (although many orange liqueurs are made from sour, rather than sweet, oranges, or from a combination). Orange fruits and peels are used in numerous desserts, jams and marmalades, candied peels, as well as cookies, cakes, and candies. Oil derived from orange peels, as well as flowers, leaves, and twigs is used as an essential oil in perfumes; orange seed oil may also be used in cooking or as a component in plastics.

4. Diseases of *Citrus Sinensis*

Sweet oranges is susceptible to a large number of diseases that can cause severe economic losses.

4.1 **Bacterial diseases**

4.1.1 **Pierce's disease (PD):** The disease is caused by *Xylella fastidiosa,* a xylem-limited bacterium, transmitted by sharp-shooter insects, contaminated budwood and natural root-grafts (Redak et al., 2004). *X. fastidiosa* also causes citrus variegated chlorosis (CVC) in plants like like alfalfa, peach, plum, almond, elm, coffee, sycamore, oak, maple and pear among other hosts (Chatterjee et al, 2008) The insect vectors contact the bacterium through feeding on the xylem of infected plants and transmit same to healthy plants. Disease symptom first begins as drying or "scorching" of leaves which become slightly yellowish

(chlorotic) along the margins before drying out, or the outer leaf may dry suddenly while still green. Typically, the leaf dries progressively over a period of days to weeks, leaving a series of concentric zones of discolored and dead tissue. Other symptoms include, premature leaf senescence, petiole 'matchsticks', incomplete periderm development and eventually death (Stevenson et al, 2005). There is extensive colonization of the xylem vessels of susceptible citrus plants. The aggregate symptoms is collectively referred to as Pierce's disease, and was initially thought to result from the accumulation of bacteria and its associated gum within the xylem vessels, causing vascular occlusions and water déficit (Purcell and Hopkins, 1996).

Table 1: Nutrient composition of sweet orange

Composition	Amount
Energy	197 kJ (47 kcal)
Sugars	9.35 g
Dietary fibre	2.4 g
Fat	0.12 g
Protein	0.94 g
Water	86.75 g
Vitamin A equiv.	11 µg (1%)
Thiamine (vit. B_1)	0.087 mg (8%)
Riboflavin (vit. B_2)	0.04 mg (3%)
Niacin (vit. B_3)	0.282 mg (2%)
Pantothenic acid (B_5)	0.25 mg (5%)
Vitamin B_6	0.06 mg (5%)
Folate (vit. B_9)	30 µg (8%)
Choline	8.4 mg (2%)
Vitamin C	53.2 mg (64%)
Vitamin E	0.18 mg (1%)
Calcium	40 mg (4%)
Iron	0.1 mg (1%)
Magnesium	10 mg (3%)
Manganese	0.025 mg (1%)
Phosphorus	14 mg (2%)
Potassium	181 mg (4%)
Zinc	0.07 mg (1%)

Source: USDA Nutrient Database (2014)

4.1.2: **Citrus Variegated Chlorosis (CVC):** Citrus variegated chlorosis like Pierce's disease (PD) is caused by xylem-limited bacterium similar to *Xylella fastidiosa* (Rossetti, et al 1990). CVC is transmitted by leafhoppers (*Homalodisca coagulata* Say), commonly called sharpshooters (Cicadellids), a subfamily that is diverse taxonomically, consisting of approximately 1,950 species representing almost 9% of the total number of Cicadellidae species with *X. fastidiosa* as the most important known vector (Knight and Webb, 1993). *X. fastidiosa* also affect coffee, almond, alfalfa, stone fruits, landscape ornamentals, and native hardwoods (Ryan et al., 2006; Siciliano et al., 2007; Koide et al., 2006). It is one of the most devastating citrus diseases in South America, France and United States, especially in the States of Florida and Oregon. In Brazil, the diease was first formally described in 1987 in the States of Minas Gerais and Sao Paulo where it was called citrus variegated chlorosis (Rossetti, 1990). It became important in the early 1990s before expanding throughout many citrus-growing areas of South America, spreading to North America (Redak, et al, 2004). A collaborative research activities between scientists from Brazil, the State of Florida and France have provided some insight into the disease. Like in Pierce's disease, strains of *X. fastidiosa* have been found to be pathogenic to other plants, where it causes serious diseases (Barnard et al., 1998). In affected citrus fruits, the size and cell wall composition of this organism resembles the bacteria in other hosts (Almeida and Purcell, 2006). The insect vector Cicadellids are also known vectors in other hosts and abundant in citrus orchards, on trees, grasses and other native plants (Lefevre et al., 1988). Serological studies for the detection and isolation of the vírus using a dot-immunobinding assay (DIBA) and polyclonal antibody preparation have been reported (Beretta et al., 1991a; 1991b; Garnier et al., 1993). Chang et al., (1992) reported that DSA-ELISA assays using CVC antisera gave high OD readings with several *X. fastidiosa* strains. In Brazil, ELISA assays conducted with fresh plant materials also gave positive results with both symptomatic and asymptomatic leaf samples from same

branch. However, root tissues of symptomatic trees, leaf tissues of asymptomatic trees in affected orchards and trees in unaffected áreas gave negative results (Garnier et al., 1993). The most prominent foliar symptoms of CVC are bright interveinal chlorosis and mottling resembling zinc deficiency, more pronounced on younger leaves, but may also be present on old leaves (Fig. 2). In newly infected trees, the foliar symptoms are restricted to individual limbs, the underside of the leaf corresponding to the chlorotic area on the upper side becomes light to dark brown (Brlansky et al., 1996). Possible long-term management strategy in areas where the vector is present include: biological control of vectors, cultural methods, chemical control of vectors and pathogen and eradication of infected citrus (Brlansky et al., 1996).

Fig. 2: Citrus Variegated Chlorosis.
(R. H. Brlansky University of Florida, Citrus Research and Education Center)

4.1.3 **Citrus greening or Huanglongbing disease**: Citrus greening also known as huanglongbing (HLB) disease is a very destructive disease of sweet orange. The disease was first noticed in China in the early 1900's but has now spread across the world (Bove, 2006), to become one of the greatest challenges for citrus growers (Cevallos-Cevallos et al., 2012). Although Koch postulates have not been confirmed, the disease has been associated with a

phloem-limited fastidious, gram-negative bacterium of the *Candidatus Liberibacter* species. *C. Liberibacter* spp. is a member of the proteobacteria group based on its 16S rDNA sequence with three known species: *Candidatus Liberibacter asiaticus* (Las), *Candidatus Liberibacter africanus* and *Candidatus Liberibacter americanus* (Gottwald et al., 2007; Sagaram and Burns, 2009). *C. liberibacter* is transmitted by grafting and sap-sucking psyllids *Diaphorina citri* and *Trioza erytreae* (Abdulla et al., 2009; Folimonova et al., 2009]. Fujikawa and Iwanami (2012) employed rDNA specific primers to identify *Candidatus Liberibacter asiaticus* (Las) as most wide spread and economically important disease in Asia, Brazil and North America, affecting more than 90% of sweet oranges in different parts of the world (Farber, 1991). Symptoms include blotchy chlorosis and/or mottling of leaves, yellowish shoot, vein corking, stunted growth, poor root growth, small, greenish malformed fruits and finally death (Bove, 2006). Other symptoms include twig dieback, poor flowering, and stunted growth. Fruits from diseased trees are small and distorted. The blotchy mottle used for HLB detection consists of blotches of yellow on dark greenish-grey leaves (Fig. 3) (Mishra et al, 2011).

In regions that are still not infested with greening, rapid identification and culling of infected trees and budwoods in quarantine is the most important control measure (Fujikawa and Iwanami 2012). As one of the most destructive disease of citrus worldwide, rapid identification of tolerant varieties is considered a critical step towards effective control. Early detection and removal of infected trees is presently encouraged to minimize damage and loss (Committee on the Strategic Planning for the Florida Citrus Industry, 2010). Also, the development of tolerant and resistant citrus varieties is being emphasized as an alternative to reducing the impact of the disease (Halbert, 2005).

Fig. 3: Citrus leaf samples showing (a) visible HLB symptoms and (b) healthy leaves.
(Mishra et al, 2011)

4.1.3: **Citrus Canker**: Citrus canker is caused by *Xanthomonas axonopodis* pv. *citri*, and originated from Southeast Asia, but is today present in all citrus growing areas in several countries. The pathogen was identified in Brazil (Sa˜o Paulo) early in 1957 where it caused erumpent lesions on fruit, leaves and younger stems (Rossetti, 1977). Increase in disease severity results to premature leaf and fruit drop, twig dieback, general decline and blemishes on fruits quality and yield (McBride et al., 2010). The disease starts as tiny blister-like lesions on the leaves and progresses to distinct necrotic-raised corky lesions that often have a yellow halo. The spots (technically called lesions) are usually surrounded by a yellow halo, and can be observed on both the upper and lower sides of the leaf. The diseased stem and twig may appear scabby or corky, often surrounded by a water-soaked margin. Even stems can have

symptoms with brown bumps or lesions. The fruit shows dark brown to black lesions that are raised and often have a yellow halo. Similar symptoms can appear on fruit (Fig. 4).

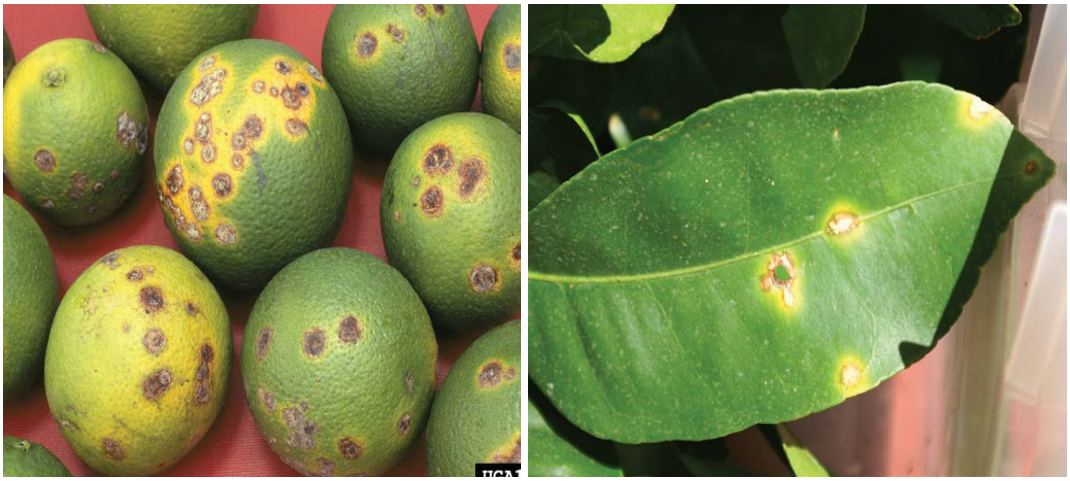

Fig. 4: Citrus Canker on fruits and leaf.
(Photo credits: Timothy Schubert, Florida Department of Agriculture and Consumer Services, Bugwood.org and Sheila McBride, Texas AgriLife Extension Service).

4.2: **Viral diseases**

4.2.1: **Citrus Tristeza Virus (CTV)**: *Citrus tristeza virus* (CTV) is one of the most important destructive agents of citrus diseases and exists as numerous strains. The spread of the pathogen to unaffected áreas is mostly through the movement and propagation of the infected plants or infected buds and then locally it is spread by a few of aphid species (Timmer *et al.* 2000). The pathogen is reported to be responsible for the death and debilitation of citrus trees. Reports in the late 1800s and early 1900s on declining sweet orange trees on sour orange rootstocks led scientists to conclude that tree failure was due to an incompatibility between certain scion varieties and sour orange roots (Philemon, 1994). Atta et al, (2012) gave a historical and geographical spread of the disease as follows: ''the most destructive epidemics of tristeza occurred in Argentina in 1930, then appeared in Brazil in 1937 and was was named tristeza. The epidemics occurred respectively in Ghana in 1938, California (1939), Florida

(1951), Spain in 1957, Israel in 1970, and Venezuela (1980). Disease outbreaks have also been reported from other citrus groves in Cyprus in 1989, Cuba in 1992, Mexico in 1995, the Dominican Republic in 1996 and Italy in 2002 (Garnsey *et al.* 2000; Gottwald *et al.* 2002; Davino *et al.* 2003). Presently CTV is widespread in Israel, Morocco, India, China, Japan, Pakistan, Iran, Syria, Egypt, southern California and Florida in USA, Argentina, Brazil, South Africa, Tanzania, Australia, and southern Spain and is moving into northern Spain (EPPO 2006), previously free of the disease''. All species, including hybrids of citrus are affected by the disease (Atta et al, 2012). Some species of the cotton or melon aphid, *Aphis gossypii*, namely: *A. spiraecola* (the spirea aphid), *Toxoptera aurantii* (the black Citrus aphid) and *Toxoptera citricida* (the Brown citrus aphid) have been associated with the natural spread of CTV (Atta et al, 2012; Rocha-Peña *et al.* 1995). Symptoms are extremely variable and depend on the viral isolate, host, environment and scion/rootstock relationships (Moreno *et al.* 2008). However, common symptoms include reduced fruit size, leaf vein-clearing, yellowing and cupping of leaves, and stem pitting. Infection on sweet orange, mandarin, or grapefruit trees on sour orange rootstock causes necrosis in the phloem of the sour orange rootstock just below the bud union. This girdling causes eventual decline and death of the infected tree. Preventitive measures include quarantine and bud wood certification programs to prevent introduction of CTV into areas where CTV does not exist yet. It can be controlled by eradication or suppression programs in orchards where disease incidence is low (Gottwald *et al*, 2002).

4.2.2 **Citrus Ringspot Virus (CRV**): The disease was first reported in *citrus* sp. from Florida and California, U.S.A. by Swingle and Webber in1896 (Fawcett, 1933) and Wallace and Drake (1968). Also, the disease hás been associated with citrus psorosis, an economically important disease of unknown etiology. It is an occasional problem in old citrus plantations, occuring mainly in orange and grapefruit trees, characterized primarily by the scaling and

flaking of the bark on the scion cultivar (Philemon, 1994). Because of these symptoms, the disease is sometimes referred to as scaly bark. Symptoms do not occur until the tree is usually over ten years old. First symptoms of scaly bark consist of small flecks of gum on the trunk and main branches. These areas become dry and scaly and as they enlarge, the tree becomes stressed and less productive. Experimentally infected plants mostly show systemic mosaics, mottles, ringspots or necrosis.

4.4 **Nematode Diseases**

Different species of parasitic nematodes have been found associated with the rhizosphere of citrus plants, but only few that can reproduce and cause damage on infected trees. Amongst these, *Tylenchulus semipenetrans, Radopholus similis, Pratylenchus coffee* and *Meloidogyne* spp. are considered be agriculturally important, because they cause significant economic losses in different regions of the world. Others are of minor importance because they rarely cause significant economic losses or are restricted to relatively small geographic areas. These include *Hemicycliophora arenaria, H. nudata, Paratrichodorus lobatus, P. minor, Pratylenchus brachyurus, P. vulnus, Xiphinema brevicolle* and *X. index* (Duncan, 1999). *T. semipenetrans* however, is the dominant pathogenic species in most citrus regions and among diverse soil textures and information on control measures is most extensive for this nematode (Duncan and Cohn, 1990). The disease has become more widespread with increasing importance because present control options are limited, following the withdrawal of the soil fumigant DBCP, which was previously used as a post plant fumigant for control (Agrio, 2005). Results from surveys indicate that infestations of citrus areas range from approximately 50 to 60 % (Nairn *et al*, 1998). Although *T. semipenetrans* is widely distributed, it has a narrow host range with occurrence restricted to citrus, grape, olive and a few additional plant species of minor importance. Thus, presence of *T. semipenetrans* is usually the result of introductions via contaminated nursery stock.

In terms of economic importance, most studies estimate yield losses due to *T. semipenetrans* to be within the range of 10% to 30%, depending on the level of infection (Duncan and Cohn, 1990). Mature trees can tolerate large population of these nematodes before exhibiting lack of vigor or decline symptoms, however younger trees exhibit poor growth, when replanted in nematode-infested soils (Duncan and Cohn, 1990). Symptom development depends on overall orchard conditions. As with other root diseases and nutrient deficiencies, above ground symptoms include stunting, slow growth, yellowing, sparse foliage, small, non-uniform fruit, defoliated upper branches and yield reduction. Dieback is particularly noticeable in the upper portion of trees. Such symptoms are not readily distinguishable from other production problems without sampling and extraction of nematodes from root and soil samples. Nematode damage to the root system affects water and nutrients absorption by the roots necessary for normal growth. In the case of below ground symptoms, feeder roots heavily infected by the citrus nematode are slightly thicker than healthy ones and have a dirty or stubby appearance because of the adhesion of soil particles to the gelatinous matrix deposited by the female nematode on the root surface. Nematode damage to the root system affects water and nutrients absorption by the roots necessary for normal growth. Damage is greatest when other root-limiting factors like fungal infections, water stress or poor growth during early development also impact nematode-infected trees. Because symptoms may not be apparent on lightly infected roots, infected nursery stocks may not be easily detected. Infected trees growing under optimum conditions may appear healthy for some time (Duncan and Cohn, 1990). For this reason, the disease is often referred to as slow-decline. Heavily infected root systems eventually cause a reduction in yield and quality of fruit. Trees in early stages of decline however, may have relatively vigorous root systems. Affected trees appear similar to stress conditions caused by *Phytophthora* root rot, poor nutrition, and inadequate

irrigation. Tree decline, which depends upon care of the grove and overall tree vigor, may not occur for three to five years after heavy infection. Infected roots may appear coarse and dirty. Female nematodes are found in small groups on the root surface. Soil adheres to the gelatinous matrix in which eggs are embedded (Vogelzang, 1999). Positive identification requires the extraction of the nematodes from soil samples taken in the feeder root zone between the trunk and the drip line of the tree. The nematodes may also be identified microscopically on infected roots in the laboratory (Vogelzang, 1999). Another nematode damaging citrus is the burrowing nematode (*Radopholus similis*), which causes a severe spreading decline disease of citrus. In addition, lesion, root-knot, sting, dagger, stubby root and other ecto-parasitic nematodes can damage citrus (Richard *et al., 1999*).

4.5. **Fungal diseases**

4.5.1: **Sweet orange scab (SOS) and citrus scab diseases:** Both diseases routinely affect citrus in different parts of the world. Citrus scab, also called common citrus scab or sour orange scab is caused by *Elsinoë fawcettii*, while sweet orange scab is caused by another related fungus *Elsinoë australis* (Kunta et al. 2013; Chung, 2010). The significance of citrus spp. fruit as a pathway for the introduction and spread of *Elsinoë australis* is well documented (Sivanesan *et al* 1998). Citrus scab disease was first discovered in Brazil in 1935 where it caused scab lesions on citrus fruit, leaves, and twigs and is present in most humid citrus producing areas (Spósito et al, 2011). Sweet orange scab differs from citrus scab primarily with respect to host range. Citrus scab rarely occurs on sweet oranges or limes, but occurs primarily on sour oranges (used primarily as rootstocks), grapefruit, lemons, mandarins, satsumas, tangerines and tangerine hybrids. In contrast, sweet orange scab occurs on sweet oranges, limes, lemons, mandarins, satsumas, kumquat, grapefruit, tangerines and tangerine hybrids. The disease is common in South America, mainly Brazil (Spósito et al., 2011), Argentina and Paraguay. A specific pathotype, *E. australis* p.v. *natsudaidai* occurs

only in Korea on *Citrus natsudaidai* (Hyun *et al.* 2001). In the United States, it has been confirmed in the states of Arizona, Florida, Louisiana, and Texas (USDA, 2010). Symptom development starts a few days after infection and is dependent on environmental conditions that promote disease development like warm temperatures and moist plant tissues. The incubation period is at least 5 days. Artificially inoculated seedlings develop scab symptoms after 7 to 14 days (Timmer, 1999). The best time to detect the disease in the field is during early spring which coincides with the formation new of tissues and fruits. Symptoms generally develop one week after tissue infection (USDA, 2010). Lesions start on the underside of leaves as water soaked spots, typically forming along the edge of the leaf or the mid-vein (Fig. 5). There is a dramatic increase in the resistance of the leaves and fruits to infection in later stages (INTA, 2010). Once established in an area, *E. australis* can spread readily to nearby hosts in the natural environment with adequate rainfall, temperatures and inoculum. Long distance dissemination of *E. australis* is most likely through the movement of infected nursery stock, including budwood (CABI, 2010). Leaves are susceptible to infection when young (flush stage), primarily in the early spring as they emerge from the bud and 'petal fall' commences; thereafter they become immune to infection (Timmer *et al*., 2000). A protuberance is formed on leaf tissue where the infection develops due to induction of cell hyperplasia and forms a depression on the opposite side. In addition to the pustules, the leaves exhibit distortions, but if infections develop close to when the leaf becomes resistant or immune, the pustules are smaller and no distortion of the leaf blade occurs (Hernandez and Mendes, 2003). There is a dramatic increase in the resistance of the leaves and fruits to infection in later physiological stages (INTA, 2010). During the six to eight weeks after 'petal fall,' fruits are highly susceptible to *E. australis* (Timmer et al, 2000) or up to 12 weeks after petal fall. Infected fruit readily express symptoms after infection, but tissue susceptibility decreases rapidly as fruit mature (Fig. 5.) (USDA, 2010).

Fruits are highly susceptible to *E. australis* during the 6 to 8 weeks after petal fall. Fruits infected in the very early stages of their development are subjected to premature fall. The initial scab forms on immature fruit is slightly raised and pinkish to light brown in color. As the lesion expands, it takes on a cracked or warty appearance and may change color to a yellowish-brown and eventually to dark- gray. The scabs typically form a pattern on the fruit like water splashes. Although there is little affect on internal fruit quality, fruit are severely blemished rendering them unsellable in the fresh produce market. Further, the disease can cause premature fruit drop and stunted young nursery trees on newly established field plantings.

Fig. 5: Citrus Scab disease Early lesions and Advanced lesions on fruit.

Source: USDA APHIS SOS – New/Science/Survey
http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus/sweet_orange.shtml

4.5.2 **Citrus Black spot (CBS) disease:** First discoverd in Australia some 80 years ago, CBS disease is now present in South Africa, Zimbabwe, Swaziland, Mozambique,China, Indonesia, Japan and Brazil (Kotze, 1981). The climatic conditions in Brazil, a leading producer of citrus favours the disease. The sexual stage of the causal organism was discovered in 1948 by Kiely in New South Wales, Australia and was called *Guignardia citricarpa* Kiely (Kotze, 1981). The imperfect stage was named *Phoma citricarpa* McAlp and was later renamed *Phyllostictina citricarpa* McAlp Petrak. CBS produces lesions on infected citrus fruits which do not cause post harvest decay, but affect consumers' acceptability. In

addition to *Citrus sinensis*, other citrus species (except sour orange) are affected by the disease and heavy losses may occur in 'Valencia' and navel orange varieties (Kotze, 1981). The disease may be present for many years before producing symptoms and this affects disease management strategies. Symptoms on fruits have been classified into three categories as hard spot or shot-hole, freckle spot and virulent or spreading spot (Kotzé, 2000). A fourth group, malonose spot was added by Kotze (1981) for citrus infected in South Africa. Hard spot is characterized by dark brown lesions surrounding a depressed, round, light brown to gray centre that contains pycnidia (Spósito et al, 2011). This symptom appears as the fruit changes colour. The second symptom is false melanose, and is characterized by small black spots distributed over the fruit, sometimes giving rise to a tear-streaked pattern when spore-laden water flows over the fruit (Fig. 6). This symptom appears on green fruit, and the lesions do not have fungal fructifications. The third symptom is freckle spot, which is characterized by small red depressed lesions that usually appear at fruit maturation and post-harvest. Pycnida are produced in the freckle spot centre ((Spósito et al 2011). Disease spread is enhanced by fallen citrus leaves that serve as source of inoculum for the production of ascospores and pynicdiospores (Kotzé, 1981). In Brazil, many attempts to control CBS by grass mulching were unsuccessful and at least five fungicide applications are necessary to reduce disease symptoms on fruit (Rossêtto, 2009).

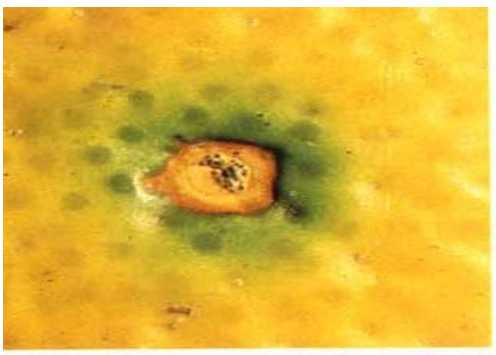

Fig.6: Typical hard spot lesion with pycnidia at the centre of necrotic tissue.

(Kotze, 1981)

4.5.3: **Powdery mildew.** This disease is caused by *Acrosporium tingitaninum* a very important disease causes the shoots to collapse and dry with a white colour. Under severe conditions, affected leaves drop off and the twigs show die-back symptoms. Young fruits also get covered by the fungus and they drop off prematurely, leading to reduction in yield. Suckers running from the base to the centre of the tree are usually affected. Damp mornings with a few hours of sunshine are the most favourable conditions for the onset of the disease (Sastra-Hidayat, 1992). Other fungal diseases of C. Sinensis are Gummosis (*Phytophthora species*), pink disease (*Pellicularia salmonicolor*), twig blight or citrus (*Diplodia natalensis*) and anthracnose or wither tip (*Colletotrichum gloeosporioides*).

5. Control measures

5.1 Chemical methods: Post-harvest treatments with chemicals effectively reduce the viability of pycnidiospores in lesions on symptomatic citrus fruits with black spot fungal disease that causes lesions on citrus fruit (Korf *et al.* 2001). Routine packinghouse treatments with chlorine dips, warm water bath, or chemical tank dip (1000 µg/ml guazatine, 503 µg/ml imazalil sulphate, 500 µg/ml 2,4-D, sodium salt) and combination of these treatments and wax treatments reduces the viability of conidia (Korf *et; al.* 2001). Imazalil (IMZ) and thiabendazole (TBZ) as systemic fungicides also have a trans-laminar effect as post-harvest surface treatments for several citrus fungi including: *Diplodia, Alternaria, Fusarium, Botrytis, Molinia, Rhizoctonia*, and apple scab (Ware and Whitacre, 2004). Thiabendazole has a range of activity similar to that of benomyl (Ware and Whitacre, 2004). Studies on the distribution of postharvest fungicides on citrus fruit show that residues are predominantly found on the surface of the fruit and in the peel and only small amounts migrate into the flesh (Friar and Reynolds, 1994). Systemic fungicides (benomyl, carbendazim) are commonly used before flushing and after petal fall (Canteros, 1998). Benomyl has been used to effectively

control *Elsinoe australis* that causes common old scab lesion disease in citrus fruits (Whiteside, 1980). Its use was however discontinued because of the emergence of resistant strains of the causal pathogen (Whiteside, 1977). Benomyl is still effective in many locations and is still recommended for scab control (Knapp 2000). No chemicals are presently recommended for post-plant control of the citrus nematode (Ehsani *et al 2007*).

5.2 Use of Bio-control agents: Strains of *Pseudomonas syringae* are very effective in controlling postharvest diseases of citrus fruits, and antagonistic activity has been correlated with *in vitro* production of lipodepsipeptides. Additionally, biocontrol agents can induce a range of defence mechanisms of resistance in citrus tissue that result in a broad spectrum of metabolic modifications, such as systemic acquired resistance, induced systemic resistance and production of reactive oxygen species (Gilchrist, 1998). Achilea *et al*, (1985) studied the expression of syringomycin (*syr*B1) and syringopeptin (*syp*A) synthetase genes from *P. syringae* pv. *syringae* biocontrol strains *in vitro* on different culture media and *in vivo* on citrus fruits (*Citrus sinensis* cv. Tarocco) and their interactions with *Penicillium digitatum*, using quantitative RT-PCR. Similarly, gene transcript levels of chitinase (CHI1), allene oxide synthase (AOS), glutathione peroxidase (GPX1) and phenylalanine ammonia-lyase (PAL1) were measured. *Syr*B1 and *syp*A genes were more actively expressed when antagonistic *Pseudomonas* strains were grown on orange peel broth as compared to NB and PDB. *Penicillium digitatum* resulted to be strongly stimulatory only to *syr*B1 expression, thus suggesting that *syr*B1 gene could be involved in biocontrol activity (Achilea *et al*, 1985).

5.3 Packaging: Grade standards for sweet oranges are based on maturity, colour intensity and uniformity, firmness, shape, size, smoothness, freedom from decay, as well as freedom from defects (bruises and abrasions), insects, fungal attack, growth cracks, chemical burns,

and physiological disorders. Well-vented polyethylene and plastic mesh bags of various sizes are also used to market oranges. Oranges may be singly sealed or wrapped with various plastic films, a practice that has not been widely adopted (Webber, 1989).

5.4 Storage conditions: Under normal weather conditions, fruits are better stored on the tree than in cold storage. Cold storage should not be attempted if the fruit storage potential has been expended by prolonged tree storage. Once harvested, fruit quality will not improve. Before placing into storage, fruit should be pre-cooled to slow respiration and treated with an approved fungicide to reduce decay. Oranges can be stored for up to 12 weeks under optimum storage conditions. Ultimate storage-life depends on cultivar, maturity, pre-harvest conditions, and postharvest handling. During storage, fruit should be inspected often for signs of decay or disorders. Such problems will advance rapidly once the fruits are removed from cold storage (Webber 1989).

6. Conclusion

We have reviewed the importance of sweet orange (*Citrus sinensis* L) in different areas of human health: treatment of arteriosclerosis, prevention of cancer, kidney stones, stomach ulcers and reduction in cholesterol level, high blood pressure and strengthening of the immune system. These health benefits are as a result of vitamins, especially vitamin C, phytochemical compounds like liminoids, synephrine, hesperidin flavonoid, polyphenols, pectin etc. A single orange is said to have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory, blood clot inhibiting and antioxidant properties. It is therefore necessary to control the impact of diverse pathogens that limits its production, nutritional value and market qualities through chemical treatment of fruits, use of biological

control agents, proper packaging and storage facilities and other disease management practices to reduce postharvest damages. Breeding for resistance, the most effective tool with which many of these diseases are managed is usually available in coevolved pathosystems, but may be uncommon if new pathogenic races evolve. Inadequate host resistance can be a significant barrier in the management of these new races, as well as those that are caused by common pathogens. In the later cases, other disease management may be employed. Considering its health benefits, there is the need for public enlightenment on the importance of sweet oranges as the fruit is relatively cheap and common almost all year round.

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