**Sudan University of Science and TechnologyCollege of Graduate Studies**

# Study on Bacterial Contamination of Some Horticultural Products in Khartoum State and their Control Using Plants Extracts

## دراسة على التلوث البكتيري لبعض المنتجات البستانية في ولاية الخرطوم ومكافحتها باستخدام المستخلصات النباتية

A thesis Submitted in Partial Fulfillment of the Requirements for the M. Sc. Degree in Plant Protection

**By:**

**Eisa Khamees Salih Tambary**

B.Sc. Agric. (Honors), September, 2008.

College of Agricultural Studies -Shambat

Sudan University of Science and Technology

**Supervisor: Dr. Ibrahim Saeed Mohammed**

Department of Plant Protection

College of Agricultural Studies -Shambat

Sudan University of Science and Technology

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## الآية

بسم الله الرحمن الرحيم

**قال تعالى:** ﴿شَهِدَ اللَّهُ أَنَّهُ لَا إِلَٰهَ إِلَّا هُوَ وَالْمَلَائِكَةُ وَأُولُو الْعِلْمِ قَائِمًا بِالْقِسْطِ ۚ لَا إِلَٰهَ إِلَّا هُوَ الْعَزِيزُ الْحَكِيمُ (١٨) إِنَّ الدِّينَ عِنْدَ اللَّهِ الْإِسْلَامُ ۗ وَمَا اخْتَلَفَ الَّذِينَ أُوتُوا الْكِتَابَ إِلَّا مِنْ بَعْدِ مَا جَاءَهُمُ الْعِلْمُ بَغْيًا بَيْنَهُمْ ۗ وَمَنْ يَكْفُرْ بِآيَاتِ اللَّهِ فَإِنَّ اللَّهَ سَرِيعُ الْحِسَابِ (١٩) فَإِنْ حَاجُّوكَ فَقُلْ أَسْلَمْتُ وَجْهِيَ لِلَّهِ وَمَنِ اتَّبَعَنِ ۗ وَقُلْ لِلَّذِينَ أُوتُوا الْكِتَابَ وَالْأُمِّيِّينَ أَأَسْلَمْتُمْ ۚ فَإِنْ أَسْلَمُوا فَقَدِ اهْتَدَوْا ۖ وَإِنْ تَوَلَّوْا فَإِنَّمَا عَلَيْكَ الْبَلَاغُ ۗ وَاللَّهُ بَصِيرٌ بِالْعِبَادِ (٢٠)﴾

**صدق الله العظيم**

سورة آل عمران

من الآية (١٨) إلى الآية (٢٠).

## DEDICATION

# TO my mother and father
# To my dear brothers and sisters
# To my family
# To teachers and friends with
# Love
# &
# Respect

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All my special and unlimited and thanks are to Allah, who offered me the health and strength to complete this work.

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# ABSTRACT

Fruits and vegetables play a vital role in improving the health of people worldwide. The crops suffer considerable losses because of bacterial soft rot. The aim of this study was to survey the level of incidence of bacterial soft rot disease in five produces of horticulture namely Banana, Orange, Mango, Potato and Tomato, in central markets of Khartoum State and to identify the bacterium associated with this disease and to explore the antibacterial potentials of some higher plants aqueous crude extract against the bacterium under in vitro. The Survey revealed that the bacterial soft rot disease is prevalent in the five fruits and vegetables with varied incidence in each produce. The disease was significantly high in Tomatoes (9.3%) fallowed by Banana (6%). The lowest incidence was in Potatoes (3.0%). Upon isolation on NA bacterium, all the isolates of causal bacterium that readily macerated the host tissue were found to be *Erwinia carotovora.* Studies on antibacterial activity of the three plants extracts against bacterium indicated that all extracts at all concentrations exhibited varying degrees of inhibition against *Erwinia carotovora* with zones of inhibition ranging from 27.4% to 100%. Among plants extracts, Damas showed more pronounced inhibitory effects on the growth of the bacterium where it completely inhibited its growth compared to control. Neem at 30% and 40% and Argel at 40% concentrations also demonstrated significantly high zone of inhibition 100%, 99.3% and 97.4% respectively. The results also indicated that was a variation in the potency of the plant extracts and their different concentrations as well as in the sensitivity pattern of the test bacterium. The findings of this study are therefore important as they highlighted the need for adhering to effective measures that aim at reducing *Erwinia* soft rot incidence in fruits and vegetables especially during storage and transit. Also, the

potentials of the plant extracts to serve as possible bio-control and antimicrobial agents for bacterial soft rot of fruits and vegetables were demonstrated in this study.

# ملخص البحث

تلعب الفواكه والخضروات دوراً هاماً في تحسين صحة الشعوب على نطاق العالم. لكنها تعاني من خسائر معتبرة جراء الإصابة بالعفن البكتيري الطري. تهدف هذه الدراسة لإجراء مسح لمعرفة مدى الإصابة بالعفن الطري البكتيري في خمسة منتجات بستانية هي الموز،المانجو، البطاطس، البرتقال و الطماطم في الأسواق المركزية بولاية الخرطوم وتعريف البكتريا المرتبطة بهذه المرض واستكشاف إمكانيات مضادات البكتيريا من المستخلصات المائية للنباتات ضد هذه البكتريا خارجياً.أوضحت نتائج المسح أن مرض العفن الطري البكتيري موجود في كل منتجات الفواكه والخضر الخمسة التي فحصت وبدرجات إصابة مختلفة في كل منتج. كانت الإصابة عالية ومؤثرة في الطماطم ( ٩٠.٣%) ويلي ذلك الموز ( ٦٠%) بعد العزل على بيئة النيترونيت أجار اتضح أن كل عزلات البكتيريا المسببة للمرض التي أدت إلى تهتك أنسجة العائل إروينيا كراتوفورا.الدراسات التي أجريت على النشاط المضاد للبكتيريا في ثلاثة مستخلصات نباتية أن كل تركيزات المستخلصات قد أظهرت تثبيطها للبكتريا بدرجات متفاوتة وتراوحت منطقة التثبيط بين ٢٧.٤% - ١٠٠%. فيما بين المستخلصات النباتية كان التأثير التثبيطي للدمس على نمو البكتريا تماماً مقارنة بالشاهد . كما أن تركيزات النيم عند ٣٠%و٤٠% والحرجلعند ٤٠% قد أدت إلى مناطق تثبيط عالية ومؤثرة ١٠٠% و٩٧.٤% على التوالي.

النتائج أوضحت أيضا أن هنالك تفاوتا في مدى فعالية المستخلصات وتركيزاتها وأيضا في نمو حساسية البكتيريا المختبرة. تكمن أهمية هذه الدراسة في أنها قد سلطت الضوء للحوجة إلى الالتزام بطرق فعالة بهدف تقليل الإصابة بالعفن الطري الارويني في الخضر والفاكهة خاصة أثناء التخزين الترحيل من مسفات بعيدة.هذه الدراسة أوضحت أيضا إمكانية استخدام المستخلصات النباتية كعوامل في المكافحة البيولوجية كمضادات بكتيرية ضد العفن الطري البكتيري في الخضر والفاكهة.

# CHAPTER ONE

## 1. Introduction

Horticultural crops in general, fruits and vegetables in particular play a vital role in improving the health of people in the developing world where more than two billion person, the vast majority of whom are women and children, suffer from micronutrient deficiencies and in return increases incomes and job opportunities.

In fact, fruits and vegetables are part of everyday meals, including special high-value diets for infants and the elderly. Fruits and vegetables besides being an important component of traditional food are also central to healthy diets of modern urban population. They form the basis of a wide array of processed or partially processed products. Increasingly innovative products use fresh fruits and vegetables and vegetables in fast foods and components of ready meals. However, China considered the top of the ten countries with most fruits production (122,184,944 tones) (FAOSTAT, 2012).

In Sudan,the total output of the horticultural sub-sector is estimated at about 12% of the total agricultural production of Sudan (Bank of Sudan, 1997-1998 and Elamin, 2001). The horticultural sub-sector can be further divided into vegetables and fruit sub-sectors.

Fruits and vegetables products are valuable and nutritious food commodities which substantially contribute to improve the social welfare and health status of the urban and rural populations (Baudoin, 1994). The vegetables and fruits sub-sector include a wide range of crops. The most important vegetables are onions, tomatoes, potatoes, eggplants, bean in additional to the various root and leaf vegetables, while the most important fruits are banana, mango, guava, grapefruits, orange and lime.

Furthermore, the diversity of climate, soil and adequacy of water resources provided the country with the opportunity to grow various types of horticulture produces from tropical to equatorial ones, including date palm, banana, guava, citrus fruits and vegetables and mango.

The estimated total fruit production in Sudan is about 1.9 million tons in a cultivated area of about 186,000 hectares where as that of vegetables is about 273,000 hectares producing 3.4 millions of tons of vegetables. (Hind and Mohamed, 2010)

One of the major constraints facing the quantity, quality and availability of healthy fruits and vegetables crops worldwide are the losses and contamination caused by post harvest diseases. The major groups of postharvest diseases are those which arise from infections initiated during and after harvest. A wide variety of bacterial pathogens cause postharvest disease in fruits and vegetables. Among these, are *Erwinia, Pseudomonas, Bacittus, Lactobacillus and Xanthomonasspp*(Coates*et al.*, 1995, Ploetz, 2007). Charles *et al.*, (1991) reported that spoilage of fruits and vegetables after harvest often causes losses as great as 25-50% of the harvested crop.Awad,( 1987) reported that in Sudan a wide variety of fruits and vegetables were found susceptible to the isolates of *Erwiniacarotovora*.

Meanwhile the withdrawal of a group of pesticides used to control post-harvest diseases of fruits and vegetables due to risk of residues initiated the search for alternative disease control methods to be sought. Among these are: the use of plant-derived pesticides; antagonistic micro-organisms;and the manipulation of resistance responses in harvested commodities. Furthermore, the presence of antimicrobial compounds in higher plants presents an attractive option. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Schmutterer,2002). However, a number of potential alternative control avenues are open for controlling post-harvest diseases of fruits and vegetables (Agrios, 2005).

Accordingly, this study was undertaken to focus on detection and identification of bacterial contaminants that cause soft rot of some horticulture produces in local markets and to examine the prospects for the antimicrobial potentials of some higher plants aqueous crude extract in control of postharvest diseases of fruits and vegetables and recommends future research directions with the following objectives:-

- To survey the occurrence of bacterial soft disease in some horticulture produces in Khartoum State;
- To assess the incidence of the disease in five produces of horticulture in Khartoum state;
- To detect and identify the most prevailing bacterial pathogen associated with soft rot disease;
- To explore the antimicrobial potentials of some higher plants crude extract against the bacterium associated with soft rot disease of horticulture produces.

# CHAPTER TWO
# LITREATURE REVIEW

## 2.1.Bacterial soft rot

Bacterial soft rot is one of the most destructive diseases of horticulture produces worldwide wherever fleshy storage tissues of fruits and vegetables are found. The disease can be found on crops in the field, in transit and in storage or during marketing resulting in great economic losses. It is primarily caused by *Erwinia carotovora* subsp. *carotovora* and sometimes by *Erwinia carotovora* subsp. *atroseptica*(Harrison, 1990;Hajhamed *et al.,* 2007).

The bacteria have a very wide host range infecting fruits and vegetable species belonging to all families. Name of the disease aroused from the characteristic soft decay of fleshy tissue which terminates into watery or slimy mass. The bacteria enter the host tissue through injuries. The decay is aggravated when high humidity is coupled with high temperature which results in fast rate of multiplication of the bacterium. For this reason much of the loss due to this disease occurs during hot weather (Agrios, 2006 and Bhat *et al*., 2010) other bacteria by several types of bacteria, but most commonly species of gram-negative bacteria that cause soft rots include *Pectobacterium*, and *Pseudomonas*. The bacteria mainly attack the fleshy storage organs of their hosts (tubers, corms, bulbs, and rhizomes), but they also affect succulent buds, stems, and petiole tissues (Agrios, 2005).

With the aid of special enzymes, the plant is turned into a liquidly mush in order for the bacteria to consume the plant cell's nutrients. Disease spread can be caused by simple physical interaction between infected and healthy tissues during storage or transit. The disease can also be spread by insects. Control of the disease is not always very effective, but sanitary practices in production, storing, and processing are something that can be done in order to slow the spread of the disease and protect yields (Plots, 2007).

**2.2*Erwinia carotovora* subsp *carotovora* Ecc.** (Jones)/Bergey

**2.2.1. Classification**

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Erwinia*

Species: *carotovora*

*Erwiniacarotovora* is a rod shaped gram negative phytopathogenic bacterium, and deadliest pathogen which affects productivity of plants (Agrios, 2005). The member of this family causes soft-rotting disease on a wide variety of crop species worldwide including fruits, vegetables and flowers (Chatterjee *et al*., 1995).

Transmission of *E. carotovora* occur either through plant to plant or insect to plant. The bacterium causes cell death through plant cell wall destruction by creating an osmotically fragile cell. Yield losses up to 98.8% have been experienced under artificial epiphytotics (Thinda and Payakab, 1985). The commercially important soft rot Erwinias are *E. carotovora subsp. carotovora* (Ecc), *E.carotovora subsp chrysanthemi,* and *Erwinia carotovora subsp atroseptic*a (Eca), which cause diseases of potato and other commercially important crops (Czajkowski *et al*., 2009; Rahmanifar *et al*., 2012). Erwinia carotovora is non-spore forming and peritrichously flagellated. It is a facultative anaerobe, catalase negative and oxidase positive (Harris *et al*., 1998). Direct and indirect crop losses due to these pathogens are considerable, especially in certain production areas (Perombelon, 1998).

### 2.2.2Hosts and symptoms

There are a variety of host plants including but not limited to; banana, beans, cabbage, carrot, cassava, coffee, corn, cotton, onion, other crucifers, pepper, potato, sweet potato and tomato. For each host there are different symptoms displayed. Most symptoms are along the lines of watery and soft decay of the tissue. Cabbage and crucifers' symptoms start where the tissue makes contact with the soil. Often there is a change in color and in the case of a carrot; the whole taproot can be decayed leaving just the epidermis. Sweet potatoes show clear lesions that grow rapidly leaving a recognizable watery and soft, oozy tissue where only the peel remains intact (Bell,2004).

Potatoes experience a cream to tan colored tuber that becomes very soft and watery. A characteristic black border separates the diseased area and the healthy tissue. Only when the secondary organism invades the infected tissue does that decay become slimy with a foul odor. Like the carrot, the whole tuber can be consumed leaving just the epidermis in the soil. The foliage becomes weak and chlorotic with upward turned leaves and lesions on the stem. The stem also rots and becomes mushy with its colorless or brown lesions (Perombelon, 1998).

### 2.2.3Dormant Symptomless Stage

Soft rots are characterized by their distinct maceration of hosts' cell walls with pectolytic enzymes, and subsequent digestion of the intracellular fluid as the bacteria grows. But, little is known about the pathogen's interaction with its host at earlier stages when it is still attaching to, and growing within, the host with no symptoms present. In fact, the bacteria may develop large populations within a plant before any symptoms can be seen. No one knows exactly why the bacteria have this dormant stage, or what factors influence the bacteria's virulence, but the research is being done (Agrios, 2005).

### 2.2.4Ecology

In addition to infected plants, *E. carotovora* can also be found in the guts of insects and bodies of water introduced by aerosols, runoff into rivers and dumping of potatoes. After rainfall upon diseased plants, an aerosol containing the bacteria is created. Fifty percent of the bacteria that become suspended in aerosols can survive for five to ten minutes and may travel for miles (Ploetz, 2007). Growth of the bacteria is possible between 32–90 °F, with the most ideal conditions between 70–80 °F. Post-harvest storage and transportation is difficult for tropical and other warm environments when the air is not properly ventilated during these processes. Higher temperatures and high humidity are ideal growing conditions for the bacteria making ventilation a big priority when trying to combat this disease (Agrios, 2005).

### 2.2.5Importance

Due to its wide range of hosts, bacterial soft rot devastates many significant crops both in the field and in storage all over the world (Elphinestone, 1987). Almost all fresh fruits and vegetables are subject to infection by bacterial soft rots. In the tropics, soft rot develops on important crops like corn, cassava, and banana even while still in the field. Specifically, soft rot of potatoes can cause a huge decrease in yield, and is the most serious bacterial disease that potatoes are exposed to. For a grower of potatoes, there is a possibility that 100% of a whole season's yield could be destroyed due to insufficient conditions in a storage facility (Agrios, 2005). All in all, bacterial soft rots cause a greater loss of produce than any otherknown bacterial disease.

### 2.2.6Management

There are very few things that can be done to control the spread of bacterial soft rots, and the most effective of them have to do with simply keeping sanitary growing practices (Harris, 1979).

Storage warehouses should be free of all plant debris, and the walls and floors disinfected with either formaldehyde or copper sulfate between harvests. Injury to plant tissues should be avoided as much as possible, and the humidity and temperature of the storage facility should be kept low using an adequate ventilation system. These procedures have proven themselves to be very effective in the control of storage soft rot of potato (Bell, 2004).

It also helps if plants are planted in well-drained soils, at intervals appropriate for adequate ventilation between plants. Few varieties are resistant to the disease and none are immune, so rotating susceptible plants with non-susceptible ones like cereals is apositive practice to limit soft rot infection (Barkai, 2001).

The control of specific insect vectors is also a good way of controlling disease spread in the field and in storage. Soil and foliage insecticide treatment helps controls the bugs that frequently cause wounds and disseminate the bacteria (Web. 26 Oct 2010). However, As per Farrar *et al*., (2009), till now there are no effective chemical controls for any of the soft-rot Erwinias.

The risk associated with use of pesticides led to withdrawal of a group of pesticides used to control post-harvest diseases of fruits and vegetable and at the same time initiated the search for alternative disease control methods to be sought. However, a number of potential alternative control avenues are open for controlling post-harvest diseases of fruits and vegetables (Charles *et al*., 1991).

Among these are: the use of plant-derived pesticides; antagonistic micro-organisms; the manipulation of resistance responses in harvested commodities. Other methods of the disease control such as hot water treatment (Shirsat *et al*., 1991) and air-drying of tuber (Bartz and Kelman, 1985) have also been tried with varying scale of success. However, the presence of antimicrobial compounds in higher plants presents an attractive option. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Schmutterer, 2002).Furthermore, the use of products of plant origin such as Neem (*Azadirachtin indica*) is much safer but these products are applied mostly in insect pest management than control of microorganisms (Stoll, 1998). However, extracts of ginger rhizomes (*Zingiber officinale*), garlic bulb (*Allium sativum* L.) and aloe vera (*Aloe barbadensis* M.) have been used in controlling fungal pathogens (Amadioha, 1999; Obagwu *et al*., 1997; Ahmed and Beg, 2001). Some bactericidal and fungicidal properties of these products had been reported(Emechebe and Alabi, 1997; Stoll, 1998).

## 2.3Horticultural crops

Sudan has great potential to produce good quality fruits and vegetables because of its natural resources and wide range of climate. Thus, horticulture is an economic sector that plays a significant role in the overall socio-economic development in Sudan. Accordingly, many types of fruits and vegetables are grown in both irrigated and rain-fed areas (Mahmoud *et al.*, 1996).

However, horticultural produces represent about 12% of the national agricultural income. The estimated total fruits and vegetable production in Sudan is about 1.9 and 3.4 million tons, in a total area of about 186,000 273,000 hectares respectively.Unfortunately, Losses of 30-40% of fruits and vegetables were reported during harvest transport and storage (Hind and Mohamed, 2010).Hence, the economic impact of fruits and vegetables is still very limited compared to their actual production potential in the country. Of the major fruits and vegetable are potatoes, tomatoes, mangoes, banana and sweet oranges.

## 2.4.Potato(*Solanum tuberosum)*

The potato plant belongs to the family *Solanaceae* which includes, among 2000 other species, tomato *(Lycopersicum esculentum*), sweet pepper (*Capsicum annuum*), eggplant (*S.Melongena var. esculentum*), tobacco *(Nicotianatabacum*), and petunia (*Petunia hybrida*) (Fernald, 1970). The genus *Solanum* is a polymorphous and largely tropical and subtropical genus containing more than 1000 species. The origin agreed to be the high elevation of South America and the area of first domestication was reasoned to be the area where wild diploids are still found and where the greatest diversity of cultivated forms can still be found, and is identified as the high plateau of Bolivia and Peru, in the general region of Lake Titicaca(Hoopes and Plaisted, 1987).

The potato is one of the world's most important crops, following wheat, maize, and rice. It is the staple food of many cultures and civilizations past and present, and is the world's fourth largest food crop. The term Potato is used to refer both to the plant, and the vegetable itself (Howard, 1970; Simmonds, 1976; De Jong, 1984 and Burton, 1989).

In Sudan, the potato is grown mainly as winter crop and the main area of production are along the Nile bank in both Khartoum and Northern Estates. Although potato cultivation in Sudan depends mainly on exotic advanced cultivars but an old introduced material is still produced in Jebel Marra in the far west and it is locally known as Zalingei potato.The estimated total potatoes production in Sudan is about 616,000 tons in a cultivated area of about 88,000 feddans (Hind and Mohamed, 2010).

## 2.5.Banana (*Musa spp.*)

*Musa* species are native to tropical South and Southeast Asia, and are likely to have been first domesticated in Papua New Guinea. (Tracing antiquity of banana cultivation in Papua New Guinea( Nelson, *etal.*, 2006).

Banana is among the most important food crops in the world (Frison and Sharrock, 1999). In 2013 bananas were fourth among the main world food crops in financial value(Holmes Bob, 2013).
Banana (*Musa spp*) is a popular and widely consumed tropical fruit that provides carbohydrates, proteins, vitamins and minerals to more than 400

million people worldwide (INIBAP,1987). Of all the fruits, banana holds first place by production volume and is amongst the five most consumed fruits on the planet (FAO, 2011). Based on the 2010 statistic from FAO, banana is grown in more than 150 countries in acreage of about 4 771 944 hectares. In fact, banana represents one of the major agricultural products traded between many regions in the world. It is a dynamic industry, which contributes substantially to nation's economy and the general welfare of many people in the world (Elamin, 2001). In additional to farmers' role, many business and millions of people are involved in the processing, transporting and marketing of this fruit (Elamin, 2001).

In Sudan banana is considered as one of the most important fruits in the Sudan. Its cultivation in the Sudan is reported to date back to the late 19$^{th}$ century or the early 20$^{th.}$. It was reported that banana production has been practiced since that time in Kassala area then its production spread to the different parts of the country wherever arable land and irrigation water are available (Abdelhai, 1995)

Banana fruits are very popular and widely consumed due to their lower prices compared with other fruit crops. The crop is grown in many parts of the country, including Khartoum and Kassala States as well as along the banks of the Blue Nile. Sennar, in Central Sudan, is one of the most important areas of banana production for local consumption and export.Is also produced in some parts of Darfur where in certain parts of the flood plains fruit growers described certain banana relatives (types) with very small edible fruit (finger type). According to Fagusan (1953), a wild banana (*Musaenseate*) occurs in southern Sudan and produces edible seed though not edible fruits. Recently,new cultivars and lines were introduced for evaluation. The Dwarf Cavendish is an old banana cultivar which has been the only variety grown in Sudan for about a hundred years. This cultivar is well adapted to Sudan conditions and is almost free of serious banana pests and diseases. In addition, local plantains are grown in southern Sudan.

The total area alloted to banana is estimated to be 68,500 feddans, producing about 822,000 tons annually (Statistics, Department of Horticulture, Ministry of Agriculture, 2013).

### 2.6. MANGO(*Mangifera indica L.*)

*Mangiferaindica* L. is a member of the family Anacardiaceae–notorious for embracing a number of highly poisonous plants. It is native to southern Asia, especially eastern India, Burma, and the Andaman Islands. The fruit is one of the most celebrated of tropical ones. Worldwide the fruit is very popular with the masses due to its wide range of adaptability, high nutritive value, richness in variety, delicious taste and excellent flavor. It is a rich source of vitamins A and C (Dawoud, 2008).

In Sudan, Mangoes are ones of the most important horticulture produce and number one in production compared to other fruits. The crop and suffers from several diseases at all growth stages. Some of these diseases are of great economic importance as they cause heavy losses in mango production (Mahmoud *et al.*, 1996).Several varieties of fruit trees are grown. Some of them are ancient in the country while others were introduced not long ago. The most well known fruit trees in Sudan include date palm, banana, guava, citrus fruits and mango. *M. indica* L. has been grown since 1904. In Southern Sudan it has probably arrived from India via Congo, while in the North, the introductions have mainly come from trees already established in Egypt, but some mango cultivars are probably imported directly from India (Bacon, 1948). More recently, a number of mango cultivars have been introduced from South Africa through the Ministry of Agriculture and Forestry and the Farmers Union.

ThisInclude: Tommy Atkins Haden, Sensation and Keitt, while Sidege cultivar was introduced from Egypt (Dawoud, 2008).

### 2.7. Orange fruit (*Citrus sinensis* Osbeck)

The orange (specifically, the sweet orange) is the fruit of the citrus species, *Citrussinensis,* in the family Rutaceae (Morton, 1987). It is assumed to have originated in southern China, northeastern India, and perhaps southeastern Asia, and that they were first cultivated in China around 2500 BC (Nicolosi, 2000).

Orange trees are widely grown in tropical and subtropical climates for their sweet fruit. The fruit can be eaten fresh, or processed for its juice or fragrant peel. As of 2012, sweet oranges accounted for approximately 70% of citrus production. In 2010, 68.3 million metric tons of oranges were produced, production being particularly prevalent in Brazil and the U.S. states of California and Florida (FAOSTAT, 2012).

In Sudan, the most important citrus fruit trees grown include sweet orange (*Citrussinensis* Osbeck), grapefruit (*C.paradisi* Macf.) and lime (*C.auranfifofia*). Other citrus trees are grown but on a limited scale, like mandarin (*C.reticulata* Blanco), lemon (*C.limon*) and pummelo (*C. grandis*). This is in addition to other types of citrus trees used mainly as rootstocks, of which the sour orange (*C.aurantium*)is the most widely used(Dawoud, 2008). The northern, eastern and central regions are important areas of production. Jebel Matra area in Western Darfur State is of a special importance in citrus production as almost all the sweet navel orange fruits (seedless fruits) are produced there. Small areas of citrus fruits are found in some parts of Kordofan states (Mahmoud*et al*., 1996).

The major old cultivars of oranges include varieties like Sinari, Beladi, Valencia, Navel and Nuri 16. All cultivars of citrus fruits grown are introduced old varieties. During the eighties, some new orange and grapefruit varieties were officially released. The estimated total potatoes production in Sudan is about 124,000 tons in a cultivated area of about 25,000 feddans (Hind and Mohamed, 2010)

### 2.8. Tomato (*Lycopersicon esculentum* mill.)

Tomato (*Lycopersicon esculentum*mill.) is member of the family Solanaceae that includes also other cultivated crops such as potato, pepper, eggplant, tobacco .The origin of tomato is believed to be central and south America, especially Mexico, from where the crop was transferred to Europe in the 16th century , then to old world continents (Hedrick , 1919 and Rick , 1977). Tomato is an important food and cash crop for the majority of the low income farmers in the tropics (Prioret*et al.,* 1994). In fact it is considered as one of the most important and popular vegetable in many countries (Shared ,1966) .This is because of its acceptable flavor, nutritive value and ability to fruit in a wide range of environments and the relative ease with which it can be cultivated. The production estimate worldwide was 95 million Mt annually (FAOSTAT, 2002).

The tomato in the Sudan is considered as one of the major vegetable crops and widely used in the processed forms as paste, ketchup, sauce and dry tomato slices. The crop presents one of the main cash vegetable crops in central and Northern Sudan. Tomato grow successfully almost in every part of the Sudan during the winter season and in close system farming (FAO, 1999). The main producing areas of tomato (*L. esculentum* Mill.) are Central and North Sudan. Tomato is also produced in gable Mara and some parts of the main rain fed areas around villages in central clay plains and utilized as sun dried slices. Summer production of tomato which ensures high profitability because of the scarcity of the crop at that time is practiced in limited areas in Blue and White Nile Khartoum state and Northern Sudan (FAO, 1999). The crop is recently produced under controlled greenhouses during summer season and this practice is extending rapidly every year(Agrios,2005). The most important grown cultivars are the canning Types such as Strain B, Strain C, Peto86, Peto111 and Castle Rock in addition to few local varieties (Marmar *et al.*, 2009).

Although a number of pests and diseases are considered one of the main limiting factors for its growing, the production of tomato was developed rapidly since 19th century. According to FAO (2005/2006) the area under tomato production has increased rapidly from 1300 hectares during 1999 to 43453 hectares in 2005/2006.

**2.9. Neem (*Azadirachta Indica* Juss.)**

*Azadirachta Indica* of the family Meliaceae is a tropical evergreen tree which thought to have originated in Asia and Burma. However, the exact origin is uncertain, some authors said Neem is native to the whole India, other attribute it to dry forest areas, throughout all south and south Asia(Ruskin, 1992).

The Neem tree is known as “the village pharmacy” in India because of its healing versatility, and it has been used in medicine for more than 4,000 years due to its medicinal properties. Neem is also called ‘*arista*’ a word that means perfect, complete and imperishable. The seeds bark and leaves contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal uses (Ruskin, 1992)

All parts of the tree have been examined by chemists which contains number of chemical compound called "triterpeness" or limonoids .there are nearly 100 proto limonoids, limonoids or triterpenoid, pentanor, hexane or triterpenoid and some none terpenioid (Ascher, 1993).

The Neem oil contains several terpenioid, steroids, alkaloids, flavonoids, glycoside and other components (Anon. 2001).Biological activities of Neem products has been used as an effective postharvest protestant for many crops .Neem is especially effective against the cow pea weevil (David *et al*., 2003).

In Sudan, Neem is introduced in the 20"century. The first one were planted at Shambat in 1916.Todaytrees are spread in town and villages along the Blue and White Nile, irrigated areas of Central Sudan, Kordofan and Darfur (Schmutterer, 1969). Research work in the Sudan dates back to the year 1987 when Siddig started some observation trials at Hodeida Research Station and he is one who recommended Neem water extracts as component of an integrated pest management package suggested for the control of potato pests such as leaf hoppers and cutworms (Siddig, 1987).

### 2.10. Damas (*Conocarpus lancifolius* Engl.)

*Conocarpus lancifolius* is one of the most important species in the family Combretaceae (Pandey and Misra, 2008). It is an evergreen tree that grows up to 20m in height and 60 - 250 cm or more in diameter. It is usually a multi-branched tree in its natural habitat (NAS, 1983).

The tree is multipurpose; wood which is the main product is used domestically for house construction, firewood and excellent charcoal. Commercially timber was more useful formerly; it was cut and exported from Somalia to Saudi Arabia for construction. Other potential uses include wood based board. Bark may be a useful source of tannins (Booth and Wickens, 1993).

The Damas tree is evergreen and its foliage makes a good fodder, also it is a good shade and roadside tree. It is used as wind breaks around irrigated agricultural areas and for avenue planting. As drought-resistant species, *C. lancijolius*is is one of the more promising trees for trials in arid areas. It is recommended for a variety of soil types including saline soils, and yields excellent charcoal and valuable wood (NAS, 1983).

Information on the importance of *C. lancifolius* in its native distribution areas relative to other species with similar wood, fuel and forage uses is lacking hence it is difficult to assess its importance. However, Somali tribe owing the Damas at the dry river valleys (wadis) containing *C. lancifolius* have restricted cutting because of the threat of overexploitation (Booth and Wickens, 1993).

Damas growsbest in areas where the mean annual temperature ranges from 20°c to 30°c,. The tree grows from sea level up to about 1000 m. The rainfall in its natural habitat is generally between 50 mm and 400 mm, but the tree grows mainly along seasonal watercourses. It can be grown in plantations in areas with less than about 400 mm but grows well only if irrigated or within reach of groundwater. It withstands drought conditions for several months when irrigation fails. Damas does well on deep soils ranging from pure sand to clays and loams, but has difficulty on shallow soils. It will tolerate moderately saline soils (NAS, 1983).

### 2.11.Argel*Solenostemma argel* (Del)

Argel *Solenostemma argel* (Del) of the family Asclepiadaceae is an aromatic and medicinal plant that contains a variety of chemicallyactive substances (Shayoub, 2003). It is widely distributed in Egypt, Libya, Chad, Algeria, Saudi Arabia, Palestine, and Central and Northern part of the Sudan (Ahmed, 2004). However, among these above mentioned countries, Sudan isregarded as the richest source of the Argel plant which grows naturally in the northern of the Sudan and extends from Berber to Abu- Hamad, especially the Rubatab area. In other Arabic countries and Sudan, the tradition name of this plant is Hargel. The part used of the plant is dried leaves and stems (El- Kamali *et al.*, 1996).

Phyto-chemicals of medicinalpropertiesfromArgel shoots had been reported by many workers (Roos *et al.*, 1980; Hamed, 2001). Suleiman*et al.*, (2009) reported that the aqueous extracts of argel have antifungal and antibacterial properties.The farmers in Kassala State put Argel shoots in porous jute sacks in the irrigation canals to be leached by water. The water was effective in controlling aphids and white flies in summer tomatoes and Egyptian bull worm in okra respectively (Elkamali and Khalid, 1996).

The pharmacological actives of different extracts of *S.argel*have been investigated by Roos*et al.,*(1980); Abdelwahab (2002) and Tharib, *et al*., (1986)confirmed the presence of antibiotic substances in the ethanol extracts of the Argelplant as well some antifungal properties of the plant.They further confirmed additionalsubstances or compounds isolated from the Argel stem, and weredemonstrated to have antibacterial properties against both gram – positive and gram – negative organisms.

# CHAPTER THREE

# MATERIAL AND METHODS

The first part of this study includes a survey which covered central markets of fruit and vegetable in Khartoum, Omdurman and Khartoum North whereas the second part was conducted in the laboratory of plant pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology during May-July, 2013.

The aim of this study isto find outsurvey the level of incidence of bacterial soft rot disease in five produces of horticulture in central markets of Khartoum State and to detect and identify the bacterium associated with this disease. The study also explored theantibacterial potentials of some higher plants crude extracts against the bacterium associated with soft rot disease of horticulture produces under *in vitro*conditions.

### 3.1.Materials, tools and equipments used in the study

- Petri-dishes
- Gloves
- Camera
- Marker pen
- Electric blender
- Sensitive balance
- Incubator
- Needle
- Flame
- Laminar flow cabinet
- Microscope
- Autoclave
- Slide

- Aluminum foul
- Water path
- Nutrient agar (NA)
- Gram stain
- Crystal violet
- Iodine
- Filter papers
- Medical cotton

All materials which used in this study were sterilized using 70% ethyl alcohol. Formalin (10%) was used for Petri plate sterilization.

### 3.2. Survey and Sampling

In order to record soft rot incidence in five horticultural produces, namely Banana, Orange, Mango, Potato and Tomato, and to collect diseased samples that showing typical symptoms of the disease, a survey of central markets of fruit and vegetable in Khartoum, Omdurman and Khartoum North were conducted.In each market,three cold store or shops (locations) were randomly selected and visited. In each location hundred pieces from each of the five produces were then randomly selected and keenly observed for soft rot symptoms. Total number of each crop pieces showing soft rot symptoms was recorded and percent disease incidence was then calculated using standard formula.

Also samples from each of the produces showing typical symptoms of soft rot disease were collected in order to isolate the causal organisms. Secured samples were put in paper bags, brought to laboratory and kept cool in Plant Pathology Laboratory for further investigations.

### 3.3. Isolation of bacterium

Diseased pieces of each horticultural produce showing typical soft rot lesions were selected for isolation of bacterium. Each sample was cleanedseparately, surface-sterilized with 0.5% sodium hypochlorite solution (for 30 seconds), washed with sterile distilled water, and ground in sterile 0.85% saline solution using sterile mortar under aseptic conditions. The resulting bacterial suspension was left undisturbed for a few minutes. A loop full of this suspension was then streaked on to plates containing nutrient agar (NA) (Bacto Agar 10gm, NaCl 5.0 gm, K2HPO4 5 gm, KH2PO4 2gm, Bacto-peptone 1.0gm),and incubated at +-2°C for 24 h. Individual colonies (transparent, circular, raised, shiny and creamy white)growing on NA were selected, re-suspended in 0.85 % saline, streaked on NA plates, and then incubated at+-2 °C for another 24 h. This was repeated several times in order to obtain pure cultures. The isolated pathogen wascharacterized on the basis of morphological and chemical reaction.

### 3.4. Characterization and identification of Isolated Bacterium

Characterizationand identification of soft rot *Erwinias*bacterium based on examination of the different characteristics with reference to existing taxa in the Bergey's Manual of Determinative Bacteriology (Safrinet, 2000; Buchaman and Gibbons, 2004). This includes examination of the culture colonies in terms of extent of growth, colour, nature of colony edge, elevation, etc.,microscopic examinationof the bacterium with a binocular microscope and of its reaction to general dye (Gram stain). Since its invention in 1884 by the Danish physician Christian Gram, Gram's Method of differential staining has been extensively used in diagnostic microbiology, The Gram reaction, which divides bacteria into two groups. Gram-positive and Gram-negative organisms, is often used as a primary criterion for their identification.

### 3.5. Collection of leaves samples

Neem and Damas leaves were collected from trees growing in the premises of the college of Agricultural study, Shambat whereas the Argel sample were obtained from local market. All samples were brought to the laboratory where they were shade dried under ambient temperature, ground and powdered separately to obtain fine powder for extraction.

### 3.6.Preparation of aqueous extracts and concentrations

Aqueous extracts of each of the plant materials were prepared as recommended by Okigbo (2006). The obtained fine powder form each plant was weighed in to 20, 30 and 40gm and placed each in a separate conical flask containing 100 ml distilled water and was placed in a shaker for 4 hours. The solutions were later filtered through layers of muslin cloth. Concentrations of 20%, 30% and 40% of the Argel, Neem and damas leaves extracts were prepared.

### 3.7.Agar well diffusion Method

The antimicrobial activity of the leaves aqueous extracts was determined by using the agar well diffusion techniqueas described by Rao and Srivastava (1994). In this method 2ml of bacterial cell suspension (10 X 105) of *Erwinia carotovora* obtained from an overnight culture of the bacterium were thoroughly mixed with 200 ml of sterile molten nutrient agar, then the medium was distributed into sterile Petri-dishes and was left to solidify at room temperature for 24 hours. In each plate a well was made by sterile standard cork borer.About 0.2 ml of each concentration of extracts was introduced into different wells. The control was filled with sterile distilled water. The plates were then arranged in the incubator following complete randomized design with three replications (Petri dishes) at +-3°C for 72 hours, after which they were observed every

24 hour for clear zones around the wells indicative of zones of inhibition, which were measured and recorded in mm.

**3.8.Statistical analysis:**

The data obtained was statistically analyzed according to analysis of variance (ANOVA), L.S.D test was used for means separation.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

The aim of this study are to investigate the level of incidence of bacterial soft rot disease in five produces of horticulture in Khartoum State and to detect and identify the bacterium associated with this disease. The study also explored the antimicrobial potentials of some higher plants crude extract against the bacterium associated with soft rot disease of horticulture produces under *in vitro.*

The results of the survey of the level of percentage incidence of bacterial soft rot disease in five produces of horticulture in Khartoum State were presented in Table (1)and Figure(1).During the course of survey it was found that the bacterial soft rot disease is prevalent in the five produces of horticulture in all the wholesale and retail markets of Khartoum State with variable incidence in each produce (Table, 1 and Fig, 1). The highest and significantly different disease incidence was observed in Tomatoes (9.3%) and the lowest incidence 3% and 3.3% was in Potato and Mango respectively.

It is worthwhile to mention that tomato is supplied to Khartoum from production areas distant. This practice is followed by tomatoes traders during hot summer months where cold storage facilities are lacking. Time elapsed in transit and lack of storage under relatively high atmospheric temperature for tomatoes supplied to Khartoum becomes the reason for higher post harvest soft rot disease in distant markets. Similar results were obtained by Bhat *et al*, (2010) who reported that damage of soft rot disease increases at higher temperatures. Rajeh*et al.,*(2000) and Raju *et al.,* (2008) also reported enhancementof soft rot disease at increased temperatures. This explanation could similarly be applied to the relatively high incidence (6.0%) and (4%) in Banana and Orange respectively.

Upon isolation, all the isolates of causal bacterium that readily macerated the host tissue were found to be *E. carotovora,* gram negative, rod shaped. Similar findings were reported by Oliveira *et al.,* (2003)who indicated that *E. carotovora subsp carotovora* favors relatively higher temperature.This report is supported by our findings that some of the bacteria isolated from samples collected from tomato exposed to high temperature do show characteristics belonging to *E. carotovora subsp carotovora* group.

The low incidences of soft rot disease in Mango could be attributed to the known low pH in this fruit which is inhibitory to the majority of bacterial plant pathogens. Moreover, other Erwinias which favors low temperature that exist during cold storage could be a reason for occurrence of the disease in orange and potato. Similar explanations were reported by (Oliveira *et al.*( 2003) who indicated that usually *E. carotovora subsp atroseptica* and *E. carotovora subsp carotovora* both are present latently in potato crop and iftemperature remains low for a considerable length of time during the growing season, *E. carotovora subsp atroseptica* will outgrow andif the temperature remains high, *E. carotovora subsp carotovora* will outgrow.

**Table 1: Percentage of Disease incidence of soft rotErwiniainfive horticultural produces in Khartoum State**

| Locations & Fruits/Veget. | Disease incidence Khart-oum | Khart. North | Omdur-man | Total | Mean |
|---|---|---|---|---|---|
| Banana | ٦ | ٧ | ٥ | ١٨ | (6.0%)b |
| Orange | ٤ | ٢ | ٦ | ١٢ | (4.0%)b |
| Potato | ٢ | ٣ | ٤ | ٩ | (3.0%)b |
| Tomato | ٧ | ١٢ | 9 | ٢٨ | (9.3%)a |
| Mango | ٢ | ٣ | ٥ | ١٠ | (3.3%)b |
| Mean% | 4.2 | 5.4 | ٥.٨ | ٧٧ | ٥.١٣ |
| CV | | | | | 33.3 |
| LSD | | | | | 2.23 |
| SE | | | | | 2.9 |

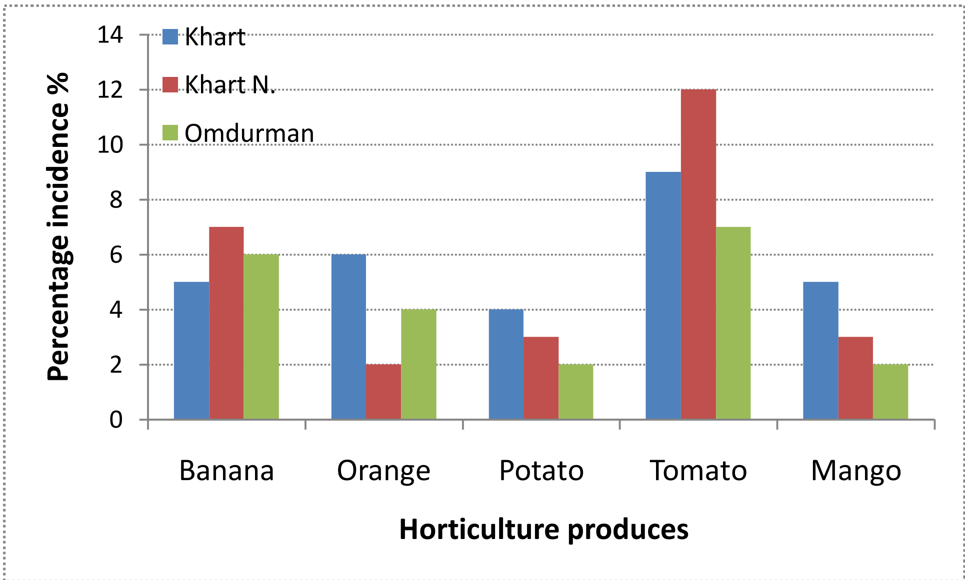


**Fig.1: Percentages incidence of Erwinia soft rote disease in five horticultural produces in Khartoum State**

The use of higher plantsextracts to control plant diseases is an environment friendly approach and an effective alternative to toxic chemical pesticides. Since very few botanical extracts have been documented to control the soft rot pathogens and many works reported the antibacterial action of higher plant extracts against a good number of bacterial pathogens (Khan *et al.*,2000 ; Sasitorn, 2003andOpara, 2010), the identification of effective plant extracts against the bacterium, *Erwiniacarotovora* was undertaken to control the soft rot disease.

In this study the results of the effect of leaves aqueous extracts of Hargel, Neem and Damas, at different concentrations, on the growth of *Erwinia carotovora* after 24,48 and 72 hours from inoculation *in vitro* was presented in Table 2, 3 and 4. After 24 hour from inoculation.The results indicated that all plants extracts at all concentrations exhibited varying degrees of inhibition against the *Erwinia carotovora*with zones of inhibition ranging from 27.4 to 100% (Tables,2, 3 and 4).Meanwhile, the Damas leaves extract showed more pronounced inhibitory effects on the growth of the bacterium where it completely inhibited its growth compared to control. Probably because of the diffusible active ingredient content; similar conclusion was made by earlier workers against some pests (Benjilal *et al*.,1984; Jones, 1985)Among plant extracts, Damas at all concentrations, Neem at 30% and 40% and Argel at 40% concentrations demonstrated significantly($P<0.05$) high zone of inhibition;100%, 99.3%and 97.4% respectively.However, this inhibitory effect continued for 72 hours to varying degree when compared with that of the control (sterile water. Similar results were obtained by Krebs and Jaggir (1999) who investigated water extract of Neem and hemp flowers against *E. carotovora* causal agent of potato soft rot. The extracts were tested *in vitro* using a pure bacterial culture and *in vivo* on potatoes latently infected with the pathogen.

Moreover, extracts of many species of plants were reported to have antimicrobial activities (Bullerman, 1974, Abdel-Rahim *et al.,* 1989 and Al-Jali *et al.,* 1997).Vlietinck *et al.,*(1995) screened about 100 medicinal plants, used by traditional healers to treat infections in Rwanda, for their antibacterial, antifungal and antiviral properties. Their study showed that

45% were active against tested bacteria, fungi and antiviral properties. In Sumatra (Indonesia),114 plant species extracts were assayed for their antibacterial activity (Ahmed,2002).

In Sudan Ahmed (2004) demonstrated the antibacterial effect of some medicinal plants against the gram-positive and gram-negative bacteria as well.Similar studies which explored the effect of extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish *et al.,* 1999; Okigbo, 2006; Shariff *et al.,* 2006). More recent results were demonstrated by Agrios,(2005) who proved the presence of antimicrobial compounds in higher plants and which has been recognized as important products in combating plant pathogenic diseases. He mentioned that such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases.

**Table 1: Effect of leaves aqueous extracts of Argel, Neem and Damas on the growth of *Erwinia carotovora* after 24 hours from inoculation *in vitro*.**

| Treatments and concentrations (%) | | Inhibition zone (%) | | | |
|---|---|---|---|---|---|
| | | R1 | R2 | R3 | Mean |
| Hargel | 20 | 23.9(4.9) | 27.0(5.2) | 31.4(5.6) | 27.4(5.2)c |
| | 30 | 89.6(9.5) | 81.0(9.0) | 75.4(8.7) | 82.0(9.0)b |
| | 40 | 97.0(9.9) | 95.1(9.8) | 100(10.0) | 97.4 (9.9)a |
| Neem | 20 | 82.9(9.1) | 86.4(9.3) | 82.9(9.1) | 84.0(9.2)b |
| | 30 | 97.8(9.9) | 100(10.0) | 100(10.0) | 99.3(9.9)a |
| | 40 | 100(10.0) | 100(10.0) | 100(10.0) | 100(10.0)a |
| Damas | 20 | 100(10.0) | 100(10.0) | 100(10.0) | 100(10.0)a |
| | 30 | 100(10.0) | 100(10.0) | 100(10.0) | 100(10.0)a |
| | 40 | 100(10.0) | 100(10.0) | 100(10.0) | 100(10.0)a |
| Control | | 0.00(0.7) | 0.00(0.7) | 0.00(0.7) | 0.00(0.7)e |
| LSD | | | | | 2.06 |
| SE± | | | | | 2.4 |
| CV% | | | | | 19.9 |

Means followed by the same letter are not significantly different at ($P<0.05$). Means between brackets are transformed according to $\sqrt{X+0.5}$ .

The results also indicated there was a variation in the potency of the plant extracts as well as in the sensitivity pattern of the test bacterium (Tables, 2,3 and 4).Moreover, the current study showed that the screened concentrations of plant extracts differ in their reactions to test bacterium. Likewise the test organisms responded differently to the different concentrations of extracts. This variability in response which expressed by test organisms to different plant extracts was also reported by Aiyelaagbe (2001). In his investigation, he explained that the majority of the studies involving plant parts or extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree.

**Table 2: Effect of leaves aqueous extracts of Argel, Neem and Damas on the growth of *Erwinia carotovora* after 48 hours from inoculation *in vitro*.**

| Treatments and concentrations (%) | | Inhibition zone (%) | | | |
|---|---|---|---|---|---|
| | | R1 | R2 | R3 | Mean |
| Argel | 20 | 17.1(4.2) | 25.6(5.1) | 3.20(1.9) | 15.6(3.7)c |
| | 30 | 82.0(9.0) | 75.6(8.7) | 58.7(7.7) | 72.1(8.5)b |
| | 40 | 94.3(9.7) | 91.5(9.6) | 100(10.0) | 95.3(9.8)a |
| Neem | 20 | 82.9(9.1) | 86.4(9.3) | 82.9(9.1) | 84.0(9.2)ab |
| | 30 | 94.3(9.7) | 100(10.0) | 100(10.0) | 98.1(9.9)a |
| | 40 | 100(10.0) | 100(10.0) | 100(10.0) | 100.0(10)a |
| Damas | 20 | 93.4(9.7) | 97.9(9.9) | 97.4(9.1) | 96.2(9.6)a |
| | 30 | 97.1(9.9) | 98.7(9.5) | 100(10.0) | 98.6(9.8)a |
| | 40 | 100(10.0) | 100(10.0) | 100(10.0) | 100.0(10)a |
| Control | | 0.00(0.7) | 0.00(0.7) | 0.00(0.7) | 0.00(0.7)d |
| LSD | | | | | 2.06 |
| SE± | | | | | 2.90 |
| CV% | | | | | 21.08 |

Means followed by the same letter are not significantly different at ($P<0.05$).Means between brackets are transformed according to $\sqrt{X + 0.5}$ .

**Table 3: Effect of leaves aqueous extracts of Argel, Neem and Damas on the growth of *Erwinia carotovora* after 72 hours from inoculation *in vitro*.**

| Treatments and concentrations (%) | | Inhibition zone (%) | | | |
|---|---|---|---|---|---|
| | | R1 | R2 | R3 | Mean |
| Argel | 20 | 22.5(4.8) | 22.3(4.8) | 10.8(3.4) | 18.5(4.3)d |
| | 30 | 79.3(8.9) | 73.9(8.6) | 54.0(7.4) | 69.0(8.3)c |
| | 40 | 86.8(9.3) | 85.6(9.3) | 98.6(9.1) | 90.3(9.2)ab |
| Neem | 20 | 80.0(8.1) | 84.4(9.2) | 85.7(9.3) | 83.3(8.7)bc |
| | 30 | 93.8(9.7) | 88.0(9.4) | 100(10.0) | 93.9(9.7)a |
| | 40 | 100(10.0) | 100(10.0) | 100(10.0) | 100.0(10)a |
| Damas | 20 | 96.3(9.8) | 98.0(9.9) | 97.8(9.9) | 97.3(9.8)a |
| | 30 | 95.0(9.7) | 98.5(9.9) | 100(10.0) | 97.8(9.8)a |
| | 40 | 100(10.0) | 100(10.0) | 93.8(9.7) | 97.9(9.9)a |
| Control | | 0.00(0.7) | 0.00(0.7) | 0.00(0.7) | 0.00(0.7)e |
| LSD | | | | | 2.06 |
| SE± | | | | | 2.40 |
| CV% | | | | | 19.2 |

Means followed by the same letter are not significantly different at ($P<0.05$). Means between brackets are transformed according to $\sqrt{X + 0.5}$ .

## CONCLUSIONS

- Bacterial soft rot causes postharvest disease in fruit and vegetables. The bacterium infects produce before harvest and then remains quiescent until conditions are more favorable for disease development after harvest. Also it may infect produce during and after harvest through surface injuries. In this study the survey revealed that the bacterial soft rot disease is prevalent in the five fruits and vegetable screened with varied incidence in each produce;

- The disease was significantly high in produces where distant transport was practiced under lack of cold storage e.g. Tomatoes and Banana;

- Upon isolation on NA medium all the isolates of causal bacterium that readily macerated the host tissue were found to be *Erwinia carotovora.*

- Investigations on antibacterial activity of the three plants extracts against bacterium indicated that all extracts at all concentrations exhibited varying degrees of inhibition against the *Erwinia carotovora.*

- Among plant extracts Damas showed more pronounced inhibitory effects on the growth of the bacterium;

- There was a variation in the potency of the plant extracts as well as in the sensitivity pattern of the test bacterium, Erwinia;
- The potentials of the plant extracts to serve as possible bio-control and antimicrobial agents for bacterial soft rot of fruits and vegetables were thus demonstrated in this study.

## RECOMMENDATIONS

Based on the foregoing results the following studies are recommended;

- To further investigate the level of incidence of soft rot disease in fruits and vegetables caused by other pathogens.
- To carry an antimicrobial properties investigations but in a group of medicinal plants against targets organism to determine their potentials as botanical pesticides,
- The inhibitory activity of plant extracts was most likely due to antimicrobial components present in plant extracts. However, the exact chemical compounds and their controlling mechanism to the soft rot bacteria need to be elucidated

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# APENDICES

**Appendix (1):**

Randomized Complete Block AOV Table for INHIBITION Table, 1: Percentages incidence of Erwinia soft rot disease in five horticultural produces in Khartoum State

| **Source** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| REPLICATI | 2 | 6.933 | 3.4667 | | |
| TREATMENT | 4 | 82.400 | 20.6000 | 7.36 | 0.0087 |
| Error | 8 | 22.400 | 2.8000 | | |
| Total | 14 | 111.733 | | | |

**Appendix (2) :**

Randomized Complete Block AOV Table for INHIBITION

Table, 2: Effect of leaves aqueous extracts of Hargel, Neem and Damas on the growth of *Erwinia carotovora* after 24 hours from inoculation *in vitro*.

| Source | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| REPLICATI | 2 | 0.243 | 0.1213 | | |
| TREATMENT | 3 | 273.298 | 91.0994 | 34.62 | 0.0000 |
| Error | 24 | 63.153 | 2.6314 | | |
| Total | 29 | | | | |

**Appendix (3):**

Randomized Complete Block AOV Table for INHIBITION

Table, 3: Effect of leaves aqueous extracts of Hargel, Neem and Damas on the growth of *Erwinia carotovora* after 48 hours from inoculation *in vitro*.

| **Source** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| REPLICATI | 2 | 1.046 | 0.5230 | | |
| TREATMEN | 3 | 244.919 | 81.6396 | 26.60 | 0.0000 |
| Error | 24 | 73.647 | 3.0686 | | |
| Total | 29 | | | | |

**Appendix (4):**

Randomized Complete Block AOV Table for INHIBITION
Table, 4: Effect of leaves aqueous extracts of Hargel, Neem and Damas on the growth of *Erwinia carotovora* after 72 hours from inoculation *in vitro*.

| **Source** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| REPLICATI | 2 | 0.203 | 0.1013 | | |
| TREATMENT | 3 | 235.202 | 78.400 | 30.70 | 0.0000 |
| Error | 24 | 61.297 | 2.5541 | | |
| Total | 29 | | | | |

**Plate 1:Banana infection by the Soft rot**

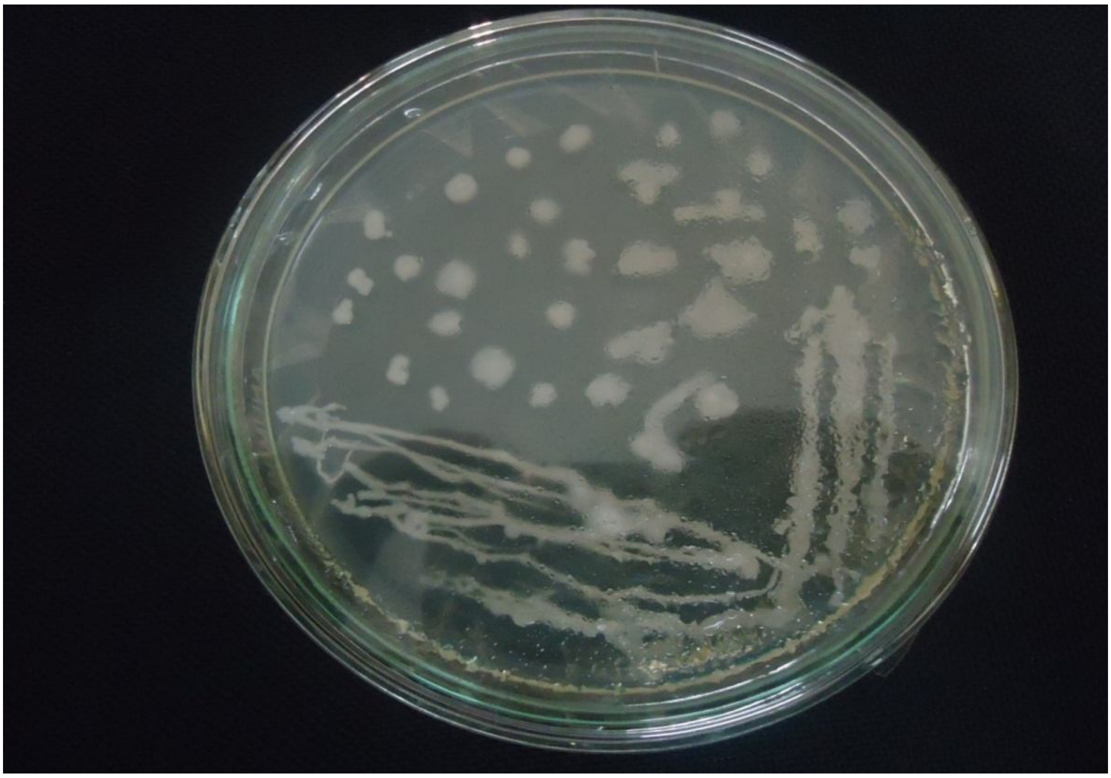

**Plate 2: Pure Cultureof Bacteria (*Erwinia carotovora*)**

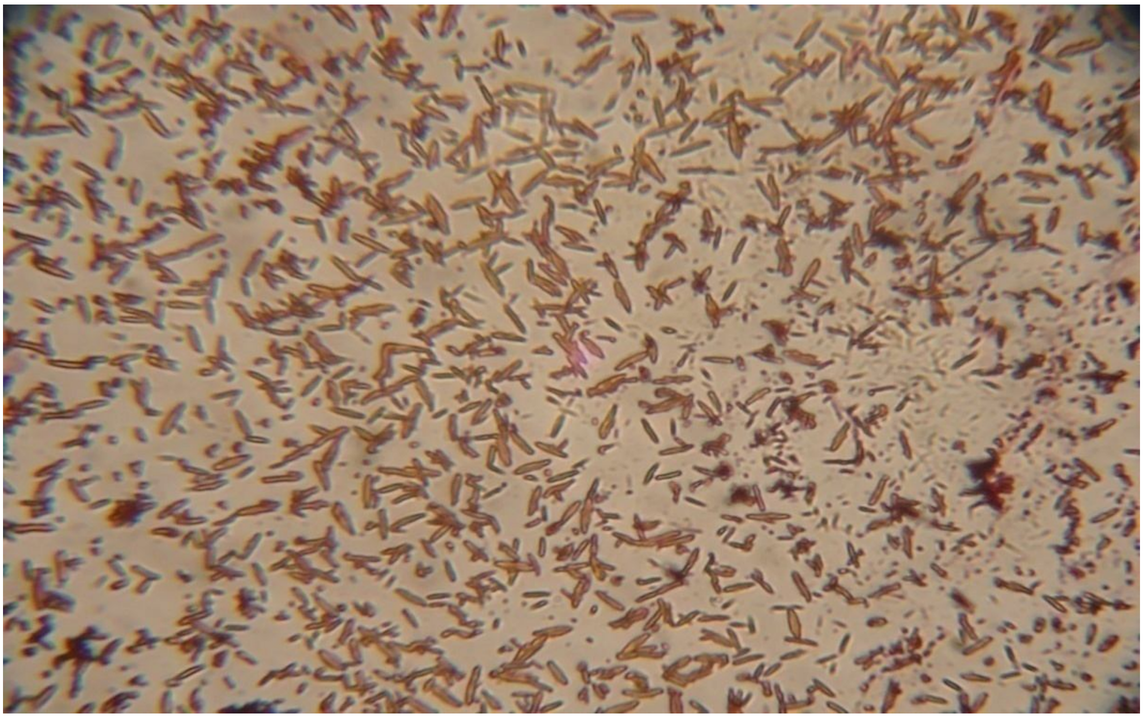

**Plate 3:Bacteria (*Erwinia carotovora*)**