



Sudan University of Science and Technology

College of Graduate Studies



**In Vitro Evaluation of Two Plants (Ginger and Cinnamon)
Aqueous Extracts against the Bacteria *Streptomyces spp* of
Potato Common Scab**

تقويم استخدام المستخلصات المائية لنباتي الجندريد والقرفة علي البكتريا
Streptomyces spp المسبب لمدى الجرب العادي في البطاطس معمليا

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قال آعالى:

(رَبَّنَا إِنَّا سَمِعْنَا مُنَادِيًا يُنَادِي لِلْإِيمَانِ أَنْ
آمِنُوا بِرَبِّكُمْ فَآمَنَّا رَبَّنَا فَاغْفِرْ لَنَا
ذُنُوبَنَا وَكَفِّرْ عَنَّا سَيِّئَاتِنَا وَتَوَفَّنَا مَعَ
الْأَبْرَارِ).

صدق الله العظيم

سورة آل عمدان الآفة 193

Dedication

To my Mother and Father,

Who encourage me to study and

to those who assisted me to attain my goals.

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First of all I want to render my praise to his almighty Allah, the most Gracious the most merciful who gave me life, health, knowledge and patience to complete this work.

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ABSTRACT

This study was conducted at the plant pathology laboratory Sudan University of Science and technology of college of agricultural studies during the period (November 2019 – April 2019). The aim of this study is to evaluate the inhibitory effect of aqueous extracts of two plants Ginger (*Zingiber officinale*) and cinnamon (*Cinnamomheum*

verumis) on the growth of the bacterium *Streptomyces* spp *in vitro*. Different concentrations (5%, 10% and 20%) of each of the tested plants, in addition to penamox 2% (Amoxicillin) antibiotic were tested and compared to the untreated control H₂O. Penamox 2% recorded the highest inhibitory effect (3.07). The result showed that all concentrations (5%, 10% and 20%) of aqueous extracts of both plants were found to be significantly different ($p \leq 0.05$) when compared to the untreated control (H₂O) and the Penamox 2% and reducing the inhibitory effect by 2.6, 2.6 and 2.6 for ginger and 2.5, 2.6 and 2.6 for cinnamon, respectively. The study concluded the antibiotic (Penamox 2%) is the most effective (3.07) followed by the different concentration by 2.6, 2.6 and 2.6 ginger and cinnamon 2.5, 2.6 and 2.6 respectively. We recommended further studies to evaluate the effect of these plants on the growth of the bacterium under field condition.

ملخص الاطروحة

أجريت هذه الدراسة في مختبر أمداء النبات بجامعة السودان للعلوم والتكنولوجيا بكلية

الدراسات الزراعية خلال الفترة (نوفمبر 2019 - أبريل 2019). الهدف من هذه الدراسة هو تقييم التأثير المثبط للمستخلصات المائية لنبات الزنجبيل (*Zingiber officinale*) والقرفة (*Cinnamomum verumis*) على نمو بكتيريا *Streptomyces spp*. تم اختبار تركيزات مختلفة (5% ، 10% و 20%) لكلا النباتات التي تم اختبارها ، بالإضافة إلى المصنوع الحيوي 2% Penamox (Amoxicillin) ومقارنته مع الشاهد (untreated control) H₂O. سجل 2% Penamox أعلى تأثير تثبيطي (3.07). أظهرت النتائج أن جميع التركيزات (5% ، 10% و 20%) من المستخلصات المائية لكلا النباتين كانت مختلفة بشكل كبير ($P \leq 0.05$) بالمقارنة مع الشاهد (H₂O) و 2% Penamox. تقلد التأثير المثبط بنسبة 2.6 ، 2.6 و 2.6 للزنجبيل و 2.5 و 2.6 و 2.6 للقرفة ، على التوالي. أثبتت الدراسة أن المصنوع الحيوي 2% Penamox هو الأكثر فعالية مقارنة بـ بالتراكيز المختلفة للزنجبيل والقرفة (2.5 و 2.6 و 2.6) (2.6 ، 2.6 ، 2.6) علي التوالي. نوصي بإجراء المزيد من الدراسات المستقبلية وتطبيقها علي نمو البكتريا تحت ظروف الحقل.

CHAPTER ONE

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to the family Solanaceae, which involves tomato, eggplant and other vegetables. This family contains 90 genera and 2000 species and named to the genera Solanum (Ahmed, 1999).

Potato is the only vegetable listed among the five principal world food crops (FAOSTAT, 2015). It comes in the forefront of tuber-crops and occupies the fourth position after wheat, sorghum and rice as the most edible and consumed crops in the world (Singh *et al.*, 2004).

Other researcher (Abdul *et al.*, 2013; Ali *et al.*, 2015) put the potato fourth most important food crop worldwide after maize, wheat and rice, with production of more than, 323 million tones. Moreover, its nutritional value is higher than most of the food crops. It is considered as the richest source of carbohydrates. It is a very rich source of nutrient contents containing good amount of starch, carbohydrates, vitamins especially C and B1, minerals, protein, fat and amino acids (Buckenhuskas, 2005).

Potato was introduced to the Sudan sometimes before the second World War, since then it has been expanding in a rapid manner both horizontally and vertically, as the crop suits wide range of conditions in the Sudan (Alameen, 2003).

In Sudan, Potato is planted as a cash and food crop and plays an important role in the agricultural economy of the country. The area under potato is around 30 thousand hectares with an annual production of 400 000 tones and an average yield of 15 t/ha (Elraiah *et al.*, 2013).

Potato production in Sudan has increased in response to high demand due to urbanization and awareness of its nutritional value (Elraiah *et al.*, 2013).

In the Sudan several potato diseases can cause severe crop losses if not properly managed. These include early blight, late blight, scab, black leg, natural occurrence of Potato virus S (PVS), potato virus X (PVX) potato virus Y (PVY) and potato leaf roll virus (PLRV) were reported in important areas of production in the country. Rhizotonia, verticillium wilt, Fusarium dry rot, and bacterial soft rot (Christ, 1998).

In the Sudan, currently *S. scabies* has become very dangerous and a devastating disease on potato. All varieties are susceptible to this disease. However, common scab disease of potatoes caused by *S.*

scabies (bacteria), cause seed decay and reduce market value. Thus, the present study was undertaken with the following main objective

I) To evaluate the relative efficacies of selected botanical extracts *Ginger (Zingiber officinale)* and *Cinnamon (Cinnamomum verumis)* against the potato common scab bacterial isolate *in vitro*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Potato crop:

Potato, (*Solanum tuberosum* "L."), is the world's fourth most important crop after after maize, wheat and rice . It is grown chiefly in the temperate zone. Potato belongs to the family Solanaceae. This family also includes several plants which are of high food value. The genus *Solanum* that includes the potato comprises 2000 species (Hawkes, 1989).

The potato species can be found throughout the Americas, from the United States to Southern Chile (Hijmans and Spooner, 2001). The potato was originally believed to have been domesticated independently in multiple locations, but later genetic testing of the wide variety of cultivars and wild species traced a single origin for potatoes. In the area of present-day southern Peru and extreme northwestern Bolivia, from a species in the *Solanum brevicaulis* complex, potatoes were domesticated approximately 7,000–10,000 years ago (Spooner, 2005).

Potato is one of the major vegetable crops grown worldwide following maize, wheat and rice with a production of 368 million tons (FAOSTAT, 2015).

Rich (1983) mentioned that it is one of humankind's most valuable foods, is produced in 130 countries where three -fourth of world's population lives. More than 90 of which are located in the tropical and subtropical zones. Although most of the production still comes from the temperate zone in the industrialized Countries, mainly in Asia (Vanderzaag, 1991).

In Sudan, potato is grown mainly as winter crop and the main area of production are along the Nile bank in both Khartoum and Northern states. Although potato cultivation in Sudan depends, mainly on exotic advanced cultivars but an old introduced material is still produced in Jabal Mara in the far west and it is locally known as Zalingei potato (Abdelgadir, 2005).

At Jebel Mara in Western Sudan the variety grown is of unknown origin. Some new other varieties with high yield include Diamond, Famoza, Ajax, Draga, Turbo, Akira, Maradona and Oblex, (Ali, 2000). The estimated total potatoes production in Sudan is about 616,000 tons in cultivated area of about 88,000 feddans (Hind and Mohamed, 2010).

2.2 Nutritional Value

A raw potato is 79% water, 17% carbohydrates (88% is starch), 2% protein, and contains negligible fat. In an amount measuring 100 grams (3.5 oz), raw potato provides 322 kilojoules (77 kilocalories) of

energy and is a rich source of vitamin B6 and vitamin C (23% and 24% of the Daily Value, respectively), with no other vitamins or minerals in significant amount. The potato is rarely eaten raw because raw potato starch is poorly digested by humans (Beazell *et al.*, 1939).

2.3 Bacterial Diseases of potato

Anonymous (1960) listed approximately 160 diseases and disorders of *Solanum tuberosum*. About 50 of them are caused by fungi, 30 by viruses, 10 by bacteria, and another 50 or so are either non parasitic or due to unknown causes. Several others are due to nematodes or insect pests, in addition to parasitic weed dodder (*Cuscuta* spp).

Streptomyces was often classified with the fungi rather than with the bacteria (Anonymous, 1960). It produces rudimentary hyphae which produce spores by the formation of separations. It is also classified with the bacteria. It belongs to the order Actinomycetales and family Streptomycetaceae (Walker, 1969).

2.3.1 *Streptomyces* spp

Scab disease of potato and certain other root crops are caused by streptomycetes. *Streptomyces* spp are aerobic, filamentous, Gram-positive prokaryotes belonging to the order Actinomycetales, suborder Streptomycineae, family Streptomycetaceae and genus *Streptomyces*. According to Kutzner (1981).

Thaxter (1892) reported that the causal agent of common scab is the

actinomycete *Streptomyces* spp Thaxter first identified and described the pathogen in 1890, calling it *Oospora scabs*.

The name was changed to *Actinomyces scabs* in (Gussow, 1914). Some writer classifies it as a bacterium (Walker, 1969). While others, including the United States Department of Agriculture, usually classify it as fungus (Anonymous, 1960; Drechsler, 1919; and Rich 1976). It produces a rudimentary, coiled hyaline mycelium characteristic of fungi, septations then it routed in one - celled hyaline spores $1 - 2 \times 0.6 - 0.7 \mu$ resembling bacteria. These spores germinate by means of one or two germ tubes. Some systematists consider the actinomycetes a connecting link between the bacteria and true fungi, but Drechsler disagrees (Rich, 1983).

Deeper pitted scab usually produces distinct brown, corky, sunken lesions of variable size, they are roughly circular, and the majority of them 1-5 mm in diameter. Thin-skinned red or white cultivars are usually susceptible (Rich, 1983).

Robert (1955) mentioned that common scab, ordinary scab, brown scab, potato scab, and scab are some of the names applied to what it is undoubtedly, the most common malady affecting Potato, as this scab is known wherever potato are cultivated. The earliest mention of the disease appears to be that in London Encyclopedia of agriculture in 1825, but the causal agent was not described until

1890 when Thaxter published his paper on it. In addition to *S. scabies*, other *Streptomyces* species have been shown to or reported to cause common scab or other scab-like diseases of potato. Three of these (*S. acidiscabies*, *S. caviscabies* and *S. turgidiscabies*) have recently been described (Lambert, 1989; Goyer *et al.*, 1996, Miyajima *et al.*, 1998).

2.3.2 Distribution

Common scab of potato is one of the oldest known plant diseases and was first described in 1890 (Thaxter, 1891). Current information suggests that common scab is almost as widely distributed as the host itself (Loria *et al.*, 1997).

Common scab occurs most frequently on light, sandy, or gravelly soils, and it is most prevalent in dry seasons. It is rare on peaty and heavy soils (Brooks, 1928).

Bacterial growth is favored by warm, moist conditions. Bacteria thrive on medium containing starches and sugars. Thus potato tubers are a good medium for their growth. Some bacteria attack only tubers, while others attack both tubers and plants (Rich, 1983).

It is present in all the potato-growing areas of North America and Europe (Keinath and Loria 1989). Tashiro *et al.* (1983) reported the incidence of the disease in the Far East, whereas Mohanty *et al.* (1980) refer to a serious common scab problem in the coastal tracts of the Cuttack and Puri districts in India. A report by Pung and Cross (2000) confirmed the presence of the disease in Victoria,

Australia, and particularly the island of Tasmania. It was previously reported in South Australia. Common scab also occurs in Austria, Denmark, (Oestergaard and Nielsen, 1979), France (Bouček-Mechiche *et al.*, 2000), Finland (Heinamies and Seppanen, 1971), Germany (Koronowski and Massfeller, 1972), Greece (Alivizatos and Pantazis, 1992), Israel (Doering-Saad *et al.*, 1992), Hungary (Elesawy and Szabo, 1979), Ireland (Dowley, 1972), the Netherlands (Janse, 1988), Sweden (Emilsson and Gustafsson, 1953), Norway (Bjor and Roer, 1980), Poland (Sadowski *et al.*, 1996), Arabia (Ali, 1987), Korea (Park *et al.*, 2003), Japan (Miyajima *et al.*, 1998) and the United Kingdom (Large and Honey, 1953; Read *et al.*, 1995). The disease is present in all potato production regions of South Africa; albeit at varying levels (Marais and Visser, 1989).

Tarr (1955) reported that the distribution of any plant disease within the Sudan is governed largely by the presence or absence of suitable host plants and suitable climatic condition. However, other factors do undoubtedly play a part. Thus soil characteristics including pH composition, temperature and moisture content are important in root diseases. . The pathogen is soil-borne.

2.3.3 The Economic Importance

The disease is widespread in all the potato growing regions of the world and reduces the marketability of table, processing and seed potatoes and was ranked as the fourth most important disease in a

1999 survey of potato growers (Loria *et al.*, 1997).

Wheeler (1972) noted common scab of potato caused by *S. scabies* unsightly pustules of the tubers reduce their market value and storage qualities.

2.3.4 Host range

Common scab pathogens not only cause disease in potato, but can attack the fleshy roots of radish, turnip, beet (*Beta vulgaris* L.), groundnut (*Arachis hypogaea* L.), carrot (*Daucus carota* L.), rutabaga (*Brassica napus* L. var. *napobrassica* (L.) Rchb.), parsnip (*Pastinaca sativa* L.), mangel (*Beta macrorrhiza* Steven) and (*Tragopogon porrifolius* L.) (Lutman and Johnson, 1915; Jones, 1953; Goth and Webb, 1986; Sherf and MacNab, 1986; De Klerk *et al.*, 1997; Goyer and Beaulieu, 1997; Boucek – Mechiche *et al.*, 2000)

In addition, groundnuts grown in rotation with potato have exhibited water-soaked lesions and *S. scabies* was consistently isolated (De klerk *et al.*; 1997). Various other monocotyledonous and dicotyledonous crops have also been reported to be negatively affected when artificially infected with *S. scabiei*

(Hooker and Kent, 1946; Hooker 1949; Leiner *et al.*, 1996), probably due to toxic effects induced by thaxtomin A, a broad-spectrum phytotoxin produced by the scab pathogen (Leiner *et al.*, 1996).

2.3.5 Description

Streptomyces spp is a Streptomycete bacteria which means it forms a mycelium made of hyphae, a growth form more usually associated with fungi. The hyphae of *Streptomyces*, are much smaller than those of fungi (0.5–2.0 μm) and form a heavily branched mycelium. They are Gram-positive and have a high proportion of the DNA bases guanine and cytosine (Loria *et al.*, 2003).

Paul Dyson (2011) *Streptomyces* spp, When cultured on agar the hyphae develop aerial fragments which bear chains of spores, giving the culture a fuzzy appearance. The chains of spores have the appearance of corkscrews and are grey in color (Loria *et al.*, 2003). These chains allow it to be differentiated from other species that are virulent on potatoes. Each chain contains 20 or more spores that are 0.5 by 0.9–1.0 μm , smooth and grey. Bacteria are often distinguished by their ability to grow on media containing different substances, which they either feed on or that inhibit their growth. Defining characteristics of strains of *Streptomyces* spp are that they grow on the sugar raffinose, are unable to degrade xanthine and when grown on media containing the amino acid tyrosine, they produce the pigment melanin, the same chemical that gives humans their skin colour. This trait is often associated with their ability to cause disease, but it is not always present and is considered a secondary trait.

2.3.6 Disease Cycle of *Streptomyces* spp

Streptomyces spp was the first to describe and isolate the causal agent of common scab in North America as *Oospora scabies* (Thaxter 1891). The change in name to *Streptomyces scabies* occurred in 1948, was revived in 1989, but then changed to *S. scabies* in 1997 to follow grammatical convention (Lambert and Loria 1989). A 2007 opinion paper published by Saddler *et al.* requests the approval of continued use of *S. scabies* in reference to the potato pathogenic *S. scabies*. While no final decision has been made on this taxonomic concern, a rebuttal to Saddler's paper was published in 2008.

Cylindrical spores (0.5 by 0.9–1.0 μm) are produced in mature spiral chains containing 20 or more spores (Lambert and Loria 1989). Spores are released from the tip of the hyphae. The bacterium is dispersed by spores and survives on seed, in soil, and in soil water (Agrios 2005). The hydrophobic characteristic of the spores allow's them to also be transported by arthropods and nematodes (Loria *et al.*, 2006).

The spores germinate and enter plant tissues through wounds, larval feeding sites, stomata, and lenticels (Locci 1994; Agrios 2005). Penetration of tubers by *Streptomyces* spp and other pathogenic *Streptomyces* species is thought to take place through young lenticels, probably because they have not yet formed a layer of protective suberin (loci 1994). Loria *et al.* (2003) demonstrated that penetration and growth occurs through the cell walls. Young tubers

are most susceptible up until three to four weeks from tuber initiation (Khatri *et al.*, 2011 and Agrios 2005).

Thaxtomin, a phytotoxin produced by *Streptomyces* genotypes that cause common scab, may aid penetration of rapidly growing plant cells, such as expanding internodes (Loria *et al.*, 2003).

Tegg *et al.* (2005) demonstrated that thaxtomin is more effective in young, physiologically active tissues, which would include expanding internodes. Studies suggest that thaxtomin results in a compromised cell wall (Scheible *et al.*, 2003) allowing for penetration.

The tuber is the only known tissue type displaying symptoms on potato (Powelson and Rowe 2008). However, Han *et al.*, (2008) demonstrated that *S. scabies* affects the emergence and growth of roots at early stages of development. When exposed to a tuber, the pathogen grows through the outer few cell layers symplastically and apoplastically (Loria 2001 and Agrios 2005). As host cells die, they provide nutrients for the bacterium. Subsequently, the living host cells around the area of the infection divide and produce layers of cork cells that eventually push outward and form a scab lesion on the tuber. Surface (russet scab), raised (erumpent scab), and pitted (pitted scab) lesions may be observed on the same tuber, and can vary in size; lesions may also coalesce to form large scab-by areas on a tuber.

2.3.7 Conditions favorite to Disease

Streptomyces species that are pathogenic on potato can potentially cause lesions on the tubers of susceptible genotypes given the occurrence of favorable conditions including a pH higher than 5.2, temperatures of 20–22 °C, and soil moisture below field capacity during early tuberization (Archuleta and Easton 1981). Water develops a microfilm around the developing tuber to limit infection (Gudmestad 2008). Streptomyces spp will generally cause disease in soils with less than 65–70% soil moisture (Gudmestad 2008). Environmental conditions may further influence the types of lesions observed (shallow, raised, or pitted) (Goyer *et al.* 1996). However, lesion type has also been associated with pathogen species and/or the presence of one or more virulence factors (Boucheck-Mechiche *et al.* 2000). Multiple species and/or strains of Streptomyces may be present within the tuber spheremicrobial community, making it difficult to discern tight associations among these factors. Environmental factors that are conducive to common scab caused by Streptomyces spp include low soil moisture during tuber initiation, daytime temperatures above 70 °F, and a soil pH range of 5.5 to 7.5. The severity of scab lesions increases as the soil pH increases from pH 5.2 to 8.0. Above pH 8.0, scab severity decreases again (Agrios 2005).

2.3.8 Diagnostic Methods

Lambert and Loria (1989) explained that *S. scabies* can be

distinguished from other scab causing Streptomyces by their use of raffinose as a sole carbon source, production of melanin, in ability to degrade xanthine and grow below pH 5, and susceptibility to Streptomycin and crystal violet. Using DNA hybridization techniques the distinctiveness of *S. scabies*

strains from other scab forming Streptomyces was confirmed, through a high degree of diversity that exists within the species (Healy and Lambert, 1991). These findings were confirmed by (Paradis *et al.*, 1994), studying fatty acid composition, protein profiling and DNA – DNA hybridization of 31 pathogenic and non pathogenic strains from potato. They found that *S. scabies* strains, whilst highly diverse, could be distinguished from other pathogenic Streptomyces.

2.3.9 Control of the Disease

2.3.9.1 Use of irrigation

Lapwood (1966) confirmed that scab is most severe when the soil is dry soon after tubers begin to develop.

In the United Kingdom and Europe, common scab is controlled largely by specified irrigation schedules (Lapwood, 1966; Lapwood *et al.*, 1970, 1971, 1973; Wellings and Lapwood 1971; Davis *et al.*, 1976; Adams *et al.*, 1987).

Western (1971) pointed out that supplementing rainfall by irrigation

were practicable to keep the soil moist for perhaps 4–6 weeks after tuber initiation seems a promising way to control scab even on the most affected land.

High soil moisture and low soil temperature after tuber initiation reduce the disease. Tubers become infected while still very young and if they can survive this period without infection they escape the disease (Lapwood and Hering, 1970).

Various theories have been proposed to explain the mechanism of scab control by irrigation (Lapwood and Adams, 1975; Adams and Lapwood, 1987). Firstly, there is a direct influence on tuber susceptibility, e.g. lenticel proliferation. Soil moisture is necessary for the development of lenticels from stomata. With adequate soil moisture, susceptible stomata rapidly develop into resistant lenticels and are therefore protected from infection (Labruyère, 1971; Adams, 1975). Secondly, there are direct effects on the growth of the pathogen due to lowering of the soil temperature and reduced oxygen availability. Thirdly, irrigation indirectly affects disease by providing an environment conducive to antagonism (Lewis, 1971). Antagonistic bacteria move faster than scab-causing *Streptomyces* spp in the water films in wet soil. The antagonistic bacteria colonise the lenticels first and compete with the pathogen for the niche. Fourthly, high moisture levels have been associated with a decrease

in calcium levels in tuber tissue and it has been implied that increased calcium leads to increased scab susceptibility (Horsfall *et al.*, 1954; Davis *et al.*, 1976).

2.3.9.2 Resistant varieties

Walker (1957) reported that resistant varieties are the ultimate solution for control of this disease. The tolerant varieties are useful in this regard, but they are not successful under conditions extremely favorable for scab. Western (1971) pointed that the resistance occurs on a big number of russetted skin varieties. Pentland crown is more resistant than Majestic, but there is no acceptable replacement for King Edward). Somewhat resistant varieties include Menonimee, Ontario, Cayuga and Seneca (Kenneth, 1969).

2.3.9.3 Crop rotation

Bollen *et al.* (1989) found that when cropped once every six years potatoes yield 7% less than a first cropping with potatoes, and when grown once every three years this reduction can be as much as 20 %. Soil borne diseases such as *Verticillium dahlia*, *Rhizoctonia solani* and *Streptomyces* spp appeared to be the main organisms responsible for the decline in yields.

2.3.9.4 Use of Fertilizers

Vanloon and Houwing (1989) mentioned that in integrated farming the amounts of N, P and K applied should be adjusted to meet needs revealed by soil or tissue analysis. It is better to use organic manure rather than artificial fertilizer, provided that the losses of NO_3 and NH_3 to the environment can be minimized. The advantages of organic manure are its relative cheapness, its beneficial effect on soil structure, its stimulating effect on soil fauna, and the extra yield increase that cannot be reached by artificial fertilizer. Organic manure is best applied in spring when the leaching of nitrate is minimum.

A disadvantage of organic manure is that it does not allow precise fertilization with N, since the proportion of the organically bound N that mineralizes during the growing season cannot be predicted. This may lead to shortage or excess of N in the crop. This problem can partly be overcome by a split application of N, provided the second application is based on the nitrogen status of the plant or the soil. Petiole nitrate content has proved to be a useful indicator of the crop nitrogen status.

2.3.9.6 Chemical Control

2.3.9.6 .1 Tuber Treatments

Relatively little research has been conducted on the effect of tuber treatments on common scab. Kulikova (1982), Singh and Soni (1987),

Somani (1988) and Fulton (1994) reported the use of copper sulphate, formaldehyde, mercuric chloride, borax, boric acid, tetracycline, plantomycin and quintozene. Fluazinam, flusulfamide, fludioxonil and mancozeb have provided control in Australia (Wilson *et al.*, 1999; Pung and Cross, 2000).

2.3.9.6. 2 Soil Treatments

Specht (1985) reported that in integrated farming, soil cultivation should be directed to improving soil structure and controlling weeds, while using the minimum of energy.

The most widely used chemical method of controlling potato common scab is soil treatment with quintozene (PCNB) before planting (Erickson, 1960; Vashisth *et al.*, 1990). However, quintozene is persistent, could be carcinogenic, and may decrease yield or impart off-flavours to tubers.

The recommendation for potato growers is to reduce soil pH to 5.2 or below (Locci 1994). Sulfur, acid-forming fertilizers, and gypsum applied before or at planting reduces the soil pH to between 5.0 and 5.2, which helps to suppress CS caused by *S. scabies* (Locci 1994; Powelson and Rowe 2008). Further, growers are encouraged to carefully monitor and manage soil moisture to field capacity during early tuberization in fields with a history of common scab or in fields planted to highly susceptible potato cultivars with the potential for carrying seed-introduced inoculums.

2.3.9.6.3 Foliar applications

McIntosh and Burrell (1980) reported that foliar sprays with ethionine (a protein synthesis-inhibiting amino acid) decreased the incidence of common scab caused by soil borne *S. scabiei* in greenhouse and field trials.

2.3.9.7 Biological control

Biological control such as the utilisation of suppressive soils (Anderson and Lorang, 1988; Wilson, 1994), antibiotic-producing *Streptomyces* strains antagonistic to potato scab pathogens (Wood & Tveit, 1955; Wilson, 1994) and biofumigation (Gouws and Wehner, 2004), offers an environmentally acceptable means of controlling common scab. It is important to realise that these methods can only be effective when applied as part of an integrated disease management system.

In further experiments 93 streptomycetes were isolated from lenticels of potato tubers grown in naturally disease - suppressive and disease -conductive soils (Liu *et al.*, 1996). Of these 22 strains showed greater antibiotic activity against virulent *S. scabies* RB 311 than pon R and pon ss II. These strains were non - pathogenic on leaf - bud tubers in green house testing and significantly reduced scab without affecting tuber yield in field-pots tests. Other

bioactive compounds have also been studied as possible control agents, antibiotic substances from a red alga, *Laurencia okamurae*.

2.3.9.7.1 Control with Natural "Pesticide"

Medical plants have a long history of use and their use is widespread in over world countries. According to the report of the World Health Organization 80% of the worlds population rely mainly on traditional therapies which involve the use of plant extracts or their active substances (Sofowora,1999). The herbal medicines may be in form of powders, liquids, or mixtures, which may be raw, or boiled, ointments, liniments, and incisions (Malu *et al.*, 2009).

Ginger (Zingiber officinale) belongs to Zingiberaceae family. The part of the plant used is rhizome (Onyeagba *et al.*, 2004).

Nutrient Composition Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fiber and 12.3% carbohydrates. The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C (Govindarajan, 1992).

In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one is the most abundant constituent in the gingerolseries. The powdered

rhizome contains 3–6% fatty oil, 9% protein, 60–70% carbohydrates, 3–8% crude fiber, about 8% ash, 9–12% water and 2–3% volatile oil (Ali *et al.*, 2008)

In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent up to biosynthesis 3–5. Oleoresin, which is isolated by acetone and ethanol extraction, contains 4–7.5% of dried powder, pungent substances namely gingerol, shogaol, zingerone and paradol (Hoffman, 2007).

In vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria. These bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted with ginger (Gupta and Ravishankar, 2005). It inhibits the growth of *Escherichia coli*, *Proteus sp.*, *Staphylococci*, *Streptococci* and *Salmonella* (Ernst and Pittler, 2000 and White, 2007).

Ginger has strong antibacterial activity and to some extent antifungal properties (Nielsen and Rios, 2000).

Cinnamon (*Cinnamomum verumis*) is the name for several species of trees and the commercial spice products that some of them produce. All are members of the genus *cinnamomum* in the family *lauraceae*.

only a few *cinnamomum* species are grown commercially for spice. *Cinnamomum verumis* sometimes considered to be (true cinnamon),

but most cinnamon in international commerce is derived from related species, also referred to as "cassia".(Iqbal, 1993 and Bell,2009). In 2016, Indonesia and china produced 75% of the world's supply of cinnamon.

A number of species are often sold as cinnamon (Chen et al., 2014). Ground cinnamon is composed of around 11% water,81% carbohydrates(including 53%dietary fiber),4% protein, and 1%fat (US National Nutrient Database2016). In a100 gram reference amount, ground cinnamon is rich source of calcium (100% of the daily value, DV), iron (64%DV), and vitamin K (30%DV).

2.3.9.7.5 Penamox (Amoxicillin)

Composition :

Each capsule contains: Amoxicilline trihydrate B.P. equivalent t Amoxicillin anhydrous 250 mg and Amoxicillin anhydrous 500mg. Each 5ml of reconstituted suspension contains: Amoxicillin trihydrate B.P. equivalent to Amoxicillin anhydrous125 mg and Amoxicillin anhydrous 250mg.

Action:

Amoxicillin is semi synthetic penicillin with a broad spectrum antibacterial activity against wide range of gram positive and gram negative pathogens.

Manufactured by: pharmland pharmaceuticals, Building 71\1 , block 2,

Al-bagair industrial area , gazeera state- Sudan.

CHAPTER THREE

MATERIALS AND METHODS

This study was conducted in the laboratory of plant pathology, Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology during the period (November 2019 – April 2019). The aim of this study is to evaluate the effect of some plant aqueous extracts Cinnamon (*Cinnamomum verum*) and Ginger (*Zingiber officinale*) against the growth of the bacterium *streptomyces scabies* under laboratory conditions.

3.1 Isolation and Purification of the Bacteria from Infected potato tubers

Potato tubers showing symptoms of common scab were obtained from potato market, Nutrient agar Media (NA) was prepared for the bacteria isolation according to the method used by Lambert and Loria (1989).

Symptoms on the scabby potato tubers (Plate 1) were recorded before the tubers were surface-disinfected in 70% ethanol for 20 seconds and then rinsed several times in sterile distilled water (SDW). Thereafter, from each tuber, a small piece of potato tissue was cut from under the surface of a single lesion, at the border between healthy and infected tissue and put on NA plate.

Plates were incubated at 28°C and checked for bacterial growth after 24h (Plate 3). Sterile loop was used to sterilely streak the plate. Then the plates were incubated at 25 – 28°C. Then the bacterial growth was checked for bacterial growth after 24 hours for further use. Colonies of bacteria were streaked several times, respectively onto the same medium until a pure culture was obtained (Plate 4) and kept in refrigerator for further use.



Plate 1 Symptoms of *Streptomyces scabies* on potato tubers



Plate 3: Growth of *Streptomyces* spp Nutrient Agar

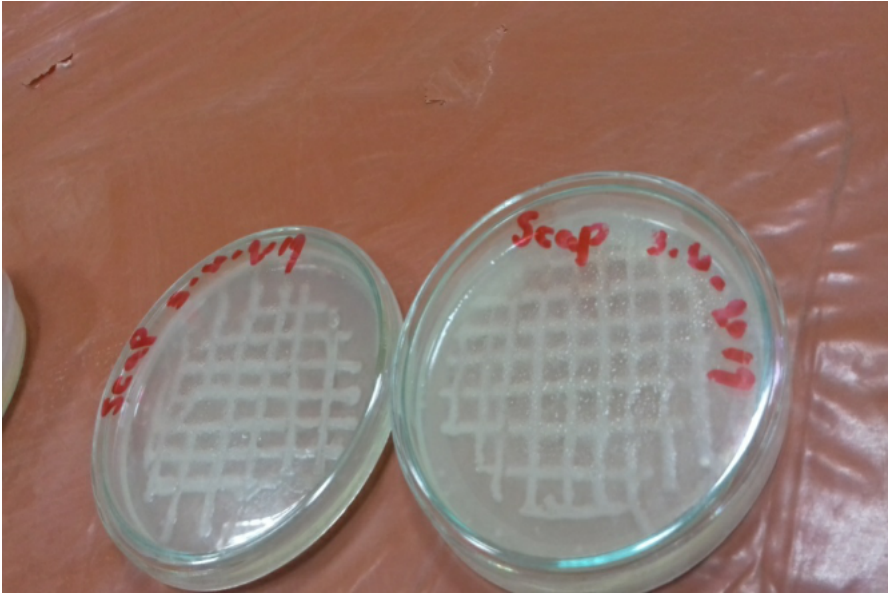


Plate4 Pure culture of *Streptomyces* spp the causal agent of potato plant

3.2 Inoculums Preparation

Pure isolates of Bacterial cell were harvested in sterile distilled water by using sterile glass rod. Control of bacteria using selected botanicals: Plant materials, equipment, tools and reagents:

3.3 Effects of botanicals

The aim of this experiment was to study the antibacterial activities of plant extracts on the growth of streptomyces spp invitro. The plants used were Cinnamon (*Cinnamomum verum*) and Ginger (*Zingiber officinale*), in addition to penamox 2% which obtained from pharmacy.

Two plant materials were used *Zingiber officinale* and *Cinnamomum verum*, the following were prepared :Penamox was buy from pharmacy Sterile distilled H2OPure bacterial culture Sterile beaker, glass rod, sterile filter paper discs, scalpel and cotton swabs.

3.3 Preparation of plant extracts

Zingiber officinale (Ginger) and *Cinnamomum verum* (Cinnamon) were obtained from Shambat local market.

Twenty gram of dried material of each plant species were placed in a 250 ml conical flask, then 80 ml of sterile distilled water were added to each flask. The mixture was placed on a shaker (orbital incubators 1500 and left to extract for 24 h at a speed of about 133rpm at room temperature 25C.

Extracts are expressed through 2 layers of cheesecloth. Filtered extracts were the collected in 50 ml round bottom flasks.

Hundred ml of bacterial suspension (1.0×10^8 CFU/) were spread on to

the surface of the NA plate using sterile cotton swabs (Casimiri and Burstein,1998). Sterile filter paper disc were dipped briefly in the respective plant extracts and were then applied on to surface of the NA plate .Discs fattened with Penamox (2%) were used as positive controls, while sterile distilled water – treated discs were use as a negative control. The treated plates were incubated at 28 c° for 48 h and the developing inhibition zones were observed and measured to determine the relative efficacy of the plant extracts against streptomyces spp.

Table1. Plant extracts used for the control of Streptomyces spp.

Common name	Botanicals name	Family	Plant parts used
Ginger	<i>Zingiber officinale</i>	Zingiberaceae	Rhizome
Cinnamon	<i>Cinnamomum verum</i> J. presl	Lauraceae	Inner bark

3.4. Statistical Analysis Procedure

The data was collected statistically analyzed using the soft ware to Statistix 8 in a one-way Completely Randomized design .

Data were transformed using the equation .

CHAPTER FOUR

RESULTS

4.1 Isolation and Purification of the Bacteria from Infected potato tubers

The pathogenic bacteria that were isolated from infected potato tuber plants showing symptoms. The results showed that the bacteria were identified based on morphological and cultural characters as *Streptomyces* spp (Plate 2).

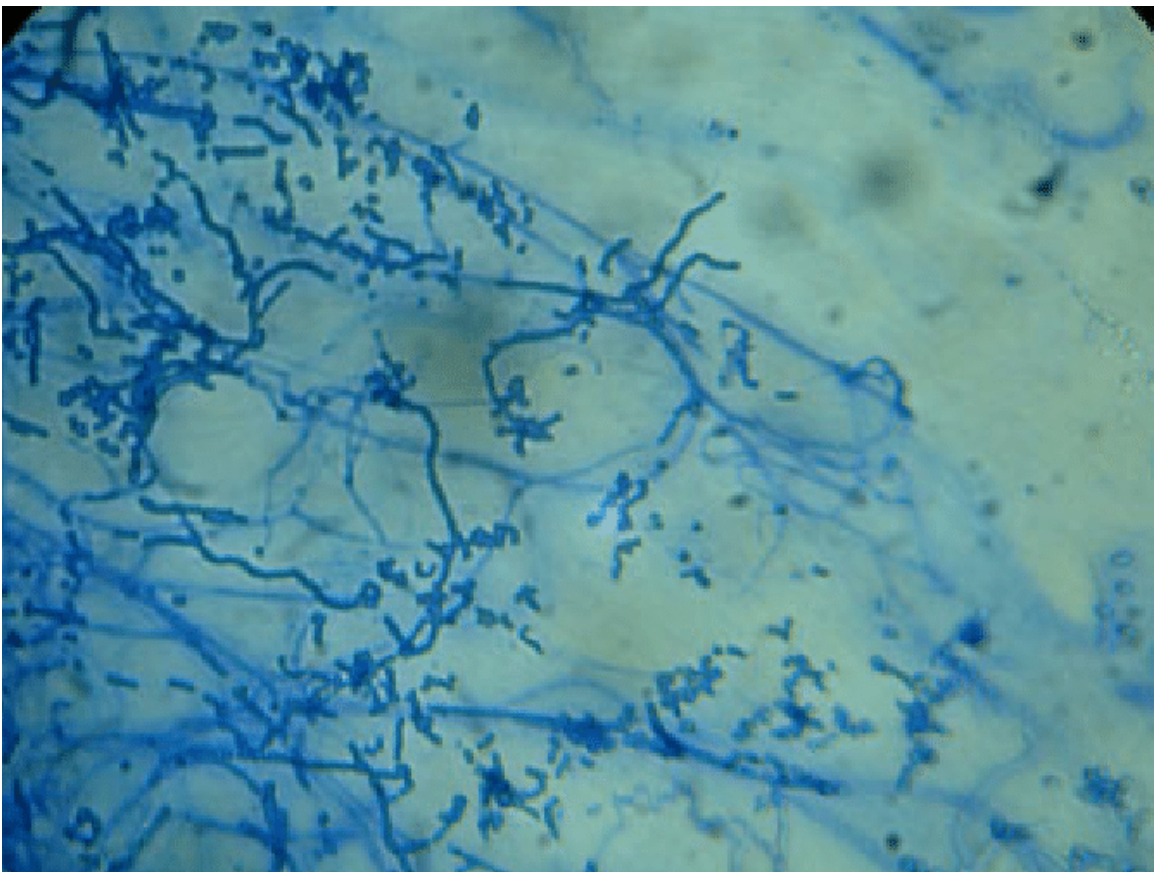


Plate 2 the isolated *Streptomyces* spp under microscope

4.2 Effects of aqueous extracts of Ginger and the antibiotic Penamox 2% against Streptomyces spp two days after inoculation *in vitro*

The results presented in Table (2) two days after inoculation, showed that all concentrations (5%, 10% and 20%) of aqueous extracts in addition to the penamox 2% tested were found to be significantly ($p \geq 0.05$) inhibited the growth of Streptomyces spp compared to the untreated control (H₂O) by (2.6, 2.6, 2.6) of 3.7 inhibitory effect respectively.

There is no significant difference between the different concentrations

Table 2 Effects of Ginger aqueous extracts and the antibiotic Penamox 2% against Streptomyces spp two days after inoculation *in vitro*

Treatments	Mean (mm)
Penamox 2%	3.07 ^a
Ginger 20%	2.6 ^b
Ginger 10%	2.6 ^b
Ginger 5%	2.6 ^b

Untreated control (H ₂ O)	0.2 ^c
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LSD 0.1
CV 3.6
SE± 0.06

Dissimilar letters on the "mean" showing significant differences data transformed using the equation



Plate 5 Effects of Ginger aqueous extracts and the antibiotic

Penamox 2% against Streptomyces spp in potato two days after inoculation *in vitro*

- A: Ginger 20%
- B: Ginger 10 %
- C: Ginger 5 %
- D: H₂O
- E: Penamox 2%

Effects of Cinnamon aqueous extracts and the antibiotic Penamox 2% against Streptomyces spp two days after inoculation *in vitro*

The result in Table (3) showed that the different concentrations of Cinnamon aqueous extracts (5%, 10% and 20%) effected the growth of the bacteria Streptomyces spp significantly ($p \geq 0.05$) when compared to the untreated control (H₂O) inhibited the growth by (2.6, 2.6, 2.5) respectively .

On the other hand no significant ($p \geq 0.05$) difference recorded between the different concentrations one to another .

Penamox 2% followed the same trend as in ginger experiment by recording the highest inhibitory effect (3.7).

Our results revealed that both tested plants aqueous extracts were significant ($p \geq 0.05$) retarded the growth of the bacteria

Streptomyces spp in vitro.

Table 3 Effects of Cinnamon aqueous extracts and the antibiotic Penamox 2% against Streptomyces spp two days after inoculation *in vitro*

Treatments/concentration	Mean(mm)
Penamox 2% (+control)	3.07 ^a
Cinnamon 20%	2.5 ^b
Cinnamon 10%	2.6 ^b
Cinnamon 5%	2.6 ^b
Untreated control (H ₂ O)	0.2 ^c

Dissimilar letters on the "mean" showing significant differences data transformed using the equation .

LSD 0.01

CV 3.02

SE± 0.05



Plate 6 Effects of Cinnamon aqueous extracts and the antibiotic Penamox 2% against *Streptomyces* spp in potato two days after inoculation *in vitro*

A: Cinnamon 20%

B: Cinnamon 10 %

C: Cinnamon 5 %

D: H₂O

E: Penamox 2%

CHAPTER FIVE

DISCUSSION

The present study revealed that the scab bacterial disease has become established and attained epidemic status in Khartoum state. The use of plant extracts for controlling scab of potato granted some favorable results that might give more options for controlling the disease.

Relatively, Ginger (*Zingiber officinale*) expounds higher inhibitory effects with inhibition zone of 2.6 mm. However the effectiveness of, *Zingiber officinal* was previously reported by (Suhad *et al*., 2012), aqueous extract of ginger roots were used for antibacterial activity against various Gram negative and Gram positive bacteria.

Cinnamomum verum (cinnamon) showed an inhibitory effect with inhibition zone of 2.5mm, while when used to control Citrus Bacteria Canker on Lime showed inhibition zone 6.50 mm (Elshafia, 2017).

On the other hand (Elrasheed, 2007) reported similar effect of the other plant extract (Neem seed extraction) , when his results showed a significantly positive effect on controlling the common scab disease as compared to check treatment. The concentration 10 % was significantly higher in inhibiting the causal organism, followed by 5 % concentration. Also the study showed that the inhibition of *Streptomyces* spp was significantly decreased with the time .

The variation in the inhibitory effect may be due to qualitative and quantitative differences between the selected plant materials or due to the diffusion of bacteria on plate .

CONCLUSION

- The two plant extracts, Gengir (*Zingiber officinale*) and Cinnamon (*Cinnamomum verum*) were expound positive inhibitory effect against the bacteria streptomyces spp of potato .

RECOMMENDATIONS

- Further investigations on plant extracts ginger (*Zingiber officinale*) and Cinnamon (*Cinnamomum verum*) because they have proved highly effective against bacteria streptomycetes spp of potato.
- Fractioning of the extracts into different substances for both tested plants is essential to determine the most effective substance responsible for the suppression of the bacterial growth.
- The selected plant species need more investigation to be considered as a promising option to IPM component to shorten the spread of disease.
- Further studies are recommended to test the effect of both tested plants under field conditions.

APPENDICES

Equipment ,tools and reagents:

Equipment ,tools and reagents used in the isolation of the bacteria from field Samples were :

- Autoclave
- Laminar flow cabinet
- Light microscope
- Cover slips
- Microscope glass slide
- Scalpel
- Tissues paper
- Sterile inoculation loop
- Petri dishes
- Flame
- Nutrient agar
- Ethanol (70-90%)
- 0.1% sodium hypochlorite (NaClO)
- 0.85% saline or sterile water.

origin data		
treatment/CONCENTRATII ON	R1(Cinnamo n)	R2(Ginge r)
20%	6.5	7
20%	7	7.5
20%	6	7.5
20%	6	6
10%	6.5	7
10%	6.5	6.5
10%	6	7
10%	6	6
5%	6.5	7
5%	6.5	6.5
5%	6	7
5%	6	6
Penamox 2%	9	10
Penamox 2%	10	9.5
Penamox 2%	9	9
Penamox 2%	9.5	9
H ₂ O	0	0
H ₂ O	0	0
H ₂ O	0	0
H ₂ O	0	0

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