Sudan University of Science and Technology

College of Graduate Studies

In vitro Evaluation of the Efficacy of Ginger and Coriander Ethanolic Extracts Against the Early Blight Disease Caused by Fungus ***Alternaria solani*** in tomato plants **(*Solanum lycopersicum*)**

تقييم المعملي لفعالية المستخلصات الايثانولية للجنزبيل والكسبرة ضد اللفحة المتأخرة المتسببه عن فطر(الالترناريا سولاني) في نبات الطماطم تحت ظروف المعمل

A Thesis Submitted in Partial Fulfillment of the Requirements for the **M. Sc.** Degree in Plant Protection. 2016.

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الآية

قال تعالي:

هُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ
رَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنْ
ها قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ
بِهًا وَغَيْرَ مُتَشَابِهٍ انْظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي
ذَلِكُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ (99)).

صدق الله العظيم

سورة الأنعام (99)

Dedication

To my mother

To my father

To my brothers and sisters

To all my family

To all my teachers

To all my colleagues and friends

With respect

Mohamed

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All thanks are due to Almighty Allah (SWT) who gave me health and strength, and helped me tremendously to complete this work.

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List of Content

Content	**Page NO**

List of List of Tables

List of Figures

List of Plates

ABESTRAC

Tomato(*Solanum lycopersicum*) is one of the most popular and widely consumed vegetables grown worldwide. It is considered as an importance source of some vitamins and mineral salts. Tomatoes are attacked by many diseases and pests in Sudan, the important diseases in Sudan include; Damping off-of seedling, Tomato yellow leaf curl virus (TYLCV), powdery mildew, bacterial spot, fuzarium wilt, late blight and **Early blight ,**but the early blight has highly significant effects in Sudan . This study which conducted under laboratory condition of Plant Pathology, College of Agricultural Studies, Sudan University of Science and Technology during January- February 2016, to isolate and identify of causal pathogen the Early blight of tomato more over to investigate , the inhibitory effect of ethanolic extracts of ginger rhizomes and coriander seeds and standard fungicide (**score $^{\mathbf{250EC}}$)** against the fungus *A. solani* (compared to control). Used Three concentrations of ethanolic extract of ginger rhizomes is 12.5% ,25% and75 %, coriander seeds 12.5% ,25% and 50% and fungicide (score $^{\mathbf{250}\mathrm{Ec}}$) 100% were used in addition to the control sure.

The results showed that all concentrations of ethanolic extracts of all plants tested and fungicide exhibited significant high inhibitory effect against linear growth of tested fungus compared to control. The highest concentration of ethanolic extract of ginger rhizomes at **75%**concentration caused the highest reduction (inhibition percentage) of mycelial growth of *A. solani* (95.6%), followed by ginger rhizomes at **25%**and**12.5%** (93.3%,84.6%),coriander seeds at **12.5%**(89%)and **50%(**87.6%), the standard fungicide and coriander seeds at **25%** (88% and 83.3%),respectively. Generally, the results showed that the antifungal activity increase with increase in extract concentration of ginger rhizomes, and decrease in extract concentration of coriander seeds .

ملخص البحث

تعتبر الطماطم من اهم الخضروات المزروعه استهلاكاً في العالم . وهي من اهم مصادر الفيتامينات والاملاح المعدنيه . تصاب الطماطم بالعديد من الامراض والافات في السودان وتشمل الامراض: موت وسقوط البادرات وفايرس التفاف اوراق الطماطم والفحه المتاخره واللفحه المبكره(Early blight) , ولكن اللفحه المتاخره ذات تاثير معنوي كبير في السودان. اجريت التجربه تحت الظروف المعمليه بمعمل امراض النبات بجامعه السودان للعلوم والتكنلوجيا وذلك اثناء الفتره من (يناير- فبراير) من عام 2016.وذلك لعزل والتعرف علي المسبب المرضي للفحه المبكره في الطماطم , ودراسة الاثر التثبيطي للمستخلص الايثانولي لرايزومات الجنزبيل وثمار الكسبره ضد النمو الخطي لفطر الالترناريا بالاضافه لمبيد اسكور (score EC250) (دايفينكونازول) مقارنة مع الكنترول . وذلك باستخدام 3 تراكيز رايزومات الجنزبيل ,(75% - 25% -12,5%) و3 تراكيز اخري من ثمار الكسبرة (50% - 25%- 12.5%)و تركيز 100% من الجرعي الموصي بها. و اظهرت النتائج المستخلصات الايثانوليه لكل النباتات المختبر ذات اثر معنوي علي نمو الفطر مقارنه مع بالكنترول واعلي تركيز من رايزومات الجنزبيل اعطي اعلي تثبيط بنسبة(95.6%) ثم 25 و12.5 بنسبة(93.3 – 84 %). ثم تركيز 12.5 من ثمار الكسبرة بنسبة(88%) وتركيز50 بنسبة (87.6%) وأخيرا المبيد وتركيز25 من ثمار الكسبره (83 – 88 %) علي التوالي . عموما اظهرت النتائج ان الزيادة الاثر التثبيطي لمستخلص رايزومات الجنزبيل تزيد بزيادة في تركيز مستخلص الجنزبيل والنقصان في تركيز مستخلص ثمار الكسبرة.

CHAPTER one
IntroductIon

Tomato(*Solanum lycopersicum*) is one of the most popular and widely consumed vegetables grown worldwide. Popularity of the crop stems from its acceptable flavor, nutritive value (high in vitamin C and A), the short life cycle, and the high productivity. In the Sudan tomato ranks second to onion among vegetable crops based on cultivated area. It is grown throughout the country where irrigation water and arable land are available and is mainly grown by small holders who employ relatively poor crop management practices.

In the arid tropical region of the Sudan the high summer and the low relative humidity limits the production of tomato to the cooler period of the year. To extend the season of production it is necessary to know the nature of growth, flowering and fruiting of the plant in relation to climatic conditions (Abdalla and Verkerk, 1968).

Tomato is the second most important vegetable crop in Sudan. of the devastating diseases of tomato is early blight caused by *Alternaria solani* (Singh, *et al.* 1980, Maiero and Barksdale, 1989).

The disease is caused by two fungi *viz. A. solani* (Jones & Grout) and *A. alternata* (Fr.) Keissler, With their Spores abundance in the atmosphere (Iglesias, *et al.* 2007) and in the soil, they are always a threat when conditions become conducive for their infection, and thus represents a serious threat to potato production (Leiminger and Hausladen, 2012).

Early blight is widespread in most areas where potatoes and tomatoes are grown, but is particularly prevalent in tropical and temperate zones. The disease is a potential threat where tomatoes are cultivated under irrigation or during times of heavy dew (Rotem , 1994). Early blight tuber rot may occur if tubers werewounded during harvest as *A. solani* spores are present on or near the soil surface Tuber rot is, however, not common and has a limited distribution (Pscheidt, 1985).

The primary damage of early blight is due to premature defoliation of the plant. Photosynthesis rates increase and respiration rates decrease in apparently healthy tissues. Physiological changes are difficult to measure and evaluation of crop loss is based on the level of disease (Rotem, 1994). Early literature (Neergaard, 1945) cites yield losses of 5–50 %. There is often a discrepancy between damage to foliage and yield loss, which is due to the increase in disease spread at the end of the season, when most of the yield has been produced (Rotem, 1994). Control of early blight has been shown to increase yield (Harrison & Venette 1970). When tomato fruit or potato tubers become infected, the quantity and quality of marketable produce decrease and the number of secondary pathogens increases (Pscheidt, 1985).

This disease causes a severe reduction in yield and high economic losses every growing season. They are controlled by chemical fungicides, long crop rotation, and pasteurization of seedbeds with steam or fumigants and by breeding resistant tomato cultivars (Spletzer and Enyedi, 1999).

The biocontrol of plant pathogens is currently regarded as a key practice in sustainable agriculture because it exploits a natural resource. Nature is a source of many different biocontrol agents, including the plant growth, promoting micro-organisms (PGPM) which promote plant growth by inducing a defense response (Siddiqui, 2006). Natural plant extracts have latterly gained importance for crop protection against pests and pathogens because of their safety and target specificity. They have also been found effective against a wide range of pathogens (Manickam and Rajappan, 2001). Many reports have been published on non-chemical means to protect tomatoes plants against plant pathogens. Among these means, plant extracts have proved effective in inhibiting soil-borne pathogens.

The objective of this study is:

i) Evaluate the effectiveness of ethanolic extracts of ginger rhizomes and coriander seeds in controlling *A. solani* in tomato plant.

CHAPTER TWO
LITEREATURE REVIEW

2.1 Alternaria solani

Alternaria solani is a fungal pathogen, which produces a disease in tomato , potato plants other and plant species called early blight. The pathogen produces distinctive "bulls eye" patterned leaf spots and can also cause stem lesions and fruit rot on tomato and tuber blight on potato. Despite the name "early," foliar symptoms usually occur on older leaves. If uncontrolled, early blight can cause significant yield reductions. Primary methods of controlling this disease include preventing long periods of wetness on leaf surfaces and applying fungicides (Olanya, *et al*. 2009).

2.1.1 Taxonomy

Kingdom: Fungi

Phylum: Deuteromycetes

Class: Dothideomycetes

Subclass: Pleosporomycetidae

Order: Pleosporales

Family: Pleosporaceae

Genus: *Alternaria*

Species: *A. solani*

Binomial name: *Alternaria solani*

(Waals, *et al*. 2001)

2.1.2 Hosts

The host plants of Solanaceous crops, include tomatoes, potatoes, peppers, chilies, and eggplants.

2.1.3 Symptoms

The symptoms show as angular, oval shape and brown-black necrotic characterized by concentric rings. The concentric ridges produce a target board effect the most characteristic symptoms of the disease, several spot coalesce & spread all over the leaf. In several attack leaves shrivel and fall down (Sanjeev kumar, 2008).

Symptoms of early blight occur on foliage , stem and fruit, of tomatoes. Initial symptoms on tomatoes leaves appear as small 1-2 mm black or brown lesions and under conducive environmental conditions the lesions will enlarge and are often surrounded by a yellow halo (Plate 1). Lesions greater than 10 mm in diameter often have dark pigmented concentric rings. This so-called “bulls eye” type lesion is highly characteristic of early blight (Plate 2). As lesions expand and new lesions develop entire leaves may turn chlorotic and dehisce, leading to significant defoliation.

Lesions occurring on stems are often sunken and lens-shaped with a light center, and have the typical concentric rings (Plate 3). On young tomato seedlings lesions may completely girdle the stem, a phase of the disease known as “collar rot,” which may lead to reduced plant vigor or death.

Infection of both green and ripe tomato fruit normally occurs through the calyx with lesions sometimes reaching a considerable size (Plate 4). The lesions appear leathery and may have the characteristic concentric rings. Infected fruit will frequently drop prematurely (Kemmitt, 2002).

2.1.4 Pathogen biology

The causal pathogen of early blight is the fungus *Alternaria solani*. which has no There is no known sexual stage and hence it is classified as a Deuteromycete. The genus *Alternaria* is a large and important group of pathogenic fungi, which cause a significant number of important diseases. The mycelium is haploid and septate (Plate 5), becoming darkly pigmented with age. Sporulation in culture can be stimulated by exposure to fluorescent light. The asexual conidia are borne singly or in a chain of two on distinct conidiophores. The beaked conidia normally possess 9–11 transverse septae (Plate 6). Morphological and pathogenic variability among isolates of *A. solani* has given rise to claims of the existence of races, although this remains unproven (Kemmitt, 2002).

Plate 1. Early blight symptoms on tomato leaf "yellow halo "

Plate 2:"Bullseye" patterned leaf lesion of *Alternaria solani*

Plate 3: Early blight symptoms on stem of Tomato

Plate 4: Early blight symptoms on fruit

2.1.5 Disease cycle and epidemiology

Alternaria solani overwinters over summer primarily on infected crop debris. Thick-walled chlamydospores have been reported, but they are found infrequently. In mild climates the pathogen can survive from season to season on volunteer tomato and potato plants as well as other weedy Solanaceous hosts such as horsenettle and nightshade.

Warm, humid (24-29°C) environmental conditions are conducive to infection. In the presence of free moisture and at an optimum of 28-30°C, conidia will germinate in approximately 40 min. Desiccated germ tubes are able to renew growth when re-wetted, and, hence, infection can occur under conditions of alternating wet and dry periods. Germ tubes penetrate the leaf epidermis directly or enter through stomata.

Time from initial infection to appearance of foliar symptoms is dependent on environmental conditions, leaf age, and cultivar susceptibility. Early blight is principally a disease of aging plant tissue. Lesions generally appear quickly under warm, moist conditions on older foliage and are usually visible within 5-7 days after infection.

A long wet period is required for sporulation but it can also occur under conditions of alternating wet and dry periods. Conidiophores are produced during wet nights and the following day light and dryness induce them to produce spores, which emerge on the second wet night. Secondary spread of the disease results from conidia being dispersed mainly be wind and occasionally by splashing rain or overhead irrigation. Early blight is considered polycyclic with repeating cycles of new infection. This is the period when the disease has the potential to spread rapidly and build up to damaging levels in the crop (Kemmitt, 2002).

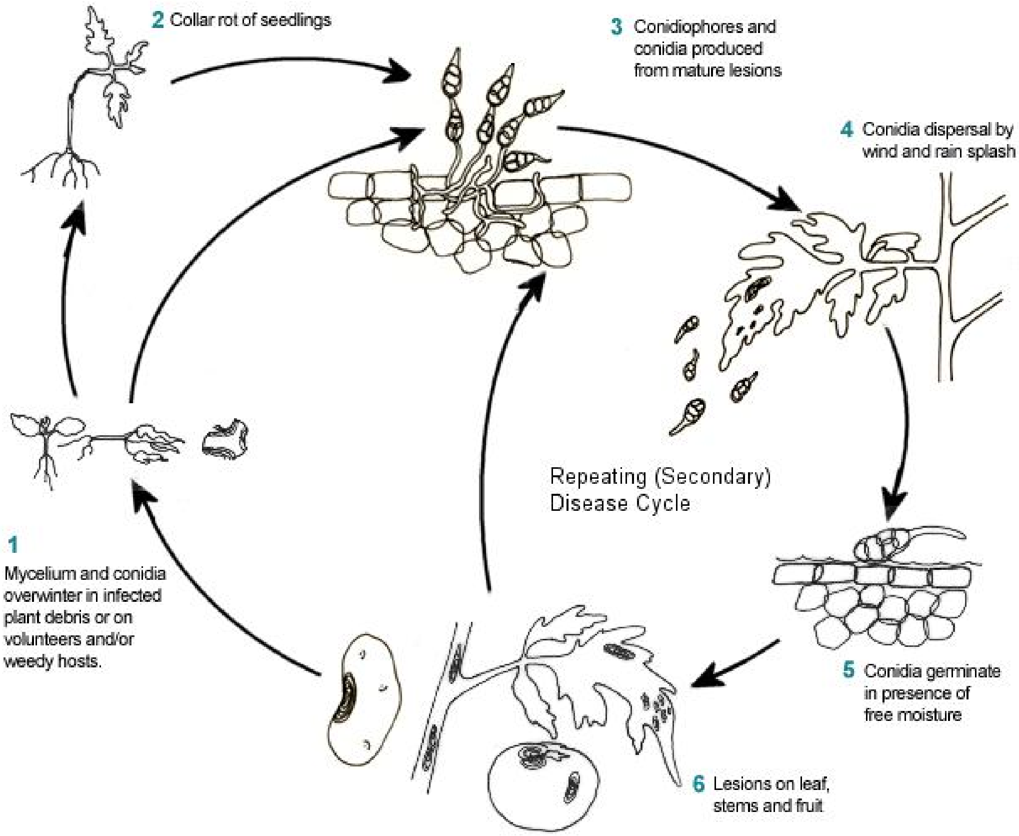

Figure 5. Disease cycle of *Alternaria solani* (Chaerani, and Voorrips, 2006).

2.1.6 Economic important

Early blight caused by *A. solani* is the most destructive disease of tomatoes in the tropical and subtropical regions. Each 1% increase in intensity can reduce yield by 1.36%, and complete crop failure can occur when the disease most severe (Pandey, 2003).

Yield losses of up to 79% have been reported in the U.S., of which 20-40% is due to seedling losses in the field (Chaerani and Voorrips, 2006). *A. solani* is also one of the most important foliar pathogens of tomato. In the U.S., yield loss estimates attributed to foliar damage, which results in decreased fruit quality and yield reduction,which can reach 20-30%. Because *A. solani* is one of numerous tomato or potato pathogens that are typically controlled with the same products, accurately estimating both the total economic loss and the total expenditure on fungicides for control of early blight is difficult. Best estimates suggest that total annual global expenditures on fungicide control of *A. solani* is approximately $32 million for tomatoes (Olanya, *et al.* 2009).

2.1.7 Control measure

2.1.7.1 Cultural practices:

In many cases employing sound cultural practices that maintain tomato plant in good health will keep early blight losses below economic levels. Because the pathogen over winters on infected crop debris, in field sanitation procedures that reduce initial inoculums in subsequent crops are beneficial. Consideration should be given to removing potentially infected material such as decaying vines and fruits from the vicinity of production fields.

Controlling volunteers and weeds, such as nightshade and horse nettle which serve as alternative hosts for the disease, prior to planting the new crop will help to reduce the risk of transmission of the disease. Ensuring seed or transplants are pathogen free before placing out in the field and rotating fields to a non susceptible host crop will also help to reduce build up of inoculums in the soil (Kemmitt, 2002).

2.1.7.2 Crop Rotation

Early blight is a soil borne disease, so rotation can be a good management tool. A good practice is to treat members of the same plant family as a group and rotate based on groups rather than individual crops. **Solanaceous** crops include tomatoes, potatoes, peppers, chilies, eggplants, and tobacco. Using a three or four years crop rotation with non- solanaceous crops will allow infected plant debris to decompose in the soil. Rotations with small grains, corn, or legumes are preferable (Watson, 2003).

2.1.7.3 Resistant cultivars

Complete resistance to early blight does not exist in commercial tomato cultivars. Using wild *Lycopersicon* species which show a high degree of resistance in breeding programs has led to the release of a number of cultivar of tomato with a degree of resistance to early blight. Apparent levels of resistance are often correlated with plant age. Immature potato and tomato plants are relatively resistant to early blight but, after tuber and fruit initiation, susceptibility increases gradually, and mature plants are very susceptible to the pathogen (Kemmitt, 2002).

2.1.7.4 Chemical control

Fungicides with curative properties are registered for use against early blight on tomato and potato. The cheaper Protestants fungicides such as mancozeb, chlorothalonil and score are the foundation of most early blight management programs. These fungicides must be reapplied every 7-10 days to provide protection of new growth as well as to counter the effects of weathering which progressively removes the chemical from the leaf surface. Timing of fungicide sprays relative to environmental conditions and subsequent potential for disease development is critical if good control is to be attained (Kemmitt, 2002).

The so-called Quin one Outside Inhibitors class of fungicides which inhibit fungal respiration is highly active against *Alternaria solani*. Molecules from this very important class of fungicides which are registered for *Alternaria* control in tomato include Azoxystrobin, Pyraclostrobin, Trifloxystrobin, Fenamidone and Devianconazol. In general Quin one Outside Inhibitors are readily taken up into the plant tissue and work preventively to stop infection by inhibiting spore germination. They are weakly curative and use rates which are considerably lower than the traditional protectant products, although cost per acre is typically higher. Quin one Outside Inhibitors are high-risk fungicides with respect to resistance development, and isolates of *A. solani* which possess the F129L mutation have been isolated from the field. These isolates show significantly reduced levels of sensitivity to the fungicides (Kemmitt, 2002).

2.1.7.5 Botanical control

This disease is controlled mainly with agro chemicals. However, the world wide trend towards environmentally-safe methods of plant disease control in sustainable agriculture calls for reducing the use of these synthetic chemical fungicides. In an attempt to modify this condition some alternative methods of control have been adopted. Recent efforts have focused on developing environmentally safe, long lasting and effective biocontrol methods for the management of plant diseases. Natural plant products are important sources of new agrochemicals for the control of plant diseases (Kagale, *et al.* 2004). Furthermore, biocides of plant origin are non-phytotoxic, systemic and easily biodegradable (Qasem, and Aau-Blan, 1996). It is now known that various natural plant products can reduce populations of foliar pathogens and control disease development. These plant extracts are environmentally safe alternatives and as components in integrated pest management programs (Bowers, and Locke, 2004). A number of plant species have been reported to possess natural substances that are toxic to several plant pathogenic fungi (Goussous, *et al.* (2010), Dushyent, and Bohra, (1997) studied the effect of 11 different plant extracts on mycelial growth of *A. solani* and found that leaf extracts of some plants *i.e. Tamarix aphylla* and *Salsola baryosma* totally inhibited the growth of the pathogen *in vivo*. Also, Wszelaki, and Miller, (2005) reported that garlic extracts significantly reduced the early blight disease on tomato. Additionally, several plant extracts have shown antimicrobial activity against fungal pathogens under ***in vitro*** and *in vivo* conditions (Kagale,*et al.* 2004).

2.2 Tomato plant

Tomato (*Solanum lycopersicum* L.) is the edible, often red fruit from the plant, commonly known as a tomato plant. The tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While it is botanically a fruit, it is considered a vegetable for culinary purposes. Tomato ranking first in the world for vegetable, accounts for 14% of World vegetable production over 100 million metric tons/year$ 1.6 billion market (Food and Agriculture Organization FAO, 2010). The total production of tomato in Sudan in the year 1999 was707715 tons and the total production of tomato for one greenhouse (350 m^2) in Khartoum reached 5ton per season (Abdol hafeez, *et al*. 2012).

2.2.1 Scientific classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Solanales

Family: Solanaceae

Subfamily: Solanoideae

Tribe: Solaneae

S.N: *Solanum lycopersicum.*

Binomial name: *Solanum lycopersicum*(L)

2.2.2 Description

Tomato is a tender a warm season perennial cultivated as an annual, it is an annual shrubby member of solanaceae .In a protected environment, tomato is a short-live herbaceous perennial(Alaa Edrees, 2014). Determinate tomato high (3-4ft), and in determinate (7-15ft), spread in 24-36 and most of roots are found in (4-8ft) (Decteau, 2000). Tomato leaves are compound, alternate, from 10-30 cm in long, and 10-15 in Diameter. The leaves arev10-15 cm in long old pinnate, with 5-9 leaflets on petioles (Acquaah, 2002). Tomato is characterized by glandular hairs (trichomes) that emit a strong aroma when broken. Tomato flowers are relative small and consist of a five lobed corolla and calyx, the staminal cone represent a fusion of five anthers around the ovary .style and stigma ensures a high level of self-pollination and homozygosis, Pollination is not a function of insect’s activity but occurs as flowers vibrate from wind currents (perice, 1987).

2.2.3 Distribution

The written literature of tomato began in 1500 when Spanish and Portuguese explorers found these plant first in Mexico and then along the west coast of South America mainly Peru, and then along on the Galapagos Island, tomato is a native to Peru-Equador regain of South America, evolving from the cherry from (*lycopersicon esculantum* Var. cerasiform) .the first historical mention of tomato by Malthiolusin in1544, placed in Italy. The plant received little notice in Spain (perice, 1987).

In Sudan are fifteen states cultivating tomato crop, but the main products area are Gezira, Khartoum, and Nile state. Tomato cultivated in both open field and greenhouses .It the second popular vegetable after onion in Sudan (Abdol hafeez, *et al*. 2012). It is grown throughout the country where irrigation water and arable land are available and is mainly grown by small holders who employ relatively poor crop management practices.

In the arid tropical region of the Sudan the high summer and the low relative humidity limits the production of tomato to the cooler period of the year. To extend the season of production it is necessary to know the nature of growth, flowering and fruiting of the plant in relation to climatic conditions (Abdalla and Verkerk, 1968).

2.2.4 Varieties

There are around 7500 tomato varieties grown for various purposes. Heirloom tomatoes are becoming increasingly popular, particularly among home gardeners and organic producers, since they tend to produce more interesting and flavorful crops at the cost of disease resistance and productivity (Redenbaugh, *et al.*1992).

The tomato is now grown worldwide for its edible fruits, with thousands of cultivars having been selected with varying fruit types, and for optimum growth in differing growing conditions. Cultivated tomatoes vary in size about 5 mm in diameter, through cherry tomatoes, about the same (1–2 cm) size as the wild tomato, up to beefsteak tomatoes (10 cm) or more in diameter. The most widely grown commercial tomatoes tend to be in the (5–6 cm) diameter range. Most cultivars produce red fruit, but a number of cultivars with yellow, orange, pink, purple, green, black, or white fruit are also available. Multicolored and striped fruit can also be quite striking. Tomatoes grown for canning and sauces are often elongated, (7–9 cm) long and (4–5 cm) diameter; they are known as plum tomatoes, and have a lower water content. Roma-type tomatoes are important cultivars in the Sacramento Valley (Redenbaugh, 1992).

The tomato varieties for summer season such as: Eloths and Sophie, beside these are local varieties like: Omdurman and Gezira.The vegetable areas in Sudan 584000 fedans in 1999 (Mohamed, *et al*. 2003). There are other resistance type breeding in Sudan against tomato yellow leaf curl virus include , Sennar(1), Sennar(2), Omdurman ,and Aljazeera (96). Variety Abed Allah and Somerset (98) are breeding to resist high temperature in Sudan (Ahmed, 2009).

2.2.5 Impotence and Nutrition value of tomato

Tomato is considered as an importance source of some vitamins and mineral salts such as; vitamin C, vitamin B , and Riboflavin, which are considered necessary for growing, and safety of skin. The external part of fruit contains high level of vitamin C. This for red tomato ,raw (per100g:energy 74kg ,carbohydrates 3.9g,fat 0.2, protein0.9, vitamins 5%,and trace metals 3%).their also others constituents such as water 94.5and lycopene 2573mg (Naika, *et al*. 2005). which are considered necessary for growing and safety of skin. The external part of fruit contains high level of vitamin C (Alaa Edrees, 2014).

2.2.6 Picking and storage

A cluster of tomatoes in order to facilitate transportation and storage, tomatoes are often picked unripe (and thus colored green) and ripened in storage with ethylene.

Tomatoes keep best unwashed at room temperature and out of direct sunlight. It is not recommended to refrigerate as this can harm the flavor. Tomatoes that are not yet ripe can be kept in a paper bag till ripening. Recently, stores have begun selling "tomatoes on the vine", which are determinate varieties that are ripened or harvested with the fruits still connected to a piece of vine. These tend to have more flavor than artificially ripened tomatoes.

Slow-ripening cultivars of tomato have been developed by crossing a no ripening cultivar with ordinary cultivars. Cultivars were selected whose fruits have a long shelf life and at least reasonable flavor. At home, fully ripe tomatoes can be stored in the refrigerator, but are best kept at room temperature. Tomatoes stored cold will still be edible, but tend to lose flavor (Parnell, 2004).

2.2.7 Diseases

Tomato cultivars vary widely in their resistance to disease. Modern hybrids focus on improving disease resistance over the heirloom plants. One common tomato disease is tobacco mosaic virus, so smoking or use of tobacco products are discouraged around tomatoes, over whether the virus could possibly survive being burned and converted into smoke. Various forms of mildew and blight are also common tomato afflictions, which is why tomato cultivars are often marked with a combination of letters that refer to specific disease resistance. The most common letters are: *V.verticillium* wilt, *F .fusarium* wilt strain I and II, *N.nematodes*, tobacco mosaic virus, and *Alternaria solani* (Mourvaki, *et al*. 2005).

Tomato attacks by many diseases and pest in Sudan, the important diseases in Sudan include; Damping off-of seedling, Tomato yellow leaf curl viruse (TYLCV), powdery mildew, Bacterial spot, Early and late blight and fuzarium wilt (Juha, 1996).

Another particularly dreaded disease is curly top, carried by the beet leafhopper, which interrupts the lifecycle, ruining a nightshade plant as a crop. As the name implies, it has the symptom of making the top leaves of the plant wrinkle up and grow abnormally (Mourvaki, *et al*. 2005).

2.3 Ginger plant

Ginger (*Zingiber ofcinale*) is widely used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibb-Unani herbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Tapsell, *et al*. 2006). Several reviews have appeared in the literature about this plant, and this may reflect the popularity of the subject and its common use as a spice and a medicinal plant (Afzal, *et al*. 2001.and Chrubasik, *et al*. 2005).

2.3.1 Scientific classification

Kingdom: Plantae
Clade: Angiosperms
Clade: Monocots
Class: Commelinids
Order: Zingiberales
Family: Zingiberace
Genus: Zingiber
Species: Z. *officinale*
Binomial name: *Zingiber officinale*

2.3.2 Plant description

Ginger (*Zingiber officinale),* is an erect herbaceous perennial plant in family Zingiberaceae grown for its edible rhizomes (underground stem) which is widely used as spice. The rhizome is brown, with a corky outer layer and pale-yellow scented center **(plate8)**. The above ground shoot is erect and reed-like with linear leaves that are arranged alternated on the stem. The shoot originates from a multiple bases and wraps around one other. The leaves can reach (7cm) in length and (1.9cm) broad on shorter stems and the plant produces cone shaped, pale yellow flowers .the ginger plant can reach (0.6-1.2 m) in height and grown as an annual plant **(plate 9)**, Ginger may also be referred to as true ginger, stem ginger, garden ginger or root ginger and it is believed to have originated in the Southeast Asia (CABI Crop protection compendium, 2012).

2.3.3 Plant Distribution

The origin of ginger is from themide-14th century, from Old English gingifer, from Medieval Latin gingber, said to be a native of Asia, Cultivated in West India, Jamaica, and Africa. In 2013, with a global production of ginger is 2.1 million tones, India accounted for (33%) followed by China (19%), Nepal, Indonesia and Nigeria (FAO, 2013).

Plate6: Shape of Ginger rhizomes

2.3.4 Medical Important

Ginger and many of its chemical constituents have been shown, in numerous clinical studies, to be useful in combating several metabolic diseases. Badreldin, *et al.* (2007) mention the document and comment on the publications that have appeared on ginger and its constituents in the last 10 years or so. Ginger and many of its chemical constituents have strong anti-oxidant actions. As several metabolic diseases and age-related degenerative disorders are closely associated with oxidative processes in the body, the use of either ginger or one or more of its constituents as a source of anti-oxidants to combat oxidation warrants further attention , and post- operative vomiting and vomiting of pregnancy.

2.3.5 Chemistry

The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry. It is not our intention in this review to cover all the many compounds reported for ginger, but to summarize the major components that have been implicated in the pharmacological activities of the crude drug.

The odor of ginger depends mainly on its volatile oil, the yield of which varies from 1% to 3%. Over 50 components of the oil have been characterized and these are mainly monoterpenoids [b-phellandrene, (+)-camphene, cineole, **geraniol**, curcumene, citral, terpineol, borneol] and sesquit- erpenoids [a-zingiberene (30–70%), b-sesquiphellandrene (15–20%), b-bisabolene (10–15%), (E-E)-a-farnesene, ar- curcumene, zingiberol]. Some of the oil components are converted into less odor-defining compounds on drying (Evans, 2002).

2.3. 6 Anti-microbial Activity of Ginger

Ginger extract (10 mg/kg) in traperitoneally had a dose- dependent anti-microbial activity against *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Escherichia coli* (Jagetia, *et al.* 2003). (Yin, and Cheng, 1998) showed that ginger had no significant action against some fungi (*Aspergillus niger* and *Aspergillus flavus*) in vitro. However, (Ficker, *et al.* 2003) found that, out of 29 plant extracts, ginger extract had the broadest range of antifungal activity measured either by the fungi inhibited or as the average diameter of the zones of inhibition.The ginger extract was the only one that was active against *Rhizopus sp.*, an organism that was not inhibited by any of the other plant extracts tested or by the anti-fungal agent ketoconazole or berberine. Using bioassay-guided isolation and identification of anti-fungal compounds from ginger, the same authors (Ficker, *et al.* 2003) reported that gingerols and gingerdiol are the main anti-fungal principles. The compounds were active against13 human pathogens at concentrations of <1 mg/ml. The gingerol content of the African land race was at least three times higher than that of typical commercial cultivars of ginger. Therefore, these authors suggested that ginger extracts standardized on the basis of the identified compounds could be considered as anti-fungal agents for practical therapy. (Iqbal, *et al.* 2006).

2.4 Coriander plant

2.4.1 Scientific classification

Kingdom: Plantae
Order: Apiales
Family: Apiaceae
Genus: *Coriandrum*
Species: *C. sativum*
Binomial name: *Coriandrum sativum* (Wikipedia.com).

2.4.2 Plant Description

Coriander (*Coriandrum sativum*), also known as **cilantro**, or **Chinese parsley** is an annual herb in the family Apiaceae and, according to the climatic conditions, is cultivated as a summer or winter annual crop. At flowering, the glabrous plants can reach heights between (0.20-1.4m). The germination is epigaeal and the plant has a tap root. The color of the more or less ribbed stem is green and sometimes turns to red or violet during the flowering period **(plate8)**. They wither before the first fruits are ripe starting from the basal leaves. The inflorescence is a compound umbel, Sometimes there are one or two linear bracts (plate9) (Diederichsen, 1996).

2.4.3 Distribution

A native of the Mediterranean region there for several thousand years, now cultivated in tropical Asia (India, Malaysia, Thailand, and china), the Middle East and Brazil. Coriander was brought to the British colonies in North America in 1670, and was one of the first spices cultivated by early settlers. (Platte, 1962, and Aggarwal and Kunnumakkara, 2009).

The International Trade Centre, (1986) estimated that in 1986, the world production of coriander essential oil was 90-100 t/ year. The main producer of coriander for export has always been the former Soviet Union, and Luk’janov and Reznikov, (1976) reported that 98% of the world’s coriander essential oil was produced by the Soviet Union. The same authors estimated the total global area of land in coriander production to be 320 000 ha.

Plate 8. The Coriander plant, leaves, flowers, stem and seeds.

Plate 9. Dried coriander fruits, often called *coriander seeds* when used as a spice

2.4.6 Chemical composition of the fruits

The essential oil content is of greatest importance, there are accessions that have almost no essential oil and others with up to 2.60% of essential oil in the air-dried fruits. The extremely leafy types from Syria have very low essential oil content in the fruits. Despite this, the essential oil content is positively correlated with the foliation of the plant.

The taste of the green leaves of the plant was more aromatic in the accessions that had high essential oil content in the fruits. The Georgian types had leaves with a very spicy taste. The Syrian types must have been subject to a selection towards plants with a mild taste more suited to use in salads than as a spice. The Ethiopian accessions show the same tendency as the Syrian, but their flavor is more aromatic.

Usually, the plants with low foliation and large fruits have allowed essential oil content. The Indian group with the lengthened fruits also belongs in this category.

This fact has been known and described in literature for a long time (Luzina and Michelsson, 1937). But the chemical composition of the essential oil of the plant is also important, as it affects its flavor.

2.4.8 Medical uses

Coriander has been used in medicine for thousands of years (Mathias, 1994). The first medicinal uses of the plant were reported by the ancient Egyptians. General references to coriander's medical uses are also found in classical Greek and Latin literature (Manniche, 1989), and instructions to cultivate coriander are contained in the German emperor Charlemagne's decree 'Capitulare de villis' in 1812 .The coriander fruits are believed to aid digestion. Many other fruits of umbel plants have been used in medicine since antiquity (French, 1971) as they also affect the digestive system and some act as an aphrodisiac. Some of these, such as hemlock (*Conium maculatum L.*), are poisonous. Coriander is also used externally to treat ulcers and rheumatism; these and several other medicinal uses are recorded by Diederichsen, (1996).

The medical uses of coriander in the modern era are described by Diederichsen, *et al.* (1996). In India, the fruits are considered carminative, diuretic, tonic, and stomachic, ant bilious, refrigerant and aphrodisiac (Diederichsen, *et al.* 1996).

CHAPTER THREE
MATERIALS AND METHODS

This study was conducted under laboratory conditions at Plant Pathology Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology during the period November to December 2015 to investigate the antifungal activity of ethanolic extracts of ginger rhizomes, coriander seeds and fungicide, **score** $^{\mathbf{250_{EC}}}$ as standard against the fungus *Alternaria solani*, the causal agent of early blight in Tomato Crop.

3.1 Isolation and Identification of the pathogen (*A. solani)*

Infected Tomato leaves showing symptom of the disease were obtained from Bahri vegetable market in November 2015 plant parts showing disease symptom were cut into small section (0.5-1.0cm), washed thoroughly with tap water, and surface sterilized by immersing in 1:4 Clorox (NaOcl) for 5 miles, rinsed three time in changes of sterilized distilled water to remove the adhering Clorox and dried on sterilized filter papers ready for culturing .A culture medium Potato Dextrose Agar,(P D A) was used . The medium was supplemented with poured 250ml in 3 Petri dishes (Petri dishes 9cm). Five sections of the dried parts were aseptically placed in Petri dish and incubated at 28c.Sub-cultures were later prepared to get pure cultures. Slides were prepared from these pure cultures, and examined microscopically (x: 40), to identify the causal pathogen which *Alternaria* Species.

3.2 Plant Material

Freshly collected seeds of Coriander were shade-dried at room temperature for 10–15 days. Dried bark and leaf samples were separately crushed and ground into fine powder with mortar and pestle.

3.3 Preparation of extracts

Extracts from seeds of small Coriander (*Coriandrum sativum)* and ginger roots (*Zingiber officinale)*,were collected from local market in Khartoum “Shambat” , Sudan and tested for their effect in reducing the mycelial growth of *A. solani in vitro* using the poisoned food technique (Schmitz, 1930).

Powdered plant materials were extracted using ethanol solvent in a Soxhlet apparatus for 8 h according to the method described by Pandey, (2007). The respective extracts were filtered and dried under reduced pressure using rotary evaporator to yield solid/semisolid residues. The crude mixture were prepared as 100% and the subsequently concentrations were prepared as 75%, 25% and 12.5% for ginger roots extract, 50%, 25% and 12.5% for Coriander extract.

The PDA media amended with five milliliters of ethanol extract, 1 and 5%, of each plant extracts individually were inoculated with mycelial discs (9 mm diameter) taken from the advancing edges of 7 day-old pure cultures of *A. solani.* The control experiments had distilled water instead of plant extracts. The inoculated media were incubated at temperature 27±1°C. Three plates for each treatment were used as a replicates. The diameter of the fungal colony was measured using a meter rule along two diagonal lines drawn on the reverse side of each Petri plate for 7 days after inoculation. Each treatment was replicated three times with four plates per replication (Pandey, 2007).

3.4 Preparation of fungicide concentrations

200µL of the score $^{250\ EC}$ (Difenoconazole 25%) were taken into 100 µl volumetric flask completed to the mark using sterilized distilled water.
5 µL of recommended fungicide doses **(**score $^{250\ EC}$**)** were added to 95 µL of molten PDA medium mixed well, the prepared media was poured into sterilized glass Petri dishes 2500 ppm fungicide concentrations.

3.5 Test procedure

The antifungal *in vitro* assays were carried out following the modified method of Okigbo, *et al.* 2005, and Chohan, and Perveen, (2006). Inhibition zone technique was used in this study to evaluate the effect of each concentration on mycelia linear growth of the fungus. Initially, fresh fungal growth was prepared from previously maintained culture of *Alternaria solani*. Prepared PDA media was amended with the required concentration from Ginger and Coriander and fungicide before being solidified in a conical flask of 250 ml, agitated and poured into sterilized glass Petri dishes. Three plates, containing 25 ml of PDA, were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

One mycelia disc of the fungus was placed in the center of PDA plates where opposite poles were marked at the back of the plate and incubated at 27±1°C in incubator and radial growth of pathogen was measured at 24 h intervals.
The Petri dishes of each concentration were arranged in a complete block design in incubator and incubated at 27±1°C for 7 days. The growth of the fungus was measured and calculated successively after 3, days after inoculation. The effect of each extract concentration on linear fungal

growth was calculated as percentage of reduction in diameter of fungal growth. The formula suggested by Vincent, (1947).

The formula Where: -

$$R = \frac{dc - dt}{dc} \times 100$$

Where:

R = Percentage reduction of the growth, dc= diameter of untreated growth and dt= diameter of treated growth.

3.6 Experimental design

The experiment was arranged in a Complete Randomized Block Design.

3.7 Statistical analyses

The obtained data was statistically analyzed according to analysis of variance (ANOVA) Duncan's Multiple Range Test (DMRT) which was used for means separation using Mstat-C statistical package (MSTAT-C .1991).

CHAPTER fouR

RESULTS

5.1 Identification of causal pathogen

Isolation of *Alternaria solani* was carried out from naturally infected tomato plants and identified on basis of cultural and morphological characteristics as *A. solani* (**plates 10and 13**) .

5.2 Effect of plant extracts on radial growth of *Alternaria solani*

All tested concentrations of ethanolic extracts of ginger rhizomes and coriander seeds exhibited different degrees of antifungal activity against *A. solani*. Two days after treatment all tested concentrations of ethanolic extracts of ginger rhizomes and coriander seeds in addition to standard fungicide resulted in similar higher non-significant inhibition percentage compared to the control treatment (**Table 1**, **Figure 1 and plate 3**). The same trend of inhibition percentage was noticed four days after treatment (**Figure 2)**. The ethanolic extract of ginger rhizomes at 75% concentration caused highest reduction (inhibition percentage) of mycelial growth of *A. solani* (95.6%), followed by ginger rhizomes at 25%and12.5% (93.3%,84.6%) , coriander leaves at 12.5%(89%) and 50%(87.6%) , the standard fungicide and coriander seeds at 25% (88% and 83.3% respectively), (**Figure 3, plate4**) . The mycelia of the control treatment grew naturally. At the end of the experiment eight days after treatment the same trend was observed as in previous count (4^{th} days after treatment) (**Table 1, Figure 4**).

Table 1. Effects of ethanolic extract of ginger and coriander, and fungicide (score $^{250\ EC}$) on the linear growth of *Alternaria solani in vitro.*

Treatment	Concs (%)	Inhibition zone (%)			
		2ndday	4th day	6th day	8th day
Ginger rhizomes	12.5%	88 (9.4) a	90.6(9.5)a	84.6(9.2) ab	87(9.4) a
Ginger rhizomes	25%	100 (10) a	100 (10) a	93.3(9.7) ab	91.3(9.6)a
Ginger rhizomes	75%	100(10) a	100 (10) a	95.6(9.8) a	91.3(9.6)a
Coriander seeds	12.5%	94(9.7) a	94.6(9.7)a	89(9.4) ab	89.3(9.5)a
Coriander seeds	25%	88.3(9.4)a	84.6(9.2)a	83.3(9.1) b	85.6(9.3)a
Coriander seeds	50%	92 (9.6) a	88.6(9.4)a	87.6(9.4) ab	84.3(9.2)a
Fungicide	100%	100 (10) a	87.3(9.3)a	88(9.4) ab	91(9.6) a
Control	-	0(0.7)b	0(0.7) b	0(0.7) c	0(0.7) b
C.V. (%)	-	4.1	15.3	3.7	4.0
SE±	-	0.2129	0.7692	0.1751	0.1915

-Means with the same letter in the same column are not significantly different ($P < 0.05$).

- Means in the parenthesis are transformed by square root transformation($\sqrt{X + 0.5}$) before analysis.

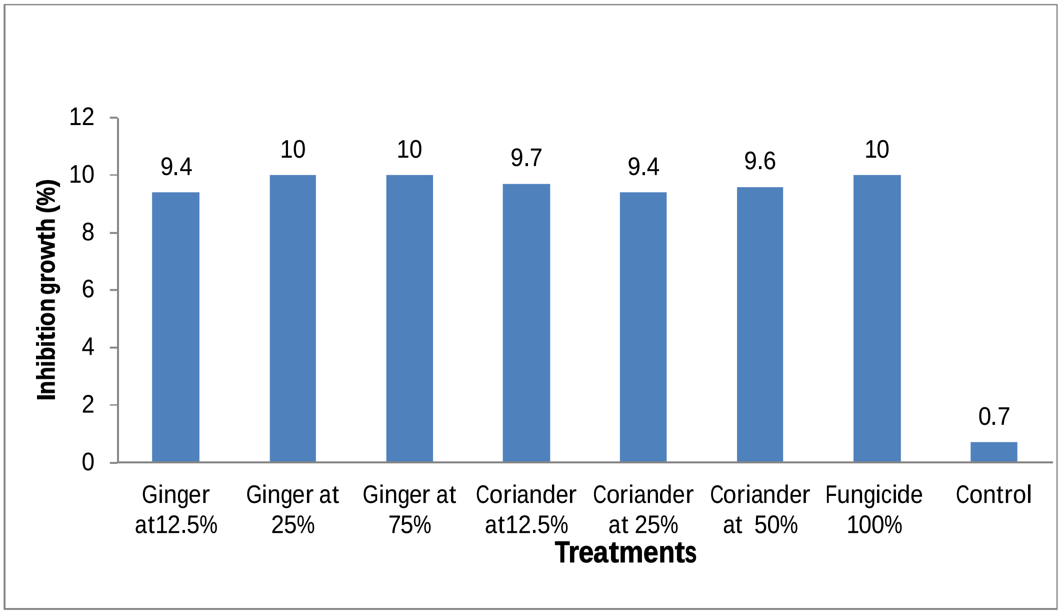

Figure 1. Effects of ethanolic extract of ginger, coriander and fungicide (**score** [250] **EC**) on the linear growth of *Alternaria solani* ***in vitro*** two days after inoculation.

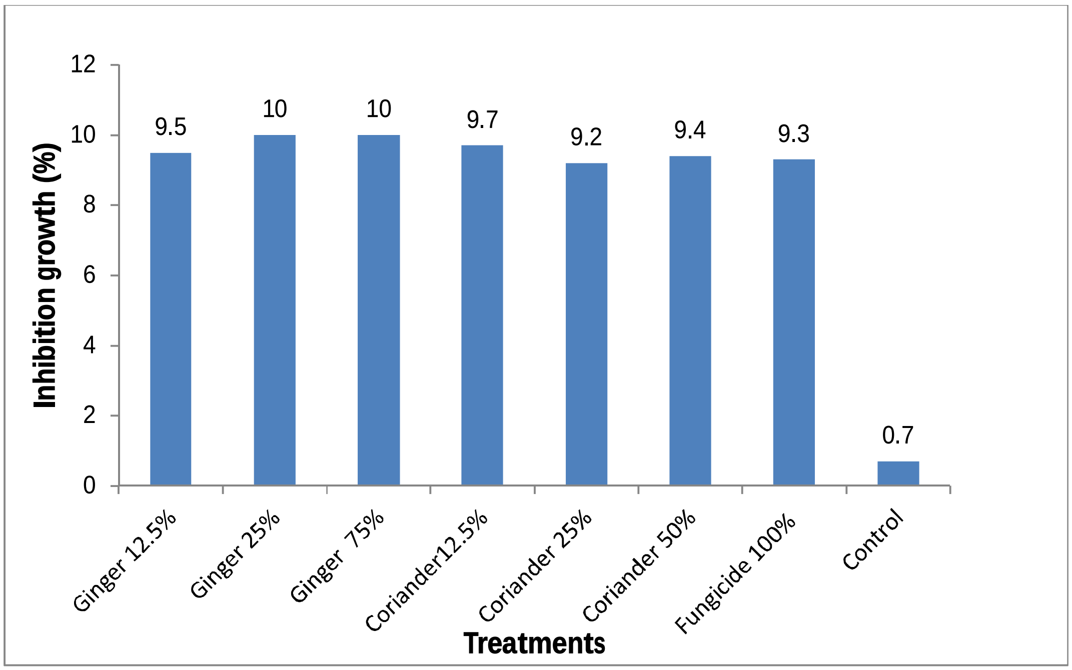

Figure 2. Effects of ethanolic extract of ginger, coriander and fungicide (**score** [250] **EC**) on the linear growth of *Alternaria solani* ***in vitro*** four days after inoculation.

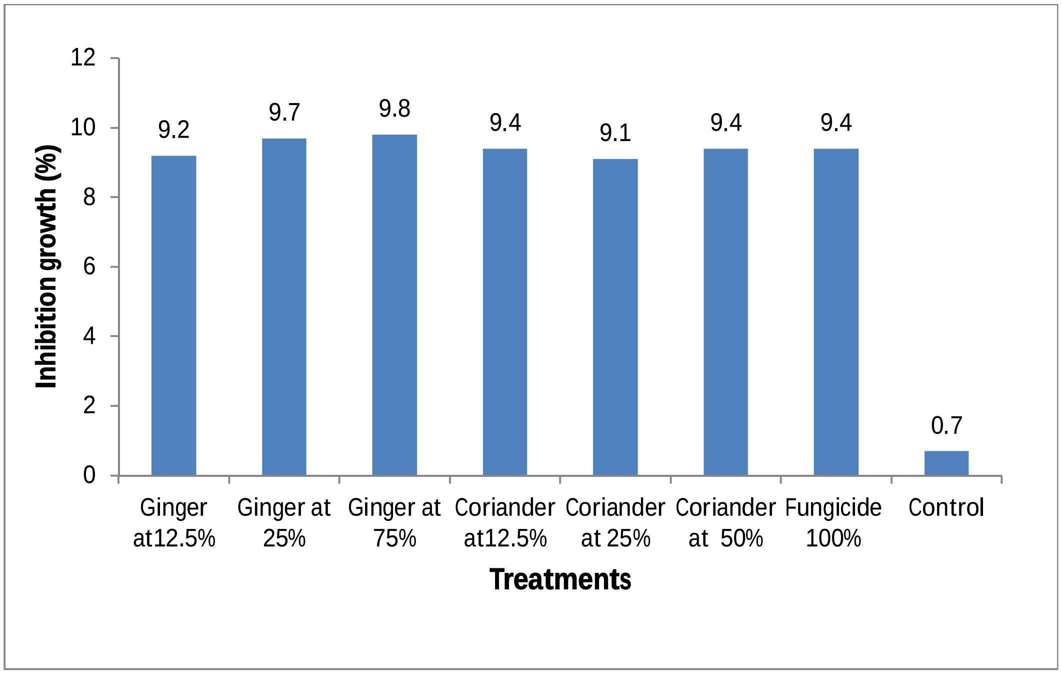

Figure3. Effects of ethanolic extract of ginger, coriander and fungicide (**score** [250] **EC**) on the linear growth of *Alternaria solani* ***in vitro*** six days after inoculation.

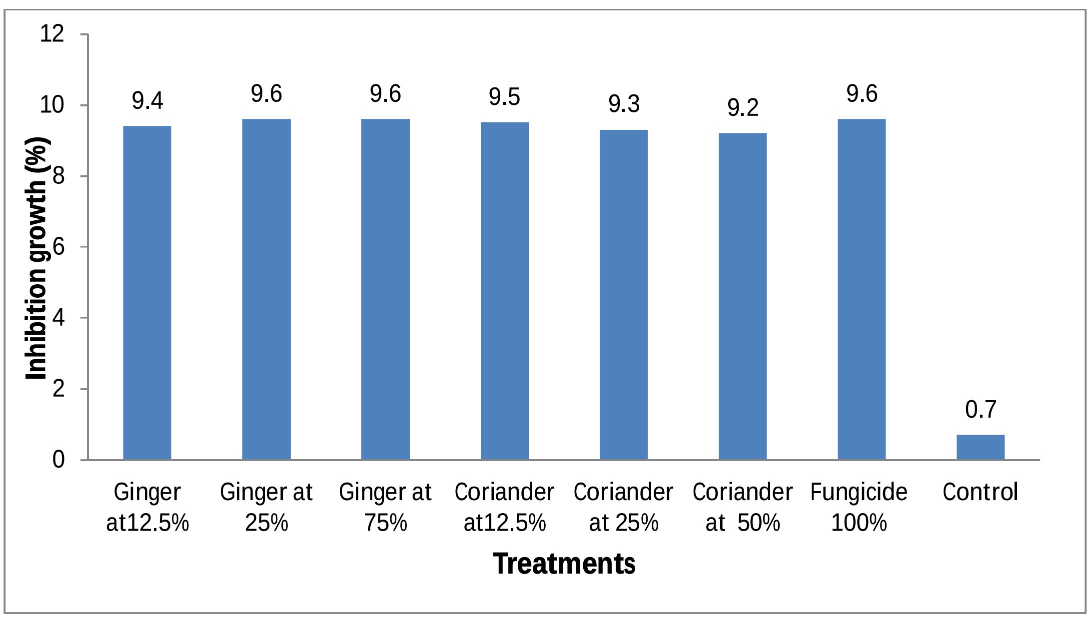

Figure 4. Effects of ethanolic extract of ginger, coriander and fungicide (**score** [250] **EC**) on the linear growth of *Alternaria solani* ***in vitro*** eigth days after inoculation.

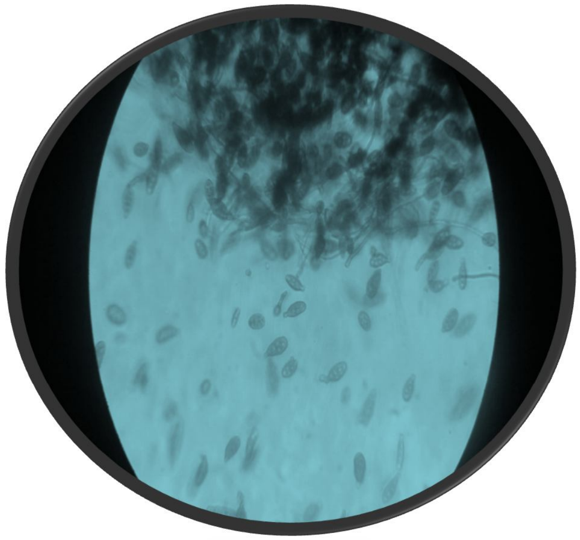

Plate 10: Shape of septate mycelium of tested fungs.

Plate 11: Shape of conidia normally transverse septate (Wikipedia).

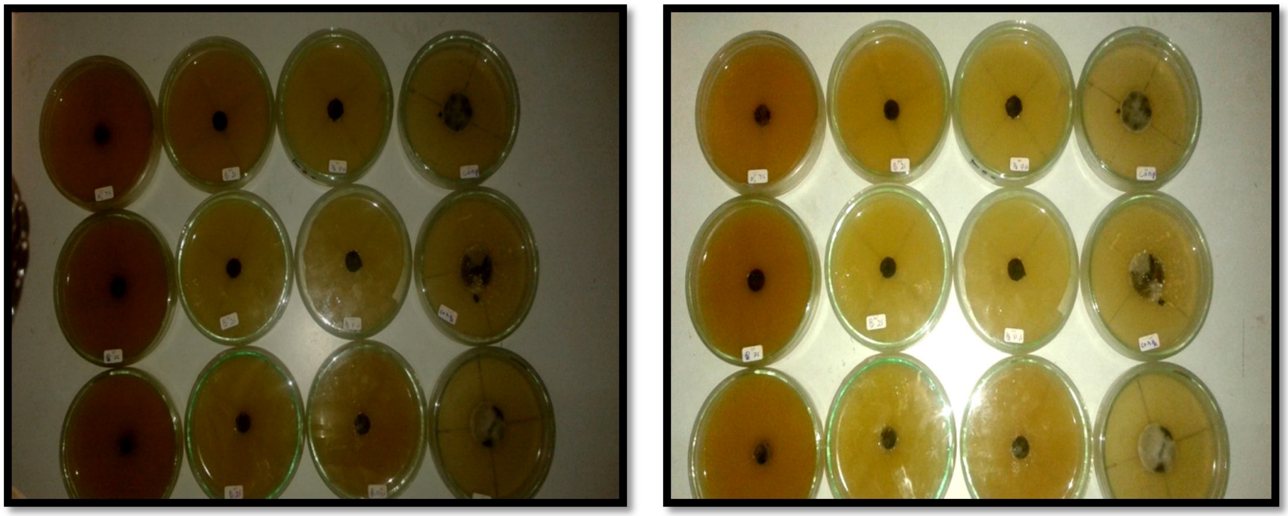

Plate 12: The experiment **four days after inoculation** (ginger rhizome and coriander seeds respectively).

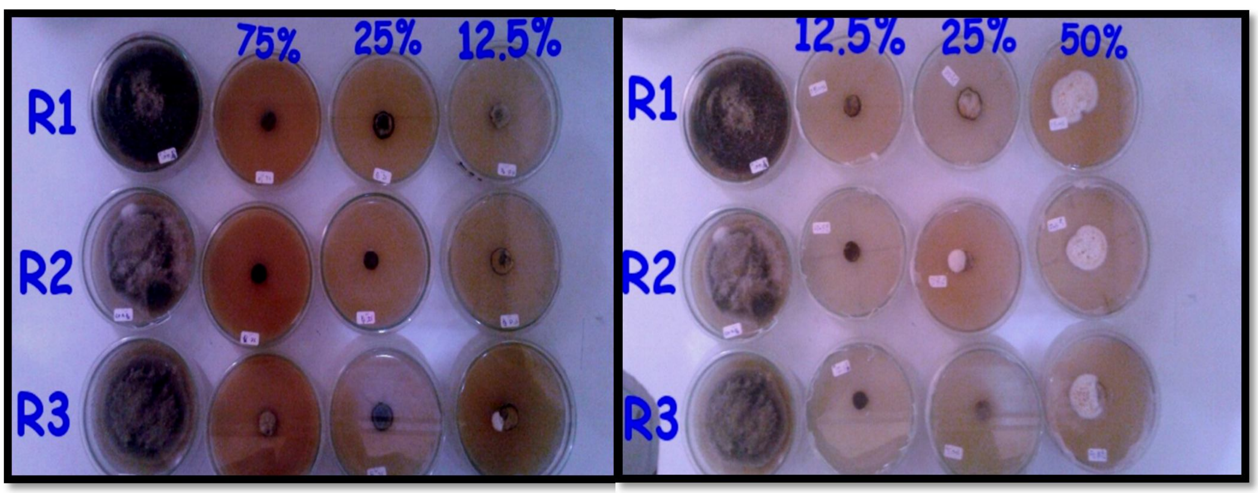

Plate 13: The experiment **eight days after inoculation** (ginger rhizome and coriander seeds respectively).

CHAPTER fivE

DISCUSSION

The results of the present study indicated that all tested ethanolic extracts of ginger rhizomes and coriander seeds, caused a significant reduction in the linear growth of *A. solani*. This reduction gradually increased by increasing the concentration of ginger rhizomes extracts in the growth medium. Similar effects of various other plant products effective against *Alternaria spp*. were reported by several authors (Latha *et al*., 2009 and Goussous *et al*., 2010) But, in leaves extract of coriander this reduction increased by decreasing the concentration.The results indicated that the highest inhibitive action of *Zingiber officinale* but coriander showed moderate activity.

Similar result was reported by Abdel-Kader, *et al*. (2012) and Fawzi, *et al*. (2009) who evaluated antifungal potential of various plant extracts against *Alternaria alternata*, *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Pythium sp*. and *Alternaria solani*. The results revealed that ginger extract had maximum inhibition effect on the growth of pathogenic fungi.

Martin Zabka . (2014) tested the efficacy of 20 essential plant oils against Four types of indoor fungi(molds) including *Alternaria alternate* , *Stochybotrys chartarum* ,*Cladosporium cladosporoides* and *Aspergillus niger*. The most effective anti-fungal essential oils were obtained from coriander (*Coriandrum sativum*), basil (*Ocimum citriodorum*) ,caraway (*Carum carvi*) and bay (*Pimenta racemosa*).

The inhibitory effect of the tested plant extracts may be due to their direct toxic effect on the pathogen as reported by Vijayan.(1989). Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly or induce systemic resistance in host plants resulting in a reduction of the disease development Kagale, *et al*. (2004).

Several authors including Curtis *et al*. (2004), Krebs, *et al*. (2006), and Latha *et al*. (2009) reported that plant extracts 20 non-host plant species caused a reduction of the early blight disease and suppressed the mycelia growth of *A. solani*. In conclusion, the study demonstrated that many plant extracts, e.g. from ginger rhizomes or coriander seeds, can be used for the biocontrol of the early blight disease. Thus, this method of control can contribute to minimizing the risks and hazards of toxic fungicides, especially on vegetables produced for fresh consumption. Further research into these extracts will identify the active compounds responsible for their fungicidal activity (Nashwa and Abo-Elyousr (2012).

CONCLUSION AND RECOMMENDATIONS

The present results indicated that the effects of the ethanolic extract of ginger and coriander seeds, as well as fungicide in inhibition the growth of the fungus *Alternaria solani* the causal agent of early blight. This reduction gradually increased by increasing the concentration of ginger rhizomes extracts in the growth medium but in seeds extract of coriander this reduction increased by decreasing the concentration. The chemical fungicides are cost effective and out of reach of the small land holders. Also, the consumers are quite doubtful on the production of synthetic chemicals and there has always been a good acceptance when it comes to natural antioxidants. This situation supplied a rationale for exploitation of plant products in disease management programs. It is envisaged that, application of plant extracts as a natural fungicides to treat different pathogens will be useful for small-scale farmers that are not able to afford costly fungicides.

The two plants are available in Sudan, According to these results of the present study, it can recommend that:

I. The ginger rhizomes and coriander seeds ethanolic extracts could represent good control against *Alternaria solani*.

II. Additional experiments should be carried out for evaluating the efficacy of these extracts against other fungi, and other diseases to determine their potential when used as biofungicides in this field

III. Further studies should be conducted to determine the active ingredients of both ginger rhizomes and coriander seeds ethanolic extracts.

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