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Sudan University of Science and Technology

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Evaluation of the Efficacy of Mesquite Extract (*Prosopis juliflora*) Against (*Alternaria solani*) as Causal Agent of Early Blight of Tomato.

تقييم فعالية مستخلص المسكيت (*Prosopis juliflora*) ضد فطر (*Alternaria solani*) المسبب لمرض اللفحة المبكرة في الطماطم

A thesis submitted in partial fulfillment of the requirements for the M.Sc. Degree in plant protection.

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الآية

بسم الله الرحمن الرحيم

قال تعالي:

وَإِذْ قَالَ رَبُّكَ لِلْمَلائِكَةِ إِنِّي جَاعِلٌ فِي الأَرْضِ خَلِيفَةً قَالُوا أَتَجْعَلُ فِيهَا مَنْ يُفْسِدُ فِيهَا وَيَسْفِكُ الدِّمَاءَ وَنَحْنُ نُسَبِّحُ بِحَمْدِكَ وَنُقَدِّسُ لَكَ قَالَ إِنِّي أَعْلَمُ مَا لا تَعْلَمُونَ (30) وَعَلَّمَ آدَمَ الأَسْمَاءَ كُلَّهَا ثُمَّ عَرَضَهُمْ عَلَى الْمَلائِكَةِ فَقَالَ أَنْبِئُونِي بِأَسْمَاءِ هَؤُلاء إِنْ كُنتُمْ صَادِقِينَ (31) قَالُوا سُبْحَانَكَ لا عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ (32)

صدق الله العظيم

الآيات (30-32 سورة البقرة)

DEDICATION

To the soul of my father and mother

To my Wife and kids

To my brothers and sisters

To all my family

To all my teachers

To all my colleagues and friends

With love and respect.

Adam

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First of all, I render my gratitude and praise to the Almighty Allah. I wish to express my sincere gratitude to my supervisor Dr: Ekhlass Hussein Mohamed for his helpful guidance, encouragement and supervision of this research.

Thanks all Thanks my best friend (Lab leader) M.sc Mawda Ibrahim Hamid Elameen and my friend TarrigAbdallaGassim.

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Last but not least, thanks are extended to my family, specially my Wife for financing my studies and helping me in many ways to finish my study.

Adam

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ABSTRACT

The present study was conducted in the laboratory of plant pathology at the College of Agricultural Studies-Sudan University of Science and Technology (Shambat). To isolate and identify the fungus caused early blight in tomato. It is an important disease of this crop and causing significant reduction in yield in tomato and to study the effect of aqueous extract of fruits and bark parts of Mesquite *(Prosopis juliflora*) at three concentrations (12.5, 25 and 50%) plus the fungicide Amistar top at standard dose on growth of the fungus. In the present study the pathogenic fungi isolated from infected plant parts of tomato showing typical symptoms of early blight. The fungus identified based on morphological and cultural characters as (*Alternaria solani in vitro*. The obtained results showed that the fungus grow best on Potato Dextrose Agar (PDA) at 28°C. The antifungal effects of medicinal plant extract of two parts of Mesquite (*Prosopis juliflora*) (Fruits and Bark) were determined by using water as solvent by Poisoned Food Technique at 28°C.The efficacy of plant extracts showed that the two plant extracts gave significant inhibition on the growth of pathogen. The results showed that all concentration of fruits and Bark aquas-extracts of Mesquite tested especially the highest one exhibited significantly high inhibitory effect against the Radial growth of *(Alternaria solani*) and the fungicide Amistar top® compared by control (44.3%, 58%, 70 %,). The current results were taking up from extracts of Mesquite considered promising and encouraging to carry out photochemical analysis of different parts of Mesquite tree by using different solvents to determine the bioactive ingredient in each part of Mesquite. In order to find an alternative botanical fungicide in place of harm and sever uses of pesticides on human, animal health and environment.

ملخص الاطروحة

اجريت هذه الدراسة بمعمل امراض النبات بكلية الدراسات الزراعية - جامعة السودان للعلوم والتكنولوجيا بغرض عزل وتعريف الفطر المصاحب لمرض اللفحة المبكرة فى نبات الطماطم بمنطقة شمبات بالسودان وامكانية مكافحته بإستخدام بعض المستخلصات النباتية الطبيعيه وهي اجزاء نبات المسكيت (*Prosopis juliflora*) (الثمار واللحاء) بنسب (50% و25% 12.5%) والمبيد الفطرى امستار توب كإستاندر باستخدام الجرعة الموصي بها مقارنة بالشاهد. تم عزل المسبب المرضي من أجزاء الطماطم المصابة ثم تم تعريف الفطر حسب الصفات المظهرية والمزرعية بأنه فطر (*Alternaria solani*). وأوضحت الدراسة المعملية لتقييم البيئة الغذائية علي نمو الفطر أن نمو الفطر في بيئة البطاطس هوالأمثل في درجة حرارة 28°م . أثرالتضادالفطر لمستخلصات شجرة المسكيت الأجزاء (لحاء وثمار) حدد بدراسة معملية أستخدم فيها المستخلص المائي بتقنية الطعم السام. أوضحت الدراسة أن كل المستخلصات ذات فعالية معنوية في تثبيت الفطر. من بين المستخلصات الاثنين وجد أن مستخلص الثمار وخاصة التركيز العالي 50% كان الأمثل في تثبيط نمو الفطر. أثر السمية للمبيد AMISTAR top® أختبر في المعمل بتقنية الطعم السام ، وجد أن هناك نقص معنوي في النمو الفطري (44.3%،58%،70%) بزيادة تركيز المبيد.النتائج الماخوذة من مستخلصات شجرة المسكيت تعتبر واعدة و مشجعة للقيام بتحاليل كيميائية لمختلف اجزاء شجرة المسكيت بأستعمال مستخلصات مختلفة لتحديد المادة الفعالة في كل من هذه الأجزاء والأنتفاع بها كبدائل لإستخدام المبيدات الضارة والمؤذية علي صحة الإنسان، الحيوان والبيئة.

CHAPTER ONE

INTRODUCTION

Tomato (*Lycopesricon esculentum* mill.) which is member of the family solanaceae that includes also other cultivated crops such as potato, pepper, eggplant, tobacco etc. The origin of tomato is believed to be central and south America, especially Mexico, from where the crop was transferred to Europe in the 16th century , then to old world continents (Hedrick , 1919 and Rick , 1976). Tomato is an important food and cash crop for the majority of the low income farmers in the tropics (Prioret, *et, al*., 1994). In fact it is considered as one of the most important and popular vegetable in many countries and this is because of its acceptable flavor, nutritive value and ability to fruit in a wide range of environments and the relative ease with which it can be cultivated (Prioret, *et, al*., 1994).

Although a number of pests and diseases (e.g. wilt diseases) are considered one of the main limiting factors for its growing, the production of tomato was developed rapidly since 19th century. According to FAO (2005/2006) and Jamal (2012), the area under tomato production has increased rapidly from 1300 hectares during 1999 to 43453 hectares in 2005/2006.

Moreover, and in most cases, in order to prevent the plant diseases and to protect the crop plants against pathogens, chemical control methods are in practice.

The major problems with the constant use of chemicals are that resistance can be induced in target organisms in addition to contamination of the environment with very toxic substances (Okigbo, 2004; Carvalho, 2004). This has initiated the exploration of safe alternate products.

Historically, the presence of antimicrobial compounds in higher plants has been recognized as important products in combating plant pathogenic

diseases. Such compounds, being biodegradable and selective in their toxicity, are considered valuable for controlling some plant diseases (Schmutterer, 2002).

Objectives of this study:

1. To Isolate and identify of the Caucal agent of Early Blight (*Alternaria solani*) in Tomato Plant in Shambat, Sudan.
2. To explore the antifungal potentials of mesquite crude extract against *Alternaria solani*.
3. To evaluate the effectives of systemic fungicide on fungal growth.
4. To assess and evaluate the effeccy of equous mesquitre (*Prosopis juliflora)* extract a gainst *Alternaria solani* of early blight disease.

CHAPTER TWO

LITERATURE REVIEW

2.1 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 14 greenhouse (350 m2) in Khartoum reached 5ton per season (Abdol hafeez, *et al*. 2012).

2.1.2 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)

2.1.3 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). Glandular hairs (trichomes) that emit a strong aroma when broken characterize tomato. 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).

2.1.4 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).

2.1.5 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).

2.1.6 Importence 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.

2.1.7 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 until 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).

2.1.8 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).

2.1.9 Economic Importance:

In the Sudan, the tomato is considered as one of the major vegetable crops and widely used in the processed forms as paste, ketchup, sauce and dry tomato slices. The crop presents one of the main cash vegetable crops in central and Northern Sudan (Jamal, 2012).

Tomato is growing successfully almost in every part of the Sudan during the winter season and in close system farming (Jamal, 2012). The main producing areas of tomato (*Lycopesricon esculentum* Mill.) are Central and North Sudan. Tomato is alsoproduced in GableMara and some parts of the main rain fed areas around villages in central clay plains and utilized as sun dried slices. Summer production of tomato which ensures high profitability because of the scarcity of the crop at that time is practiced in limited areas in Blue and White Nile and Khartoum state (FAO, 1999).

Although a number of pests and diseases (e.g. wilt diseases) are considered one of the main limiting factors for its growing, the production of tomato was developed rapidly since 19th century. According to FAO (2005/2006) and Jamal (2012), the area under tomato production has increased rapidly from 1300 hectares during 1999 to 43453 hectares in 2005/2006.

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2.1.10 Distribution in the world:

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2.1.11 Poblems and diseases:

Tomato (*Lycopersicon esculentum* Mill.) is of the important crop worldwide (Suarez *et al.,* 2007). Many diseases affect tomatoes during the growing season, both in greenhouse and field (Pelta, 2001). Among these is the early blight disease, caused by pathogenic fungus; *Alternaria solani*. In fact, thefungusis one of the most important known pathogen of tomato plant (Suarez *et al.,* 2007).

The major constraints facing the productivity of tomato worldwide are the losses caused by diseases, insects, nematodes and parasitic weeds. Among these, the most important are fungi, affecting roots, stems, leaves, flowers, and pods. The threat to plants from fungal infections has now reached a level that outstrips that posed by bacterial and viral diseases (Berger, 1977).

2.2 Early blight:

Early blight is the major disease symptom caused by the fungus *Alternaria solani* and Martin (1987). This disease, which in severe cases can lead to complete defoliation, is most damaging on tomato (Solanumlycopersicum L. (Peralta *et al*. 2005) syn. Lycopersiconesculentum Mill.) in regions with heavy rainfall, high humidity, and high temperatures (24°–29°C). Epidemics can also occur in semi-arid climates where frequent and prolonged nightly dews occur (Rotem and Reichert 1964).

Apart from the leaf symptoms that are known as early blight *Alternaria solani* can cause less economically important symptoms on tomato, including collar rot (basal stem lesions at the seedling stage), stem lesions on the adult plant, and fruit rot (Walker 1952). Yield losses up to 79% from EB damage have been reported from Canada, India, the United States, and Nigeria (Basu 1974), Datar, and Mayee (1981). Collar rot can cause seedling losses of 20% to 40% in the field.

2.2.1 Classsification of (*Alternaria solani*)

Kingdom: Fungai

Phylum: Ascomycota

Class: Ascomycetes

Order: Pleosporales

Family: Pleosporaceae

Genus: Alternaria

Species: Solani

2.2.2 Symptoms and Diagnosis

The appearance of circular or irregular dark spots on the lower, more mature leaves is one of the first symptoms of infection. Eventually, the spots enlarge into a series of concentric rings surrounded by a yellow ar. The entire leaf may be killed and will drop off the plant. Early blight can result in extensive defoliation, exposing fruit to sunscald and reducing yields. This disease typically progresses from the base of the plant, upward (Agrios, 2005).

2.2.3 Symptoms

Symptoms of early blight occur on fruit, stem and foliage of tomatoes and stem, foliage and tubers of potatoes. Initial symptoms on 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. Lesions greater than 10 mm in diameter often have dark pigmented concentric rings. This so-called "bullseye" type lesion is highly characteristic of early blight. 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. 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. The lesions appear leathery and may have the characteristic concentric rings. Infected fruit will frequently drop prematurely. Symptoms on potato tubers are characterized by sunken, irregular lesions which are often surrounded by a raised purple border. Beneath the surface of the lesion the tuber tissue is leathery or corky with a brown discoloration. Early blight lesions on tubers tend to be dry and are less prone to invasion by secondary organisms than lesions of other tuber

rots. After prolonged storage severely diseased tubers may become shriveled (Kemmitt, 2002).

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. (Kemmitt, 2002).

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. (Kemmitt, 2002).

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).

2.2.4 Pathogen biology

The causal pathogen of early blight is the fungus Alternaria solani. 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 fungus is readily cultured on artificial media such as juice where it produces a deeply pigmented gray/black hairy colony. The mycelium is haploid and septate, 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 .Morphological and pathogenic variability among

isolates of *A. solani* has given rise to claims of the existence of races, although this remains unproven.

2.2.5 Disease Cycle and Epidemiology

Alternariasolani overwinters primarily on infected crop debris. The dark pigmentation of the mycelium increases resistance to lysis which extends the survival time in the soil to several years. 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 horse nettle and nightshade.

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. Infection of potato tubers usually occurs through wounds in the tuber skin inflicted during harvest. Wet conditions at harvest provide a favorable environment for spore germination as well as causing swollen lenticels on the tubers which are easily invaded.

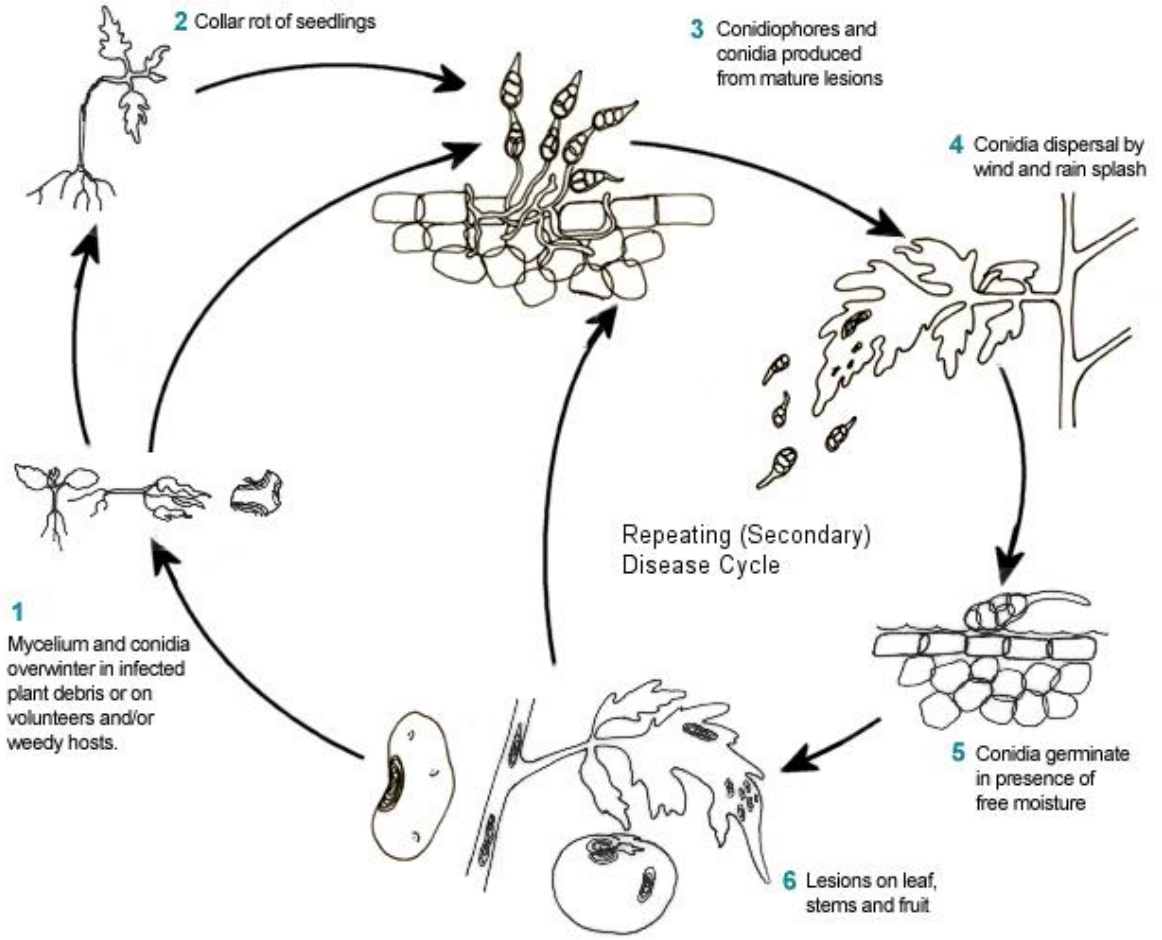


Figure Life cycle of the Pathogen as General in Tomatoe and Potato

Early blight spores survive on old plant debris or in the soil. Spores are spread by wind and rain, but occasionally, flea beetles transmit this disease. Fungal spores enter a host through wounds in the plant cuticle. Spores thrive in moist, warm temperatures (80–90 degrees F) and can persist in partially decomposed garden waste for at least a year. (Agiose 2005).

2.2.7 Control measure

2.2.7.1 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).

2.2.7.3 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). Resistant cultivars are potentially the most economical control measure because they can extend the intervals between fungicide sprays while maintaining control of the disease (Shtienberg *et al*. 1995; Keinath*et al*. 1996). Progress in breeding for EB resistance has been limited by the lack of effective resistance genes in cultivated tomato (Vakalounakis 1983; Poysa and Tu 1996; Banerjee *et al*. 1998; Vloutoglou 1999) and by the quantitative expression and polygenic inheritance of the resistance (Barksdale and Stoner 1977; Maiero*et al*. 1989; Nash and Gardner 1988a; Maiero*et al*. 1990a; Thirthamallappa and Lohithaswa 2000). Sources for Early blight resistance have been identified in wild relatives of tomato. Some of these have been utilized through traditional breeding approaches, but an increased level of resistance is negatively correlated with earliness (Nash and Gardner 1988a; Maiero*et al*. 1989; Foolad and Lin 2001; Foolad*et al*. 2002a) and yield (Barrat and Richards 1944). The most resistant breeding lines and hy-brid cultivars with acceptable horticultural characteristics that are currently available have moderate resistance to early blight and mature slightly later (Gardner 1988; Gardner and Shoe-maker 1999; Gardner 2000).

Hence, resistant cultivars with better horticultural traits are still needed. Chaerani Classical quantitative genetic analyses have provided es-timates of the number of quantitative trait loci for Early blight resistance, of the average gene action and of the herita-bilities from which the prospects for progress in

breeding programs based on phenotypic selection can be estimated (Nash and Gardner 1988a; Maiero*et al*. 1990a, b).

2.2.7.4 Chemical control

Fungicides with curative properties are regitered 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).

2.2.7.5 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 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 *invivo*. 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).

2.3 Mesquite:

2.3.1 Botanical Mesquite:

Prosopisspp is ever green leguminous trees or sharps. The genus comprises 44 species of which 40 are native to the Americas, of the remaining species 1929kdeziana ,P Africana is indigenous to Africa ,where is P kodzina , P farcta and P. cineraria are native to middle east and Pakistan (Borun and Messey ,1929 and Bukarat, 1976). Prosopis species grow in arrays of

environment and are not restricted by soil type, pH, salinity or fertility (Sid Ahmed, 2005 and Babiker 2006)

2.3.2 Scientific classification

Kingdom: Plantae

Sub kingdom: Tracheobionta

Super division: Spromalophyta

Division: Mangyoliophyta

Class: Magnoliopsida

Sub class: Rosidae

Order: Falales

Family: Fabaceae

The name *Prosopisspicigera* was selected by Linnaeus to describe the only species he was aware of *P.spicigera* in 1776 (Felker, et *al*., (2001).

Stated that genus Prosopis Linnaeus Burkat is the family Leguminose (Fabaceae). Sub family Mimosoidae. The placing of Prosopis in the wider taxonomy classification system given below based on Lewis and Elias 1981:

Family: Leguminosae

Sub family: Mimosoideae

Tribe: Mimoseae

Group : Prosopis

Genus : Prosopis

The history of taxonomic confusion within the genus was largely settled with authoritaive monograph of Arturo (Barkat 1976) ,who defined the genric

limits and divided the genus ino five section based on floral characterstices ,each with marked vegetative differences in aramture.

Forty four species and anumber of varieties of Prosopis were discribed many as seperate n species or varities , even though several are known to hybrdise (Felker *et al.*, 2001). The taxonomy of Brakat (1976), has been genarally accepted and this is used as benchmark with which other taxonomiesare compared, (Felker *et, .al* 2001).

2.3.3The benefit uses of mesquite

The tree has some benefits that include combating desertification ,nitrogen fixation as legumenous plants increasing the global green coverage using its timber for furniture ,fencing and fuel , also as animal

Feed ,however ,resently it was realized that the problems caused by the plants for more than benifits derived from them (Sidahmed ,2005 and El khalifa 2010).

2.3.4 Allelopathy

The leaves of P juliflora contain variouse chemicals inclouding tannis, flavonoids, steroids ,hydrocarbons ,waxes , and alkaloids (Felker, 2000).

These are known to affect paletability to livestock but also have effect on the germenation and growth of Prospis , weeds and other trees .

Leaf extract were also noted to kill some insect ,bacteria .and fungi . However there is some depate as to the importance of allelopathy in tree ,crop interaction and the applicability of results from pot trials to field condition.Alkaloids and flavonoids are known to degrade rabidly folowing Leaf senescence but other chemicals may accumulate under tree crowns.

Most studies have utilised leaf extract or dry leaves incorporated in to soil for analysis in pot trials.This often exoggerates the concenteration of chemicals leading to misleading results.

Reduction in crop seed germination due to chemicals inhibition was noted with *P. juliflora* leaf concenteration of mowre than 3 % but it was thought that this would not be naticeable under field condition (El Fadil ,1997).

Effects of these allelochemicals my be indirect ,upone the seed and seedling, or my be in directly via effects on other soil organisms.

Extracts from plant parts of P. juliflora decreased germination and growth of almost all plant tested in several studies , indicating that allelopathic effects are 1992 . afects atr important in the ecology of the P. juliflora, P. pollide complex (Felkar *et al*., 2007), however assessed the effects of P juli flora extracts on germination of Euphorbia sp and concluded that the phytotoxicity was withought ecologicaSl significant (Felker ,2000).Sola *et al*.,1992) .Thought that the Accumulation of steroids ,hydro carbons and waxes in P. ruscifolia leaf letter affected hydrophlic constituents and soil moisture ,capacity , where all other auther discuss only allelo chemicals effects.

However , Noor *et al*. , 1995) , observed agreater affect from fruit and seed extracts of Mesquite than from root or leaf or flower extracts . Bark extracts Have also proved effective in inhibiting germination Velu *et al*., 1996) .

2.4 AMSTAR TOP

Scientific Name: **Azoxystrobin**

Commercial Name: **Amstar Top**

Chemical Structure: $\mathbf{C_{22}H_{17}N_3O_5}$

Amistar Top contain azoxystrobin, broadspectrum fungicide,from the strobilurin group.It has systemic ,translaminar and protectant properties.

Azoxystrobin inhibit fungal respiration , its mode of action is different from the action of other fungicidal groups.It should always be used in mixture with fungicide with other mode of actions.

Amstar top shows good crop safety, diseases control and maintenance of green leafe area which result in significant yield benifits.

Highly effective broadspectrum systemic fungicide. It controls a wide range of Ascomycetes, Basidiomycetes, Deutormaycetes,including important diseases such as powdery mildews , karnal Bunt, Rusts and leaf spots occuring in wheat rice, tea, groundnut. It has consistent formulation quality and results.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study location

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 July to October, 2018. The aim of this study was to isolate and identify the fungus (*Alternaria solani*) that caused leaf blight. Tomato plant samples collected from Shambat area, Sudan and to explore the methods of control under laboratory conditions where temperature around 250C.

3.2 Collection, isolation and identification of fungus

Infected parts of tomato plant (Leaves) showing typical symptoms of early blight were collected from the College Farm at Shambat area. Then, they were put in plastic bags and transferred to the laboratory. The secured plant material (Leaves) were cut into small bits (0,5and 1, 0 cm) and washed well in tap water to remove the adhering dirty particles. The pieces were surfaces sterilized by sodium hypochloride (Clorox) Naocl (1% concentration) for 5 minute, rinsed three time in sterilized distilled water to remove traces of NaCl and dried on sterilized filter paper.

The sterilized leave sections were then plated at the rate of 5 sections per plate on Potato Dextrose Agar (PDA) medium and incubated at 28°C for 7 days. After incubation the isolated fungus was sub-cultured on PDA media for further purification of the fungus.

The identification of the fungus was based on visual culture characteristics of the hyphae and compound microscopic examination were also carried out for hyphae and conidia structure based on the method of Booth, (1977) to

confirm that the fungus is*Alternariasolani*. Standard books and research papers were also consulted during the examination of this fungus (Aneja, 2004). The purified isolates were maintained on PDA for further studies.

3.3 Preparation of Plant extract.

3.3.1 Plant extracts collection.

The obtained fine powder form the two parts of mesquite was weighted (12.5, 25 and 50 gm) and placed in 100 ml conical flask each and completed to 100 ml sterilized distilled water to obtain the three concentrations and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 12.5 %, 25% and 50% concentrations. Aqueous extracts of each parts of the plant materials were prepared as they recommended by Okigbo (2006).

3.4. Preparation of Fungicide Concentrations

One ml of the Amstar top® 250 EC fungicide was dissolved in 100 ml of sterilized distilled water of which it is the statndard dose.

3.5. Bioassay Test

Inhibition zone technique was used in this study (Rao and Srivastava, 1994) to evaluate the effect of each concentration on linear fungal growth*(AlteranariaSolani*). Initially, fresh fungal growth was prepared from previously maintained culture of the fungus. Prepared PDA media was amended with the required concentration from different parts of Mesquite extracts and fungicide Amstar top® 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 *(Alteranaria solani*) was placed in the centre of PDA plates (Rao and Srivastava, 1994) where opposite poles were marked

at the back of the plate and incubated at 28°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 28 C^0 for 5 days. The growth of the fungus was measured and calculated successively after 3, 4 and 5 days after inoculation. The effect of each extract concentration on linear fungal growth was calculated as percentage of reduction in diameter of fungal growth (R). where:

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

Where:

R = Percentage reduction of the growth.

dc= diameter of controlled growth.

dt= diameter of treated growth.

3.6. Experimental Design and Statistical Analyses

The experiment was arranged in a Complete Randomized Design, the obtained data was statistically analyzed by MSTATC software computer program according to analysis of variance (ANOVA). Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

RESULTS

This study which conducted under laboratory conditions of plant protection Department, College of Agricultural Studies, Sudan University of science and Technology during august and September 2018, was to detect and identify the fungus associated with early blight symptoms in tomato in Shambat area and to explore the effect of some mesquite parts on fungal growthof *Alternaria solani in vitro*.

4. Isolation and Identification of the Fungus.

The infected parts of tomatoe plant show typical symptoms of early blight from Shambat area isolate *Alternaria solani* according to the Condia Shape and Mycelium.

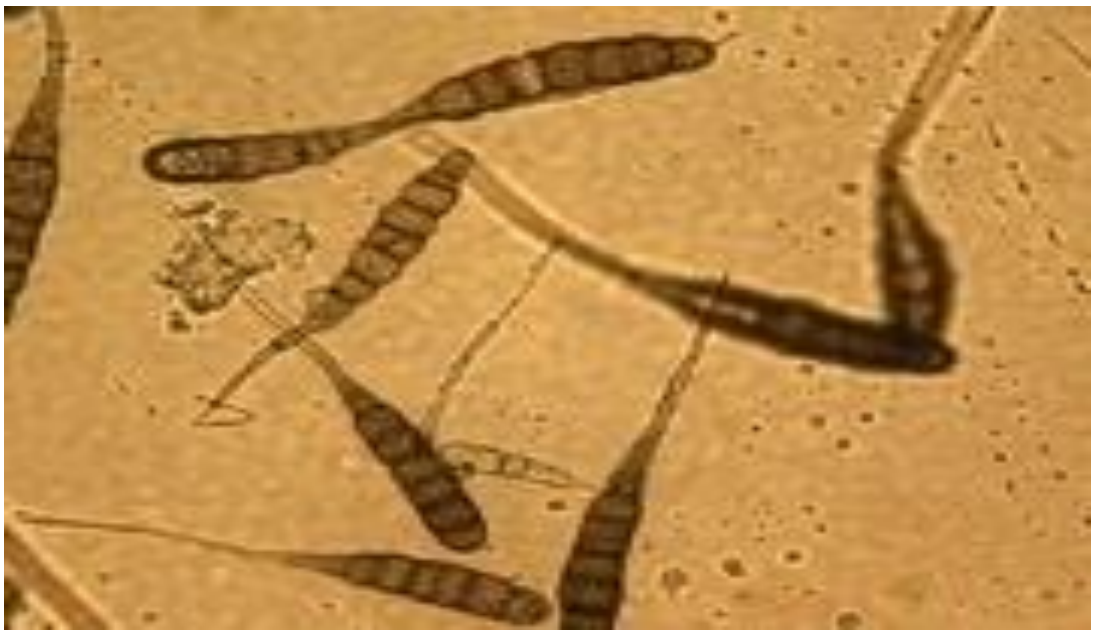
4.1 Effect of Mesquite Bark Extracts and Fungicide Amistar top® on radial growth of *Alternaria solani in vitro.*

Starting from three days from inoculation the results indicated that plant extracts at all, concentrations reduced the fungal growth compared to control (Table, 1). This trend started from day three (6.7) and continued until day six where the fungicide exhibited complete inhibition of fungal growth (10.0), demonstrating the highest inhibition rate among treatments.

The bark and fruits extracts concentration started inhibiting the fungal growth from day three and continued until day six. Their effects on fungal growth vary according to concentrations and days of growth. The lowest concentration (12.5) in bark or fruits demonstrated inhibition value ranged from 4.3 and 3.7 in day three 4.8 to 4.9 respectively. While with their concentration of 25% (5.6 and 6.11, 6.2) introdeuced.

Infected Leaf

Infected Fruit



Spores of *Alternaria solani*

The isolate were obtained from naturally infected tomatoe plant and leaves and fruit showing blight symptoms and identified as *AlternariaSolani*based on the Morphologocal Characteristic (Ellis 1976).

However, their highest concentration (50%) gave the more inhibition which reached 70% in bark and 66% in fruits. Moreover, the screened concentrations of mesquite extracts differ in their reactions to test fungus. Likewise the test organism responded differently to the different concentrations of treatments.

4.2 Effects of Mesquite Bark:

Bark of Mesquite was selected to evaluate the Antifungal activity against *AlternariaSolani*. All the bark extract at 12.5% and 25% and 50% and concentration were effective in inhibition the radial growth of *AlternariaSolani*compared to the control.

The bark extract of Mesquite at 50% and 25% caused the highest reducdtion of the Mycelial growth of *Alternaria solani* (72%, 60%) respectively followed by the (12.5%) concentraion caused the lowest inhibition of the Mycelial growth of the Pathogen (43%).

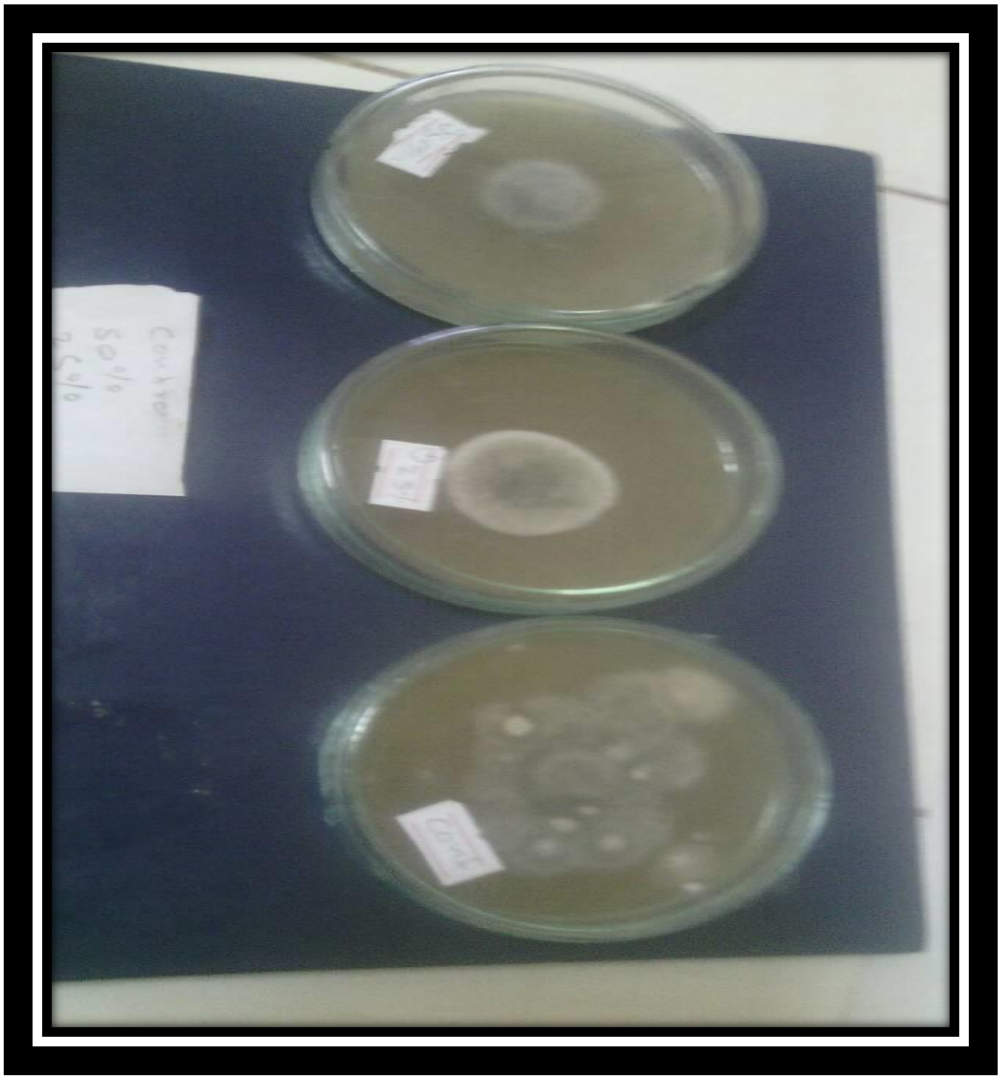


Plate 1 Effect of Mesquite Bark Extracts on radial growth of *Alternariasolani* in *vitro* .

Table 1: Effect of Mesquite Bark Extracts and Fungicide Amistar top® on radial growth of *Alternariasolani* in *vitro* after three, four, five and six days from inoculation

Trem	Threeday	Fourday	Fiveday	Sexday	Mean
12.50%	4.3±0.35[d]	4.6±0.84[d]	4.5±1.04[c]	4.8±0.28[c]	4.5
25%	5.6±0.31[c]	6.1±0.36[c]	6.1±1.01[c]	6.2±0.36[c]	6.0
50%	7.3±0.45[b]	7.2±0.36[b]	6.7±1.01[b]	6.9±0.20[b]	7.2
Fingicide	10.0±0.0[a]	10.0±0.0[a]	10.0±0.0[a]	10.0±0.0[a]	10.0±0.0[a]
Control	0.00	0.00	0.00	0.00	0.00
CV%	4.1	8.4	5.2	1.4	
SE±	0.23	0.35	0.55	0.1846	
$LSD_{0.05}$	0.53	0.78	1.23	0.4112	

Mean Coloney diameter inhibition and reduction

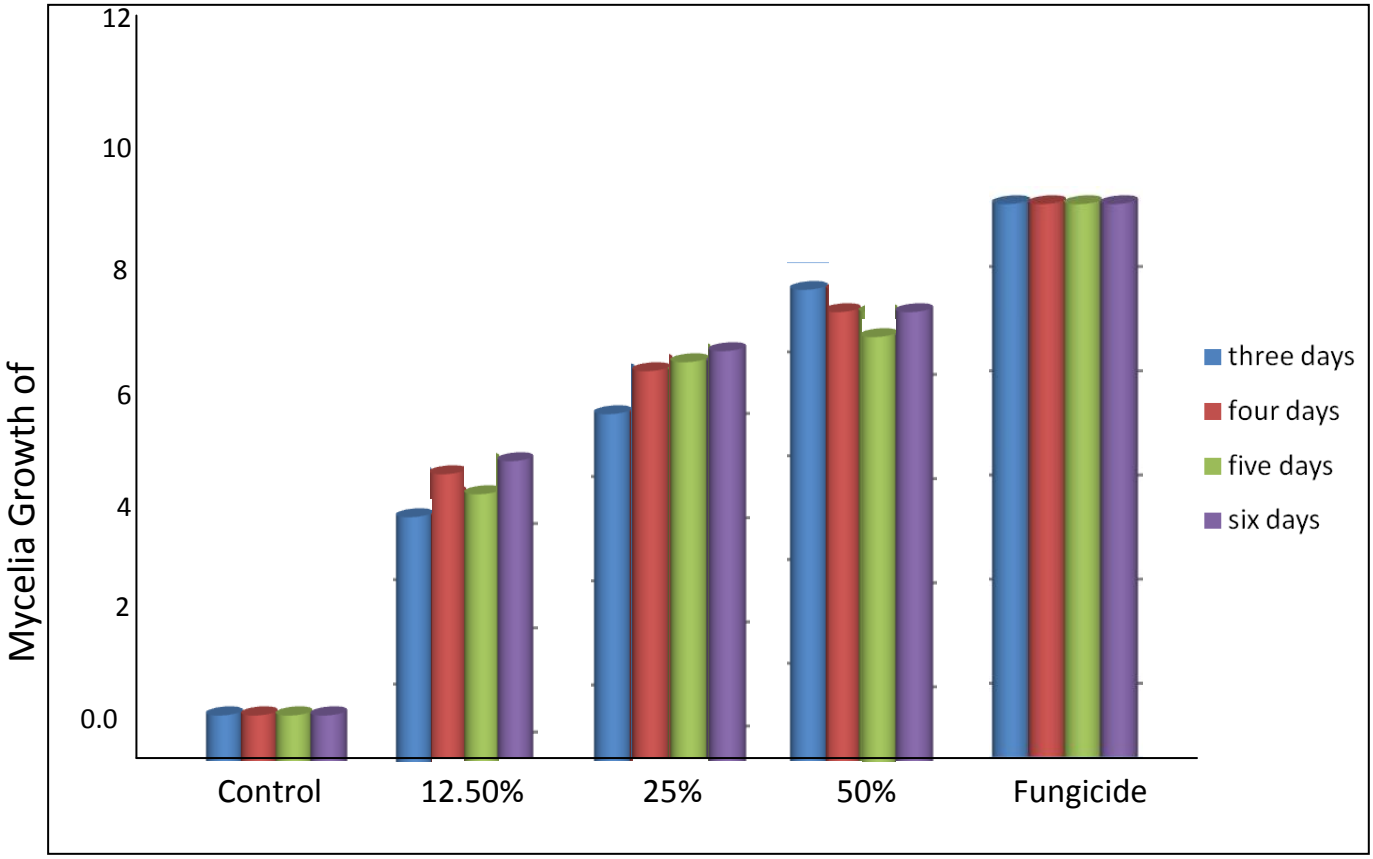


Figure 1 Effect of Mesquite Bark Extract and Fungicide A mister top® on radial growth of *Alternariasolani* in vitro after three, four , five and six days of inoculation.

Plate 2 Effect of Mesquite Bark and fruts Extracts on radial growth of *Alternariasolani* in *vitro* .

Table 2: Effect of Mesquite Fruits Extracts and Fungicide Amistar top® on radial growth of Alternariasolani in vitro after three, four, five and six days from inoculation

Trem	Threeday	Fourday	Fiveday	Sexday	Mean
12.50%	3.68 ± 2.14^{b}	4.59 ± 1.1^{b}	4.22 ± 0.67^{c}	4.94 ± 0.161^{d}	43.5
25%	6.11 ± 1.573^{ab}	5.69 ± 0.68^{b}	4.76 ± 0.42^{b}	4.94 ± 0.10^{c}	53.7
50%	7.39 ± 0.49^{ab}	7.20 ± 0.34^{b}	6.53 ± 0.33^{b}	6.62 ± 0.14^{b}	69.4
Fungicide	10.02 ± 0.00^{a}	10.02 ± 0.00^{a}	10.02 ± 0.00^{a}	10.02 ± 0.00^{a}	0.00
Control	0.00	0.00	0.00	0.00	0.00
CV%	3.94	5.74	9.57	3.09	
SE±	2.33	2.17	0.32	0.09	
$Lsd_{0.05}$	5.18	4.83	0.70	0.20	

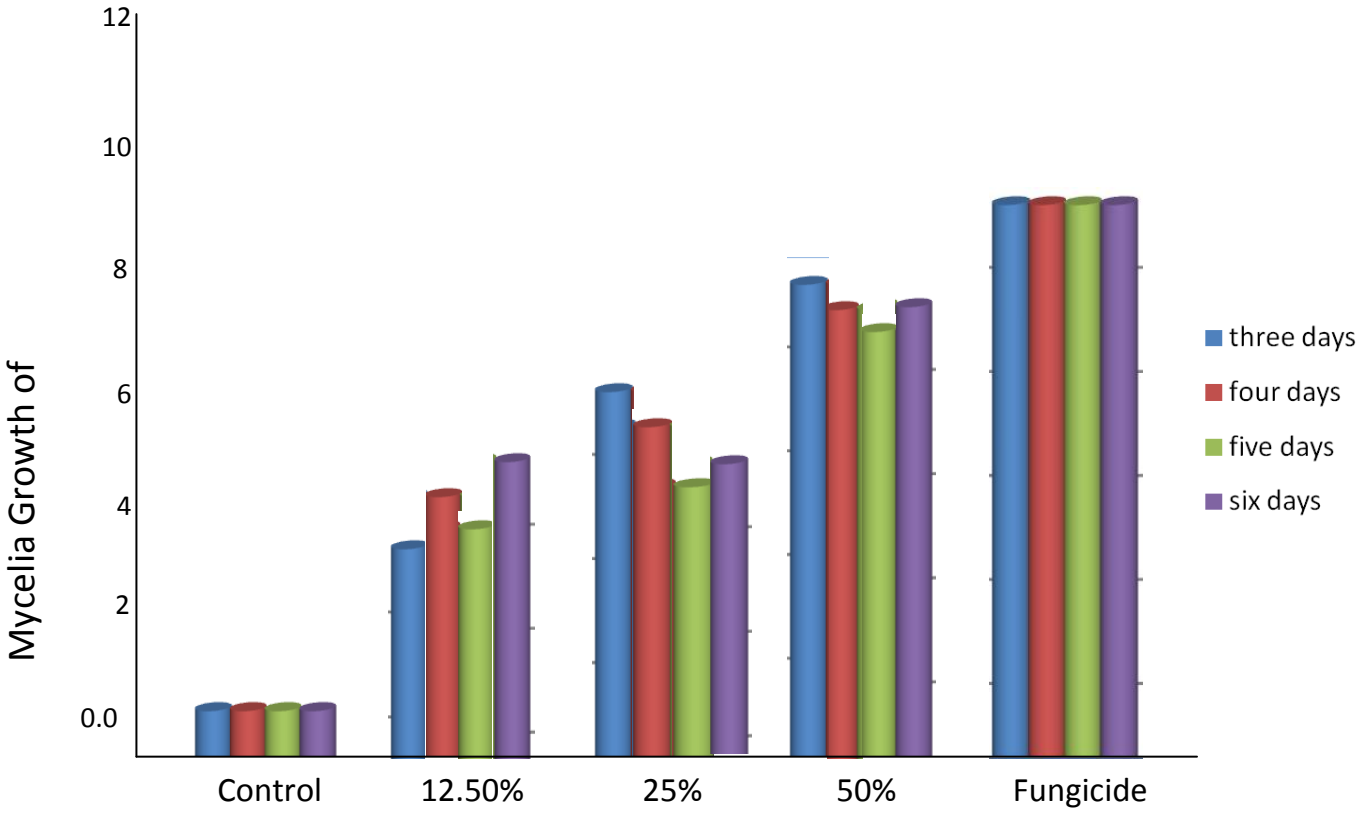


Figure 2 Effect of Mesquite Fruits Extracts and Fungicide A mister top® on radial growth of *Alternaria solani* in vitro after three, four, five and six days of inoculation.

CHAPTER FIVE

DISCUSSTION

Theses study was cared out to investigate the effect of Mesquite aqueous extracts (bark and fruits) and fungicide (Amistar Top) against *Alternaria solani* as the causal agents of earlt blight diseases.In thes study the Mesquite parts aqueous extracts (bark and fruits)consistently exhibited an inhibitory effect on fungal growth of *Alternaria solani* with significantly higher inhibition zones percent. Similar studies which explored the effect of extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Satish*et. al*., 1999; Okigbo and Ogbonnaya, 2006; Shariff*et. al*., 2006; Ergene*et. al*., 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006). Moreover, this finding is in agreement with Shimaa, Huda (2016) who tested the bioactivity of Mesquite extract against fungi and demonstrated its suppressing effect on the fungal growth *in vitro*. Also similar results were obtained by FodlElmola*et al*., (2010) who reported that the extracts of Mesquite different plant parts were highly effective in suppressing bacterial growth. Also Zainal *et al*. (1988) reported that *Prosopis. juliflora* contain antimicrobial compounds.

The results on effect of the Amistar top on the fungus showed that the fungicide expressed suppressive ability on the growth of the *Alternaria solani* with significantly high inhibition zones percent compared to other treatments. These results confirmed what had been reported by Runkhsana *et al*., (2010) who indicated that the effectiveness of systemic fungicide against fungal diseases.

Moreover, the data on concentrations from each plant aqueous extract exhibited different inhibitory abilities on fungal growth.

The highest concentrations from the two parts was the most suppressive followed in a descended order by lowest ones. Likewise, the *Alternaria solani* responded differently to the different concentrations of extracts. This variability in response which expressed by test organism to different mesquite extracts was also reported by Aiyelaagbe (2001). In his investigation, he explained that the majority of the studies involving plant extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree. However, these results confirmed that obtained by (Reem, 2012; Alhadi 2012 and Fayza, 2012)

Conclusions and Recommendations

Conclusions

The Fruit and bark aqueous extracts of mesquite (*Prosopis juliflora*) tested exhibited an inhibitory effect on fungal growth. Thus the two components plus fungicide Amistar top could be applied as part of an integrated approach to control early blight disease in Tomato.

The screened concentrations of seeds and bark aqueous extracts differ in their reactions to test fungus. Likewise the test organism responded differently to the different concentrations of extracts. This variability in response which expressed by test organism may be used to adjust an optimum dose for controlling *Alternaria solani* in Tomato.

Recommendations:

Based on the foregoing result the following studies were recommended;

- To further investigate the antimicrobial properties but in a group of medicinal plants against targets organism to determine their potentials as botanical pesticides.
- To carry out a phytochemicals analysis of different parts of mesquite plant using different solvents so as to determine the bioactive ingredient in each of these parts.

CHAPTER FIVE

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APPENDICES

1 Materials, tools and equipments used in the study

- Gloves
- Camera
- Marker pen
- Electric blender
- Petri-dishes glass
- Needle
- Autoclave
- Corcopuran
- Sensitive balance
- Incubator
- Flame
- Laminar flow cabinet
- Microscope
- Slide and cover slide
- Aluminum foul
- Water path
- Potato dextrose agar(PDA)
- Filter papers
- Medical cotton
- Ethanol
- Sodium hypochlorite

All materials except infected plant, which used in the experiments, were sterilized using 1% sodium hypo chloride , water distil and filter paper was used for Petri plate sterilization. Lactphenol Cotton blue was used for staining of the fungal cytoplasm and for providing a light blue background, against which the walls of hyphae can readily be seen (Aneja, 2004) according of Mawda, 2014).