Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Antifungal Effect of Mesquite *(Prosopis juliflora*) Extracts and Fungicide (Revus top®) against Fungus

(*Neofusicoccum mangiferae*) of Mango Branch Wilt Disease

أثر التضاد الميكروبي لمستخلصات المسكيت والمبيد (ريفص توب®) ضد الفطر (*Neofusicoccum mangiferae*) المسبب لمرض ذبول الأفرع في المانجو

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

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بسم الله الرحمن الرحيم

الآية

قال تعالي:

(وَسِيقَ الذِينَ اتقَوْا رَبهُمْ إِلى الْجَنةِ زُمَرًا حَتى إِذَا جَاءُوهَا وَفُتِحَتْ أَبوَابُهَا وَقَالَ لَهُمْ خَزَنَتُهَاسَلَمٌ عَلَيْكُمْ طِبْتُمْ فَادْخُلُوهَا خَالِدِينَ (73) وَقَالُوا الْحَمْدُ لِلهِ الذِي صَدَقَنَا وَعْدَهُ وَأَوْرَثَنَا الأَرْضَ نَتَبوأُ مِنَ الْجَنةِ حَيْثُ نَشَاءُ فَنِعْمَ أَجْرُ الْعَامِلِينَ (74) وَتَرَى الْمَلَائِكَةَ حَافِينَ مِنْ حَوْلِ الْعَرْشِ يُسَبحُونَ بِحَمْدِ رَبهِمْ وَقُضِيَ بَيْنَهُمْ بِالْحَق وَقِيلَ الْحَمْدُ لِلهِ رَب الْعَالَمِينَ(75)).

صدق الله العظيم

سورة الزمر الآيات (75.74.73)

قال رسول الله صلي الله عليه وسلم:

(ما من رجل يسلك طريقا يطلب فيه علما إلا وسهل الله به طريق إلى الجنة ومن أبطأ به عمله لم يسرع به نسبه) .

DEDICATION

Decide in which direction you are going

It is important to one and day on building your success and accepting full responsibility for your life. Step up from your comfort zone and if it is uncomfortable, you are probably moving on the right rood

To soul of my Dearest Raida my grandmother

To soul of my grandfather

Soul of my father

My Patiently mother

Sisters and brothers

To my Supervisor Dr: Ibrahim Saeed

I dedicate this work with sincerer love

Shimaa

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With respectful

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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 study the effect of water solvent extracts of Root, Brak and Leave of Mesquite tree (*Prosopis juliflora*) and Fungicide REVUS top® on Wilt Branch disease , which is considerate one of most important disease that infectous Mango trees (*Mangifera indica*), and caausing significant reduction in yield.

In present study the pathogenic fungi isolated from infected plant parts of Mango (stems,branches and leave). The fungus identified based on morphological and cultural charaters as *Noefusicoccum mangiferae* in vitro. The study conducted to evaluate the effect of media on fungus growth of *N.mangiferae*. the obtianed results showed that the fungus grew best on Potato Dextrose Agar (**PDA**) at 28ºc. the antifungal effects of medicinal plant extracts of Mesquite (Root,Bark and Leave), were determined by using water as solvent by Poisoned Food Technique at 28ºc.Three concentration of aqueous (Root,Bark and Leave), of Mesquite for at the same dose above were used in addition to Control as showed in Tables (1,2 and 3).The result showed that all aqueous extracts have significant efficacy in fungus growth inhibitory compared with Control. Root and Bark extracts was the best in fungus inhibitory by 96.1%,94.9% successively, espacially in high concentration 100% and Fungicide REVUS top® showed 99.1%. Among the results of different extracts, Root and Bark of Mesquite and Fungicide REVUS top® were geven the high inhibitory dgree to *N. mangiferae* .this results promising and encouregement to carry out photochemical analysis to determine the bioactive ingregiants in each part of Mesquite and use as alternative botanical to control Wilt Branch disease in future.

ملخص البحث

أجريت هذه الدراسة بمعمل أمراض النبات بكلية الدراسات الزراعية ، جامعة السودان للعلوم و التكنولوجيا –شمبات . لدارسة تاثير المستخلصات المائية لجذور و لحاء و أوراق أشجار المسكيت (*Prosopis juliflora*) و مبيد REVUS top® علي مرض ذبول الأفرع و الذي يعتبر من الأمراض المهمة التي تصيب أشجار المانجو (*Mangifera indica*) و تتسبب في نقص الأنتاجية.

في هذه الدراسة تم عزل المسبب المرضي من أجزاء أشجار المانجو المصابة (سيقان، أفرع وأوراق). تم تعريف الفطر حسب الصفات المظهرية و المزرعية بأنه فطر *Neofusicoccum mangiferae* . أجريت الدراسة المعملية لتقييم نمو الفطر علي البيئة الغذائية و أوضحت النتائج أن نمو الفطر الأمثل في بيئة البطاطس(**PDA**)علي درجة حرارة 28م بعد ذلك تم أختبار أثر التضاد الفطري للمستخلصات المائية لجذور ، لحاء و أوراق المسكيت و ذلك بتقنية الطعم المسلم في درجة حرارة 28م . في هذه التجربة تم استخدام ثلاث تركيزات 25% ، 50% و 100% لكل من المستخلصات الثلاثة و مبيد REVUS top® .

أظهرت النتائج ان كل المستخلصات لها فعالية معنوية في تثبيط نمو الفطر مقارنة بالشاهد و كانت مستخلصات جذور و لحاء المسكيت هي الأفضل في تثبيط الفطر بنسبة 96.1% ، 94.9% علي التوالي خاصة في التركيزات العالية 100% و كذلك تم أختبار مبيد REVUS top® علي الفطر بطريقة التطبيق السالفة حيث أعطي نسبة تثبيط 99.1% .

من خلال هذه النتائج أعطت كل من مستخلصات جذور و لحاء المسكيت و مبيد REVUS top® نسب تثبيط عالية لفطر *N. mangiferae* و هي نتائج مشجعة و واعدة للقيام بتحاليل كيميائية لهذه المستخلصات لمعرفة المواد الفعالة الموجودة في الاجزاء المختلفة لشجرة المسكيت مما يمكن من أستخدامها كبدائل أمنة لمكافحة هذا المرض الفطري المدمر في المستقبل

CHAPTER ONE

INTRODUCTION

Mango, *Mangifera indica* belongs to the dicotyledonous family Anacardiaceae. The tree is believed to be indigenenous to India and Southern Asia and originated from the Indian/Burmese border region where it has been cultivated for many centuries (Kwee and Chang., 1985). Today Mangoes are cultivated in most tropical and subtropical parts of the world where they are commonly eaten fruits (Prakash and Srivastava., 1987 and Schroeder., 1990). Countries that cultivate Mangoes commercially, but primarily local consumptions include India, Pakistan, Indonesia, Mexico, Brazil and the Philippines. The most important Mango exporting countries are Australia, South Africa, Israel, Egypt and the United States of America (Johnson *et al*., 1992).

A wide diversity of pathogens attacks various parts of nursery and adult Mango trees. Anthracnose, Blossom blight, Powdery mildew, Flower malformation, Cankers, Branch wilt disease, Twigs die back and Bacterial black spot are some of the main problems facing by Mango producer's worldwide (Prakash and Srivastava.,1987 and Wolstenholme and Whily., 1995). Of these diseases those caused by fungi contribute most to production and economic losses (Singh., 1960; Prakash and Srivastava., 1987 and Johnson *et al*., 1992).

Neofusicoccum mangiferae (Nattrass). Crous, *et al*.,(2006) is a known species that attacks mango at different stages of growth. The genus was first described from Plum, Apricot and Apple isolated by Nattrass, but has since been reported from many woody plants in various tropical and subtropical countries worldwide (Sutton and Dyko., 1989). The fungus is a cosmopolitan

polyphagus that attacks a multitude of tree flora. The earliest reports of plant diseases caused by *N. mangiferae* were from India on Mango trees in1908 (Sutton and Dyko., 1989). *Neofusicoccum* is a polymorphic fungus that has two spore stage the pycnidial and the arthoconidial (Scytalidium state). (Sutton and Dyko., 1989).

In Sudan Mango is one of the essential horticultural furit trees. It has economic significance being cultivated in different parts of the country. It has regional and international demand to its palatable and adored taste with great nutritive value. The branch wilt disease in Sudan was firstly reported by (Giha., 1975). on banyum trees (*Ficus benghalensis* L.). Shade trees that line the streets of the capital Khartoum. Since then, the disease has spread all over the country.It has very wide host range. It attacks trees in forests, orchards, ornamental, and shade trees it is one of the most hazardous diseases that spread in Sudan.

Obviously**,** *Neofusicoccum* is one of the most hazardous diseases that widely spread. There is a limited information or lack of effective control measures of the disease. Accordingly, an effective control measures should be developed to control this devastating disease that represent real threat to fruit, forest and ornamental trees. The aim of this study was to explore the antifungal activity of extracts of different parts of mesquite plants and the efficacy of systemic fungicide in suppressing the growth of this fungus in *vitro* with the following objectives**:**

1. To confirm that the causal agents is *Neofusicoccum mangiferae.*
2. To explore the inhibitory effect of aqueous extracts of different parts of Mesquite (Roots, Bark and Leaves) on fungal growth.
3. To evaluate in *vitro* the efficacy of systemic fungicide REVUS top® in suppressing the growth of the fungus.

CHAPTER TOW

LITERATURE REVIEW

2.1 Mango, *Mangifera indica*

Mango, *Mangifera india* L. belongs to the dicotyledonous family Anacardiaceae. The genus *M. indica* consists of 62 invariably arborescent species, which includes Mango and other economically important trees. The Mango forms an erect, branched evergreen tree with awide crown (Singh,1960 and Samson,1986). The fruit is a large fleshy drupe, embedding a laterally compressed fibrous and woolly stone (Singh., 1960 and Ploetz., 1994). Although the Mango originated from India . it is currently grown throughout the tropic and subtropic regions of the world (Salunkhe and Desai., 1984). It is rated as the world's third most crops in the tropic proceeded by citrus and banana (Nakasone and Poull., 1998). The popularity of the fruit in international markets is due to its excellent flavor. attractive fragrance. beautiful color. Delicious taste and health giving properties (Salunkhe and Desai. 1984. and Arauz., 2000). Importance of Mango production is currently reflected in the following production volumes; India producing 120 000 tons followed by Pakistan with 937 705 tons and Philippines with 931 500 tons, South Africa 115 152 tons (FAO STAT,2010).Eventhough India produces 70% of the worlds Mangoes, only 0.3% is exported compared to South Africa that exports 27.9% of fresh fruit (FAO,2001).

2.1.1 Scientific Classification

Kingdom : Plantae

Division : Mangoliophyta

Class : Mangoliopsida

Order : Sapindales

Family : Anacardiaceae

Genus : *Mangifera*

Species : *indica* L.

2.1.2 General Characteristics

The Mango is an evergreen, symmetrical tree ranging in height from 8 to30m, bearing leathery, simple, leaves in a compact canopy. The leaves are alternate, elliptic or spear, shaped, spirally located and fairly leathery, and 10 to 40 cm long. The leave persist on the tree for up to 4 to 5 years before being shed. Leave of young flashes are usually copper-red or purplish, gradually turning to dark green.

Mango forms an erect, well-branched evergreen tree with dense crown. The leaves are spirally arranged and come out in reddish flushes that initially hang straight down (Samson, 1986).

2.1. 3 Nutritional Properties of Mango

Mango fruit contain amino acids, carbohydrates, fatty acids, minerals, organic acids, proteins, and vitamins (Litz, 1997). During the ripening process, the fruit are initially acidic, astringent and rich in ascorbic acid (vitamin C). Ripe mangoes contain moderate amount of vitamin C but are fairly rich in pro-vitamin A and vitamin B1 and B2. The pulp of the mango fruit contains as much vitamins as butter. Fruit acidity is primarily due to the presence of malic and citric acids. In addition, oxalic, malonic, succinic, pyruvic, adipic, galacturonic, glucuronic, tartaric, glycolic and mucic acids are also present. Following fruit set, starch

accumulates in the mesocarp. Free sugars including fructose and sucrose generally increase during ripening, however, the sucrose 14 content increases three to fourfold due to hydrolysis of starch. Sucrose is the principal sugar of ripe Mango.

Mangoes are rich source of beta - carotene, a provitamin A carotenoid that is converted to vitamin A in the body. Vitamin A is an essential nutrient required for normal growth, reproduction, vision and immune health, in less developed countries. Mangoes can provide this much needed vitamin to prevent deficiencies that often develop during the off-season.Mangoes are also an important source of the essential nutrient vitamin C. Vitamin C is necessary for normal collagen breakdown and the disorder scurvy. It also serves as a cofactor for some enzymes in the body and is a powerful water soluble antioxidant that prevents free radical damage to cells.(Pal, 1998).

2.1.4Economic Important of Mango in Sudan

Mango Trees is number one fruit Tree in terms of production, followed by Banana, Date palm and Lime. Sudan produce about 5.7% of the total Arab World production.

Mango is main friut produced in the western darfur,southern kordofan,northern and khartuom states.(PAB.,2003).

2.1.5 Varieties of Mango in Sudan

In Sudan, there are more than thirty varieties of mango, divided into two main groups namely, "Baladi" or fibrous group and the "Introduced" Indian group. The last group includes many varieties such as Alphonso, Abu Samaka, Dibsha, Shendi, Gulb El-Tour,Taymour, Dr. Night, Millogoba, Tow C7ambo,

Tow Berri, Bashieri,Zibda, Mahamodi, Mabruka, Gabalia, Nailam, Biari Musri, Pyro, Aida, Khartoumi, etc.(Osman,1999)

2.1.6 Mangoes Fungal Disease

- Blossom blights **:** are common in most Mango growing countries (Kwee and Chang, 1985). In florescence's extensively colonized by *Botryosphaeria* sp**.**
- Tiwg dieback **:** (Johnson *et al*., 1992).(Ramos *et al.*, 1991). by *Botryosphaeria dothida*.
- Tip dieback **:** by *Botryosphaeria ribis*.
- Cankers **:** Usually appear as longitudinal cracks in the bark with a brown to black discoloration of the infected area (Jayasinghe and Silva., 1994).by *Botryosphaeria* sp.
- Stem rot **:** by *Botryosphaeria* sp**.**
- Fruit rot **:** by *Botryosphaeria* sp**.**
- Anthracnose **:** *Colletotrichum gloeosporiodes*.

The anthracnose is a disease, which affects the flowery parts and fruits. The disease appears in the form of small brown or black spots.Then it spreads and gradually becomes bigger and bigger. The disease is treated by collecting the flowery parts and the affected fruit and burns them by spraying with solution of yurdo (Altomy., 1990).

- Branch wilt **:** different symptoms were found to be caused by this fungus depending on the part of tree affected, by *Neofusicoccum mangiferae***.**

2.2.1 Mango Branch Wlit Fungus (*Neofusicoccum mangiferae*)

Neofusicoccum. is a genus of fungi in the family Botryosphaeriaceae for which there is the single species *N. mangiferae* (Sutton and Dyko., 1989). The fungus is a cosmopolitan and polyphagus that attacks a multitude of tree flora. More recently this species has been reclassified into the family Neofusicoccum as *Neofusicoccum mangiferae* (Crous *et al*., 2006).

2.2.2 Scientific Classification

Kingdom : Fungi

Division : Ascomycota

Class : Dothideomycetes

Order : Botryosphaerials

Family : Botryosphaeriaceae

Genus : *Neofusicoccum*

Species : *mangiferae* (Syd. & Syd, P.,1916) (Crous *et al*., 2006).

Neofusicoccum mangiferae is an anamorphic species of fungus in Ascomycota. It is a plant pathogen. Originally named *Dothiorella mangiferae* by Sydow *et al*., (1916), it was given its current name *Neofusicoccum mangiferae* by Crous *et al*., (2006).

2.2.3 Synonyms

Apparently, there is great confusion regarding the taxonomy, classification and identification of this anamorph species as reported by U.S. Department of

Agriculture, Agricultural Research Service Systematic Mycology Laboratory – Nomenclature Fact Sheets, March 9, 2015.

- *Torula dimidiate* Penz, (1887).
- *Dothiorella mangiferae* Syd. & Syd, P. (1916).
- *Fusicoccum eucalypti* Sousa da Câmara, (1929).
- *Hendersonula toruloidea* Nattrass (1933).
- *Hendersonula cypria* Nattrass (1937).
- *Exosporina fawcettii* Wilson, E.E. [as 'fawcetti'] (1947).
- *Hendersonula agathidis* Young, H. E. [as 'agathi'], (1948).
- *Scytalidium lignicola* Pesante [as 'lignicolum'] (1957).
- *Nattrassia mangiferae* (Syd. & P. Syd.,1916) Sutton and Dyko (1989).
- *Scytalidium dimidiatum* (Penz.) Sutton and Dyko (1989).
- *Fusicoccum dimidiatum* (Penz.) Farr *et al.,* (2005).

2.2.4 Biology

The fungus is able to grow on temperatures ranging from 20 to 40°C with an optimum temperature between 30-35°C. Mycelia growth is best at pH 6. The best medium for mycelial growth is Potato Dextrose Agar (PDA). Maximum conidial germination occurred at relative humidity higher than 90%. It was also reported that systemic fungicide Benomyl (Benlate) was less effective than the non-systemic fungicides: Mancozeb (Dithane M45) and Maneb (Manèbe 80) (Calavan and Wallace., 1954; Davison., 1972; Giha., 1975; Nouri., 1996; Elshikh., 2004 and Mohammed ,*et al*., 2009).

On PDA *N. mangiferae* grows readily and formed whitish mat which within 2 to 3 days turned to blackish in colour. The arthrospores were spherical to cylindrical in shape (Nour.i, 1996 and Mohammed, *et al*., 2009). Pynospores

are biseptate with terminal cells hyaline to subhyline and the middle cell light to dark brown (Calavan and Wallace., 1954).The colony is effuse and dark blackish brown to black. Hyphae are mid to dark brown and septated. Arthrocondia was mid to dark brown, smooth mostly aspected but occasionally with one or more very dark transverse septa (Ellis., 1971 and Mohammed ,*et al*., 2009).

2.2.5 Life cycle

The fungus has a very simple life cycle. The small conidia are produced in black powdery masses under bark, and are easily wind disseminated. These spores which arise from segment hyphae are carried to damage bark tissue where they germinate and initiate infection. Most active fungal growth occurs during summer where the temperature is very high and the trees are prone to infection under drought condition (Giha, 1975). The mycelium grows into living tissues infecting sap wood which become stained grey to black in color. Research in California simulated sunburn damage on bark of walnut trees with use of a blowtorch to induce infection (Olsen., 1998).

2.2.6 Epidemiology

Elliot and Edmonds (2003). demonstrated that *N. mangiferae* attacked drought-stressed trees. They also indicted that the fungus is primarily wound–invading. According to Mirzaee *et al*., (2002). this fungus attack trees growing in high humidity or high temperature where the temperature are not as extreme but high humidity is common and disease is not as prevailing in Claremont, Whittier and other more inland localities.

2.2.7 Distribution and Host Range in Sudan

The fungus was found on *Citrus* spp. and other fruit such as Mangoes and date palm in River Nile and Northern States (Giha., 1996; Elshikh., 2004and Mohammed ,*et al*., 2009).

It was also observed on shade and ornamental trees like *Ficus* spp, neem, rain tree (*Samania saman***)**. in Khartoum state and Wed Medani Town in Elgezira State (Giha., 1975, Giha., 1996; Nouri., 1996, Elatta and Nouri., 1999). The host range fungus also included forest trees such as *Senegal Acacia*, *Eucalypus camaldulensis* and *Khaya senegalensis* (Nouri., 1996, El Atta and Nouri., 1999, Elshafie and Ali., 2005).

2.2.8 Symptoms

The characteristic symptom of *N mangifere* on the host plant is bark cracking and, peeling off beneath the sooty layer are seen. (Giha., 1975, Nouri., 1996; Mohukker and Yassin , 2001; Elshikh, 2004 and Mohammed ,*et al*., 2009).

Initially *N. mangiferae* causes leaf chlorosis, necrosis, blight defoliation and die back (Nouri, 1996). Brown rot was observed on white yam tuber (Sangoyomi., 2002). and Mango fruit (Lonsdale, 1996). It causes fruit rot in banana and sweet orange (Giha, 1996). However**,** *N. mangiferae* is considered as a facultative parasite that often grows on dead branches or wound parasite (Paxton. Wilson and Davis., 1964; Giha, 1975; Roux, 1993 and Polizzi *et al*., 2009).

2.2.9 Effect on the Human

The fungus *N. mangiferae* was defined previously as *Hendersonula toruloidea* which cause onychomycosis (nail infection) and superficial skin

infection especially in tropical region. *Scytilidium dimidiatum* is synanamorph (Medical dictionary, 2009).

The fungus has been also implicated in case of eye infection (endophthamistis) in a healthy 34 year old man, resident in Spain. The infection was due to corneal penetrating Trauma in the left eye with a vegetal foreign body (wooden mallet). (Blazquez *et al*., 2000).

The infection was also observed on a 60-year old immuno competent patient, due to injury by a piece of grass in Zimbabwe (Gumbo *et al*., 2002). It was also reported the case of backbone discs infection (Spondy lodiscitis) and granular skin lesions due to *N. mangiferae* in a 62 year old male of Turkish origin had been living in Austria (Willinger *et al*., 2004).

2.3 Control Methods

2.3.1 Chemical Control

Themis *et al*., (2005). reported the effective fungicides against *N. mangiferae* that infects limb dieback of figs in California such as Tebuconazole, Propiconazole. Elshikh (2004). demonstrated that Tilt completely inhibited *N. mangiferae* in *vitro* at 200 ppm and 100 ppm.

2.3.2 Cultural Practices

In Arizona State (2007) they were recommended some methods for control of sooty canker disease in citrus such as:

1. Preventing sunburn of the bark is usually accomplished by avoided over-pruning of trees.
2. Good pruning practices, it is observed that the correct pruning should be employed to conserve branch collar trees e.g. *Ficus* spp. and others.

3. Good sanitation by removing all infested materials.
4. Maintenance of vigor with proper fertilizer and watering. (Calavan and Wallace, 1949) found that the blight on marsh grapefruit was more severe on weak trees than healthy ones. Downer (2008) recommended removal and replacement of the dead trees of *Ficus nitida* in California.

2.3.3 Biological Control

It was reported that *Trichoderma viride* had inhibitory effect on radial growth of *N. mangiferae* in *vitro* (Nouri, 1996; El-shikh, 2004 and Mohammed.*et.al*., 2009). On the other hand, Taheri *et al*., (2005). mentioned that no antagonistic mechanism i.e. coiling, vacuolization and lyses occurred but when they added the volatile metabolism of *Trichoderma* spp. It did not inhibit growth of *N. mangiferae*. Bioactivity of soil-borne Streptomyces sp. against *N. mangiferae* had shown antifungal properties. (Sadeghy[a] and Hatami[a,] 2013).

2.3.4 Resistance of *N. mangiferae*

Resistance plant to the fungus infections are due to:

1. The callus tissues formation (Elliot & Edmond, 2008).
2. Phenolic and related compounds (Zine El Abidine *et al*., 2010; Prabha and Choudhary, 1998 and Hassan *et al*., 2011).
3. Alkaloids compounds (Rakoto-Ratsimanga *et al*., 1997).
4. Cuticle thickness (Curtis, 1928).

2.3.1 Botanical Mesquite,*Prosopis* spp

Prosopis spp. (Mesquites) is ever green leguminous trees or shrubs. The genus comprises 44 species of which 40 are natives to the Americas. Of the remaining species *P. Africana* is indigenous to Africa, whereas *P. kodziana*,

P. farcta and *P. cineraria* are natives to the Middle East and Pakistan (Broun and Massey, 1929 and Bukart, 1976). *Prosopis* spp grow in arrays of environments and are not restricted by soil type, pH, salinity or fertility (Sidahmed, 2005 and Babiker, 2006).

2.3.2 Scientific Classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Mangoliophyta
Class	:	Mangoliopsida
Subclass	:	Rosidae
Order	:	Fabales
Family	:	Fabaceae

The name *Prosopis* was selected by Linnaeus to describe the only species he was aware of, *P. spicigera,* in 1767 (Felker, *et. al.,* 2001). Felker *et. al.,* (2001). stated that genus *Prosopis* Linnaeus emends Burkart is in the family Leguminosae (Fabaceae), sub-family Mimosoideae. The placing of *Prosopis* in the wider taxonomic classification system is 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 the authoritative monograph of Arturo Burkart (Burkart, 1976), who defined the generic limits and divided the genus into five sections, based on floral characteristics, each also with marked vegetative differences in armature. Forty-four species and a number of varieties of *Prosopis* were described by Burkart (1976) . The existence of populations of *Prosopis* with distinct characteristics led Burkart (1976) to describe many as separate species or varieties, even though several are known to hybridise (Silva, 1986 and Felker, *et. al.*, 2001). The taxonomy of Burkart (1976) has been generally accepted, and this is used as the benchmark with which other taxonomies are compared (Felker, *et. al.*, 2001).

2.3.3 Allelopathy

The leaves of *P juliflora* contain various chemicals including tannins, flavonoids, steroids, hydrocarbons, waxes and alkaloids (Felker, 2000). These are known to affect palatability to livestock but also have effects on the germination and growth of *Prosopis***,** crops, weeds and other trees. Leaf extracts were also noted to kill some insects, bacteria and fungi (Felker, 2000). However, there is some debate as to the importance of allelopathy in tree-crop interactions and the applicability of results from pot trials to field conditions. Alkaloids and flavonoids are known to degrade rapidly following leaf senescence but other chemicals may accumulate under tree crowns (Sola, *et.al.*, 1992). Most studies have utilised leaf extracts or dry leaves incorporated into soil for analysis in pot trials. This often exaggerates the concentrations of chemicals leading to misleading results. Reduction in crop

seed germination due to chemical inhibition was noted with *P.juliflora* leaf concentrations of more than 3%, but it was thought that this would not be noticeable under field conditions (El Fadl, 1997).

Effects of these allelo-chemicals may be direct, action upon the seeds and seedlings, or may be indirect, via effects on other soil organisms. Extracts from plant parts of *P. juliflora* decreased germination and growth of almost all plants tested in several studies, indicating that allelopathic effects are important in the ecology of the *P. juliflora - P. pallida* complex (Felker *et. al.*, 2001). However, Sen and Chawan (1970). assessed the effects of *P. juliflora* extracts on germination of a *Euphorbia* sp and concluded that the phytotoxicity was without ecological significance (Felker, 2000). Sola, *et. al.*, (1992). thought that the accumulation of steroids, hydrocarbons and waxes in *P. ruscifolia* leaf litter affected hydrophobic constituents and soil moisture capacity, whereas all other authors discuss only allelo-chemical effects. Autotoxicity of *P. juliflora* has been observed on seed germination and subsequent seedling development (Lahari and Gaur 1969, Warrag, 1994). Most studies have concentrated on effects on germination and growth of crop plants. Lahari and Gaur (1969). found decreased shoot and, particularly, root growth of a range of plants following treatment with *P. juliflora* leaf extract. Fresh leaf extracts of *P. juliflora* were found to have greater negative effects on germination than extracts from stems, dry litter or fruit by Sen and Chawan (1970). However, Noor, *et. al.*, (1995). observed a greater effect from fruit and seed extracts than from root, leaf or flower extracts. Bark extracts have also proved effective in inhibiting germination (Velu, *et. al.*, 1996).

2.3.4 The Benefit Uses of Mesquite

The tree has some benefits that include combating desertification, nitrogen fixation as a leguminous plant, increasing the global green coverage, using its timber for furniture, fencing and fuel, also as animal feed. However, recently it was realized that the problems caused by the plants far more than the benefits derived from them (Sidahmed, 2005 and Elkhalifa, 2010).

2.4.1 The Fungicide (REVUS top ®)

REVUS top® is a broad spectrum product containing two fungicides. It has preventative, systemic and curative properties and is recommended for the control of many important plant diseases. REVUS Top® provides excellent disease control of many Leaf spots, Powdery mildews, and Downy mildews. REVUS top® is applied as a foliar spray and can be used in block, alternating spray, or tank mix programs with other crop protection products. All applications must be made according to the use directions that follow.

2.4.2 Resistance Management

REVUS top® contains two fungicides - mandipropamid, and Carboxylic Acid Amide (CAA). fungicide in Group 40 and difenoconazole, a triazole fungicide in Group 3. Fungal pathogens can develop resistance to products with the same mode of action when used repeatedly. Because resistance development can not be predicted, use of this product should conform to resistance management strategies established for the crop and use area. Consult your local or State agricultural authorities for resistance management strategies that are complementary to those in this label. Resistance management strategies may include rotating and/or tank mixing with products having different modes of action or limiting the total number of applications per season.

Manufactured for: Syngenta Crop Protection, North Carolina

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, Shambat, Sudan University of Science and Technology during the period February to April 2015, to evaluate the antifungal effect of different parts of Mesquite (Root, Bark and Leaves). aqueous extracts and efficacy of fungicide REVUS top® against the fungus *Neofsicoccum mangiferae.*

3.2 Equipments, Tools and Materials used in the Study

- Incubator Laminarflowcabinet
- Autoclave Compound microscope
- Needle Injection
- Slide Marker pen
- Petri-dishes Conical flask
- Sensitive balance Aluminum foul
- Gloves Face mask
- Regestration form Camera
- Potato Dextrose Agar (PDA).
- Mesqiute root Mesqiute leave
- Mesqiute park Infested Mango leave and small branches
- Ethanol 95% Soap
- Filter paper Medical cotton
- Fungicide REVUStop® Clorax

All Tools, which used in the experiments, were sterilized.

3.3 Isolation and Identification of Fungus

Infected parts of Mango *M indica* (Stems, Branches and Leave). showing typical symptoms of Canker on stem and Necrosis of leaves at the College Farm at Shambat area were collected. Thereafter, they were put in poly bags then transferred to the laboratory. The secured plant material (Stems, Branches and Leaves). were cut into small bits (0,5and 1,0 cm). and washed well in tap water to remove the adhering dirty particles. The cuted 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 stems, branches and leave sections were then plated at the rate of 5 sections per plate on Potato Dextrose Agar (PDA). media 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 *Neofusicoccum mangiferae.* 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.

3.4.1 Collection and Preparation of Plant Sample

Different parts of Mesquite (Roots,Bark and Leaves). were obtained from Aljaili zone, Northern Bahri (Assegai Alansar). All the plant parts were cleaned from dust and foreign material by hand and washed with distilled water and Clorox, and dried under shade. After complete dryness, plant samples were crushed separately to obtain fine powder for extraction.

3.4.2 Extraction Method

The obtained fine powder form different parts of mesquite was weighted (25, 50 and 100 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 25 % 50% and 100% concentrations. Aqueous extracts of each of the plant materials were prepared as they recommended by Okigbo (2006).

3.5 Preparation of Fungicide Concentrations

One ml of the REVUS top® 250 EC fungicide was dissolved in 100 ml of sterilized distilled water of which 25, 50 and 100ppm was prepared.

3.6 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. Initially, fresh fungal growth was prepared from previously maintained culture of *Neofusicoccum.* Prepared PDA media was amended with the required concentration from different parts of Mesquite extracts and fungicide REVUS top® before being solidified in a conical flask of 250 ml, agitated and poured into sterilized glass Petri dishes. Three plates, containing 30 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 centre of PDA plates where opposite poles were marked at the back of the plate and incubated at 25°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 25 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**:**

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

Where**:**

R = Percentage reduction of the growth.

dc= diameter of controlled growth.

dt= diameter of treated growth.

3.7 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

4.1 Laboratory Experiment

This study which conducted under laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology, during February to April 2015 was to confirm that the fungus associated with stem, branch and leaves disease of Mango trees collected from Shambat Research area is *Neofusicoccum mangiferae* and to explore the antifungal potentials of different parts of mesquite plant and efficacy of fungicide REVUS stop® against the fungus. The results cover effect of plant extracts on growth of *N mangiferae* and confirmation of the causal agent.

4.2 Identification of the Fungus

As presented in Plates 1 and 2**,** the isolated fungus has typical shape of spore and conidia of the fungus *Neofusicoccum mangiferae*as described by Sutton and Dyko,(1989). Giha, (1996). and Abbasher *et al.,* (2013).

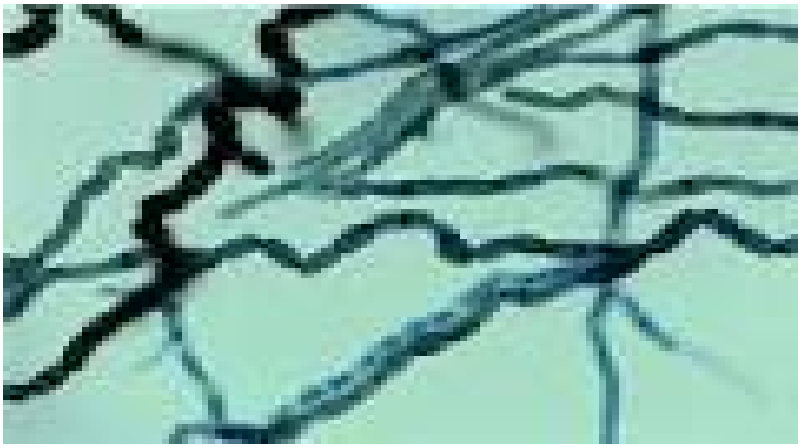

Plate 1: Typical Shape of Spores and Conidia of *N. mangiferae*

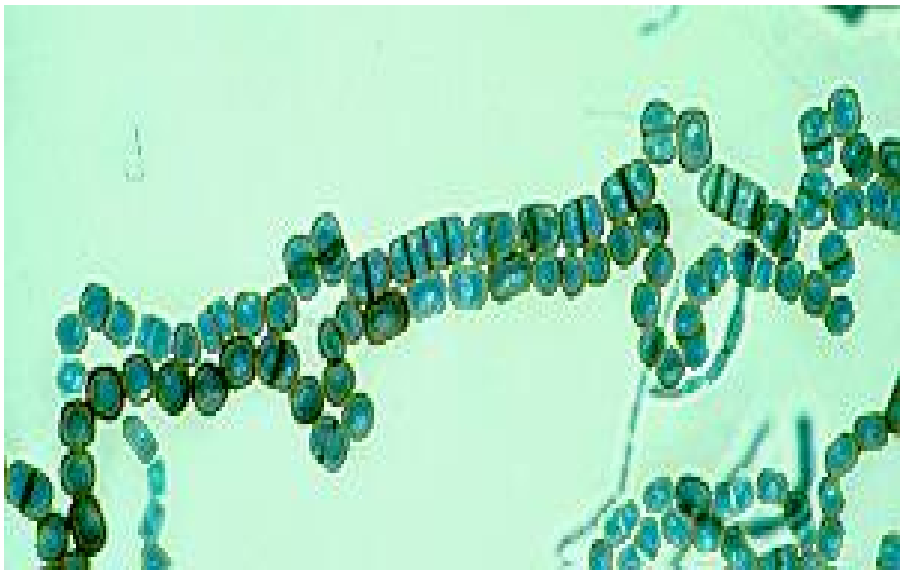

Plate 2: Present Shape of Arthrospore of *N. mangiferae*

4.3 Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N. mangiferae* in *vitro* after three days from inoculation

After three days from inoculation the results indicated that plant extracts at all concentrations reduced significantly ($P<0.05$). the fungal growth compared to control. (Table1 and Figure1). Moreover the Root and Bark extracts and Fungicide at 100% and 50% concentration near to completely inhibited the growth of fungus. in fact , among all Mesquite plant parts extracts, Bark at all concentration (100% ,50% and 25%). demonstrated the highest inhibition of fungal growth (100%,86% and 84.6%). followed in descending order by Mesquite Root extract (94.4%,96.1% and 78.6%). The Leaves extract was the lowest one in inhibiting the growth of fungus (56.7%, 45.5% and 46,7%). However, the suppressing effect of fungicide REVS top® (100%, 99.1% and 98.9%). was more pronounced at all concentration.

Table 1. Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N. mangiferae* in *vitro* after three days from inoculation

Treatments		Inhibition Zone %			
Concentrations %		R1	R2	R3	Mean%
Root	25	84.5(9.1)	77.4(8.8)	74(8.6)	78.6(8.8)C
	50	91.2(9.8)	74.3(8.6)	92.6(9.6)	86.0(9.2)Bc
	100	96.6(9.8)	94(9.7)	92.6(9.6)	94.4(9.7)Ab
Bark	25	86.5(9.3)	85(9.2)	85.2(9.2)	84.6(9.2)Bc
	50	93.2(9.6)	92.5(9.6)	92.6(9.6)	92.8(9.6)Ab
	100	100 (10.0)	100 (10.0)	100 (10.0)	100.0(10)A
Leaves	25	55.8(7.4)	39.8(6.3)	44.4(6.7)	46.7(6.8)E
	50	50.7(7.1)	41.3(6.4)	44.4(6.7)	45.5(6.7)E
	100	64.2(8)	54.9(7.4)	51.9(7.2)	56.7(7.5)D
REVUS top®	25	96.6(9.8)	100(10)	100(10)	98.9(9.9)A
	50	98(9.9)	100(10)	99.3(10)	99.1(9.9)A
	100	100(10)	100(10)	100(10)	100(10)A
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	00.0(0.7)F
SE±				0.1623	
C.V. (%)				3.38	
LSD				0.4717	

Any two mean value (s) bearing different superscripts (s) are differing significantly at ($p<0$-0.5). Data in the parentheses transformed using square root transformation$\sqrt{x + 0.5}$ before analysis.

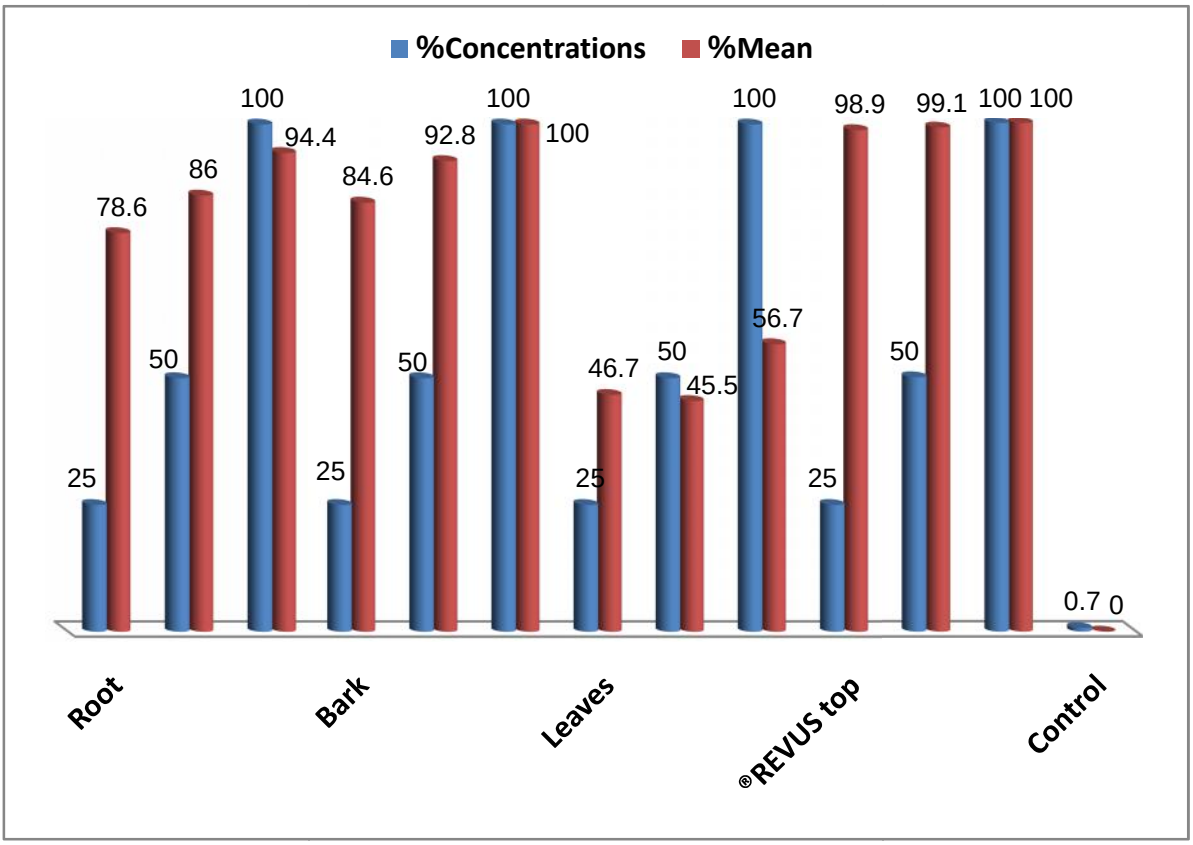

Fig 1. Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N. mangiferae* in *vitro* after three days from inoculation

4.4 Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N .mangiferae* in *vitro* after four days from inoculation

The results in Table, 2 and Fig. 2 showed that the aqueous extracts of different parts of mesquite and fungicide REVUS top® at all concentrations continued exhibiting an inhibitory effect against fungal growth after four days from inoculations. The percentages fungal growth inhibition was significantly high compared to the control.

Moreover, the highest inhibitory effect was demonstrated by concentration of Root and Bark extracts at 100% concentration and that of fungicide REVUS top® at all concentrations (96.1%, 94.9%, and REVUS top® 100%). respectively compared to the untreated control. The inhibitory effect was significantly ($P<0.05$). high against test fungus. Among the plant extracts screened that of Root and Bark were the most effective in suppressing the fungus growth at all concentration. However, the results showed that the inhibitory effect increase with increased concentration.

Table 2. Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N. mangiferae* in *vitro* after four days from inoculation

Treatments		Inhibition Zone (%)			
Concentrations%		R1	R2	R3	Mean
Root	25	85.4(9.3)	84.5(9.2)	82.3(9)	84(9.1)C
	50	90.1(9.5)	88(9.4)	90.9(9.6)	89.7(9.5)B
	100	97.5(9.9)	95.9(9.8)	94.9(9.8)	96.1(9.8)A
Bark	25	85.2(9.3)	84.5(9.2)	84.8(9.2)	84.8(9.2)C
	50	90.1(9.5)	89.6(9.5)	89.6(9.5)	89.9(9.5)B
	100	95(9.8)	94.8(9.8)	94.9(9.8)	94.9(9.8)A
Leave	25	36.9(6.1)	31(5.6)	32.8(5.8)	33.6(5.8)F
	50	40.9(6.4)	37.8(6.1)	40.4(6.4)	39.7(6.3)E
	100	53.2(7.3)	53.4(7.3)	49.5(7)	52(7.2)D
REVUS top®	25%	97.5(9.9)	98.4(9.9)	97.5(9.9)	97.6(9.9)A
	50%	97.5(9.9)	98.4(9.9)	98.9(9.9)	98.1(9.9)A
	100%	100(10)	100(10)	100(10)	100.0(10)A
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	00.0(0.7)G
SE±			0.06583		
C.V. (%)			1.36		
LSD			0.1914		

Any two mean value (s) bearing different superscripts (s) are differing significantly at (p<0-0.5). Data in the parentheses transformed using square root transformation$\sqrt{x + 0.5}$ before analysis.

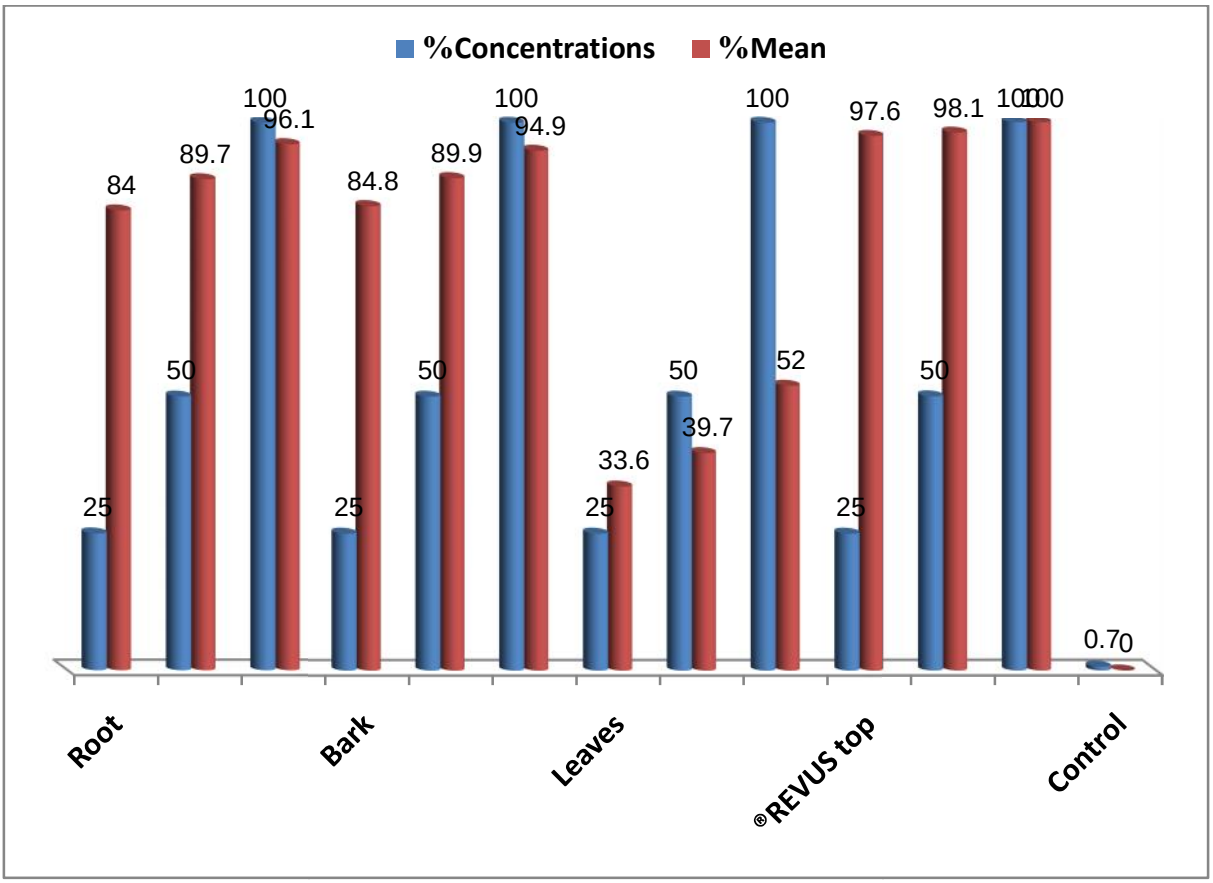

Fig 2. Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N. mangiferae* in vitro after four days from inoculation

4.5 Effect of Mesquite plant parts and Fungicide REVUS top® on redial growth of *N. mangiferae* in *vitro* after five days from inoculation

In day five after inoculation the treatments of Fungicide REVUS top® , Roots, Bark and Leaves at all concentrations (100% , 50% and 25%). were invariably continued suppressing significantly the fungal growth (100%, 97.6%, 97.3% 96.2% ,89.5% ,86.8% ,92.2% ,89.8% ,84.2% ,48.9% ,41.3% ,36.6%). respectively. However, the inhibitory effects of Roots and Cortex were consistently more pronounced than that of Leaves which showed decreasing inhibitory effect with time. Furthermore, the fungicide irrespective of concentration (100%, 50% and 25%). effected significant reduction of fungal growth (97.6%, 97.3%). respectively throughout the course of the experiment compared to control.

Moreover, the screened concentrations of all treatments differ in their reactions to test fungus. Likewise the test organism responded differently to the different concentrations of treatments.

Table 3. Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N. mangiferae* in *vitro* after five days from inoculation

Treatments		Inhibition Zone (%)			
Concentrations%		R1	R2	R3	Mean%
Root	25	88.4(9.4)	85.9(9.3)	86.1(9.3)	86.8(9.3)De
	50	88.4(9.4)	87.2(9.4)	92.9(9.7)	89.5(9.5)Cd
	100	98(9.9)	95.7(9.8)	94.9(9.8)	96.2(9.8)Ab
Bark	25	85.3(9.3)	83(9.1)	84.2(9.2)	84.2(9.2)E
	50	91(9.6)	90.2(9.5)	88.1(9.4)	89.8(9.5)Cd
	100	93(9.7)	91.5(9.6)	90.9(9.6)	92.2(9.6)Bc
Leave	25	34.4(5.9)	43.4(6.6)	32.8(5.8)	36.9(6.1)H
	50	41.9(6.5)	41.3(6.5)	40.7(6.4)	41.3(6.4)G
	100	53.5(7.3)	44.7(6.7)	48.6(7)	48.9(7)F
RRVUS top®	25	96.1(9.8)	97.9(9.9)	98(9.9)	97.3(9.8)Ab
	50	96.9(9.9)	97.9(9.9)	98(9.9)	97.6(9.9)Ab
	100	100(10)	100(10)	100(10)	100(10)A
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	00.0(0.7)I
SE±				0.09487	
C.V. (%)				1.98	
LSD				0.2758	

- Any two mean value (s) bearing different superscripts (s) are differing significantly at ($p<0$-0.5).
- Data in the parentheses transformed using square root transformation $\sqrt{x + 0.5}$ before analysis.

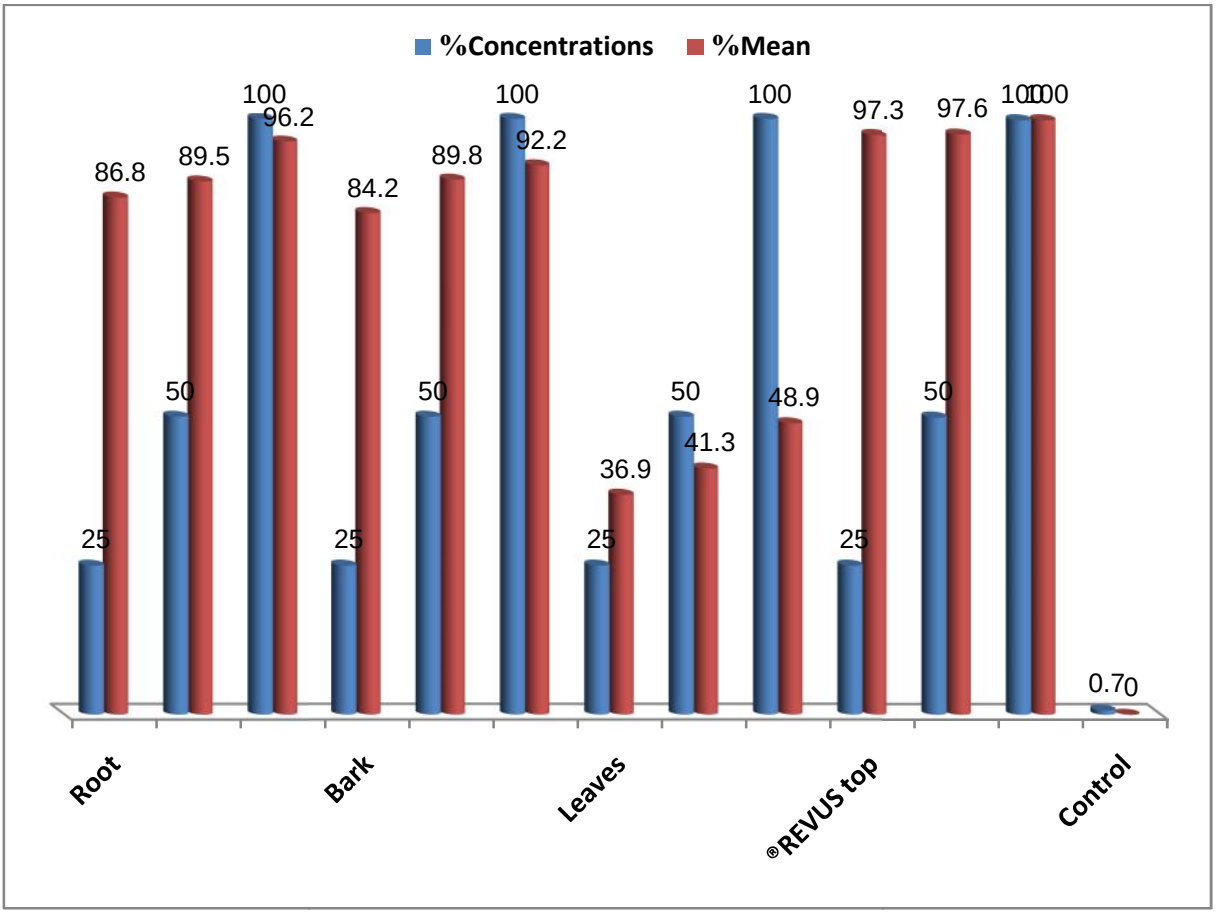

Fig 3. Effect of Mesquite Extracts and Fungicide REVUS top® on redial growth of *N. mangiferae* in *vitro* after five days from inoculation

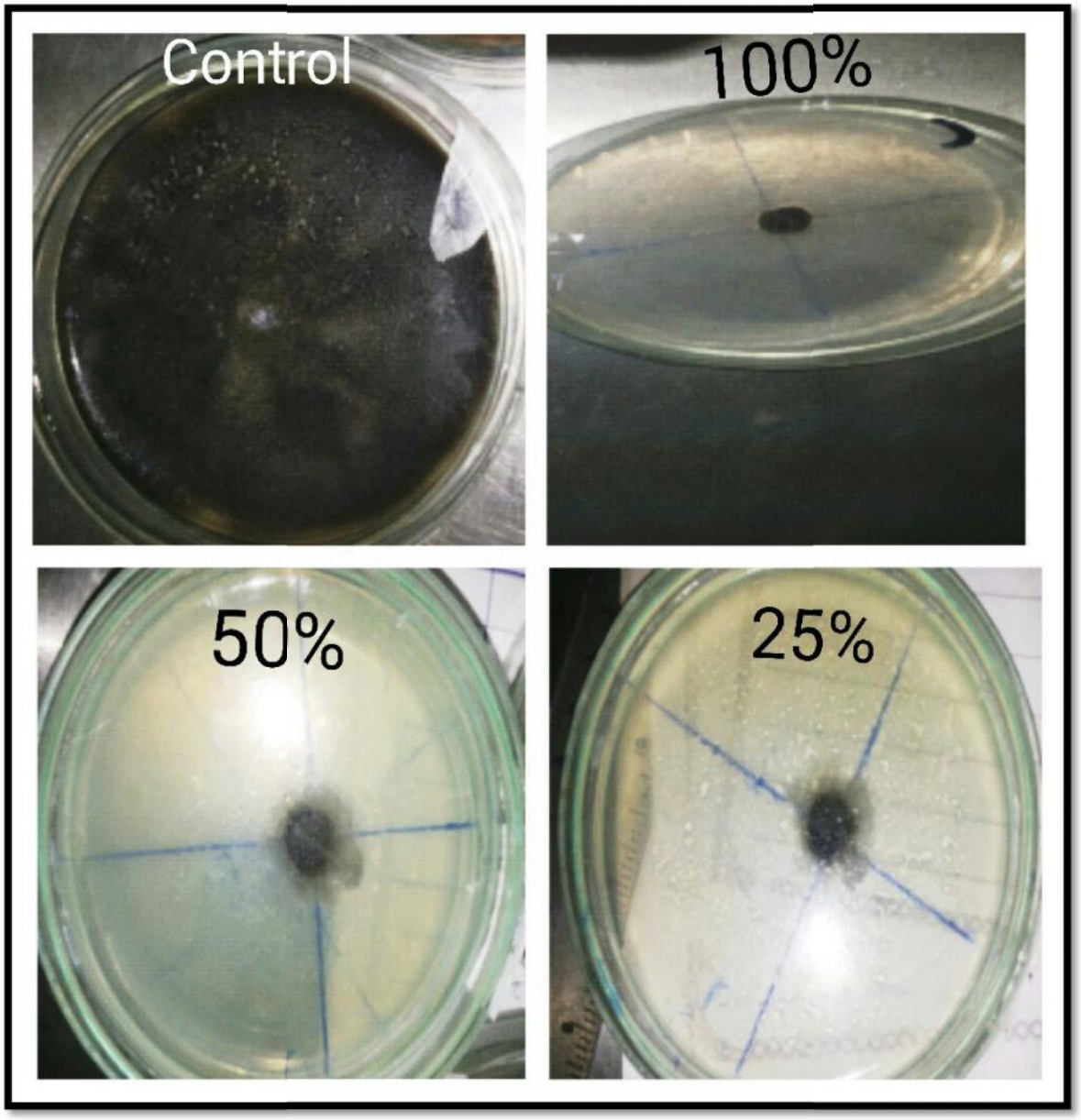

Plate 3. Effect of fungicide REVUS top® on the growth of *N. mangiferae* compare with untreated Control in *vitro*

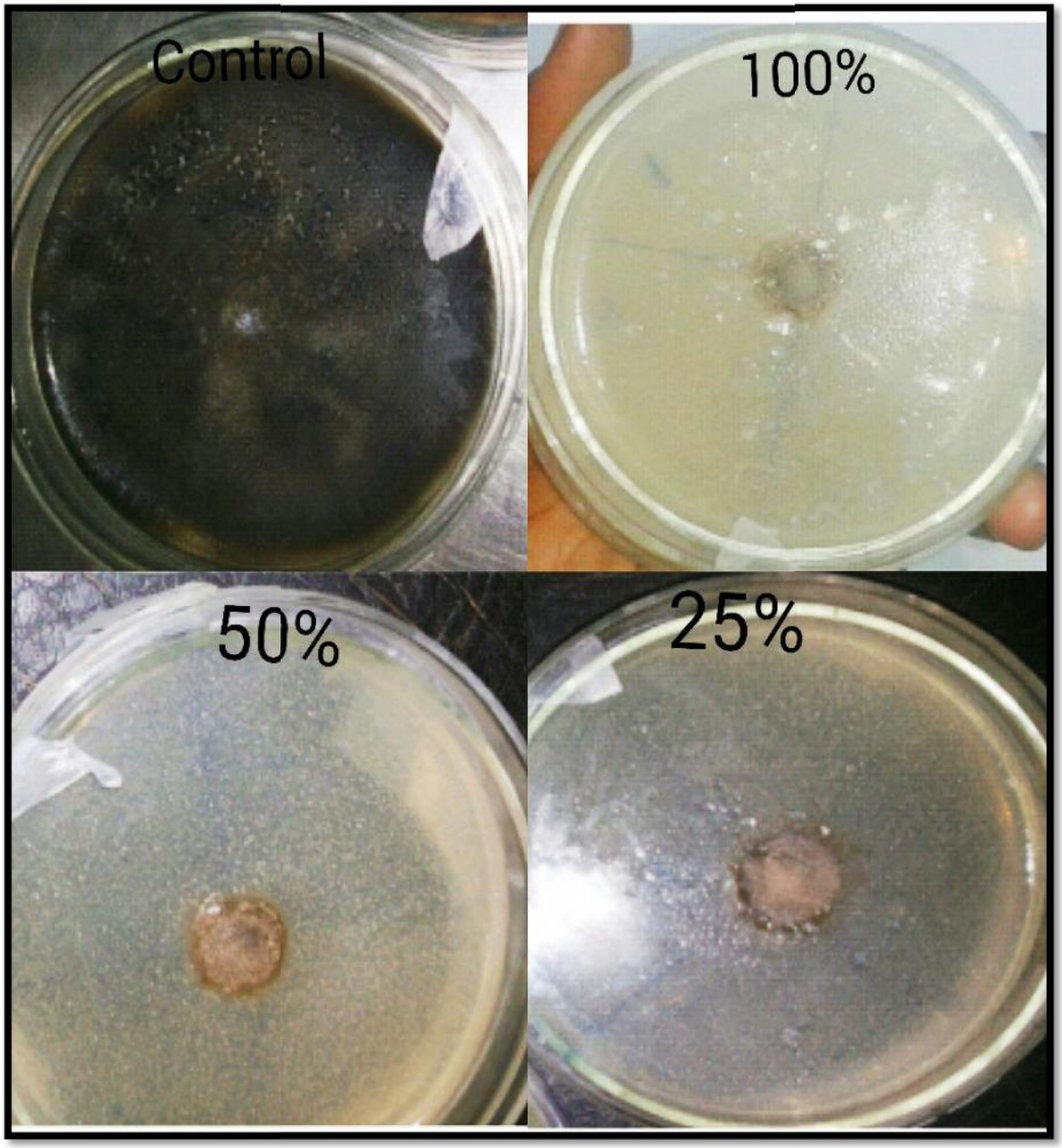

Plate 4. Effect of Root aqueous extract on the growth of *N. mangiferae* compare with untreated Control in *vitro*

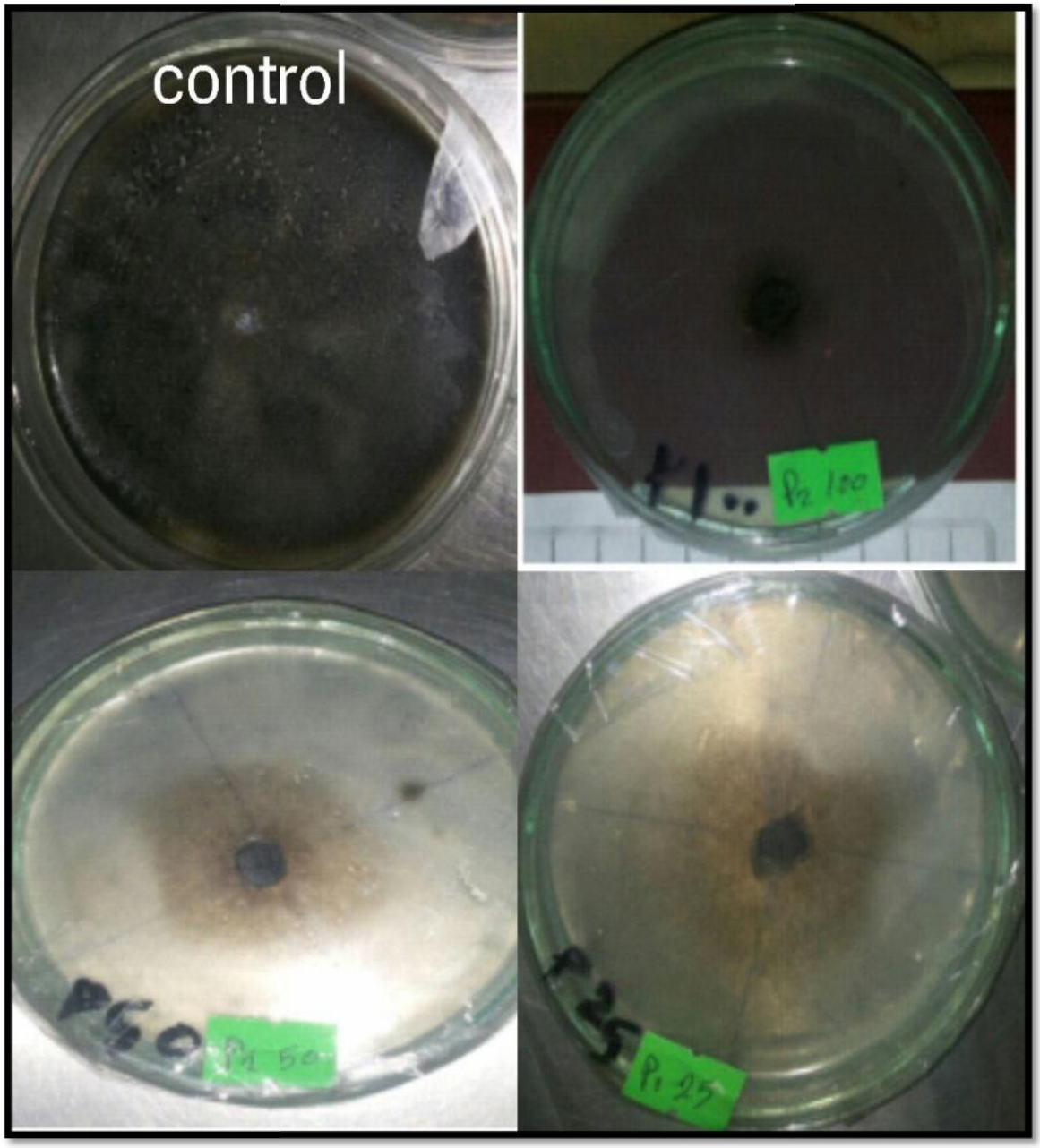

Plate 5. Effect of Cortex aqueous extract on the growth of *N. mangiferae* compare with untreated Control in *vitro*

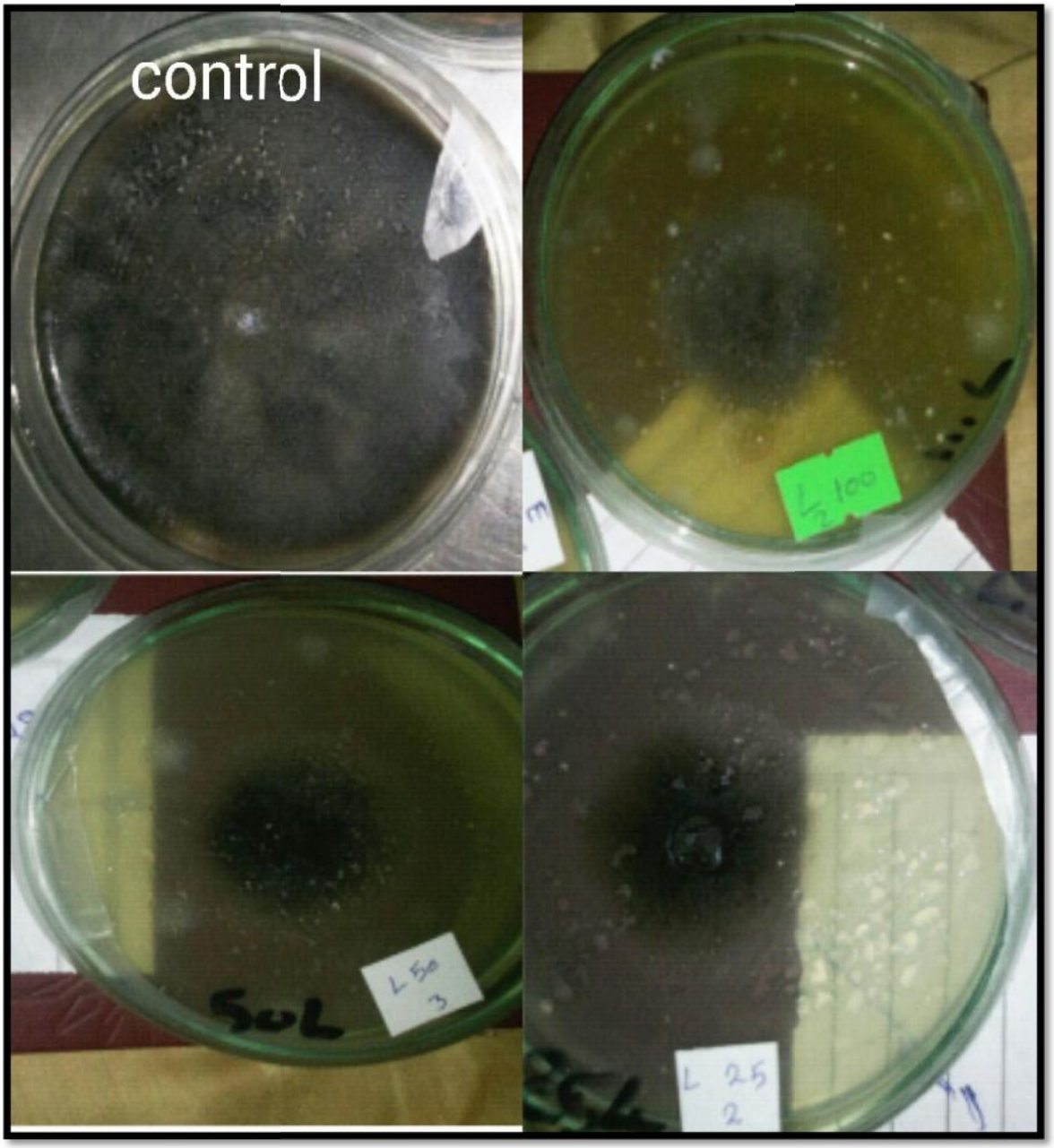

Plate 6. Effect of Leave aqueous extract on the growth of *N. mangiferae* compare with untreated Control in vitro

CHAPTER FIVE

DISCUSSION

Neofusicoccum is a cosmopolitan and polyphagus genus of fungi that attacks a multitude of fruits and forest trees (Crous, *et. al*., 2006). The damage caused by the fungus *Neofusicoccum mangiferae* differ depending on the part of the plant affected. In Sudan the disease has spread all over the country, and was reported in as many as 29 different plant species that include shade, ornamental, timber and orchard trees (Ahmed and Yassin, 1992; Nouri, 1996; Mohamed, 2000; Ahmed, 2005 and Abbasher *et al*., 2013). The well established fact is that non rational uses of synthetic pesticide have caused serious problems to human and animal health in addition their negative impact on environment. These problems include contamination of the biosphere, toxicity to man, animal and beneficial insects and other non target organisms. This have drawn the attention of the Reasershers and Public to adopt new pest management strategies based on safe alternate products of low environmental persistence, highly specific, cheep, available and biodegradable (Sanixa, *et, al*., 198). This was further highlighted by Agrafotis (2002). who reported that the development of new different antimicrobial agents more safe is very important step.

As demonstrated by many researchers there are a considerable interest in the use of biopesticide. In fact, plant 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; Sharif, *et, al*., 2006; Ergene, *et, al*., 2006; Kiran and Raveesha, 2006 and Mohana and Raveesha, 2006). More recent results were demonstrated by Siva, *et, al*., (2008). They proved the presence of antimicrobial compounds in higher

plants and which has been recognized as important products in combating plant pathogenic diseases and selective in their toxicity and are considered valuable for controlling some plant diseases. Makker, and Becker., (1997). reported that the high concentration of phorbol esters present in Mesquite parts are high.

In this study the differences among Mesquite plant parts extract in respect of their in *vitro* effects on growth of *N. mangiferae* and fungicide efficacy was investigated experimentally. The obtained results revealed that the aqueous extracts of different parts of mesquite as well as fungicide at all concentrations consistently throughout the course of the experiment exhibited an inhibitory effect on mycelia radial growth of the fungus with significantly higher inhibition zones percent compared to control. As demonstrated by many researchers there are a considerable interest in the use of mesquite extract to control pathogens. In this study the bioactivity of different parts of Mesquite extract at all concentrations tested was demonstrated against *N. mangiferae*. Similar results were obtained by Fadl Elmola *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 *P. juliflora* contain antimicrobial compounds.

Moreover, the obtained results are in line with similar studies which explored different plant extracts and plant essential oils and reported to be effective antimicrobials against fungi, foliar pathogens and soil borne pathogens (Garibaldi *et al.,* 1990; Alabouvette, 1999 and Bowers and Locke 2000). These phytofungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants and could be applied inform of extract, powders and cakes or as plant exudates (Owino and Wando, 1992 and Anjorin and Salako, 2009).

Currently, there is great reliance on uses of synthetic fungicides to combat the negative impact of plant diseases. In this study the fungicide REVUS top® consistently and throughout the course of the experiments, inhibited the radial mycelia growth of the test fungus and its suppressing effect was more pronounced at all concentrations tested compared to control. These results confirmed reported by Runkhsana *et al*., (2010). who indicated the effectiveness of systemic fungicide against fungal diseases.

Likewise in this study, the test fungus (*N mangiferae)*. responded differently to the different concentrations of extracts. This variability in response which expressed by the test fungus to different Mesquite extracts was also reported by Aiyelaagbe (2001). In his investigation, the Outher 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 (Fayza, 2012 and Reem, 2012).

CONCLUSION

In conclusion, the findings presented in this study indicate promising potentials of Mesquite, (*Prosopis juliflora*). Root and bark as sources of new antifungal in future that help in management of plant fungal diseases.

- The isolated fungus from Mango tree showing typical symptoms of branch wilt, and leaves necrosis presents typical shape of spore and conidia of the fungus *Neofusicoccum mangiferae*as described by(Sutton and Dyko, 1989). Giha, (1996). and Abbasher *et al.*, (2013).

- The aqueous extracts of different parts of Mesquite plant and fungicide REVUS top® 250 EC at all concentrations exhibited inhibitory effects against the radial mycelia growth of the test fungus. The percentages zone of inhibition was significantly high compared to the Control.

- Among different parts of Mesquite, Roots and Bark and fungicide at all concentrations tested (25, 50 and 100%). exhibited consistently the highest inhibitory effect throughout the test period than the other equivalents.

- The screened concentrations of all treatments differ in their reactions to test fungus. Likewise the test organism responded differently to the different concentrations of extracts.

RECOMMENDATIONS

It is recommended that, the following studies to be carried out as continuation to current studies.

1. Mesquite Root and Bark extracts show highly effective against the fungus *N. mangiferae* it can be serve as one of the promising condicate for contorling Wilt Branch disease in Mango trees in Sudan.
2. Further studies should be made to confirm these results and improve this botanical in the future.
3. Phytochemical screening should be made to identify the active ingredients found in this plant extracts.

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APPENDICES

Appendix 1. ANOVA

A) Variable 3 (inhibition in third day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	240.016	20.001	251.630	0.0000
Within	26	2.067	0.079		
Total	38	242.083			

Coefficient of Variation = 3.38%.

B) Variable 4 (inhibition in four day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	258.257	21.521	1712.927	0.0000
Within	26	0.327	0.013		
Total	38	258.584			

Coefficient of Variation = 1.36%

C) Variable 5 (inhibition in fifth day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	253.153	21.096	791.104	0.0000
Within	26	0.693	0.027		
Total	38	253.847			

Coefficient of Variation = 1.98%.

D) Variable 6(inhibition in sixth day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	251.176	20.931	1118.253	0.0000
Within	26	0.487	0.019		
Total	38	251.663			

Coefficient of Variation = 1.66%.

E) Variable 7 (inhibition in seventh day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	257.750	21.479	598.349	0.0000
Within	26	0.933	0.036		
Total	38	258.684			

Coefficient of Variation = 2.33%.

Appendix 2. Reduction of Growth by cm in third day after inoculation

Treatments Concentrations %		Growth diameter by (cm)		
		R1	**R2**	**R3**
Root	25	0.23	0.3	0.35
	50	0.13	0.23	0.1
	100	0.05	0.08	0.1
Bark	25	0.2	0.2	0.2
	50	0.1	0.1	0.1
	100	0	0	0
Leave	25	0.83	0.8	0.75
	50	0.73	0.78	0.75
	100	0.53	0.6	0.65
REVUS top®	25	0.05	0	0
	50	0.03	0	0.01
	100	0	0	0
Control		1.48	1.33	1.35

Reduction of Growth by cm in fourth day after inoculation

Treatments Concentrations%		Growth dimeter by (cm)		
		R1	**R2**	**R3**
Root	25	0.3	0.3	0.35
	50	0.2	0.23	0.18
	100	0.05	0.08	0.1
Bark	25	0.3	0.3	0.3
	50	0.2	0.2	0.2
	100	0.1	0.1	0.1
Leave	25	1.28	1.33	1.33
	50	1.2	1.2	1.18
	100	0.95	0.9	1
REVUS top®	25	0.05	0.03	0.06
	50	0.05	0.03	0.03
	100	0	0	0
Control		2.03	1.93	1.98

Reduction of Growth by cm in fifth day after inoculation

Treatments Concentration%		Growth diameter by (cm)		
		R1	**R2**	**R3**
Root	25	0.3	0.33	0.35
	50	0.3	0.3	0.18
	100	0.05	0.1	0.13
Bark	25	0.38	0.4	0.4
	50	0.23	0.23	0.3
	100	0.8	0.2	0.23
Leave	25	1.2	1.3	1.3
	50	1.5	1.38	1.5
	100	1.6	1.33	1.7
REVUS top®	25	0.1	0.05	0.05
	50	0.08	0.05	0.05
	100	0	0	0
Control		2.58	2.35	2.53

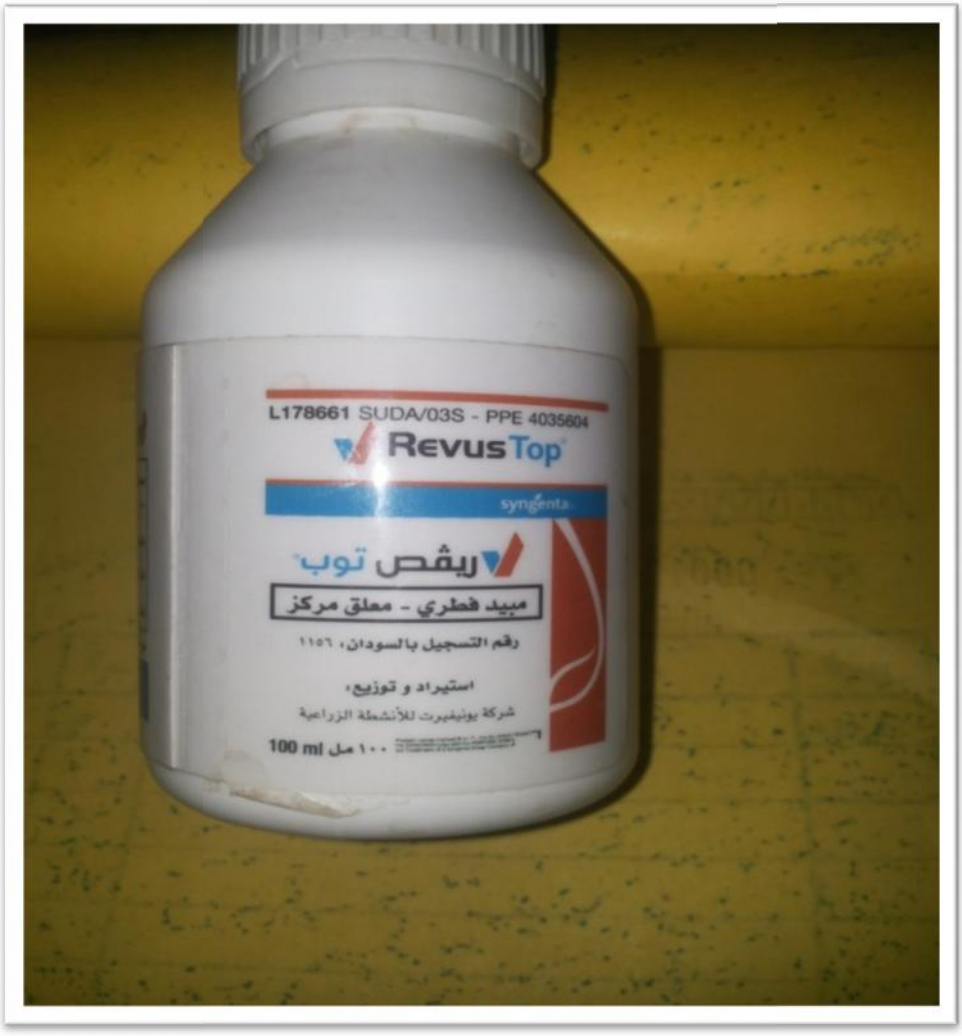
Appendix 3. FUNGICIDE

Name: REVUS top®

Active ingredients: Mandipropamid + Difenoconazole

Manufactured for: Syngenta Crop Protection, North Carolina

Mode of action: reduction the germination of fungal spores (zoo spore and sporangia spores), inhibit germination of hyphae and inhibit form of spores.



Fungicide REVUS top®

Appendix 4. Mangoes trees

Mango field which isolates the fungus *Neofusicoccum mangiferae* from it.

Shambat Research Farm.

Appendix 5. Mesqiute tree (*Prosopis juliflora)*