Sudan University of Science and Technology

College of Graduate Studies

Efficacy of some Natural Product, Sodium Bicarbonate and Fungicide (Revus top®) on *Neofusicoccum mangiferae*, fungus

فعاليه بعض المنتجات الطبعية و بيكربونات الصوديوم والمبيد الفطرى (ريفص توب ®) على الفطر نيو فزكوكم مانقفيرا

A thesis submitted in partial fulfillment of the requirements for the M.Sc. Degree in Plant Protection

By:

Khansa Alfashamim Ali Mahadi

Sudan University of Science and Technology

Department of Plant Protection

B.Sc. Agric. (Honors), 2011.

Supervisor:

Dr. Ibrahim Saeed Mohamed

College of Agricultural Studies

Sudan University of Science and Technology

2015

الآية

قال تعالي:

﴿قل لو كان البحر مدادا لكلمات ربي لنفد البحر قبل أن تنفد كلمات ربي ولو جئنا بمثله مددا (١٠٩) قل إنما أنا بشر مثلكم يوحى إلي أنما إلهكم إله واحد فمن كان يرجوا لقاء ربه فليعمل عملا صالحا ولا يشرك بعبادة ربه أحدا (١١٠)﴾

صدق الله العظ

سورة الكهف

Dedication

To my mother

To my father

To my sisters and brothers

To my family

To my teachers

To my colleagues and friends

With love and respect.

khansa

ACKNOWLEDGMENTS

All thanks are due to Almighty Allah who gave me health and strength, and helped me tremendously to produce this work.

I would like to express my thanks to my supervisor Dr. Ibrahim Saeed Mohamed College of Agricultural studies, Sudan University of Science and Technology, who devoted most of his time to teach us various disciplines of scientific research.

Thanks are also extended to my dear teachers Mawada Ibrahim and Waleed Elamin for their continuous and help statistical analysis during this study.

Also Special Thanks are due to my colleagues at the M.Sc. who helped me during the experiments. Grateful thanks are due to all the staff of the Department of Plant Protection, College of Agricultural Studies, Sudan University of Science and Technology.

khansa

Contents

List of tables

List of figures

List of plates

ABSTRAC

Neofusicoccum mangiferae considered as one of most important disease of forest and horticulture trees in worldwide and Sudan. The present investigation was undertaken under laboratory conditions of Plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology, to study the antifungal activity of aqueous leaves extracts of basil, bulbs of garlic plants, NaHCO3 salt and efficacy of fungicide (Revuse top) on growth, of the fungus *Naeofusicoccum mangiferae*. Three concentrations of aqueous extract of basil and garlic and fungicide, each of 25, 50 and 100%, NaHCO3 salt at three concentrations 10, 20 and 40% and at were used in addition to control. The results showed that all concentrations of the aqueous extracts of all plants tested, NaHCO3 and fungicide exhibited significantly high inhibitory effect against the linear growth of test fungus compared to control. However, the effect of both fungicide and garlic extracts was more pronounced against test fungus than the NaHCO3 and basil extract. The highest concentration of the fungicide revues top, garlic extract, NaHCO3 at(100%, 40%) and basil at gave significantly higher inhibition zones percent (83.6, 83.3, 60, and 44.6%) respectively compared to the untreated control in day six after inoculation. Among the plant extracts tested that of garlic was invariably the most effective in suppressing the fungus growth than its equivalent NaHCO3 and basil. Generally, the results showed that the antifungal activity increase with increase in extract concentration.

ملخص البحث

يعتبر مرض نيوفزكوكم مانقفيرا من اهم امراض الاشجار الغابيه و البستانيه فى العالم. اجريت هذه الدراسه فى تحت ظزوف المختبز بقسم وقاية النبات ،(معمل امراض النبات) كلية الدراسات الزراعه ، جامعه السودان للعلوم و التكنولوجيا (شمبات) لدراسه تاثير المستخلص المائى لاوراق نباتات الريحان وبصيليات نبات الثوم و المبيد الفطرى ريفص توب على نموا فطر النتراسيا مانقفيرا. استخدمت ثلاثه تراكيز من المستخلص المائى للريحان و التوم و المبيد الفطري (٥٠،٢٥و١٠٠%)وملح بيكربونات الصوديم في الثلاثه تراكيز(٢٠,١٠ و٤٠%) أضافة الى الشاهد. تم تقيم الاثر التثبيطى لهذه التراكيز بتسجيل نسبه تثبيط نمؤ الفطر. اوضحت النتائج ان كل تراكيز المستخلص المائى للنباتات و ملح بيكربونات الصوديم و المبيد الفطرى قد اظهرت تاثير معنوى ضد الفطر المختبر مقارنه بالشاهد. علي أية حال كلتي المبيد الفطري و مستخلص الثوم كان اكثر وضوحا ضد الفطر المختبر من ملح بيكربونات الصوديم و الريحان. التراكيز الاعلى فى كل من المستخلصات النباتيه المائى لي كل من المبيد و الثوم (١٠٠%) و بيكربونات الصوديم(٤٠%) و الريحان (١٠٠%) اعطت اعلى نسبة تثبيط مقارنه بالشاهد (٨٣,٦ و ٨٣,٣ و ٦٠ و ٤٤,٦)على التوالى مقارنه بي الشاهد الغير معامل في اليوم السابع بعد التلقيح . فيما بين المستخلصات النباتيه مستخلص الثوم دائما الاكثر فعاليه فى تثبيط نمؤ الفطرمن ملح بيكربونات الصوديم و مستخلص الريحان. عموماً اظهرت النتائج ان الفعاليه ضد الفطر تذداد بزيادة تركيزالمستخلصات .

CHAPTER ONE

INTRODUCTION

Neofusicoccum mangiferae (Sutton and Dyko, 1989) is a cosmopolitan fungus of the family Botryosphaeriaceae for which asexual stage has not been described (Elliott and Edmonds, 2004). *Neofusicoccum* was originally described by Nattrass (1933) in Egypt as the main cause of die back on deciduous trees based on cultural characteristics and reproductive morphology of the fungus then Calavan and Wallace (1949), Punithalingam and Waterston (1970). The name *Nattrassia mangiferae* has been given by Sutton and Dyko (1989) who revised the genus *Hendersonula.*

Neofusicoccum is a polymorphic fungus that has two spore stages, the pycnidial and the arthroconidial (*Scytalidium*state) (Sutton and Dyko, 1989). Earlier names for this fungus have been *Dothiorella*there *mangiferae*, *Exosporina fawcettii*, *Fusicoccum eucalypti*, *Hendersonula cypria*, *H. agathidis*, and *H. toruloidea*. The syanamorph is known by the name *Scytalidium dimidiatum*, also *Torula dimidiata*, and *S. lignicola*. More recently this species has been reclassified into the family *Neofusicoccum* as *Neofusicoccum mangiferae* (Elliott and Edmonds, 2004).

The fungus *Neofusicoccum* is a polyphagus fungus that attacks different types of trees. The earliest reports of plant diseases caused by *N. mangiferae* were from India on mango trees in 1908 and on citruses in California in 1923 (Sutton and Dyko, 1989). Recently, the disease was reported on *Eucalyptus* spp. in Iraq (Alzarari *et al*., 1979), on madrone (*Arbutus menziesii*) in USA (Elliott, *et al*., 2002), on strawberry trees

(*Arbutus unedo*) in Europe (Tsahouridou and Thanassoulopoulos, 2000) and on cassava in West Africa (Msikita *et al*. 1997).

According to FAO (2007), symptoms caused by *Neofusicoccum* differ depending on the part of the plant affected. The disease is mostly documented to cause stem and branch dieback, Cankers with external cracks are produced on stems and branches from which oozing exudates may be observed. Blossom blight and asymptomatic trees can produce fruit which develop stem-end rot or soft brown rot after ripening (Ahmed, 2005).

In Sudan *Neofusicoccum* is an introduced disease that firstly reported by Giha (1975) on *Ficus benghalensis* L. Since then, 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; Nour 1996; Mohamed, 2000 and Ahmed, 2005). *Neofusicoccum* has a wide host range, occurring on many trees in forests, orchards, ornamental, and shade trees including *Ficus* spp., *Acassia* spp., *baobab* spp., apple spp., *Citrus spp., Eucalyptus pp., Citrus* spp., *Mangifera* spp. and *Arbutus* spp. (Giha, 1975 and Abbasher *et al.*, 2013).

Based on the foregoing, *Neofusicoccum* is one of the most hazardous diseases that spread widely. There is limited information or lack of effective control measures about the disease control. 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 is to explore the potential of some higher plants extracts and systemic fungicide in suppressing the growth of this fungus *in vitro* with the following objectives:-

1. To explore the potentials of crude aqueous extract of some plants in inhibiting the growth of *Neofusicoccum* mangiferae.
2. To study the effect of Sodium bicarbonate solution on growth of *Neofusicoccum* spp
3. To evaluate the efficacy of systemic fungicide in suppressing the growth of the fungus *Neofusicoccum mangiferae*

CHAPTER TWO

LITREATURE REVIEW

2. 1. *Neofusicoccum mangiferae*

Nattrassia is a genus of fungi in the family Botryosphaeriaceae for which there is a single species *Nattrassia 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).

2.1.1 Classification:

Kingdom: Fungi

Division: Ascomycota

Class: Dothideomycetes

Order: Botryosphaeriales

Family: Botryosphaeriaceae

Genus: *Neofusicoccum*

Species: *N. mangiferae* (Sutton & Dyko 1989).

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

2.1.2 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 and Microbiology 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.) Sutton & Dyko (1989)
- *Scytalidium dimidiatum* (Penz.) Sutton, B. & Dyko (1989)
- *Fusicoccum dimidiatum* (Penz.) Farr, D. F. (2005)

2.1.3 Biology:

The fungus is able to grow on temperatures ranging from 20-40°C with an optimum between 30-35°C. Mycelia growth was best at pH 6. The best medium for mycelial growth was 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; Nori, 1972; Giha, 1975; Davison, 1996 and Elshikh ,2004).

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

2.1.4 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 colour. Research in California simulated sunburn damage on bark of walnut trees with use of a blowtorch to induce infection (Olsen, 1998).

2.1.5 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.

2.1.6 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, 1975; Elshikh, 2004; and Mohammed Elamein *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; Nori, 1996, Elatta and Nori, 1999). The host range fungus also included to forest trees such as *Acacia Senegal*, *Eucalypus camaldulensis* and *Khaya senegalensis* (Nori, 1996; El Atta & Nori, 1999, Elshafie & Ali, 2005).

2.1.7 Symptom:

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

Initially *N. mangiferae* causes leaf chlorosis, necrosis, blight defoliation and die back (Nori, 1996). Brown rot was observed on white yam tuber (Sangoyomi, *et al.*, 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 (Paxon *et al*., 1964; Giha, 1975; Roux, 1993 and Polizzi *et al.*, 2009).

2.1.8 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 immunocompetent 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).

2.2 Control Measures:

2.2.1. Chemical Control:

Themis *et al*., (2005) mentioned 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.

2.2.2. Cultural practices:

In Arizona State (2007), it was recommended for control of sooty canker disease in citrus to apply the following methods:

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.

2.2.3. Bio-control:

It was reported that *Trichoderma viride* had inhibitory effect on radial growth of *N. mangiferae* in vitro (Nori, 1996; El-shikh, 2004 and

Mohammed Elamien, *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 and Hatami, 2013).

2.2.4. 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 (Prabha and Choudhary1998,; Zine El Aabidine *et al*., 2010; and Hassan *et al*., 2011).

3-Alkaloids compounds (Rakoto-Ratsimanga *et al*., 1997).

4-Cuticle thickness (Curtis, 1928).

2.3 Botanical extract:

2.3.1Garlic (*Allium sativum*)

2.3.1.1 Scientific classification

Kingdom: *Plantae*

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Amaryllidaceae

Subfamily: Allioideae

Genus: *Allium*

Species: *A. sativum*

Binomial name

Allium sativum (NGRP,2006).

The crop which is commonly known as garlic is a species in the onion genus, Allium of the family Alliaceae. Its close relatives include the onion, shallot, leek, chive (Block, 2010) With a history of human use of over 7,000 years, garlic is native to central Asia, and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. It was known to Ancient Egyptians, and has been used for both culinary and medicinal purposes.

3.2.1.3 Uses:

Garlic is central to the cuisines of Mexico, the Caribbean, South America, the Middle East, India and China and can impart flavor to many different type of dishes. Economically, garlic is used in commercial food flavoring (Wiersema et al., 1999). Garlic is also used in folk medicine including treatment of bronchitis and respiratory problems, gastrointestinal problems, flatulence, leprosy, menstrual cramps, high blood pressure, diabetes and externally for warts, corns, arthritis, muscle pain, neuralgia and sciatica (Grieve and Mrs.M., 1971; Simon *et al*., 1984; Heinerman and John, 1995 and PFAF, 2002). Sangoyomi (2004) reported that aqueous extract of garlic effectively inhibited mycelia growth, conidia, pycnidia and sclerotial production of *Butryodiplodia theobromae, Aspergillus niger, Sclerotium rolfsii , Rhizoctonia solani and Neofusicoccum mangiferae* fungal pathogen in yam storage.

2.3.2 Sweet basil (*Ocimum basilicum* L.)

2.3.2.1Classification:

Kingdom: Plantae

Unranked: Asterids

Order: Lamiales

Family: Lamiaceae

Genus*: Ocimum*

Species*: O. basilicum.*

Sweet basil (Wikipedia 2015)

2.3.2.2 Origin and Distribution

Basil is native to areas in Asia and Africa and grows wild as a perennial on some pacific islands). The crop was brought from India to Europe through the Middle East in the sixteenth century, and subsequently to America in the seventeenth century (Muenscher *et al.,* 1987).

2.3.2.5 Uses to Control plant disease:-

Muntasir (2014) who investigated the antifungal effect of aqueous extract of ryhan (*Ociumum basilicum)* on growth of *N. mangifera* the cause of sooty canker in date palm reported significant inhibition of fungal mycelia growth.

2.4 Mineral Salt, Sodium Bicarbonate ($NaHCo_3$)

2.4.1 General properties

This salt has Molecular mass of 84,007 g/mol and CAS Number: 144-55-8. It is also known as aerated salt, widely used in 19th century for both sodium and potassium bicarbonate. The salt ($NaHCo_{3)}$ is mainly prepared

by the Solvay process, which entails the reaction of sodium chloride, ammonia, and carbon dioxide in water.

$CO2+2NaOH \rightarrow Na2CO3+H2O$

$Na2CO3+CO+H2O \rightarrow 2NaHCO3$

2.4.2 Uses:

It is used as a fabric softener in laundry. Sodium bicarbonate is used also as BC dry chemical fire extinguishers and as an alternative to the corrosive ammonium phosphate in extinguishers. The alkali nature of sodium bicarbonate makes the agent forms a crust over the surface similar to the effect of wet cosmetic. It is also used as a cleaning agent and as absorbent for moisture and odor. In the insect's pest control, the product is used repeatedly to kill fleas, ants, and other insect pest (GUN, 2007). In diseases control (sodium bicarbonate, (NaHCO3) and potassium bicarbonate (KHCO3) have been of considerable interest in the use of baking soda for controlling various plant fungal diseases (Karabulut *et al.*, 2003 and Smilanick *et al.*, 2006). In study by Prasannath and Mahendran (2013), the results revealed that potassium or sodium bicarbonate showed high inhibitory effect on the mycelia growth of *A. solani, w*here complete inhibition was obtained with potassium or sodium bicarbonate.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Location of the study

This study was conducted under laboratory conditions at Plant pathology Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology within the period of September to December 2014, it was intended to investigate the effect of aqueous extracts of some higher plants, Sodium bicarbonate and efficacy of fungicide (Revuse top®), against *Neofusicoccum mangiferae*.

3.2. Collections of infected plant material

Random samples were collected from bark and leave of infected mango trees (*Mangiferae indicia*) showing typical symptoms of canker on stem and necrosis of leaves at the college farm at Shambat area. Collected samples were kept in plastic bags and brought to laboratory for further isolation and identification of the fungus Neofusicoccum.

3.3. Collection of botanical and chemicals materials

Basil leaves were collected from Shambat area and brought to the laboratory where they were shade dried. After complete drying, plant samples were crushed separately to obtain fine powder for extraction. The Garlic sample was collected from local market. Sodium bicarbonate ($NaHCo_3$) was brought from soil laboratory and the fungicide was obtained from Professor Mohamed Osman laboratory, Khartoum University.

3.4. Isolation, characterization and identification of *Neofusicoccum mangiferae*

Previously collected samples of infected stem bark and mango leaves showing typical symptoms of the disease were cut into small part approximately 0.5 to 1.0 cm, washed thoroughly with the tap water, surface sterilized with Clorox (NaOCl) (1% concentration) for 1 minute, rinsed three times in sterilized distilled water and dried on sterilized filter paper. The sterilized stem and leaves sections were then plated at the rate of 6 sections per plate on sterilized Petri-dishes containing potato dextrose agar medium (PDA). The inoculated Petri dishes were incubated at 25^0C for 7 days. Growing fungus was further sub-cultured on PDA medium for further purification of the fungus. Purified growing fungus was examined under compound microscopic based on the method of (Giha, 1975 and Nori, 1996) to confirm that the fungus is *Neofusicoccum mangiferae*.

Fungus identification by growth habit character and spores using microscopic examination to confirm that the fungus is *Neofusicoccum mangiferae* was supplemented by other identification aids such as Sutton and Dyko (1989), Giha(1996) and Abbasher *et al.,* (2013). Standard books and research papers were also consulted during the examination of this fungus (Elliott, and Edmonds, 2004). The purified isolates were maintained on PDA medium for further studies.

Aqueous extracts of each of the plant materials were prepared as recommended by Okigbo (2006). The obtained fine powder form basil and grinded garlic bulb plant was weighed (25, 50 and 100 gm) and placed in 100,ml conical flask each and completed to 75, 50 and 100 ml sterilized distilled water respectively to obtain the three concentrations and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 25, %(L, Lowest) 50%(M, middle) and 100% concentrations(H highest). The granules of $NaHCo_3$ was weighted (10, 20

and 40 gm.) and dissolved in 90, 80 and 60 ml sterilized distilled water respectively in 100 ml conical flask each and completed to 100 ml sterilized distilled water to obtain the three concentrations of the salt, 10%(L, lowest), 20% (M, middle)and 40%(H, highest).

3.6. Preparation of fungicide concentrations:

The recommended dose of the commercial product, 2ml/l which represent 100% as higher dose) and two lower other concentrations {50% middle and 25% lower dose) were used. Accordingly, two ml of the Revus top fungicide was dissolved in one litter of sterilized distilled water to obtain highest (H) concentration 100% and it was diluted to give the middle (M) 50% and lower 25% (L).

3.7. Test procedure

Reduction 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 garlic, basil, $NaHCO_3$ and fungicide revues before being solidified in a conical flask of 100 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 served as control.

One mycelial 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 colony diameter measure after 7 day as mean growth along two axes on two pre-drawn perpendicular line on the reveres side of the plate. 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 inhibition in diameter of fungal growth according to Awuah(1989): -

$$MP = \frac{M1 - M2}{M1} \times 100$$

Where MP = Percentage inhibition of mycelial growth, M1= Mycelial growth in control petri dish without extract/fungicide , M2= Mycelial growth in extract/fungicide petri dish.

3.8. Experimental design.

The plates were arranged in a Complete Randomized Block Design with three replications.

3.9. Statistical analyses

The obtained data was statistically analyzed by computer software MSTATC according to analysis of variance (ANOVA); Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

RESULTS

This study which was conducted under laboratory condition of plant pathology, College of Agricultural Studies, Sudan University of science and Technology during the period September to December 2014 to investigate the inhibitory effect of basil leaves and garlic blub aqueous extracts, $NaHCo_3$ salt and fungicide revues top efficacy against the fungus *Neofusicoccum mangiferae.*

4.1 characterization and identification of the fungus

Neofusicoccum mangiferae as described by Giha (1996); (Sutton and Dyko, 1989) and Abbasher *et al.,* (2013).

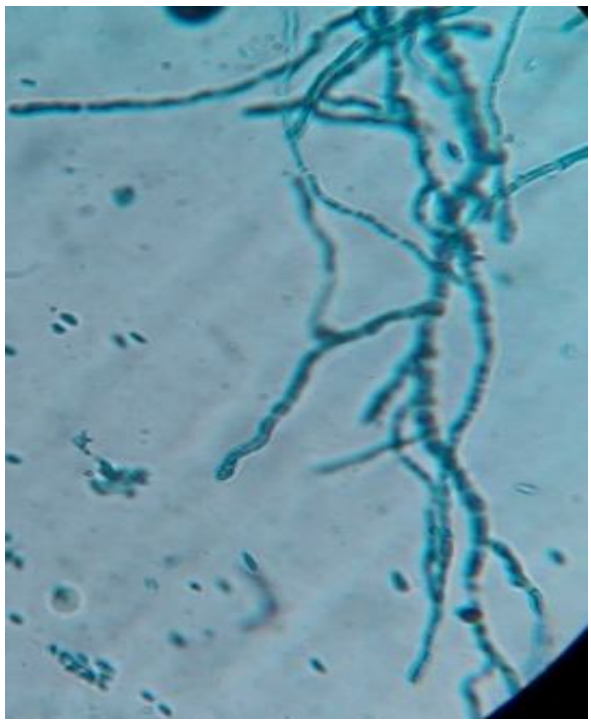

Plate (1) presents typical shape of spore and conidia of the fungus under the microscope

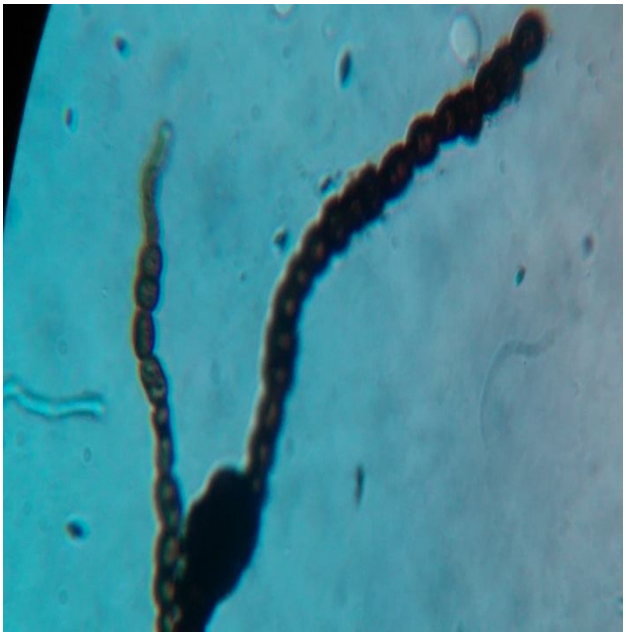

Plate (2) shape of Arthrospores

4.2. Effect of aqueous extracts of basil leaves, garlic blub, $NaHCo_3$ and fungicide on radial mycelial growth *of Neofusicoccum mangiferae* three days post inoculation

The results (Table 1 and Figure 1) showed that almost all plant aqueous extracts, salt and fungicide had negative effects on the fungal growth after three days from inoculation. Moreover, the garlic extract at 100% concentration completely inhibited the growth of the fungus. In fact, among plant extracts, garlic at the three concentrations (25, 50, and 100%) demonstrated the highest inhibition of mycelial growth (57, 70.3 and 100%) followed in descending order by fungicide (92, 85 and 75%), basil (40, 58,3 and 82%) and $NaHCO_3$ in (10, 20 and 40) are (35, 49 and 70.3%) respectively (Table, 1). However, the suppressing effect of fungicide was more pronounced at all concentrations tested.

Table 1: Effect of different concentrations of aqueous extracts of basil, garlic, $NaHCO_3$ and fungicide on radial mycelial growth of *Neofusicoccum mangiferae* three days post inoculation.

Treatments		Inhibition Zone (%)			
Concentrations		R1	R2	R3	Mean
Basil	25	21(4.6)	45(6.7)	54(7.3)	40.0(6.2)^{fg}
	50	87.3(9.3)	40(6.3)	46(6.8)	58.3(7.4)^{def}
	100	71(8.4)	82(9.0)	93(9.6)	82.0(9.0)^{abc}
Garlic	25	60(7.7)	54(7.3)	57(7.5)	57.0(7.5)^{def}
	50	64(8.0)	67(8.2)	80(8.9)	70.3(8.1)^{cde}
	100	100 (10.0)	100 (10.0)	100 (10.0)	100.0(10)^{a}
NaHCo3	10	25 (5.0)	47 (6.8)	33 (5.7)	35.0(5.8)^{g}
	20	39 (6.2)	59 (7.7)	49 (7.0)	49.0(6.9)^{efg}
	40	63 (7.9)	71 (8.4)	77 (8.8)	70.3(8.1)^{cde}
Fungicide	25	61 (7.8)	77 (8.4)	87 (9.3)	75.0(8.5)^{bcd}
	50	80 (8.9)	87 (9.3)	88 (9.4)	85.0(9.2)^{abc}
	100	92 (9.6)	91(9.5)	94 (9.7)	92.0(9.6)^{ab}
Control		0.00 (0.7)	0.00 (0.7)	0.00 (0.7)	00.0(0.7)^{h}
SE±		0.39			
C.V. (%)		10			
LSD		1.259			

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.*

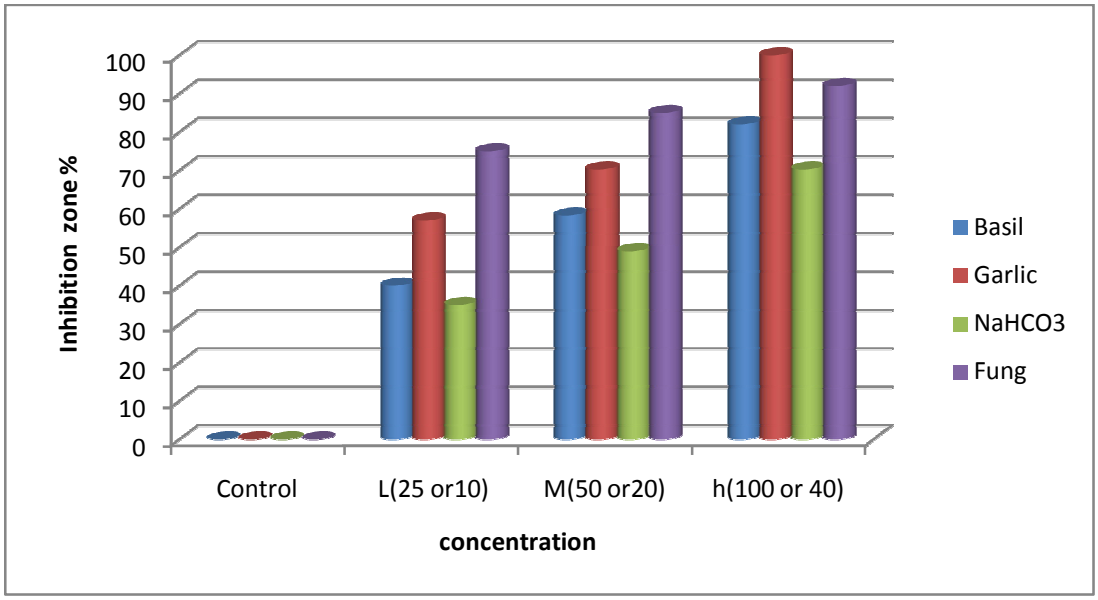

Fig. 1: Effect of aqueous crude extracts of Basil, Garlic, NaHCO3 and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* third day post inoculation.

4.3. Effect of different concentrations of aqueous extracts of basil leaves, garlic blub, NaHCo3 and fungicide on radial mycelial growth of *Neofusicoccum mangiferae* four day post inoculation

In day four after inoculation, all plant extracts concentrations as well as that of ($NaHCO_{3)}$ salt and fungicide continued exhibiting inhibitory effects against the fungal growth (Table, 2 and fig. 3). The percentages fungal growth inhibition was significantly high compared to the control.

Moreover, the highest concentration (100%) of the plant extracts, fungicide, and salt ($NaHCO_{3)}$ gave significantly higher inhibition zones percent against test fungus in all treatments (92.6, 85.6, 50.2 and 45.5%) respectively compared to the untreated control.

Furthermore, among plant extracts Garlic extract at all concentrations tested continued to be the most suppressive, followed in descending order by Basil and $NaHCO_3$ Salt whereas the fungicide at all concentrations maintained its superior suppressive effect (Table, 2).

Generally, the results showed that the antifungal activity of treatments increase with concentration. However, generally all treatments at all concentrations expressed an inhibitory effect less than the third day.

Table 2: Effect of different concentrations of aqueous extracts of basil, garlic, $NaHCO_3$ solution and fungicide on the linear mycelial growth of *Neofusicoccum mangiferae* four days post inoculation.

Treatments		Inhibition zone (%)			
Concentrations %		R1	R2	R3	Mean
Basil	25	17.6(4.2)	27.9(5.3)	14.7(3.8)	20.0 (4.4)^{f}
	50	23(4.5)	50(7.1)	49(7.0)	40.6(6.3)^{d}
	100	53(7.3)	45.5(6.7)	38(6.2)	45.5(6.7)^{c}
Garlic	25	50(7.1)	50(7.1)	47(6.8)	49 (7.0)^{cde}
	50	55(7.4)	55(7.4)	66(8.1)	58 (7.6)^{bcd}
	100	100 (10.0)	83 (9.1)	95 (9.7)	92.6 (9.6)^{a}
NaHCo3	10	29 (5.4)	53 (7.3)	8.6 (3.0)	30. (5.2)^{ef}
	20	19 (4.4)	22(4.7)	64 (8.0)	35. (5.7)^{ef}
	40	64 (8.0)	65 (8.0)	72%(8.5)	67 (8.1)^{abc}
Fungicide	25	76 (8.7)	76 (8.7)	81 (9.0)	77. (8.8)^{ab}
	50	77.0 (8.8)	83 (9.1)	82 (9.0)	80. (8.8)^{ab}
	100	87.0 (9.3)	86 (9.3)	84 (9.1)	85.6 (9.2)^{a}
Control		0.00 (0.7)	0.00 (.7)	0.00 (.7)	0.00
SE±		0.40			
C.V. (%)		13.9			
LSD		1.598			

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

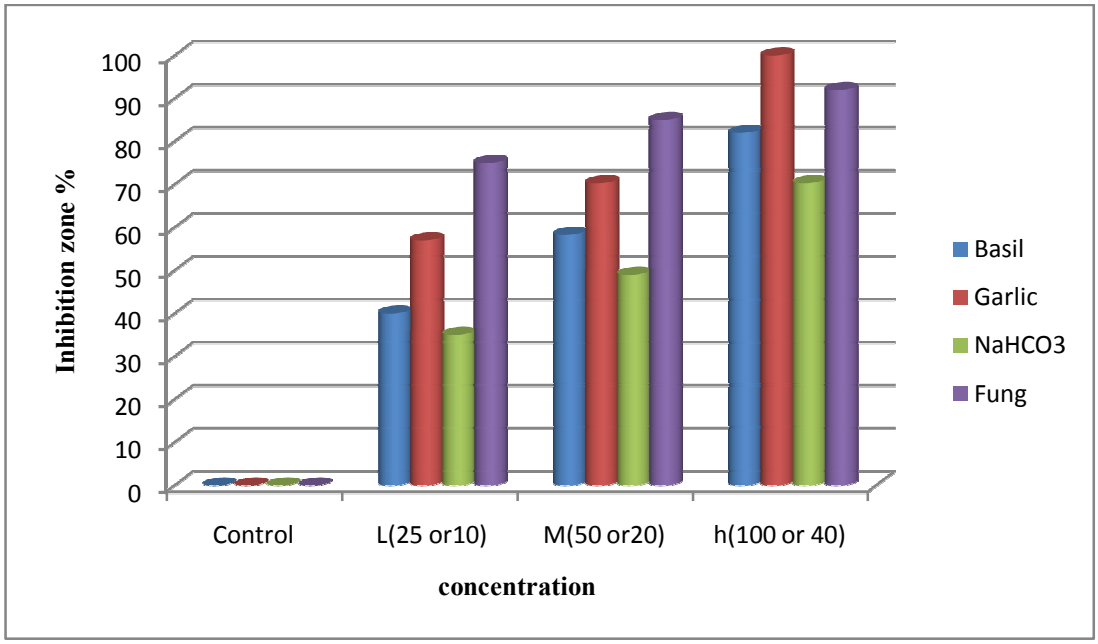

Fig. 2: Effect of aqueous crude extracts of Basil, Garlic, NaHCO3 and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* four days post inoculation.

4.4. Effect of different concentrations of aqueous extracts of basil, garlic, $NaHCO_3$ solution and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* five day post inoculation.

In day five after inoculation (Table, 3 and fig. 4), treatments of Garlic, basil, $NaHCO_3$ and fungicide at all concentrations invariably continued exhibiting significant inhibitory effects against the fungal growth (87, 55 and 51,6%),(85.6 ,78.6 and 77.6%) ,(63.3, 37.6 and 33%) and (48.3, 22.3 and 19%) respectively. However, the inhibitory effects of $NaHCO_3$ and Garlic were more pronounced than that of basil which showed decreasing inhibitory effect against test fungus compared to day three and four (Table, 1 and 2). 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: Effects of different concentrations of aqueous extracts of garlic, basil, NaHCO3 and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* five days post inoculation.

Treatments		Inhibition zone (%)			
Concentration		R1	R2	R3	Mean
Basil	25	17(4.3)	27(5.2)	13(3.6)	19.0 (4.2)^{ef}
	50	18(4.3)	16(4.0)	33(5.7)	22.3 (4.6)^{f}
	10	36 (6.0)	48 (6.9)	61 (7.8)	48.3 (6.9)^{cd}
Garlic	25	47(6.8)	57(7.5)	51(7.1)	51.6 (7.1)^{cd}
	50	52(7.2)	52(7.2)	61(7.8)	55.0 (7.4)^{c}
	10 0	96 (9.8)	74 (8.6)	91 (9.5)	87.0 (9.3)^{a}
NaHCo3	10	29 (5.4)	20 (4.9)	50 (7.1)	33.0 (5.8)^{de}
	20	30 (5.5)	61 (7.8)	22 (4.7)	37.6 (6.0)^{d}
	40	61 (7.8)	61 (7.8)	68(8.2)	63.3 (7.9)^{bc}
Fungicide	25	78 (8.8)	75 (8.6)	80 (8.9)	77.6 (8.7)^{ab}
	50	74 (8.6)	82 (9.0)	80 (8.9)	78.6(8.8)^{ab}
	10 0	86 (9.3)	86 (9.3)	85 (9.2)	85.6(9.2)^{ab}
Control		0 (.7)	0 (.7)	0 (.7)	0(0.7)^{g}
SE±			0.39		
C.V. (%)			10.9		
LSD			.4231		

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

❖ *Data in parentheses transformed using square root transformation* $\sqrt{X + 0.5}$*) before analysis.*

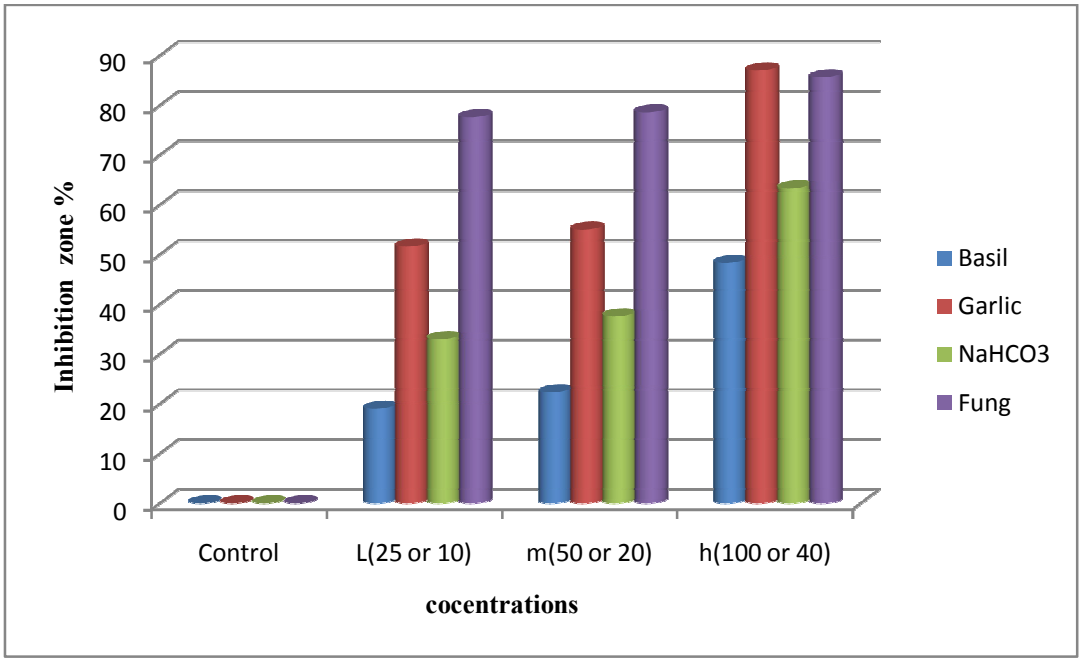

Fig. 3: Effect of different concentrations of aqueous crude extracts of Basil, Garlic, NaHCO3 and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* five day post inoculation.

4.5. Effects of different concentrations of aqueous extracts of garlic, basil, $NaHCO_3$ and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* after six days from inoculation.

Generally, it could be seen from the results (Table, 4 and Fig 4) that after six days from inoculation, extracts of the two plants (Garlic and Basil) expressed continued bioactivity at all concentrations. Meanwhile, $NaHCo_3$ and fungicide at all concentration suppressed the fungal growth at variable levels. In fact, the effects induced by all treatments are significantly high compared to control (Table, 4).

Moreover, among all treatments Garlic extract and fungicide at all concentrations tested (25, 50 and 100%) exhibited consistently the highest inhibitory effect throughout the days of recording (Table, 1, 2, 3 and 4) than the other equivalents. However, the inhibitory effect of basil

plant extracts reduced with time of recording. Obviously, in all tested products, growth inhibition increased with the concentration

Table, 4: Effects of different concentrations of aqueous extracts of garlic, basil, $NaHCO_3$ and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* six days post inoculation.

treatments		Inhibition zone (%)			
Concentrations %		R1	R2	R3	Mean
Basil	25%	28 (5.3)	25 (5.0)	26 (5.1)	26.3(5.1)^{f}
	50	28 (5.3)	48 (6.9)	25 (5.0)	33.6(5.7)^{def}
	100	35 (5.9)	44 (6.6)	55 (7.4)	44.6(6.6)^{cd}
Garlic	25	44 (6.6)	45 (6.7)	50 (7.1)	46.3(6.8)^{cd}
	50	48 (6.9)	64 (8.03)	50 (7.1)	54(7.3)^{c}
	100	87 (9.3)	71 (8.4)	92 (9.6)	83.3(9.1)^{a}
NaHCo3	10	21 (4.6)	20 (4.5)	48 (6.8)	29.6(5.3)^{ef}
	20	27 (5.2)	42 (6.5)	24 (8.0)	31(6.3)^{cde}
	40	57 (7.5)	59 (7.7)	64 (8.0)	60(7.7)^{bc}
Fungicide	25	80 (8.9)	69 (8.3)	76 (9.7)	75(8.9)^{ab}
	50	70 (9.3)	78 (8.8)	79 (8.9)	75.6(9.0)^{ab}
	100	81 (9.03)	85 (9.2)	85 (9.2)	83.6(9.1)^{a}
Control		0 (.7)	0 (.7)	0 (.7)	0(0.7)^{g}
SE±				0.37	
C.V. (%)				10.6	
LSD				٠.٤١٥	

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

❖ *Data in parentheses transformed using square root transformation ($\sqrt{X+0.5}$) before analysis.*

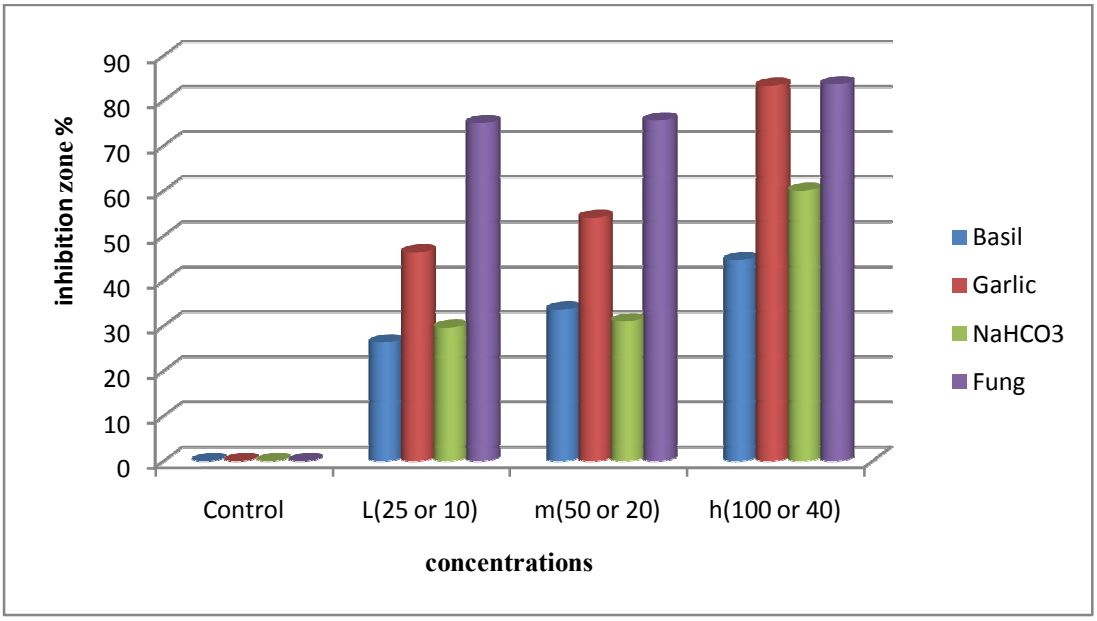

Fig. 4: Effect of different concentrations of aqueous crude extracts of Basil, Garlic, $NaHCO_3$ and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* six days post inoculation.

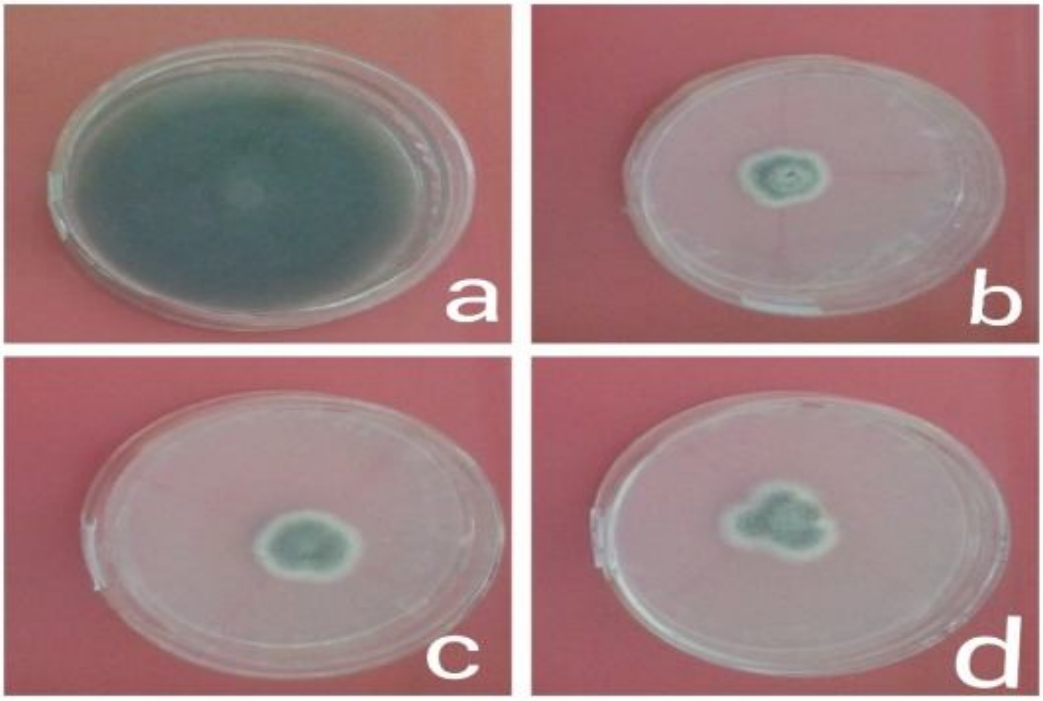

Plate (3) Effect of fungicide revus top on the growth of *Neofusicoccum mangiferae* invitro. (a) Control untreated, (b) concentration (100%h) (c) concentration (50%m) (d) contraction(25%L).

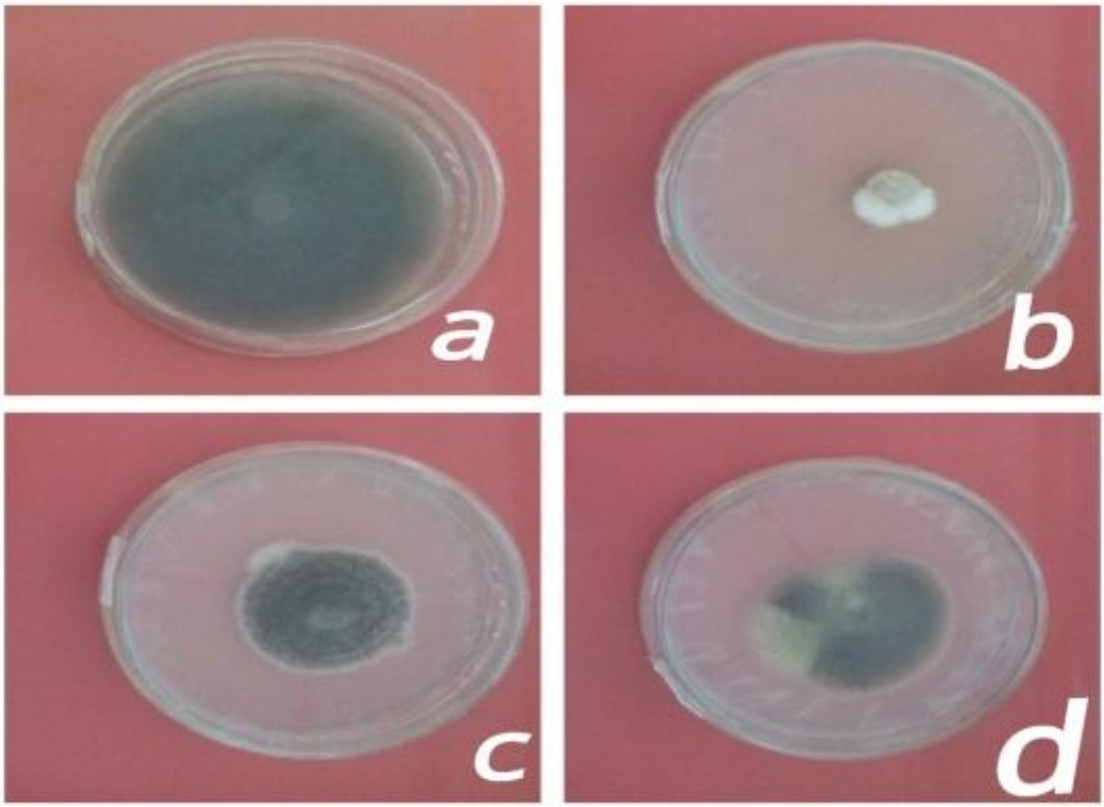

Plate (4) Effect of aqueous extract of garlic on the growth of Neofusicoccum mangiferae invitro. (a) Control untreated, (b) concentration 100% (c) concentration 50% (d) contraction 25%.

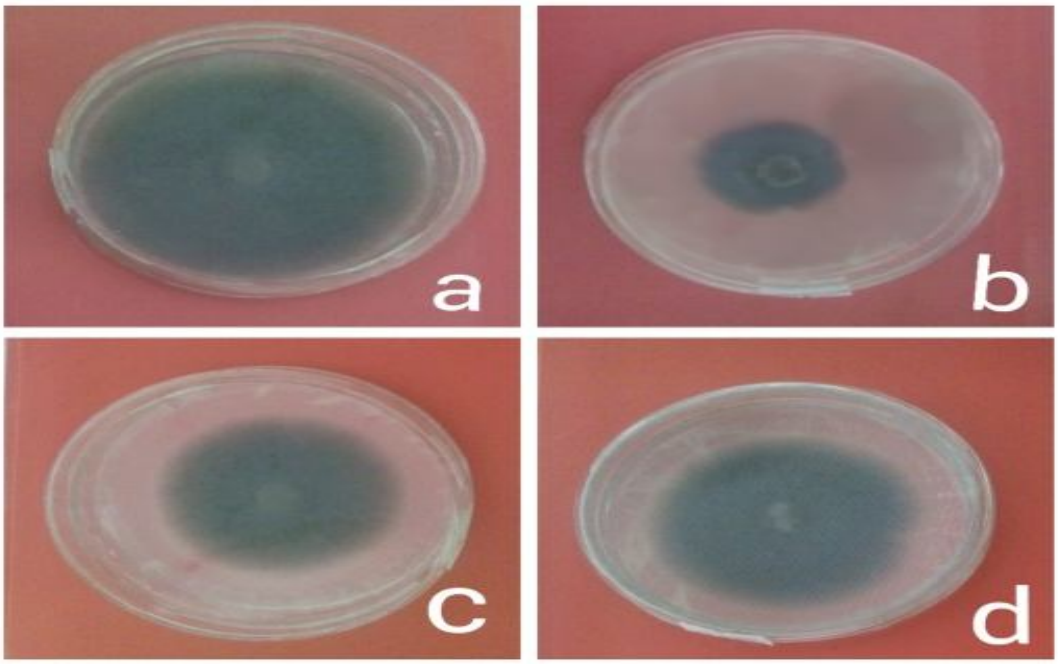

Plate (5) Effect of sodium bicarbonate solution on the growth of Neofusicoccum *mangiferae* invitro. (a) Control untreated, (b) concentration 40% (c) concentration 20% (d) contraction 10%.

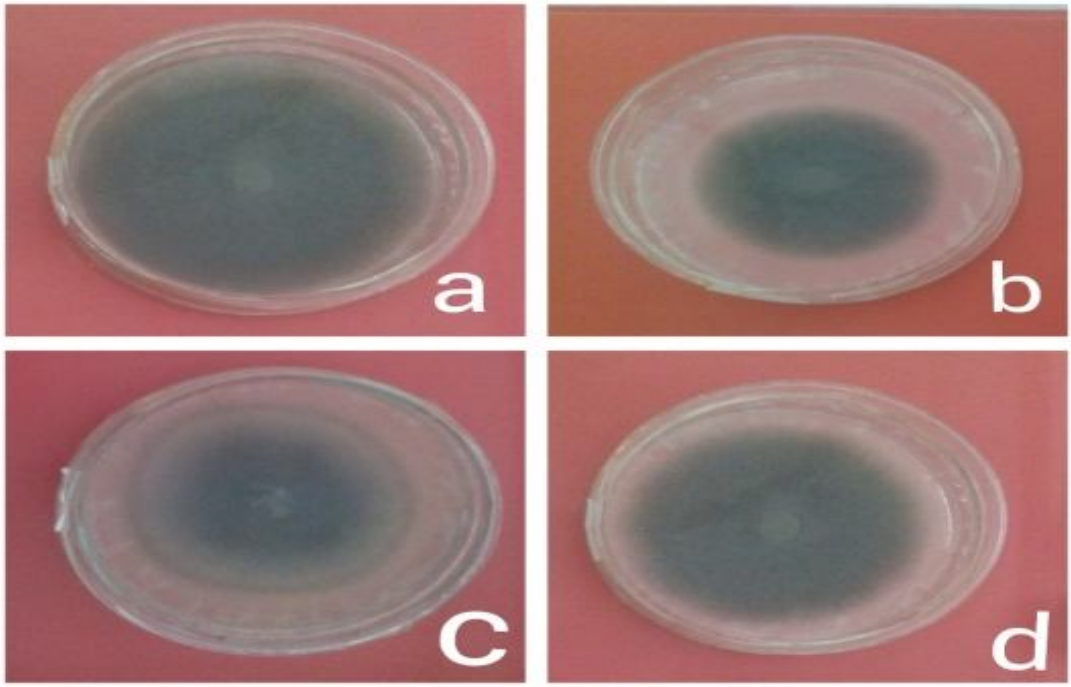

plate (6) Effect of aqueous extract of basil on the growth of Neofusicoccum *mangiferae* invitro. (a) Control untreated, (b) concentration 100% (c) concentration 50% (d) contraction 25%.

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). Limited success in controlling this disease which poses a threat to a wide range of fruit, ornamental and forest trees emphasizes the need and importance of developing an alternative and effective control measures. This study was conducted under laboratory condition of plant pathology, College of Agricultural Studies, Sudan University of science and Technology during the period from September to December 2014 to investigate the effect of basil leaves and garlic blub aqueous extracts and the $NaHCo_3$ salt and fungicide revues top efficacy against the fungus *Neofusicoccum mangiferae.*

A number of control strategies were presented to combat this disease (Karabulut *et al*., 2003; Sangoyomi, 2004; Themis *et al.,* 2005; Smilanick *et al.,* 2006; Mohammed *et al.,* 2009 and Muntasir, 2014). Historically, numerous phytochemicals have been isolated from different plants which are now being prescribed by medical practitioners all around the world (Newman, *et al.,* 2000). In fact, higher plants are extremely abundant with biologically active secondary metabolites. Over 80% of all known Alkaloids, Terpenioid, Phenols and other secondary metabolite were

produced by higher plants (Siddig, 1993). Pest control strategies, therefore, need to proper regulation in the interest of human health and environment. In recent years there has been considerable pressure on consumers to reduce or eliminate chemical fungicide in the food .there is increased the public awareness over the level of pesticide residue in food. This concern to encouraged researchers to look for alternative solutions to synthetic pesticides (Sharma and Meshram, 2006)

The results of this study (Tables 1-4 and Figures 3-6) revealed that the Garlic and basil aqueous extracts consistently throughout the course of the experiment exhibited an inhibitory effect on mycelial radial growth of the fungus with significantly higher inhibition zones percent compared to control. Similar studies which explored the effect of extracts of many higher plants have been reported to exhibit antifungal properties under laboratory condition. In fact, this finding is in agreement with Muntasir (2014) who tested the bioactivity of basil extract to control *N. mangiferae* the cause of sooty canker in date palm. Similar results of Garlic antifungal properties demonstrated by this study were also reported by Sangoyomi (2004) who proved that aqueous extract of garlic effectively inhibited mycelia growth, conidia, pycnidia and sclerotial production of *Butryodiplodia theobromae, Aspergillus niger*, *Sclerotiumrolfsii, Rhizoctonia solani,* and *Neofusicoccum mangifera*, fungal pathogens of yam in storage. These results also confirm the findings of Islam *et al*., (2001) who reported the control of *Colletotrichum spp.* and *M. phaseolina* in jute using garlic extract at rational 1:2. The result suggests common white garlic extracts a suitable biofungicide against cowpea fungal pathogens. It also explains the inter-specific biodiversity among the garlic spp.

As demonstrated by many researchers there is a considerable interest in the use of sodium bicarbonate (NaHCO3) for controlling various fungal diseases in plants (Karabulut *et al.*, 2003 and Smilanick *et al.*, 2006). As well known Bicarbonates are widely used in the food industry and were found to suppress several fungal diseases of cucumber plants. Spraying plants with NaHCO3 solution has provided good control of several plant diseases as *Colletotrichum musae* (De Costa *et al.*, 2012) and inhibit the colony growth of *Botrytis cinerea* in vitro (Palmer 1997).

The data presented in this study showed that the use of NaHCO3 solution *in vitro* expressed an inhibitory effect against the mycelial growth of *Neofusicoccum mangiferae* and the percentage zone of inhibition was significantly higher than the control. The obtained results were in line with that of Prasannath and Mahendran (2013) who test the effect of NaHCO3 on the mycelial growth of *Alternaria solani* that cause early blight of disease in tomato in vitro. Similar results where NaHCO3 was used against fungal diseases were reported by (Abd-Elkareem *et al.*, 2012) who investigate the effect of NaHCO3 on late blight in potato under filed condition where the treatment significantly reduced the disease incidence.

Generally, uses of synthetic fungicides considerably reduce the impact of this disease. In this study the fungicide Revues top® consistently inhibited the radial mycelial growth of *N. mangiferae* and its suppressing effect was more pronounced at all concentrations tested throughout the time of the investigation. These results confirm that which reported by Themis *et al.*, (2005) who indicated the effectiveness of fungicides against *N. mangiferae* that infects limb dieback of figs in California. Elshikh (2004) as well demonstrated that the fungicide Tilt completely inhibited *N. mangiferae* in vitro at 200 ppm and 100 ppm.

CONCLUSION

- 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 is *Neofusicoccum mangiferae* as described by Giha (1996); (Sutton and Dyko, 1989) and Abbasher *et al.,* (2013).

- The crude aqueous extracts of Garlic and Basil as well as that of ($NaHCO_{3)}$ salt and fungicide Revuse top at all concentrations exhibiting inhibitory effects against the radial mycelial growth of the test fungus. The percentages zone of inhibition was significantly high compared to the control.

- Among all treatments Garlic extract 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

Based on the foregoing results the following studies are recommended:

- To further investigate the antimicrobial properties in a group of medicinal plants and sodium bicarbonate solution against targets organism to determine their potentials as pesticides,

- To study different components of bicarbonates using different solvents so as to determine the efficacy of these components in controlling plant diseases.
- Further research may be needed to look into on-field trial of the garlic spp. before embarking on large scale production of the active constituents as biopesticide.
- The variability in response which expressed by test organism towards the different concentrations of treatments could be investigated to adjust an optimum dose for controlling *Neofusicoccum mangiferae*.

REFERENCES

Abbasher, A. Abbasher, Majdolin M. Husian, Mohamed S. Zaroug, and Eldur B. Zahran (2013). Prevalence and Etiology of Branch Wilt Disease (Nattrassia mangiferae) on Tree Species in Wad Medani Area, Sudan Department of Plant Pathology, Faculty of Agriculture and Natural Resources Abu Haraz, University of Gezira. P.O. box 42 Wad Medani, Sudan ,Journal homepage:http:// journals.sustech.edu.

Abd-El-Kareem1 and Fatten, M. Abd- El- latif (2012). Using bicarbonates for controlling late blight disease of potato plants under field conditions Life Science Journal;9(4) Plant Pathology Dept., National Research Centre, Giza, Egypt 2 Agriculture Botany Dept., Fac. Agric. Benha Un Life Science Journal 2012;9(4)iv. Egypt fadldeeb@yahoo.com http://www.lifesciencesite.com .

Awuah RT(1989).fungitoxic effect of extracts of some West African Plants. *Annals Applied. Biology*. 115:451-435.

Ahmed, M. I. (2005). Etiologies of branch wilt disease in selected forest, fruit and ornamental trees in central Sudan. Ph.D. Thesis. University of Gezira, Sudan.

Ahmed, N. E.; and Yassin, A. M. (1992). An epidemic wilts disease effect on date palm in northern Sudan. Technical Bulletin No. 5 Agricultural Research corporation (A R C), Wed Medani Sudan.

Alzarari, A. J.; Attrackchi, A. A.; Tarabeib, A. M. and Michail, S. H. (1979). New hosts of Hendersonula toruloidea. *Pakistan Journal of Scientific and industrial Research* 22: (5), 251.

Blazquez, R. Gurrero, C. Menasalvas, A.; Nova, A.; Cester, R. and Segovia, M.(2000). abstract. Intersci. Conf. Antimicrob. Agent chemother., Sep. 17-20, 40:390. Ho. Gen. Morales Meseguer, Maurica, Spain.

Block, E. (2010). Garlic and Other Alliums: The Lore and the Science. *Royal Society of Chemistry*, ISBN 0-85404-190-7.

Boxer, Arabella and Philippa Back.(1980) The Herb Book. London: Octopus Books, Limited, 1980.branch wilt of Pisidium guajava in Iran. *Phytopathology* 150: 244- 247.

Calavan, E.C. and Wallace (1949). Exporine Branch Wilt of grapefruit in Southern California: *Phytopathology*. 44: 635-639.

Calavan, E.C. and Wallace JM. (1954). Hendersonula toruloidea Nattrass on citrus in California. *Phytopathology*. 44: 635-639.

Callery and Emma (1994). The Complete Book of Herbs. Philadelphia, PA: Courage Books.

Crous, P.W.; Slippers, B.; Wingfield, M.J.; Rheeder, J.; Marasas, W.F.O.; Philips, A.J.L.; Alves, A.; Burgess, T.; Barber, P.; Groenewald, J.Z. (2006). "Phylogenetic lineages in the Botryosphaeriaceae". *Studies in Mycology* 55: 235–54. doi:10.3114/sim.55.

Curtis, K. M (1928). The morphological aspect of resistance to brown rot in stone fruiste. *Annals of Botany* 42:39-68.

Davision, A.D.(1972). Factor Affecting Development of Madrone Canker. *Pl.Dis.Reptr*. 56 (1): 50-52.

De Costa, D.M. and Gunawardhana, H.M.D.M. (2012). Effects of sodium bicarbonate on pathogenicity of Colletotrichum musae and potential

for controlling postharvest diseases of banana. *Postharvest Biology and Technology*, 68: 54-63.

Downer, J (2008). The Visit Report of Ficus nitida in Santa Manica, Los Angelos, California.

El-Atta, H.A. and Nouri, W.M , (1999). Phytotoxicity and antimicrobial activity of the culture filtrate of Nattrassia mangiferae Nattrass . *Univ. KJ Agric. Sci*., 7: 130-138.

Elliot, M. and Edmonds, R. L. (2008). Injected treatments for mangemant of Madrone Canker. *Arboriculture & Urban Forestry,* 34(2):110-115.

Elliot, M. and Edmondsm R.L.(2003). Systematical Study of Nattrassia mangifera the cause of madrone canker*, WIFDWC* 51:59-62.

Elliott, M. and Edmonds, R. L. (2004).Systematic study of Nattrassia mangiferae,the cause of madrone canker. In: Proceedings of the 51stwestern International Forest Disease Work Conference; 2003August 18–22(B. W. Geils ed.). Pp 59-62. Grants Pass, OR. Flagstaff, AZ: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Elliott, M., Edmonds, R. L. and Mayer, S. (2002). Role of fungal diseases in decline of Pacific madrone. *Northwest Science* 76:293–303.

Ellis, M.B (1971). Dematiccous, Hyphomyeets. CAB, CMI, Kew, Surrey Engeland.

Elshafie, M. E. and Ali, O. M. M(2005). First report of Ficus bengahalnsis dieback by Scytalidium dimidiatum in Sudan. Cab abstracts.

Elshikh, A.M.E (2004) investigation on the causal agent of sooty canker disease at date plant trees in Northern State. Sudan.

Food and Agriculture Organization of the United Nations, FAO (2007) working paper FBS/32E forest related health and biosecurty overview of forest pests, Rome, Italy.

Giha,O. H. (1975). Hendersonula toruloidea associated with a serious wilt disease of shade trees in Sudan. *Plant Disease Reporter* 59: 52-52.

Giha,O.H. (1996).Introduction in Plant Pathology .First Edition ,Publisher: Dar El-Assaala for journalism and publication, Khartoum, Sudan.

Grieve, M. A (1971). Modern Herbal; The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-Lore of Herbs, Grasses, Fungi, Shrubs, & Trees with All Their Modern Scientific Uses. New York: Dover Publications, 1971.

Gumbo, T. N.; Mkanganwi, N.; Robertson, V. J. and Masvaire, P. (2002). Case report of Endophthalitis, mycoses (Germany), 45: 118-119.

GUN Free Documentation license, 2007 http://cquconcepts.com/chem-sodiumbicarbonate .

Hassan, W. A., Haleem, R. A., Hassan, P. H. (2011). Effect of Heat-stress Predispostion on Development of Sooty Canker Caused by Neoscytalidium dimidiatum (Penz) Corus & Slipper. *ACTA Agrobotanic* 64(4); 207- 212.

Heinerman and John(1995). The healing benefits of garlic. New York: Wings Books. (*HSA Library).*

Herb Society of America 9019 Kirtland Chardon Rd. Kirtland, Ohio 44094 ©2003 The Herb Society of America.

http://cals.arizona.edu/PLP/plpext/diseases (2007) .

http://medilexicon.com/medical dictionary PLP(2009) *N. mangifera* .

http://en.m.wikipedia.org./wiki/basil.last modification in march 2015.

Islam S.M.A, I. Hossain, G.A Fakir & M. Asad-Ud-Doullah (2001). Effect of physical sorting, seed treatment with garlic extract and Vitavax -200 on seedborne fungal floral and seed yield of jute (Corchorus capsularis L). *Parkistan Journal of Biological Information.* 4(12):1509-1511.

Johnson, G.I.; Cook, A.W. ; Mead ,A.J. and Wells I.A.(2009). Stem End Root of Mango in Austeralia : Causes and Control.

Karabulut, O. A.; Bursa, G. and Mansour,M., (2003). Near-Harvest Applications of Metschnikowia fructicola, Ethanol, and Sodium Bicarbonate to Control Postharvest Diseases of Grape in Central California. *Plant Diseases* .87:1384-1389.

Lonsdale, I. H.(1996) Preliminary results on the made of infection of N. mangifera in mango. South Africa Mango Growth Association Year Book 13: 97-99.

Mirzaee, M. R.; Mohammedi, M.; and Radiom, H. (2002). Nattrassia mangiferae, the cause of dieback and trunk cankers of Ficus religiosa andbranch wilt of Pisidium guajava in Iran.*Phytopathology* 150: 244- 247.

Misikita, W. J.; James, S. Yaniniek B.;Ahounou M.; Baimey H.; and Fagbemissi, R. (1997) First report of Nattrassia mangiferae root and stem rot of cassava in West Africa. *Plant Diseases*, 81: 1332.

Mohamed, R. I. (2000). Studies on the ecology, etiology and pathology of Nattrassia mangiferae on tree flora from central Sudan. M.Sc. Thesis University of Khartoum.

Mohammed, M. E., Banihashemain S. M; Dafalla, G.; Bove, J. M. and Duran –Vila, N.(2009). Occurrence and Identification of Citrus viriods from Sudan .*Journal of Plant Pathology* 91 (2): 185-192 E.

Mohukker, R. I. M. and Yassin, A. M. (2001) A note on resistance of four tree species to branch wilt epidemic caused by Nattrassia mangiferae (Hendersonula toruloidea Nattrrass) in the Sudan.*University of Khartoum Journal of Agricultural Science,* 9 (2): 306-310.

Muenscher, Walter Conrad and Myron Arthur Rice(1978). Garden Spice and Wild Pot-Herbs. Ithaca, NY: Cornell University Press, 1978.

Muntasir Adam (2014). Third conference of pest management in Sudan 2-3february CPRC-ARC Wed Madani.

Nattrass, R. M. (1933). A new species of Hendersonula (H. toruloidea) on deciduous trees in Egypt. *Transactions British Mycological. Society,* 18: 189-198. 93.

Newman D J., Cragg GM., and Snader KM. (2000).The influence of natural products upon drug discovery. *Natural product reports*, 17(3), 215-234.

National Genetic Resources Program NGRP(2006). Germplasm resources information n*etwork -(GRIN)* [online]. Beltsville, Maryland:

National Germplasm Resources Laboratory. [accessed July 27, 2006]. Available from World Wide Web (http://www.ars-grin.gov/cgi-bin/npgs/html/taxgenform.pl).

Nori, W.M(1996) .Branch wilt and mortality of some shade trees caused by N.mangiferae in Khartoum State .M.Sc. Thesis ,University of Khartoum ,Sudan.

Nour, W. M. T. (1996). Branch Wilt and Mortality of some Shade Trees in Khartoum State Caused by Nattrassiamangiferae.Pp 84, M.Sc. thesis,Khartoum university.

Olsen, M.W; (1998). Sooty canker in Horticulture Crop. The University of Arizona. College of Agriculture Plant disease mangemant. *Plant Sci.* 1 9-4.

Palmer, C.L., Horst, R.K. and Langhans, R.W. (1997). Use of bicarbonate to inhibit in vitro colony growth of Botrytis cinerea. *Plant Disease*, 81: 1432-1438.

Paxton JD, Wilson EE and Davis JR. (1964). Branch wilt disease of fig caused by Hendersonula toruloidea. *Plant Dis Rep*. 48: p 142.

PFAF. (2002)Plants for a future database [accessed November 17, 2003]. Available from (http://www.ibiblio.org/pfaf/D_search.html).

Polizzi. G.: Aiello, Vitale, A; Frida. F. G.; Groenwaldm, J.Z. and Crous, p. W(2009). First Report of Shoot Blight, Canker and Gummosis caused by Neoscytalidium dimiditum. *Plant Disease*, 93(11) :1215 .

Prabha, C. and Choudhary, S. S.(1998). Phytochemical relationship among some Members of Caesalpinaceae. *Plant Physiology and Biochemistry*, India 14(2):220-226.

Prasannath . K and. Mahendran. S (2013) In Vitro Inhibition of OF Tomato Early Blight Pathogen in Response to Application of Selected Inorganic Department of Agricultural Biology, Faculty of Agriculture, Eastern University, Sri Lanka, Chenkalady

Punithalingam E. and Waterston J.M. (1970). CMI descriptions of pathogenic fungi and bacteria no. 274. Hendersonula toruloidea. ommonwealth Mycological Institute, Kew, United Kingdom.

Rakoto-Ratsimanga, A.; Ratsimanga-Urverg, S. and Rasoanaivo, P.(1997). Research breakthrough at NAPRECA-M adagascar. NAPRECA Newsletter, Issue No. 856-6283 Vol. 14 no. 1.

Roux C. (1993). Nattrassia mangiferae, a fungus causing several diseases of mango. SA Mango Growers Yearbook.

Sadeghya B and N.Hatamia N. (2013) Bioactivity of soil-borne Streptomyces sp. against N.mangiferae Department of Agriculture, Payamnoor University (PNU) of Kermen, Karmen ,Iran. Published online: 02 sep 2013).

Sangoyomi (2002) TE and Find all citations by this author (default). Or filter your current search Ekpo EJA. 2002. First report of Nattrassia mangiferae as a postharvest fungal pathogen of white yam (Dioscorea rotundata) in Nigeria. *Plant Diseases* . 86(8): p 919.

Sangoyomi, T.E. (2004). Post-harvest fungal deterioration of yam (Dioscorea rotundata Poir)and its control. PhD thesis, University of Ibadan Nigeria. 180pp.

Sharma, K. and Meshram, N.M. (2006) Bioactivity of essential oils from *Acorus calamus* Linn. And *Syzygium aromaticum* Linn against

Sitophils oryza linn. In pest stored wheat. *Biopestiide International* 2, 144-152.

Siddig, S.A. (1993). Evaluation of neem seed and leaf water extracts and powder from the control of insect pest insect in the Sudan / Agric. Res. Cro Tech .Bull.NO. 6.

Simon, James E., Alena F. Chadwick and Lyle E. Craker (1984). Herbs: an indexed bibliography1971-1980: the scientific literature on selected herbs, and aromatic and medicinal plants of the temperate zone. Archon Books. (HSA Library).

Smilanick, J.L.; Mansour, M.F. and Sorenson, D. (2006). Pre- and Postharvest Treatments to Control Green Mold of Citrus Fruit During Ethylene Degreening. Plant Disease .90:89-96.

Sutton, B.C. and Dyko, B.J. (1989). Revision of Hendersonula. Mycological Research, 93: 466-488.

Sydow, H.; Sydow, P.; Butler, E.J. (1916). "Fungi Indiae orientalis pars V". *Annales Mycological*14 (3–4): 177–220.

Themis J.; Michailides, D. P.; Morgan, D and Reyeslt, H. (2005). Etiology and Management of Limb Dieback of Figs in California . project report, 2005).

Tsahouridou, P. C. and Thanassoulopoulos,C. C. (2000). First report of Hendersonula toruloidea as a foliar pathogen of strawberry-tree (Arbutus unedo) in Europe. *Plant Disease* 84: p 487.8.

Tucker, Arthur O. and Thomas DeBaggio. (2000). The big book of herbs: a comprehensive illustrated reference to herbs of flavor and fragrance. Loveland, CO: Interweave Press. (HSA Library).

U.S. Department of Agriculture, March 9, (2015). Agricultural Research Service Systematic Mycology and Microbiology Laboratory - Nomenclature Fact Sheets.

Wiersema, John H. and Blanca Leon. (1999). World economic plants: a standard reference. Boca Raton: CRC Press. (HSA Library).

Willinger B, Kopetzky G, Harm F, Apfalter P, Makristathis A, Berer A, Bankier A and Winkler S. (2004). Disseminated infection with Nattrassia mangiferae in an immunosuppressed patient. J. *Clinic. Microbiol*. 42(1):478-80.

Zine El Abidine, A., Baissac; Y; Moukhi, A., Jay – Allemand, C.; Khadari, b; El Modfar, C(2010). Resistance of Olive –tree to Spilocea oleagina is mediated by the synthesis of phenolic *international journal biology* compounds. Int. J. Biol. 12: 61-67.

APPENDIXES

Appendix 1: ANOVA

a) Variable 3 (inhibition in third day after inoculum)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	206.712	17.226	30.579	0.0000
Within	26	14.647	0.563		
Total	38	221.359			

Coefficient of Variation = 10.01%

b) Variable 4 (inhibition after fourth day after inoculum)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	215.361	17.947	19.800	0.0000
Within	26	23.567	0.906		
Total	38	238.928			

Coefficient of Variation = 13.99%

c) Variable 5 (inhibition after fifth day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	214.808	17.901	33.339	0.0000
Within	26	13.960	0.537		
Total	38	228.768			

Coefficient of Variation = 10.95%.

d)Variable 6 (inhibition after sixth day after inoculums)

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	12	194.630	16.219	31.345	0.0000
Within	26	13.453	0.517		
Total	38	208.084			

Coefficient of Variation = 10.61%.

Appendix 4:

a (Reduction of growth by cm in 3th day after inoculation):

Treatments	Growth diameter by (cm)		
Concentrations %	R1	R2	R3

Basil	25	1.4	.975	.8
	50	.225	1.7	.95
	100	.5	.8	.11
Garlic	25	.625	.5	.35
	50	.7	.8	.775
	100	0	0	0
NaHCo3	10	1.325	.925	1.1
	20	1.075	.725	.9
	40	.65	.5	.4
Fungicide	25	.675	.5	.225
	50	.35	.225	.2
	100	.125	.15	.1
Control		1.775	1.775	1.775

b (Reduction of growth by cm in 4th day after inoculation):

Treatments		Growth diameter by (cm)		
Concentrations %		R1	R2	R3
Basil	25	2.15	1.885	2.225
	50	.2	1.3	1.325
	100	1.2	1.4	1.6
Garlic	25	1.115	1.115	.125
	50	1.3	1.3	1.375
	100	0	.425	.875
NaHCo3	10	1.825	1.2	2.375
	20	2.1	2.025	1.575
	40	.925	.9	.725
Fungicide	25	.6	.6	.475
	50	.575	.425	.45
	100	.325	.35	.4
Control		2.6	2.6	2.6

c (Reduction of growth by cm in 5th day after inoculation):

Treatments		Growth diameter by (cm)		
Concentrations %		R1	R2	R3
Basil	25	2.8	2.45	2.925
	50	2.775	2.85	2.25
	100	2.15	1.75	1.325
Garlic	25	1.8	1.45	1.65
	50	1.625	1.625	1.3
	100	.125	.875	.275
NaHCo3	10	2.4	2.7	1.7
	20	2.375	1.35	2.625
	40	1.325	1.3	1.075
Fungicide	25	.725	.85	.675
	50	.875	.6	.65
	100	.475	.45	.5
Control		3.4	3.4	3.4

d(Reduction of growth by cm in 6th day after inoculation):

Treatments		Growth diameter by (cm)		
Concentrations %		R1	R2	R3
Basil	25	2.725	2.925	2.85
	50	2.775	2	2.925
	100	2.525	2.15	1.75
Garlic	25	2.175	2.125	1.925
	50	2	1.675	1.95
	100	.5	2.125	.275
NaHCo3	10	3.05	3.1	2.025
	20	2.825	2.25	2.95

	40	1.675	1.575	1.4
Fungicide	25	.775	.55	.55
	50	1.15	.85	.8
	100	.725	1.75	.925
Control		3.9	3.9	3.9

Appendex 3: (PDA)

Potato Dextrose Agar 93.5g/litter

Potato infusion 4.0g, Dextrose 20g, Agar 15g, Distilled water 1000ml

Appendix 4: (Fungicide)

Name: Revus top^{R}

Active ingredients: Mandipropamid + Difenoconazole

Manufactured for: Syngenta Crop Protection, North Carolina

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

Appendix 5:

Mango tree was isolated form it fungus *Nattrassia mangiferae*