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

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

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قال تعالى:

(قولوا لان البحر منادانا ك لند البحر قليبتين ت كم تيد يليكم و كم كم يليكم و كم كم يليكم)

و لاجتمعت يمشيه صدا (١٠) ق لينما لبل شئكم يوما يليكم له واجد ف من كن يروح لمقاء يليه ففعل عمل صلى حلا ولا يشك بعبادة يليه

١٠١ (١١)

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

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Neofusicoccum mangiferae considered as one of the 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 Neofusicoccum 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 نيوفزوكوم مانقيفا من أهم أمراض الأشجار الغابية و البستانية في العالم. اجريت هذه الدراسة تحت ظروف المختبر بقسم وقاية النبات (عمل مراض النبات - كلية الدراسات الزراعية، جامعات السودان للعلوم والتكنولوجيا - شمبا) لدراسة تأثير المستخلص المائي لإوراق نباتات الريحان وبصليات نباتات الثوم و المهيد الفطرى على نمو فطر النتراسيا مانقيفا. استخدمت ثلاث تراكيز من المستخلص المائي للريحان فطور و الثوم و المهيد الفطرى (0.25% و 0.5% و 1%) و بيكربونات الصوديوم في الثلاث تراكيز (1% و 2% و 4%) إضافة إلى الشاهد. تم تقييم الافتقادات لهذه التراكيز بتسجيل نسبة تثبيط نمو الفطر. أوضحت النتائج أن كل تراكيز المستخلص المائي للنبيات و بيكربونات الصوديوم و المهيد الفطرى قد اظهرت تأثير على الفطر المختبر مقارنته بالشاهد. على أي حال كلتي المهيد الفطري و مستخلص الثوم كان أكثر وضوحاً ضد الفطر المختبر من ملح بيكربونات الصوديوم و الريحان.

التراكيز الأعلى في كل من المستخلصات النباتية المائية لي كل من المهيد و الثوم (100%) و بيكربونات الصوديوم (40%) و الريحان (100%) أعطى أعلى نسبة تثبيط مقارن بالشاهد (83.6 و 83.6 و 83.6 و 60 و 60 و 44.4). على النتائج مقارنة بي الشاهد الغير معامل في اليوم السابع بعد التلقيح. فيما بين المستخلصات النباتية مستخلص الثوم دائما الأكثر فعالية في تثبيط نمو الفطر من ملح بيكربونات الصوديوم و مستخلص الريحان. عموماً أظهرت النتائج أن الفاعليه ضد الفطر تزداد بزيادة تركيز المستخلصات.
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 *Dothiorellathere manniferae*, *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 citrus 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
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* spp., *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:-

*Arbutus unedo* in Europe (Tsahouridou and Thanassoulopoulos, 2000) and on cassava in West Africa (Msikita *et al.* 1997).
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
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 subhyaline 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*, *Eucalyptus 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 (Spondylo-discitis) 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 Choudhary 1998; 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:

2.3.1 Garlic (*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.)

2.3.2.1 Classification:

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 (*Ocimum 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 (NaHCO₃)

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 (NaHCO₃) is mainly prepared
by the Solvay process, which entails the reaction of sodium chloride, ammonia, and carbon dioxide in water.

\[
\text{CO}_2 + 2\text{NaOH} \rightarrow \text{Na}_2\text{CO}_3 + \text{H}_2\text{O}
\]

\[
\text{Na}_2\text{CO}_3 + \text{CO} + \text{H}_2\text{O} \rightarrow 2\text{NaHCO}_3
\]

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, where 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 indica) 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 (NaHCO₃) 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°C 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 NaHCo₃ 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 for 5 days. The colony diameter measure after 7 day as mean growth along two axes on two pre-drawn perpendicular line on the reverses 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):

\[
MP = \frac{M1 - M2 \times 100}{M1}
\]

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, NaHCo₃ 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
4.2. Effect of aqueous extracts of basil leaves, garlic blub, NaHCo₃ 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 NaHCO₃ 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inhibition Zone (%)</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
</tr>
<tr>
<td>Basil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>21(4.6)</td>
<td>45(6.7)</td>
</tr>
<tr>
<td>50</td>
<td>87.3(9.3)</td>
<td>40(6.3)</td>
</tr>
<tr>
<td>100</td>
<td>71(8.4)</td>
<td>82(9.0)</td>
</tr>
<tr>
<td>Garlic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>60(7.7)</td>
<td>54(7.3)</td>
</tr>
<tr>
<td>50</td>
<td>64(8.0)</td>
<td>67(8.2)</td>
</tr>
<tr>
<td>100</td>
<td>100 (10.0)</td>
<td>100 (10.0)</td>
</tr>
<tr>
<td>NaHCo3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25 (5.0)</td>
<td>47 (6.8)</td>
</tr>
<tr>
<td>20</td>
<td>39 (6.2)</td>
<td>59 (7.7)</td>
</tr>
<tr>
<td>40</td>
<td>63 (7.9)</td>
<td>71 (8.4)</td>
</tr>
<tr>
<td>Fungicide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>61 (7.8)</td>
<td>77 (8.4)</td>
</tr>
<tr>
<td>50</td>
<td>80 (8.9)</td>
<td>87 (9.3)</td>
</tr>
<tr>
<td>100</td>
<td>92 (9.6)</td>
<td>91(9.5)</td>
</tr>
<tr>
<td>Control</td>
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<td>0.00 (0.7)</td>
</tr>
<tr>
<td>SE±</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>1.259</td>
<td></td>
</tr>
</tbody>
</table>

*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.*
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 (NaHCO₃) 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 (NaHCO₃) 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 NaHCO₃ 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inhibition zone (%)</th>
<th>Concentrations %</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td>17.6</td>
<td>27.9</td>
<td>14.7</td>
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<td></td>
<td>53(7.3)</td>
<td>45.5(6.7)</td>
<td>38(6.2)</td>
<td>45.5(6.7)</td>
</tr>
<tr>
<td>Garlic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
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<td>55(7.4)</td>
<td>55(7.4)</td>
<td>66(8.1)</td>
<td>58 (7.6)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>100(10.0)</td>
<td>83 (9.1)</td>
<td>95 (9.7)</td>
<td>92.6 (9.6)</td>
</tr>
<tr>
<td>NaHCo$_3$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>8.6 (3.0)</td>
<td>30. (5.2)</td>
</tr>
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<td></td>
<td></td>
<td>19 (4.4)</td>
<td>22(4.7)</td>
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<td></td>
<td>64 (8.0)</td>
<td>65 (8.0)</td>
<td>72% (8.5)</td>
<td>67 (8.1)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td>76 (8.7)</td>
<td>76 (8.7)</td>
<td>81 (9.0)</td>
<td>77. (8.8)</td>
</tr>
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<td>50</td>
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<td>77.0 (8.8)</td>
<td>83 (9.1)</td>
<td>82 (9.0)</td>
<td>80. (8.8)</td>
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<td>86 (9.3)</td>
<td>84 (9.1)</td>
<td>85.6 (9.2)</td>
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<td>0.00</td>
<td>0.00</td>
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</table>

SE±: 0.40
C.V. (%): 13.9
LSD: 1.598

*Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0.05). Data in parentheses transformed using square root transformation ($\sqrt{x + 0.5}$) before analysis.*
4.4. Effect of different concentrations of aqueous extracts of basil, garlic, NaHCO₃ 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, NaHCO₃ 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 NaHCO₃ 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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inhibition zone (%)</th>
<th>Concentration</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basil</td>
<td></td>
<td>25</td>
<td>17(4.3)</td>
<td>27(5.2)</td>
<td>13(3.6)</td>
<td>19.0 (4.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>18(4.3)</td>
<td>16(4.0)</td>
<td>33(5.7)</td>
<td>22.3 (4.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>36 (6.0)</td>
<td>48 (6.9)</td>
<td>61 (7.8)</td>
<td>48.3 (6.9)</td>
</tr>
<tr>
<td>Garlic</td>
<td></td>
<td>25</td>
<td>47(6.8)</td>
<td>57(7.5)</td>
<td>51(7.1)</td>
<td>51.6 (7.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>52(7.2)</td>
<td>52(7.2)</td>
<td>61(7.8)</td>
<td>55.0 (7.4)</td>
</tr>
<tr>
<td></td>
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<td>87.0 (9.3)</td>
</tr>
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<td>20 (4.9)</td>
<td>50 (7.1)</td>
<td>33.0 (5.8)</td>
</tr>
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<td></td>
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<td>61 (7.8)</td>
<td>22 (4.7)</td>
<td>37.6 (6.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>61 (7.8)</td>
<td>61 (7.8)</td>
<td>68(8.2)</td>
<td>63.3 (7.9)</td>
</tr>
<tr>
<td>Fungicide</td>
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<td>75 (8.6)</td>
<td>80 (8.9)</td>
<td>77.6 (8.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>74 (8.6)</td>
<td>82 (9.0)</td>
<td>80 (8.9)</td>
<td>78.6 (8.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>86 (9.3)</td>
<td>86 (9.3)</td>
<td>85 (9.2)</td>
<td>85.6 (9.2)</td>
</tr>
<tr>
<td>Control</td>
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<td></td>
<td>0 (.7)</td>
<td>0 (.7)</td>
<td>0 (.7)</td>
<td>0(0.7)</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V. (%)</td>
<td></td>
<td></td>
<td>10.9</td>
<td></td>
<td></td>
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<td>LSD</td>
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</table>

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, NaHCO3 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, NaHCO3 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, NaHCO₃ and fungicide on the radial mycelial growth of *Neofusicoccum mangiferae* six days post inoculation.

<table>
<thead>
<tr>
<th>treatments</th>
<th>Concentrations %</th>
<th>R1 (Inhibition zone, %)</th>
<th>R2 (Inhibition zone, %)</th>
<th>R3 (Inhibition zone, %)</th>
<th>Mean (Inhibition zone, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil</td>
<td>25%</td>
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<td>26 (5.1)</td>
<td>26.3(5.1)¹</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>28 (5.3)</td>
<td>48 (6.9)</td>
<td>25 (5.0)</td>
<td>33.6(5.7)def</td>
</tr>
<tr>
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<td>100</td>
<td>35 (5.9)</td>
<td>44 (6.6)</td>
<td>55 (7.4)</td>
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</tr>
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<td>46.3(6.8)cd</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>48 (6.9)</td>
<td>64 (8.03)</td>
<td>50 (7.1)</td>
<td>54(7.3)c</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>87 (9.3)</td>
<td>71 (8.4)</td>
<td>92 (9.6)</td>
<td>83.3(9.1)a</td>
</tr>
<tr>
<td>NaHCo3</td>
<td>10</td>
<td>21 (4.6)</td>
<td>20 (4.5)</td>
<td>48 (6.8)</td>
<td>29.6(5.3)er</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>27 (5.2)</td>
<td>42 (6.5)</td>
<td>24 (8.0)</td>
<td>31(6.3)cde</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>57 (7.5)</td>
<td>59 (7.7)</td>
<td>64 (8.0)</td>
<td>60(7.7)bc</td>
</tr>
<tr>
<td>Fungicide</td>
<td>25</td>
<td>80 (8.9)</td>
<td>69 (8.3)</td>
<td>76 (9.7)</td>
<td>75(8.9)ab</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>70 (9.3)</td>
<td>78 (8.8)</td>
<td>79 (8.9)</td>
<td>75.6(9.0)ab</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>81 (9.2)</td>
<td>85 (9.2)</td>
<td>85 (9.2)</td>
<td>83.6(9.1)a</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0 (.7)</td>
<td>0 (.7)</td>
<td>0 (.7)</td>
<td>0(0.7)g</td>
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</tbody>
</table>

**SE±**: 0.37
**C.V. (%):** 10.6
**LSD**: .415

*Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0.05).*

- 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, NaHCO₃ 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%.
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 bulb aqueous extracts and the NaHCo₃ 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

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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).


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.


APPENDIXES

Appendix 1: ANOVA

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

<table>
<thead>
<tr>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>Prob.</th>
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</thead>
<tbody>
<tr>
<td>Between</td>
<td>206.712</td>
<td>17.226</td>
<td>30.579</td>
<td>0.0000</td>
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<tr>
<td>Within</td>
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<td>0.563</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>221.359</td>
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<td></td>
<td></td>
</tr>
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</table>

Coefficient of Variation = 10.01%

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

<table>
<thead>
<tr>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>215.361</td>
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<td>19.800</td>
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<tr>
<td>Within</td>
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<td>Total</td>
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</table>

Coefficient of Variation = 13.99%
c) **Variable 5 (inhibition after fifth day after inoculums)**

<table>
<thead>
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<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-value</th>
<th>Prob.</th>
</tr>
</thead>
<tbody>
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Coefficient of Variation = 10.95%.

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

<table>
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<th>Prob.</th>
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<td>38</td>
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</table>

Coefficient of Variation = 10.61%.

**Appendix 4:**

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Growth diameter by (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations %</td>
<td>R1</td>
</tr>
<tr>
<td>R3</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td></td>
</tr>
<tr>
<td>R1</td>
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</tr>
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</table>


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

<table>
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<th>Treatments</th>
<th>Growth diameter by (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>R2</td>
<td>R3</td>
</tr>
<tr>
<td>Concentrations %</td>
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<td></td>
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c (Reduction of growth by cm in 5th day after inoculation):

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<th>R3</th>
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d (Reduction of growth by cm in 6th day after inoculation):

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**Appendix 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 from the fungus *Nattrassia mangiferae*