بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

College of Agriculture Studies

Department of Plant Protection

Antifungal Activity of Garlic Extracts against *Neofusicocum mangiferae*

A graduation project submitted in partial fulfillment of the requirements for the degree of B.Sc. in Plant Protection.

By:

Almaldein Ibrahim Abudli

Supervisor:

Dr.Ekhlass Hussien Mohammed

Department of Plant Protection

College of Agricultural Studies, Shambat

Sudan University of Science and Technology (SUST).

Sep.2016

الآية

بسم الله الرحمن الرحيم

(قالَ تَزْرَعُونَ سَبْعَ سِنِينَ دَأَبًا فَما حَصَدْتُمْ فَذَرُوهُ فِي سُنْبُلِهِ إِلاَّ قَلِيلًا مِمَّا تَأْكُلُونَ) (47)

صدق الله العظيم

سورة يوسف الآية

DEDICATION

To all those who encouraged and guided me in my life

To my family

To my dear parents,

To my brothers and sister,

To my teachers,

And to all my dear friends and colleagues

ACKNOWLEDGEMENTS

All my thanks and prays to "Allah", who gave me strength and Patience to complete this research.

I would like to express my profound gratitude and appreciate to my Supervisor **Dr.Ekhlass Hussien Mohammed** for his interest, expert guidance and his relentless effort to make me produce this work. Thanks are also due to the staff members of Department of Plant Protection, College of Agricultural Studies, Sudan University of Sciences and Technology (SUST) for their co-operation.

Sincere thanks also to **Ms. Mawada Ibrahim** and **Ahmed Khalid** and Ms. **Madiha Soliman** and **Mohamed younis Ramadan** for their assistance and help during the study. Special thank are extended to all my friends and colleagues and I wish to see them happy and successful in their future life.

List of contents

CHAPTER THREE

MATERIAL AND METHODS

ABSTRACT

Neofusicoccum mangiferae considered as one of most important disease of forest and horticulture trees in worldwide, and in Sudan. The present investigation was undertaken under laboratory condition of plant production Department, college of Agricultural study, Sudan University of Science and Technology, to study the anti fungal activity of alcoholic of extracts of bulbs of garlic plant, and efficacy of fungicide (score) on linear growth, of the fungus *Neofusicoccum mangiferae*. Three concentration of alcoholic extract of garlic and fungicide, each of 25, 50 and 100%, at were used in addition to control. The results showed that all concentrations of the alcoholic extract of garlic tested 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. The high concentration of the fungicide score, garlic extract. At gave significantly higher inhibition .Among the plant extracts tested that of garlic was invariably the most effective in suppressing the fungus growth than fungicide. Generally, the results showed that the anti fungal activity increase with increase in extract concentration.

ملخص الاطروحة

يعتبر مرض النتراسيا (نيوفزكوكم مانقفيرا) من اهم امراض الاشجار الغابيه و البستانيه في العالم والسودان . اجريت هذه الدراسه تحت ظروف المختبر بقسم وقايه النبات (بمعمل امراض النبات) كلية الدراسات الزراعيه ' جامعه السودان للعلوم و التكنولوجيا (شمبات) لدراسه تاثير المستخلص الكحولي لبصليات نبات الثوم و المبيد الفطري (اسكور) علي نمو فطر نتراسيا مانقفيرا. استخدمت ثلات تراكيز من المستخلص الكحولي للثوم والمبيد الفطري (25, 50, 100%) اضافه الي الشاهد. تم تقييم الاثر التثبيطي لهذه التراكيز بتسجيل نسبه تثبيط نمو الفطر. اوصحت النتائج ان كل تراكيز المستخلص الكحولي و المبيد قد اظهرت تاثير معنوي ضد الفطر المختبر مقارنة بالشاهد. كما اثبتت الدراسة ان كل من المبيد الفطري و مستخلص الثوم كانا اكثر وضوحا ضد الفطر المختبر. اوضحت الدراسة ان التركيزات الاعلي للمستخلص الكحولي للثوم (100%) اعطت اعلي نسبه تثبيط مقارنته بالشاهد. كما اوضحت الدراسة ان المستخلص الكحولي للثوم له فعالية عالية في تثبيط نمو الفطرمقارنة بالمبيد. عموما اظهرت النتائج ان الفعاليه ضد الفطر تزداد بزيادة التراكيز.

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 deciduaus trees based on cultural Characteristics and reproductive morphology of the fungus then colavan and Wallace(1949),punithalingam and Waterston (1970).The Name Nattrassiamangiferae has been given by Sutton and Dyko (1989) who revised the genus Hendersonula.Neofusicoccum is a polymorphic fungus that has two spore stage , the pycnidial and the arthroconidial (scytalidium state)(Sutton and Dyko,1989).Earlier names far this fungus have been Dothiorellatheremangiferae , Exosporinafawcettii ,fusicocccum eucalypti ,Hendersonulacypria ,H. agathidis ,and H.torulaidea .the syanamorph is known by the name scytalidiumdimidiatum ,also toruladimidiata ,and S.lignicola .more recently this species has been reclassified in to 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 tree in (1908), and on citruses in California in (1923)(Sutton and Dyko,1989).Recently ,the disease was reported on Eucalyptys spp. In lraq (Alzarari et al, 1979), on madrone (Arbutus menziesii) in USA (Elliott, et Al, 2002),on strawberry trees (Arbutus unedo) in Europe (tsahouridou and thanassoulopoules ,2000) and on cassava in west Africa (misikta et al,1993).

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 crack 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 ficusben ghalensis L. since the disease has spread all over the country, and was reported in as many as 29 different plant species that include shade, ornamented, timber and orchard trees (Ahmed and Yassin 1992.,Nour 1996 ., Mohamed ,2000 and Ahmed ,2005).

Neofusicoccum has a wide hast 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*., *mangifera spp*.,and *arbutus spp.,* (Giha, 1975 and Abbasher et al,2013).Based on the foregoing , Neofusicoccum is one of the most hazardous disease that spread widely . there is limited information or lack of effective control measures about the disease control accordingly ,on effective control measures should be . developed to control this devastating disease that represent real threat to fruit , forest and ornamented 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 plant in inhibiting the growth of *Neofusicoccum mangiferae*.

2- To evaluate the efficacy of systemic fungicide (Score) in suppressing the growth of the fungus *Neofusicoccum mangiferae*.

CHAPTER TWO
LITRATUREREVIEW

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 magiferae* (Crous , *et al.,* 2006).

2.1.1 Classification

Kingdom: fungi
Division: Ascomycota
Class: Dothideomycetes
Order: Botryosphaeriales
Family: Botryosphaeriaceae
Genus: Neofusicoccum
Species: mangiferae (Sutton and Dyko1989).

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

2.1.2 Synonyms

There is great confusion regarding the taxonomy, classification and identification of this anamorph species as reported by U.S. Department of agricultural Research Service Systematic Mycology and Microbiology Laboratory-Nomenclature fact sheet, March 9, 2015.

2.1.3 Biology

The fungus is able to grow on temperatures ranging from 20-40C with an optimum between 30-35C. 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 fungicide: Mancozeb (Dithane M45) and Maneb (manebe80) (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 color.

The arthro spores were spherical to cylindrical in shape (Nori, 1996). Pyno-spores 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. Arthroconidia was mid to dark brown, smooth mostly a septated 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 conidia are produced in black powdery masses under bark, and are easily wind disseminated .These spores which arise from segment hyphae 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 Califfornia 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 Ficusspp, Neem, rain tree (samaniasaman) 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, Eucalypuscamaldulensis and Khayaseneglensis (Nori, 1996; ElAtt and Nori, 1999, Elshafie and Ali, 2005).

2.1.7 Symptom

The characteristic symptom of *N. mangiferae* 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 sweed orange (Giha, 1996). However, mangiferae is considered as a facultative parasite that often grows on dead branches or wound parasite (Paxon *et al;* 1964; Giha, 1975; and polizzi *et al*., 2009).

2.1.8 Effect on the Human

The fungus *N. mangiferae* was defined previously as Hendersonulatoruloidea 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 immune competent patient, due to injury by a piece of grass in Zimbabwe (Gumbo *et al*., 2002). It was also reported the case of backbone discs infection (Spondylodiscitis) 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 fungicide 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-preveting 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 collar trees e.g. ficus spp. And other

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 Ficusnitida in California.

2.2.3. Bio-control

It was reported that *Trichoderma viride* had inhibitory effect on radial growth of *N. mangiferae* (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 the added the

volatile metabolism of Trichodermaspp It did not inhidit 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 reported by (Elliot and Edmond, 2008).

2-phenolic and related compounds reported by (Prabha and Choudhary 1998, Zine El Aabidine *et al.,* 2010; and Hassan *et al*., 2011).

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

4-Cuticle thickness reported by (*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 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.

2.3.1.2 Uses

Garlic is central to 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.

2.4.1 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 insects 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 showed 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
MATERIAL AND METHODS

3.1 Location of the study

This study was conducted under laboratory of Plant Pathology department college of Agriculture studies "Shambat, Sudan university of science and technology within the period of September to December 2014, this study was investigate the effect of aqueous extracts of Garlic (*Allium satavim*), and efficacy of fungicide (ScorEc250) /against the growth of *Neofusicoccum mangiferin vitro.*

3.2 Collection of infected plant material

Random samples were collected from bark and leave of infected mango trees (*Mangifera indicia*) showing typical symptoms of canker on stem and necrosis of leaves at the college form 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 Isolation characterization and identification of *Neafusicoccum mangifera*

Previously collected sample of infected stem bark and mango leaves typical symptoms of the disease were cut into small part approximately 0.5 to 1.2 cm , washed thoroughly with the top water surface sterilized for 1 minute. Rinsed three times in sterilized distal water then dried on sterilized filter paper the sterilized stem and leaves sections were than plated at the rate of 5 sections per plate an sterilized Petri-dishes containing palate dextrose ager medium (PDA). The inoculated Petri- dishes were incubated at 25^{o}C for 7 days.

Growing fungus was further subculture on PDA medium for further purification of the fungus purified growing fungus was examined under compound microscopic based on the method of (Gina, 1975 and Nori, 1996) to confirm that the fungus in *Neafusicoccum mangifera*. The identification of the fungus growth was habit character and spores using microscopic examination to confirm Sutton and Dyko (1989). Gina, (1996) and abbashem *et.al,* (2013).

Standard books and research paper were also consulted during the examination of this fungus (Elliott, 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 grinded garlic bulb plant was weighed (25. 50 and 100gm) and placed in 100ml conical flask each and completed to 75-50 and 100ml sterilized distilled water respectively to obtain the three concentrations and it was placed in a shaker for 4hrs.The extracts were filtered overnight to obtain 25,50 and 100% concentrations.

3.5 Preparation of fungicide concentrations

The recommended of the commercial product, 2ml l1 which represent 100% as higher dose and two lower other concentrations (50% middle25 Lower dose) were used according (two ml) of the Scorefungicide was distilled water to obtain highest (H) concentrations 100% and it was diluted to give middle (M)50% and lower 25%(L) concentrations.

3.6 Test procedure

Poisoning Plating Technique was used in this study (Rao and Srivastava, 1994) to evaluate the effect of each concentration on linear growth of the fungal. Pure culture of the fungal growth was prepared from previously maintained culture of

neofusicoccum mangfera prepared PDA medium was amended with the reguired concentration from garlic and fungicide revues before being solidified ina conical flask of H 100ml, agitated and paured into sterilized glass petri dishes .

Three plates, containing 30 ml of PDA, were assigned for each concentration and left to solidify the other three plats with PDA medium served as control

One mycelial disc of the fungus was placed in the back of the plate and incubated at 25c in incubated and radial growth of pathogen was measured at 24 n intervals

The petri dishes of each concentration were arranged in a complete block design in incubator and incubator at 25c for 5 days as mean growth along two axes on the two pre-drawn perpendicular line on the reverse 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 the liner fungal growth was calculated as percentage of inhibition in diameter of fungal growth according to Awuan (1989)

MP = M1-M2

Where MP = percentage inhibition of mycelia growth M1 = mycelia growth in central Petri dishes without extract fungicide, M2 = mycelia growth in extract fungicide Petri dishes.

3.7. Experimental design

The plate were arranged in a complete Randomized block Design with three replications

3.8. Statistical analyses

The obtained data was statistically analyzed by computer software Statistic 08 according to analyses of variance (ANOVA), Duncan multiple Range test was used for mean separation.

CHAPTER FOUR

RESULTS

This study was conducted under laboratory conditions of plant protection Department, College of Agricultural Studies, Sudan University of science and Technology (During March and April 2016).The aim ofthis study was to investigate the effect of aqueous extracts of Garlic (*Allium satavim*), and efficacy of fungicide (Score) /against the growth of *Neofusicoccum mangiferin vitro* .

4.1 Identification of the pathogen *Neofusicoccum mangiferin vitro*

Isolation of *Neofusicoccum mangifer* frommango trees (*mangifera indicia*)was carried out from naturally infected leaves showing symptoms and identified on basis of cultural and morphological characteristics as *Neofusicoccum mangifer* according to the shape of spores and conidia.

4.2 The Effects of Garlic extractsand Fungicide on the liner growth of *N. mangiferin vitro* two day's afterincubation.

The results (Table 1) showed that the garlic aqueous extracts of all concentration and fungicide exhibited an inhibitory effect on the fungal growth 2 days after inoculation.The percentage inhibition ranged from (0.65, 0.73 and 0.00%) at the three concentrations (25, 50 and 100%) respectively. Moreover, the fungicide Tilt demonstrated 100% inhibition. The inhibitory effect from all concentrations tested was significantly different from control (1.8%).

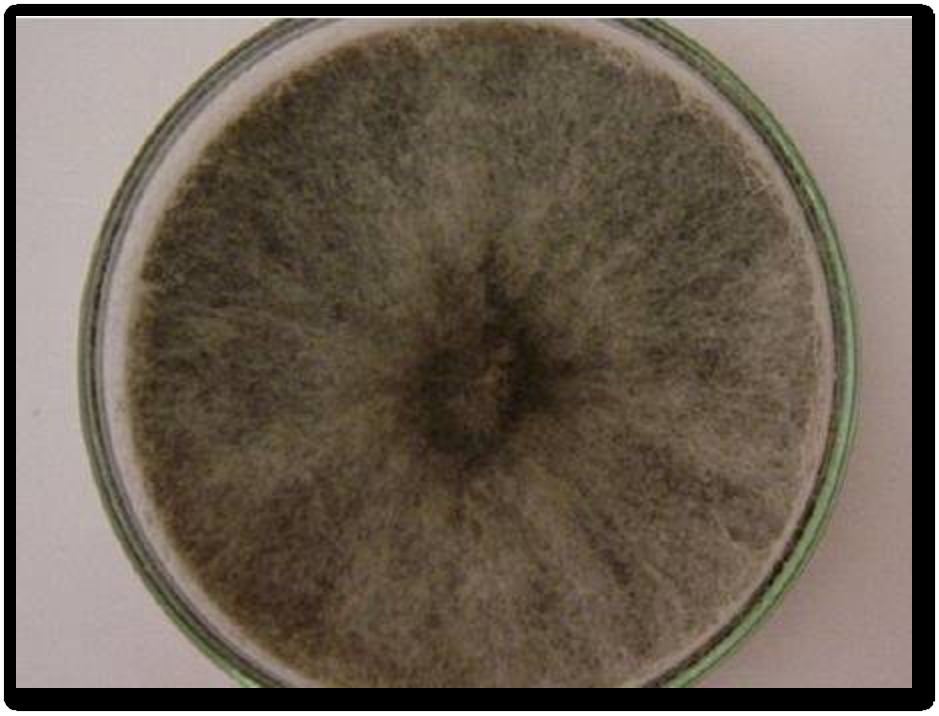

Plate 1 *Nattrassia mangiferae* hyphae

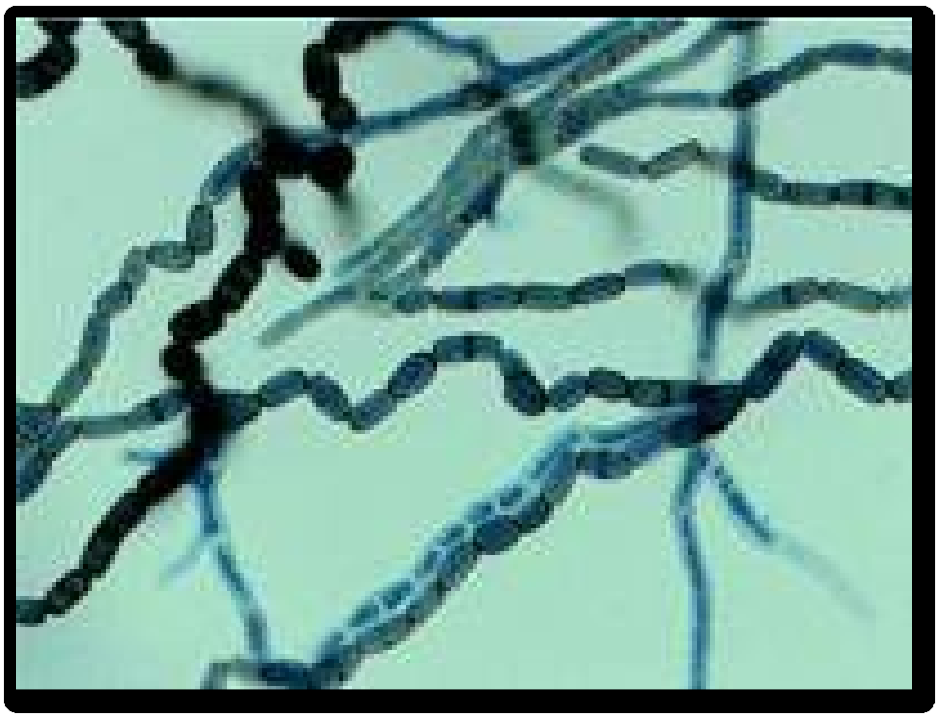

Plate 2 *Nattrassia mangiferae* Spores

Plate 3 Garlic Bulbs

Table 1Effects of aqueous extractof Garlic and fungicide (Score) on the linear growth of *Neofusicoccum mangiferin vitro.*

Treatments	Concentration (%)	Inhibition zone (%)		
		2th day	4th day	6th day
Garlic	25	0.65^{BC}	0.95^{B}	1.01^{B}
	50	0.73^{B}	1.1^{B}	1.21^{B}
	100	0.00^{C}	0.00^{C}	0.00^{C}
Fungicide (Tilt)		0^{C}	0^{C}	0^{C}
Control		1.8^{A}	4.4^{A}	4.6^{A}
C.V. (%)		0.54	0.38	0.36
SE±		0.121	0.28	0.29

Means followed by the same letter are not significantly different (P< 0.05). Data in parenthesis are transformed using square root transformation)$\sqrt{X + 0.5}$) before analysis.

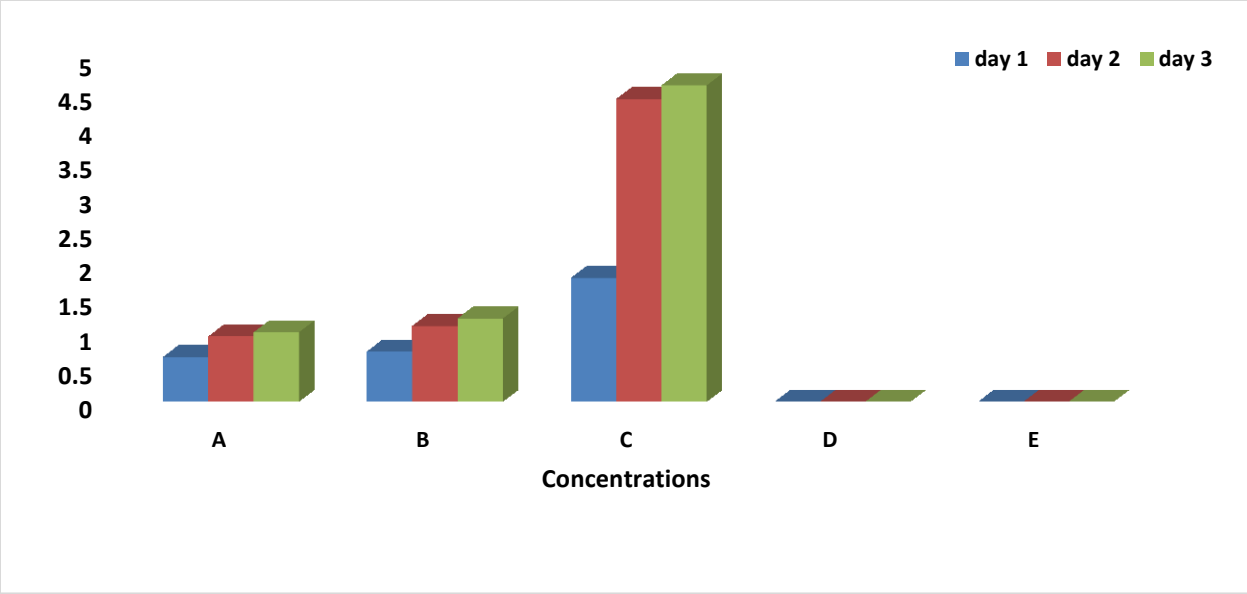

Fig. 1Effects of aqueous extractof Garlic and fungicide (Score) on the linear growth of *Neofusicoccum mangiferin vitro.*

4.3 The Effects of Garlic extractsand Fungicide on the liner growth of *N. mangiferin vitro* four days after incubation.

Four days after incubation, all concentration of garlic extracts as wellas that of the fungicide were invariably continued exhibiting suppressing effects against the fungal growth. The percentage inhibition ranged from (0.95, 1.1 and 0.00%) at the three concentrations (25, 50 and 100%) respectively. Moreover, the fungicide Tilt demonstrated 100% inhibition.The inhibitory effect from all concentrations tested was significantly different from control (4.4%).

4.4 The Effects of Garlic extractsand Fungicide on the liner growth of *N. mangiferin vitro* six days after incubation.

Sixdays after incubationthe results showed that all concentration as well as the fungicide in suppressing the fungal growth (1.01, 1. 21 and 0.00) respectively and the fungicide gave (0.00).

In fact, all tested concentrations of Garlic continued inducing a significant inhibition zone percentage against the test fungus compared to control (Table, 1).

Obviously, *N. mangifer* differs in its response to the different concentrations but on the whole, growth inhibition increased with increasing concentration. This inhibitory effect from all concentrations was significantly different from control.

CHAPTER FIVE

DISCUSSION

Nofusicoccum Mangiferae is cosmopolitan and polyphagas genus of fungi that attacks a multitude of fruits and forest trees (crocus, *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 , 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 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 Pathogen, college of agricultural studies, Sudan University of Science and Technology during the period from Après to may (2016). To investigate the effect of garlic bulbs alcoholic extract and fungicide score 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*., 2009 and Muntasir.2014). Historically, numerous phytochemicals have been isolated from different plant which is now being prescribed by medical partitions 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.

The results of this study (Tables and Figures) revealed that the garlic alcoholic extract and fungicide score consistently throughout the course of the experiment exhibited an inhibitory effect on mycelial radial growth of the fungus with significantly higher inhibition tones percent compared to control.

Similar studies which explored the effect of extract 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 *Neofusicoccum mangiferae* the cause of sooty canker in date palm similar results of garlic antifungal properties demonstrated by this study were also reported by (Saangoyomi, 2004).who proved that alcoholic extract of garlic effectively inhibited mycelia growth, conidia, *Pycnidia and Scleromae*, production of *Butryodiplodia Theobromae , Aspergillus niger*, *Sclerotium rolgsii*, *Rhizoctonia solani and Neofusicoccum mangiferae*, fungal pathogens of yam in storage .

These results also confirm the findings of (Islam *et al*., 2001) who reported the control of *Colletrichum Spp* and *M. phaseolina* in jute using garlic extract at rational 1:2.the result suggest common white garlic extract a suitable bio fungicide against cowpea fungal pathogens.

It also explains the inter-species bio diversity among the garlic spp.

Generally, uses of synthetic fungicide considerably reduce the impact of this disease.

In this study the fungicide score consistently inhibited the radial mycelia growth of *Nattrassia mangiferae* and its suppressing effect was more pronounced at all concentration tested throughout the time of the investigation.

These result confirm that which reported by (Themis *et al*,2005) who indicated the effectiveness of fungicides against *Nattrassia mangiferae* that infects limb die back of figs in California (Elshik., 2004) as well as demonstrated that the fungicide Score completely inhibited *Nattrassia 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 *Neofuscoccum mangiferae*as described by Giha (1996); (Sutton and Dyco, 1989). and Abbasher*, et al*., (2013).

The crude alcoholic extract of garlic and fungicide score at all concentrations inhibiting effects against the radial mycelia 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 extract.

RECOMMENDATIONS

Based on the foregoing results the following studies are recommended:

1- To further investigates the antimicrobial properties in a group of medicinal plants and fungicide score against targets organism to determine their potentials as pesticide.

2- Further research may be needed to look into on-field trial of the garlic ssp. Before embarking on large scale production of the active concentrations as bio pesticide

3- 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 *Nattrassia mangiferae.*

REFERENCES

Abbasher, A. Abbasher, mgjdolin M. Husiam, Mohamed S. Zaraug, and Eldur B. Zahram (2013). Prevalence and Etiology of Branch wilt Disease (*Nattrassiamangiferea*) 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 home page: http:// journal. Sustech. Edu.

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

Ahmed, N. E.andyassin, A. M. (1992). An epidemic wilts disease effect on date plam in nnorthern Sudan. Technical Bulletin No. 5 Agricultural Research Corporation (ARC), wed medani Sudan.

Calavan, E. C. and Wallace (1949).Exporine Branch wilt. Of grapefruit in Southern California: Phyto pathology.

Calavan, E.C. and Wallac J. M.(1954). TendersonulaToruloideaNattrass on Citrus in California. Phyto pathology. 44: 635- 639.

Davision, A. D. (1972). Factor Affectin Development of Madrone conkerPL.Dis. Reptr. 56 (1): 50-50.

Elliot, M. and Edmonds R. L. (2003). Systematical study of Nattrassiamangiferae the cause of madroneconker, WIFDWC 51:59-62.

Elliot, M. and Edmonds, R. L. (2008). Injected treatments for management of matron's conker. Arboriculture and urban forestry, 34(2):110-115.

Elliott, M., Edmonds, R. L. and Mayer, S. (2002).Role of fungal disease in decline of pacific madrone. North West science 76: 293- 303.

Elliott. M. and Edmonds, R. L. (2004). Systematic study of *Nattrassiamangiferae*, the cause of the modroneconker. In: proceedings of the 51 western international forest Disease work conference; (2003) August 18-22(B. W. Gil Ed).Pp 59. 62. Granter pass, OR. Flagstaff, A Z: U. S. Department of Agriculture, forest service, Rocky Mountain Research station.

Ellis, M. D. (1971).Demotic cause, Hyphmyects. CAB, CM, Kew, surrey England.

Elshafie, M. E. Ali, O. M. M (2005). First report of ficusbengahalnsis dieback by scytalidiumdimidialum in Sudan. Cab abstracts.

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

Food and Agriculture organization of the united Nations, FAO (2007) Working paper FBS/32 E Forest related heath and biosecurty overview of forest pests, Rome, Italy.

Giha, O. H. (1976). Hendersonalatoruloideaassociated with as serious with disease of shade trees in Sudan. Plant Disease Reporter 59:52:52

Giha, O. H. (1996). Introduction in plant pathology. First Edition, published: Dar EL. Assaala for journalism and publication, Khartoum, Sudan.

Hassan, W. A., Haleem, R. A., Hassan, P. H. (2011).Effect of Heat. Stress predis potion on Development of sooty canker caused by Neoscytalioliumdimidiatum (penz) Corus and slipper ACTA Agrobotaic 64(4); 207-212.

Islam, S. M. A, L. Hossain, G. A. fakir and M. Ased. Ud. Daullah (2001). Effect of physical sorting, seed treatment with garlic and vitavax.200 on seed borne fungal floral and seed yield of jute (*corchoruscapsularis* L).Parkistan journal of Biological information. 4(12): 1509-1511.

Mirzaee, M. R., Monommedi, M.; and Radiom, H. (2002).*Nattrassiamangiferae*, the cause of dieback and trunk cankers of *ficusreligiosa* and branch with of pisidiumguajava in Iran. Phyopathology 150: 244-247.

Olsen, M. W; (1998).sooty comker in Horticulture Crop. The University of Afrizona. College of Agriculture plant disease management. Plant Sci 19-4.

Paxton JD, Wilson EE and Davis JR.(1964). Branch wilts disease of fig caused by HendersonulaToruloidea. Plant Dis Rep.48: P142.

Punithalingan E. and Waterston J. M. (1970). C M I.descriptions of pathogenic fungi and bacteria no. 274 HendersonulaToruloidea. Commonwealths mycological institute, Kew, United Kingdom.

Sadeghya B and N. Hatamia N. (2013). Bioactivity of soil. Borne *streptomyces* sp. Against *N. magnifier* Department of Agriculture, payomnoor university (PNU) of Kerman ,Iran. Published online: 02 sep (2013).

Sangoyomi (2002) TE and find all citations by this anther (default). Or filter your weren't search EKPO EJA. (2002). First report of *Nattarassiamangiferae as* a postharves*t* fungal pathogen of white yam (Dioscorearotundata) in Nigeria. Plant Disease. 86 (8): Pg 919.

Sangoyomi, T. E. (2004). Post-Harvest fungal deterioration of yam (Discorearatundata) and its control. PhD thesis, university of Ibadan Nigeria .180 pp.

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

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

Themis J.; Michailides, D. P.; Morgan, D and Retest, H.(2005). Etiology and management of limb Dieback of figs in California. Project report, (2005).

Tsahourion, P. C. and thanassulopoulos, C. C. (2000). FIST REPORT OF Hander sonata toruloiea as foliar pathogen of straw harry –tree (Arbutus unwed) in Europe. Plant Disease 84: p 487.8

Tucker, Arthur, O. and Thomas pebagio, (2000). the big book of herbs: a comprehensive illustrated reference to herbs of flavor and fragrance. Loveland, CO: Inter weare press. (HAS Library)

U S. Department of Agriculture, March. (2015). Agricultural Research service systemic Mycology and Microbiology Laboratory - Nomenclature.

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

Willinger B, KopetZky G, Harm F, A falter P, Makristathis A, Barer A, Bankier A and winkle S. (2004) Disseminate infection with *nattrassiamangiferea* in an Immune suppressed patient. J. clinic Microbial. 42(1): 478-80.

Zine EL. Abidine, A., Baissac; Y; Moukhi, A., Jay- Allemand, C., khadari, B, E L Modfar, C. (2010). Resistance of olive – tree to *spiloceaoleagina*is mediated by the synthesis of phenolic

International journal biology compounds .into. J. Biol .12: 61-67