



بسم الله الرحمن الرحيم

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College of Agriculture Studies

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The Antifungal Activity of Garlic Extracts against *Nattrassia mangiferae*

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

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INCEPTION

الآية

بسم الله الرحمن الرحيم

(وَاضْرِبْ لَهُمْ مَثَلِ الْحَيَاةِ الدُّنْيَا كَمَا أَنْزَلْنَا مِنْ السَّمَاءِ فَاخْتَلَطَ بِهِ نَبَاتُ الْأَرْضِ فَأَصْبَحَ هَشِيمًا تَذْرُوهُ الرِّيَّاحُ وَكَانَ اللَّهُ عَلَى كُلِّ شَيْءٍ مُقْتَدِرًا)

صدق الله العظيم

سورة الكهف الآية (45)

DEDICATION

To my father

And mother

To my brothers, Sisters,

And to my friend Aziza,

Madiha and Mohammed Younis

ACKNOWLEDGEMENT

Firstly, thanks to God for giving me health and kept me well to finish this work.

Grateful thanks are due to my supervisor Dr. EKHLASS HUSSIEN for mentoring me and participation throughout the study

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List of contents

Thesis.....	I
Inception.....	II
Dedication.....	III
Acknowledgment	IV
List of contents.....	V
List of tables	VI
List of figures.....	VIII
Abstract.....	IX
Arabic abstract	X
CHAPTER ONE	
1.1. Introduction.....	1-2
CHAPTER TWO	
LITRATUREREVIEW	
2.1. <i>Neofusicoccum mangiferae</i>.....	3
2.1.1 Classification.....	3
2.1.2 Synonyms.....	4
2.1.3 Biology.....	4-5
2.1.4 Life cycle.....	5

2.1.5 Epidemiology.....	5
2.1.6 Distribution and host Range in Sudan.....	5-6
2.1.7 Symptom.....	6
2.1.8 Effect on the Human.....	6
2.2 control Measures.....	7
2.2.1. Chemical Control.....	7
2.2.2. Cultural practices.....	7
2.2.3. Bio-control.....	7
2.2.4. Resistance of <i>N. mangiferae</i>.....	8
2.3 Botanical extract.....	8
2.3.1 Garlic (<i>Allium sativum</i>).....	8
2.3.1.1 Scientific classification.....	8
3.2.1.2 Uses.....	8-9
2.3.2.2 Origin and Distribution.....	9
2.3.2.5 Uses to control plant disease.....	9
2.4.1 Uses.....	10

CHAPTER THREE

MATERIAL AND METHODS

3.1. Location of the study.....	11
3.2. Collection of infected plant material.....	11
3.3. Collection of botanical and chemicals, materials	11
3.4. Isolation characterization and identification of <i>Neofusicoccum mangifera</i>	12
3.5. Preparation of fungicide concentrations.....	13
3.6. Test procedure.....	13-14
3.7. Experimental design	14
3.8. Statistical analyses.....	14

CHAPTER FOUR

RESULTS

4.1 Identification of the pathogen <i>Neofusicoccum mangiferin vi</i>	15
4.2 The Effects of Garlic extractsand Fungicide on the liner growth of <i>N. mangiferin vitro</i> two days afterincubation.....	15
4.3 The Effects of Garlic extractsand Fungicide on the liner growth of <i>N. mangiferin vitro</i> four days afterincubation.....	17
4.4 The Effects of Garlic extractsand Fungicide on the liner growth of <i>N.mangiferin vitro</i> six days afterincubation.....	17

CHAPTER FIVE

5.1. Discussion	18-20
5.2. Conclusion.....	21
5.3. Recommendation	22
5.4. References	23-31

ABSTRACT

Nattrassia mangiferae (Neofusicoccum) considered as one of most important disease of forest and horticulture trees in worldwide and 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 leaves extracts of bulbs of garlic plant, and efficacy of fungicide (score) on growth, of the fungus *nattrassia 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 البحث

يعتبر مرض النتراسيا (نيوفزكوكم مانقفيرا) من اهم امراض الاشجار الغابيه و البستانيه في العالم .

اجريت هذه الدراسه تحت ظروف المختبر بقسم وقايه النبات (معمل امراض النبات) كلية الدراسات الزراعيه ' جامعه السودان للعلوم و التكنولوجيا (شمبات) لدراسه تاثير المستخلص الكحولي لبصيليات نبات الثوم و المبيد الفطري (سكور) علي نمو فطر نتراسيا مانقفيرا . استخدمت ثلاث تراكيز من المستخلص الكحولي للثوم و المبيد 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 . علي اي حال كلتي المبيد الفطري و مستخلص الثوم كانا اكثر وضوحا ضد الفطر المختبر . التركيز الاعلي في كل من المستخلص الكحولي للثوم (100%) اعطه اعلي نسبه تثبيط مقارنة بالشاهد . فيما بين المستخلص النباتي الكحولي للثوم دئما الاكثر فعاليه في تثبيط نمو الفطر من المبيد . عموما اظهرت النتائج ان الفعاليه ضد الفطر تزداد بزياده تركيز المستخلص .

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 Colavan 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 mangiferae*, *Exosporina fawcettii*, *Fusicoccum eucalypti*, *Hendersonulacypria*, *H. agathidis*, and *H. torulaidea*. The synonym is known by the name *scytalidium dimidiatum*, also *toruladimidiata*, and *S. lignicola*. More recently this species has been reclassified into *mangiferae* (Elliott and Edmonds, 2004). The fungus *Neofusicoccum* is a polyphagous 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 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 (*Arbutus unedo*) in Europe (Tsaouridou and Thanassoulopoulos, 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 *ficusbenghalensis* 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 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 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. *Dothiorella mangiferae* Syd, and Syunder (1916).

Fusicoccum eucalypti Sousa da Camara,(1929)

Hendersonu latoruloidea Nattrass(1933)

Hendersonu lacypria Nattrass (1937)

Exosporina fawcettii Wilson,[as fawcetti] (1947)

Hendersonu laagathidis Young, [as agathi], (1948)

Scyalidiumlig nicolapesante [as lignicolum] (1957)

Toruladimi datepenz (1887)

Nattrassia mangiferae (Syd. and p syd.)Sutton and Dyko (1989)

Scytalidium dimidiatum (penz.)Sutton , B. and Dyko (1989)

Fusicoccum dimidiatum (Penz.) Farr, D .F. (2005)

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 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. 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 *Ficus* spp, 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*, *Eucalypusca maldulensis* and *Khayase neglensis* (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 sweet 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 *Hendersonul atoruloidea* which cause onychomycosis (nail infection) and superficial skin infection especially in tropical region. *Scytidium 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. mangifera et 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 *Trichoderma spp* 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 (Elliot and 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 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*, *aspergillusniger*, *Sclerotium rolfsii* *Rhizoctonia solani* and *Neofusicoccum mangiferae* fungal pathogen in yam storage.

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 insects pest control, the product is used repeatedly to kill fleas, ants and other insect pest (GUN, 2007). In study by prasannath and Mahendran (2013), the results that potassium or sodium bicarbonate showed high inhibitory effect on the mycelia growth of *A. solani*, where complete inhibition was obtained .

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 (Score Ec250) /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 indica*) 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. Collection of botanical and chemicals, materials

Garlic 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.

3.4. 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 Rinsed three times in

sterilized distal water then dried on sterilized filter paper the sterilized stem and leaves sections were then plated at the rate of 5 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 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 is *Neofusicoccum 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 from 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% middle 25 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 required concentration from garlic and fungicide revues before being solidified in a 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 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 An 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 mycelial growtM1 = mycelial growth in central petri dishes without extract fungicide, M2 = mycelial growth in extract fungicide petri dishes.

3.7. Experimental design

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

3.8. Statistical analyses

The obtained data was statistically analyzed by computer software Statistix 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 of this 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* from mango 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 extracts and Fungicide on the linear growth of *N. mangiferin vitro* two days after incubation.

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%).

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

Treatments	Concentration (%)	Inhibition zone (%)		
		2 th day	4 th day	6 th 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 (Score)		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.

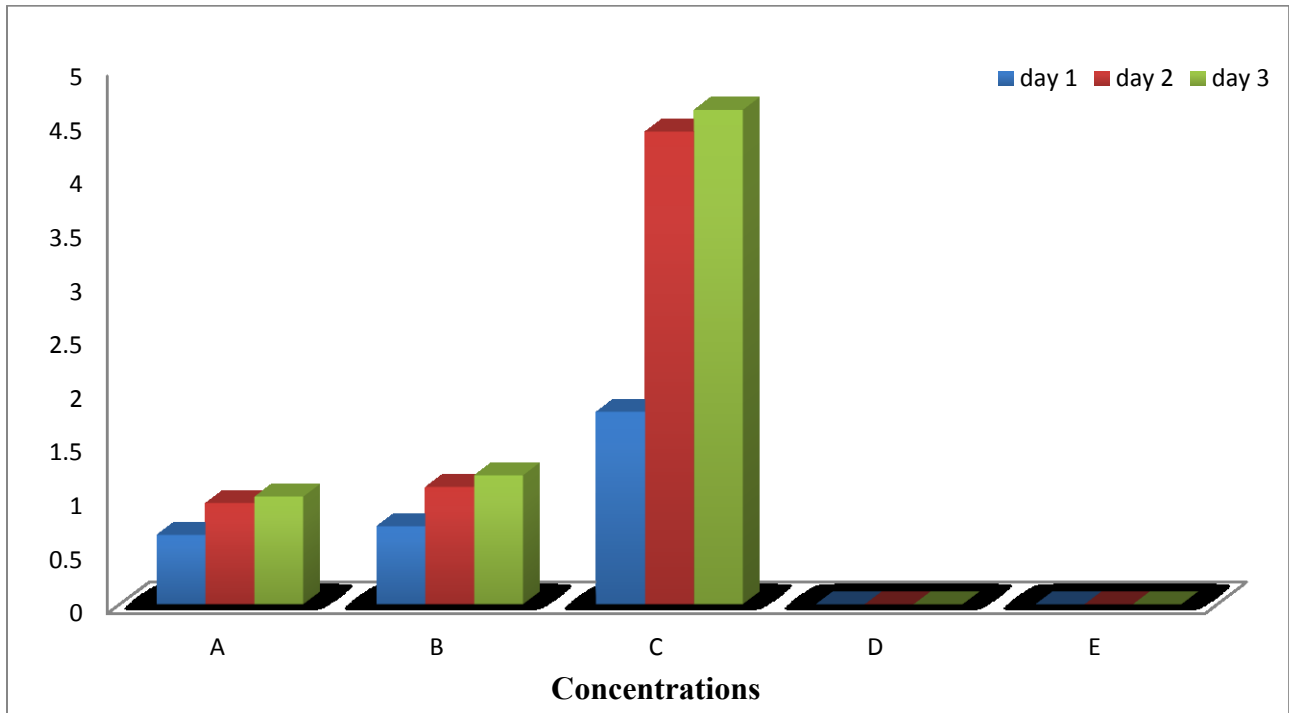


Fig. 1 Effects of aqueous extract of Garlic and fungicide (Score) on the linear growth of *Neofusicoccum mangiferin vitro*.

4.3 The Effects of Garlic extracts and Fungicide on the linear growth of *N. mangifer* in vitro four days after incubation.

Four days after incubation, all concentration of garlic extracts as well as 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 extracts and Fungicide on the linear growth of *N. mangifer* in vitro six days after incubation.

Six days after incubation the 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

Nattrassia mangiferae (*Neofusicoccum*) is cosmopolitan and polyphagous 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 April 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 plants which is 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, terpenoids, phenols and other secondary metabolites 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 pesticide (Sharma and Meshram, 2006).

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*, *Aspergillu sniger*, *Sclerotiumrolgsii*, *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 thatwhich 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 demonstrated that the fungicide Score completely inhibited *Nattrassia mangiferae* at 200PPm and 100PPm.

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 *Neofuscoaccum mangiferaeas* described by Giha(1996); (Sutton and Dyco, 1989) and Abbasher, *et al.*, (2013).

The crude alcoholic extract of garlic and fungicide score top at all concentrations exhibiting 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 investigate the antimicrobial properties in a group of medicinal plants and fungicide score against target 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 *Nattractia mangiferae*.

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