EVALUATION OF SOME PLANT EXTRACTS FOR THE CONTROL OF EARLY BLIGHT OF TOMATO INDUCED BY *Alternaria solani* (Ell. and Mart.) IN YOLA, ADAMAWA STATE

BY

DASO, Ibrahim Bayaso B. Tech. (Agric.), FUT, Yola

(M. TECH. /CP/07/0404)

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Title Page

Evaluation of Some Plant Extracts for the Control of Early Blight of Tomato induced by *Alternaria solani* (Ell. and Mart.) in Yola, Adamawa State

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DASO, Ibrahim Bayaso B. Tech. (Agric.), FUT, Yola

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M. Tech. Thesis Submitted to the Department of Crop Production and Horticulture, School of Agriculture and Agricultural Technology, Federal University of Technology Yola in partial fulfillment of the Requirements for the Award of Master of Technology (M. Tech) Degree in Crop Protection.

October, 2010

DECLARATION

I declare that this work was carried out in its original form by DASO, Ibrahim Bayaso of the Department of Crop Production and Horticulture, Federal University of Technology Yola, Nigeria.

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Sign Date

APPROVAL PAGE

We certify that the thesis "Evaluation of Some Plant Extract for the Control of Early Blight of Tomato induced by *Alternaria solani* (Ell. and Mart.) in Yola, Adamawa State" was carried out by DASO, Ibrahim Bayaso in partial fulfillment of the requirements for the award of Master of Technology (M.Tech.) Degree in Crop Protection, Federal University of Technology, Yola for its contribution to knowledge and literary presentation.

---------------------- ----------------
Dr. Hycenth Nahunnaro **Date**
(Supervisor)

---------------------- ----------------
Dr. (Mrs.) A.M. Malgwi **Date**
(Internal Examiner)

---------------------- ----------------
Prof. D.M. Gwary **Date**
(External Examiner)

---------------------- ----------------
Dr. B. B. Jakusko **Date**
(Head of Department)

---------------------- ----------------
Prof. A. Nur **Date**
(Dean, School of Postgraduate Studies)

DEDICATION

This work is dedicated to my parents Mr. and Mrs. Bayaso Daso

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ABSTRACT

Tomato production in Nigeria is principally constrained by several factors such as late blight, Fusarium wilt, damping-off and the early blight induced by *Alternaria solani*. To ameliorate these problems the use of synthetic pesticides was adopted which has resulted in increased crop production, however, these chemicals are hazardous to man and the environment. It is in this regard that the search for safer alternative is now being made. This experiment on evaluation of some plant extracts for the control of early blight of tomato was carried out in January 2008. The study consisted of two experiments conducted in the Laboratory of the Crop Production and Horticulture Department and the Landscape Unit of the Federal University of Technology, Yola. The objectives of the study were to evaluate the effectiveness of some plant extracts in the control of early blight pathogen induced by *Alternaria solani* (Ell. and Mart.) and also to determine which concentration of the extracts is most effective. The laboratory experiment was laid out using a Completely Randomized Design while the potted experiment was laid using a Randomized Complete Block Design. Five plant extracts of *Ageratum conyzoides, Chromolaena odorata, Cleome viscosa, Ricinus communis and Eucalyptus camaldulensis* were evaluated at three (3) concentrations (25 %, 50 % and 100 %) and replicated three times, to make a total of sixteen treatments including the control. The laboratory experiment involved isolation and identification of the early blight pathogen and evaluation of the extracts in the control of the early blight of tomato. Data collected were on fungal biomass, radial growth and percent inhibition. While in the potted experiment, data were collected on plant establishment, plant height, percentage disease severity, number of fruits and fruits weight as well as days to 95 % flowering. The data collected were subjected to analysis of variance using SAS Statistical package (1999) and treatment means were separated using Duncan's Multiple Range Test (DMRT). The result revealed that the plant extracts had effect on fungal biomass at high concentration as revealed by the fungal biomass recorded in *Cleome viscosa* 100 %, at 72 and 96 hours after inoculation in the combined result with 0.021 g and 0.016 g respectively. However, at 120 hours *Chromolaena odorata* 25 % concentration recorded the highest mean (0.011 g). The lowest fungal biomass (0.004 g) recorded in the control could be attributed to the pathogen using up the medium without inhibition and reaching its death phase (lysis) earlier than those in the plant extracts. The radial growth result also revealed that *Ricinus communis* at 100 % concentration recorded the lowest radial growth at 24, 48 and 72 hours respectively in the first, second and combined results. The combined results further revealed that the lowest radial growth 1.43 cm, 2.00 cm and 2.72 cm were recorded in *Ricinus communis* treatment at 24, 48 and 72 hours respectively. The potted experiment result showed that the lowest percentage disease severity at 6 WAT was recorded in *Chromolaena odorata* 100 % with 39 % severity. Also *Chromolaena odorata* 100 % had the highest fruit weight (14.92 g) and the least percentage number of infected fruits; while the control had the highest percentage number of infected fruits with 71 %. It was then concluded that the various plant extracts used at different concentrations tested on *Alternaria solani* showed promising prospects for plant disease control and has potential for utilization as ecofriendly botanical fungicides. It is hereby recommended that *Ricinus communis* 100 % and *Chromolaena odorata* 100 % should be put to further actual field trial to evaluate their effectiveness in the control of early blight.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AVRDC	Asian Vegetable Research and Development Centre
Br	Fungal biomass
Ca	Calcium
Cm	Centimeter
CRD	Completely randomized design
CSU	Columbia State University
DAI	Days After Inoculation
DMRT	Duncan's Multiple Range Test
E	East
EC	Emulsifiable concentrate
et al	And others
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
Fig	Figure
G	Gram
HCl	Hydrochloric acid
Kg	Kilogramme
Kr	Radial growth
L	Linnaeus
M	Metre
Ml	Milliliter
Mm	Millimeter
MT	Metric tonnes
N	North
^{O}C	Degrees Celsius
P	Potassium
PDA	Potato Dextrose Agar
pH	Hydrogen ion concentration
Ppm	Part per million
R_0	Final radius
R_1	Initial radius

RCBD	Randomized complete block design
SAMCOT	Samaru Cotton
SAS	Statistical Analysis System
T_0	Final time
T_1	Initial time
USA	United States of America
USDA	United State Department of Agriculture
W_0	Final weight
W_1	Initial weight
WAS	Weeks after sowing
WAT	Weeks after transplanting
%	Percentage
µm	Micro metre

CHAPTER ONE

1.0 INTRODUCTION

Tomato (*Lycopersicon lycopersicum* L. Mill., Karst.), originated from Western South America from there it spread to other parts of the world, and then to Central America. The plant was probably domesticated in Mexico and from there that it was taken to Europe by the Spanish explorers in the early 16th century (Hobson and Davies, 1971). *Lycopersicon lycopersicum* belongs to the family of *solanaceae*. It thrives well where there are long sunny periods with evenly distributed rainfall and temperatures between (32.4 - 64.8 ^{o}C) (Peet, 2003). Tomato can be grown both on high altitudes and at sea level, but it does better on high latitudes. The tomato plant requires light, free, well drained loamy soils with pH range 5-7. Nevertheless, tomato can be grown on a wide variety of soils (Purseglove, 1974). China is the largest producer of tomatoes in the world, followed by the USA, Italy and Turkey (USDA, 2003). World tomato production is mainly concentrated in the Mediterranean Sea area, North America, China, Australia, Japan, South Africa, Argentina, and Chile; while in Africa the leading producer is South Africa (USDA, 2003). World tomato production in 2001 was almost about 105 million tonnes of the fresh fruit from an estimate 3.9 million hectares (Shankara *et al.*, 2005). It is economically attractive and the area under cultivation is increasing daily.

Tomato (*L. lycopersicum* L. Mill., Karst) is a good source of vitamin A, B, C and D, minerals, Ca, P and Fe (Tindall, 1983). Tomato is used for a variety of purposes one of which is salad as well as processed products like tomato sauce, pickle, ketchup, puree, dehydrated and whole peeled tomatoes. However, tomato that is produced seem to be inadequate due to several factors such as insect pests, and pathogens e.g. nematodes which has caused great damage to tomato production (Villareal, 1980). Peet (2003) reported that tomato is attacked by many diseases which constitute a serious set back to its production. Some of these diseases include; early blight (*Alternaria solani*); late blight (*Phytophthora infestans*); damping off (*Phythium solanacerum*); bacterial wilt (*Burkholderia solanacearum*); fungal wilt (*Fusarium oxysporum*);

and nematode (*Meloidogyne javanica*). In addition Villareal (1980), reported that some of these diseases that affect tomato on the field are carried over even after harvest.

The attempt by man to improve crop yield in order to produce enough food for consumption in the face of the increasing population is a decision in the right direction although it is being hampered by many constraints (Amusa *et al.,* 1994). The most important and interesting problem encountered by scientist in this attempts, is how to drastically reduce or wholly prevent plant diseases. Even though, there is increasing armoury of weapons such as resistant varieties and chemicals to control the diseases, the control has becomes a continual battle because the attack on the crop by these enemies occur when least expected (Akinbode and Ikotun, 2008).

Efforts have been made by researchers to discover new antimicrobial compounds from various sources such as micro-organisms, animals and plants and one of such resources is folk medicines (Okereke and Wokocha, 2007). Hence, systematic screening of plants has resulted in the discovery of novel effective compounds (Tomoko *et al.*, 2002). The increasing prevalence of resistant new strains of bacteria, fungi and the recent appearance of strains has reduced susceptibility to chemical control and raises the specter of bacterial and fungal infections which consequently results in search for new disease-fighting strategies (Sieradski *et al.*. 1999). Furthermore, it has been reported that more than one hundred species of plant pathogens have become resistant to fungicides while some resistant varieties have become susceptible (Zitter *et al.,* 2005). The effect of these pathogens with resistant traits now has negative consequences on the crop producer. *Alternaria solani* has in no small measure contributed to the reduction in the yield of tomato both in the field and after harvest (Damicone *et al.*, 1986). Yield losses of up to 79 % have been reported due to early blight damage from Canada, India, USA, and Nigeria (Sherf and MacNab 1986; Gwary and Nahunnaro, 1998).

Several attempts have been made to control early blight of tomato through cultural, physical, chemical, and biological methods (Adekunle *et al.,* 2001). However, because the early blight fungi of tomato have very large host range, cultural control method have been advocated and is the most widely acceptable means of controlling the disease as one species has many hosts (Amusa *et al.,* 1994). More so, absence of durable resistance of tomato cultivars in the field is a major problem, which has been attributed to the high levels of virulence in pathogen populations (Ghaffer, 1988). Another limitation stems from the fungicides themselves when they are used intensively, they place enormous selection pressure on the fungi, and the pathogens rapidly develop resistance (Alabi *et al.,* 2005). In the recent past control of plant-parasites, essentially, involves the use of synthetic pesticides. However, apart from its very high cost, indiscriminate and unsafe use, increased concern for the environment and the inherent danger pose to man and his livestock calls for caution in its utilization (Adegbite and Adesiyan, 2005).

1.1 Objectives of the Study

Consequently, it becomes desirable to search for alternatives, such as plants extracts to ascertain their effectiveness in the control of early blight of tomato. Hence, the study was aimed at identifying plant extracts that would control early blight of tomato.

This was intended to be achieved through the following objectives:

(i) To determine the effectiveness of some plant extracts in the control of early blight of tomato induced by *Alternaria solani in vitro* and *in vivo,* and,

(ii) To determine the most effective concentration of the plant extracts in controlling the pathogen.

(iii) To recommended the most effective plant extracts and concentrations for control of early blight of tomato in Yola, Adamawa State.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of the Early Blight of Tomato

Early blight disease is caused by the fungus *Alternaria solani* (Ell. and Mart.) and the symptoms are usually observed on the plants as small, black lesions mostly on the older foliage. The fungus belongs to the Phylum: Ascomycota, Class: Euascomycetes, Order: Pleosporales, Family: Pleosporaceae and Genus: *Alternaria* (Doctorfungus, 2007). The early blight fungus is transmitted through seeds (seedborne) and through soil. The fungi colonize the seed coat during the seed development stage and when the seed germinates, they become active (Cornell University, 1995). Similarly, the Asian Vegetable Research and Development Council, AVRDC (2005) reported that the fungal disease causes damping-off and stunted seedling. Infection develops so slowly that a symptom only appears when the seedlings are transplanted in the field. The leaf spots vary in sizes from very tiny spots up to 5 cm in diameter. The spots begin as small yellow or brown spots that slowly enlarge to about 5 cm in diameter, dark colored spots with concentric rings (AVRDC, 2005). When all the spots join together, the leaves will turn yellowish or blackish and drop off. Alternari*a* stem canker (lesion) on tomato appears on stems, leaves, and fruits. An infected stem near the soil line has dark-brown to black cankers with concentric rings. AVRDC (2005) reported that when the lesions enlarge, they girdle the stem and eventually kill the plant. Fruits that are harvested from infested plants have brown or black necrotic sunken (submerged) lesions.

While Damicone *et al.* (1986) stated that the conditions that favor development are infected seeds and transplanting materials, presence of alternate hosts like weeds, infected plant debris that are left in the field to rot, cool and wet weather conditions with high relative humidity.

2.2 The use of plant extracts in the control of fungal diseases of crops

The hazards to human health through intake of pesticides residues in food stuff, potential poisoning of users during application and ecological hazards has necessitate the search for alternatives and to minimize use of pesticides. Several reports have indicated that there is a worldwide distribution of plants with fungicidal and fungistatic properties which may have the potential of being used in plant disease control (Kaitisha, 1995). Bhowmick and Vardhan (1982) have evaluated the antimycotic activity of leaf extracts of some medicinal plants on *Dreschslera turcica* (Pars) and observed that extracts from *Vitex negundo* and *Catharanthus roseus* can completely inhibit the growth of the fungus *in-vitro.* Similarly, Neem (*Azadirachta indica*) oil and *Chromolaena odorata* preparations have been reported to have inhibited growth of *Alternaria alternata* by 61.1 and 100 % at a concentration of 1 and 10 % respectively (Dharam and Sharma, 1985). While *Ryania speciosa*, family Flocourtiaceae, prevalent in Northern parts of South America and the Amazon basin are known to be effective against maize smut and stalkborers when dried roots and leaves powder where use in treating maize plants (Stoll, 1986).

In Japan, extract of *Portulaca oleracea* L. show activity against the Japanese pear pathotype of *Alternaria alternata in vitro* and the antifungal substances determined from the extracts are isobutyric acid, butyric acid, isovaleric acid, valeric acid and caproic acid (Park *et al.*, 1986). Spraying rice leaves with extracts from *Lawsonia inermis* is also reported to give better control of *Dreschslera oryzae* than seed treatment without extracts as reported by Natarajan and Lalithakumari (1987). *Alternaria alternata* and *Fusarium oxysporum* are also inhibited by extracts of young and mature leaves of *Codieum variegatum* with the extracts of young leaves being more active against *A. alternata* and the old leaves against *F. oxysporum* (Naidu, 1988). Manoharachary and Gourinath (1988) have determined the efficacy of some tropical plant extracts against four pathogenic fungi. Plant extracts from roots, stems, leaves, flowers and fruits of one hundred plants belonging to 37 families were screened for fungitoxicity against *Curvularia lunata* (Wakker) Boedijn, *Cylindrocarpon lichenicola* (*C. maassal*) Hawksworth, *Fusarium solani* (Mart sacc.) and *Myrothecium leucotrichum* (peck) Tulloch. The effect of the plant water extracts was evaluated by using a spore germination test which involved an evaluation of growth and sporulation of the fungi. Plants tested included *Calatropis, Datura,* *Ocimum*, *Ricinus* and *Tridax* species. Among the plant parts tested, extracts of roots and flowers were found to be more effective followed by leaf extracts.

Bandara *et al.* (1989a) at the University of Peradeniya in Sri Lanka also evaluated three rhizomatous perennial herbs used in native medicine for their antifungal and antibacterial properties. The herbs tested were: *Acorns calamus* (Araceae), *Zingiber zerumbet* and *Curcuma longa* (Zingiberaceae). He then evaluated the crude extracts of the rhizomes at various dilutions for their effect on growth and sporulation of *Cladosporium* sp., *Botryodiplodia theobromae*, *Fusarium solani*, *Phytophthora infestans, Pythium* sp. and *Pyricularia oryzae*. Their inhibitory action was compared to that of Benlate. Their findings revealed that extracts of *A. calamus* and *Z. zerumbet* had profound effect on growth of all fungi tested. It was particularly observed that the inhibition of growth of *F. solani* was significantly higher in *A. calamus* extract than Benlate, sporulation of *B. theobromae, F. solani*, *P. oryzae* was also inhibited. Bandara *et al.* (1989b) furthermore screened 36 medically used plant species in Sri Lanka for their activity against *Cladosporium cladosporioides*. Plant species whose extracts displayed significant activity were, *Butea monosperma* (stem bark), *Costus specious* (rhizome), *Curcuma zedoaria* (tuber), *Eupatorium riparium* (whole plant, root), *Pleisospermium alatum* (stembark, rootbark) and *Z. zerumbet* (tuber).

Mishra *et al.* (1989), have isolated essential oils from leaves of *Chenopodium ambrosioides, Cinnamomum zeylanicum, Citrus medica, Melaleuca lucadendron, Ocimum canum* and *O. grattissium.* Their findings revealed that these oils have demonstrated fungitoxicity against *Aspergillus flavus* at 200, 300, 400 and 500 ppm and most of them have shown to be more effective than synthetic fungicides viz; Agrosan, Copperoxychloride, Ceresan, Thiovit and Dithane M45. Asthana *et al*. (1986) also found that the leaf extract of *Ocimum adscendens* to be fungitoxic against *Aspergillus flavus.*

Adeleye and Ikotun (1989) of the Department of Agricultural Botany at the University of Ibadan have found that adding 0.1 % concentration of *Dioscorea bulbifera* L. extracted using HCl has some antifungal activity against five plant pathogenic fungi namely: *Sclerotium rolfsii*, *Curvularia lunata, Fusarium moniliforme, Macrophomina phaseolina* and *Botryodiplodia theobromae*. Wang *et al.* (1990) have been able to isolate antifungal and larvicidal polyacetylenes for *Artemisia borealis* (*B. campestris* subsp. *borealis*). Dichloromethane extracts for the whole plant have shown antifungal activity against *Cladosporium cucumerinum.* Work by Upadhyaya and Gupta (1990) have demonstrated the inhibitory effect of some medicinal plants on the growth of *Curvularia lunata* (*Cochliobolus lunatus*). Ethanol extracts of garlic followed by those of *Ocimum santum, Datura alba* and hemp were found to be most inhibitory to growth of the fungus. Aqueous extracts were less effective. Garlic extracts have shown to be inhibitory on the growth of a number of fungi (Tansey and Appleton, 1975).

From methanol extracts of twigs of *Oxymitra velutina* - a West African plant, 12 alkaloids; 5 aporphinoids including lysicamine, which is active against *Bacillus subtilis, Botrytis cinerea, Saprolegnia asterophora* and *Rhizoctonia solani*, have been isolated (Achenbach and Hemrich, 1991). Alternaria leaf blight one of the major diseases of pigeon pea (*Cajanus cajan*) an important legume was evaluated using *Tiliacora racemosa* for their antifungal activity against *Alternaria termissina* and was found to reduced the germination of the fungus at concentrations greater than 100 ppm (Tripathi and Dwivedi, 1989). Yegen *et al.* (1992) have studied the fungitoxic effect of extracts of six selected plants from Turkey. Results indicate that aqueous and essential oils of *Thymbra spicata, Satureja thymbra, Laura nobilis, Mentha spicata, Salvia futicasa* and *Inula viscosa* are fungitoxic to *Fusarium moniliforme, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Phytophthora capsici*.

2.3 Management of the Early Blight of tomato induced by *Alternaria solani*

This disease can be managed or prevented by ensuring proper seed selection and warm seed treatment, removing and destroying infected leaves and sometimes by ensuring application of fungicides (Damicone *et al.*, 1986). Other methods of management include:

2.3.1 Use of plant extracts

Akinmoladun *et al.* (2007) reported that the antifungal activities of *Ricinus communis* leaf extract, was due to the chemical contents of ricin and other active ingredients, showed inhibitory effect on *Fusarium oxysporum* and *Alternaria solani*. Similarly *Ageratum conyzoides* have been found to be effective in reducing the populations of soil pathogenic fungi *Phytophthora citrophthora, Pythium aphanidermatum, A. solani* and *Fusarium solani* (Amienyo and Ataga, 2007). Furthermore, Alabi *et al.* (2005) reported that *Eucalyptus globolus, Bryophyllum pinnatum* and *Ocimum gratissumum* have been use in the control of cowpea pathogen *Phythium alphanidermatum* and *Sclerotium rolfsii in vitro* and *in-vivo* in the field and laboratory.

2.3.2 Proper seed selection and treatment

Proper selection of seeds for sowing should be made so as to make sure that they are disease-free and not taken from plants that were previously infested by the early blight disease. In addition, it has been recommended that only clean seeds saved from disease-free plants are used or seeds should be treated in hot water (65 ^{O}C) for 45 minutes (Watt, 2004).

2.3.3 Crop rotation and farm hygiene

Tisserat (1993) reported that early blight control requires field sanitation measures after harvest to reduce the amount of inoculum available for infection the following year. Crop rotation should be practiced where fields should not be planted with tomato, potato, pepper, or eggplant for at least two cropping seasons so that these hosts are not present for the spores to thrive on. Proper crop rotation is important to ensure infected plant debris decomposes (Davies *et al.*, 2008). In addition, removal and destruction of crop residues at the end of the season is another management strategy. Where this is not practical, residues should be ploughed into the soil to promote breakdown by soil microorganisms and to physically remove the spore source from the soil surface. Volunteer tomato and potato plants and nightshades should be destroyed.

2.3.4 Application of fungicides

The preventive fungicide, chlorothalonil (Bravo) has been used on a seven to ten day schedule and has been reported to give good control in California (Davies *et al.,* 2008). Generally, leaf spots start developing around the time of the first blossom set. Fungicides such as Bordeaux mixture, mancozeb and chlorothalonil are recommended for the control of early blight (CSU, 2002).

2.3.5 Spacing and Trellising Plants

CSU (2002) reported that spacing and trellising plants to allow for good air circulation promotes rapid drying of leaves and hence reduce disease infection. Research has demonstrated that crowding plants will not increase yields, but does increase disease problems. The American Society for Horticultural Science recommends trellising of tomato plants. Trellising also increases the distance of the upper leaves from the sources of inoculum on the soil and lower leaves.

2.3.6 The use of mulch

Mulching has also been reported to help in reducing early blight incidence on tomato (Davies *et al.,* 2008). Black plastic polyethylene mulch has been reported to help protect the plant from inoculum splashing from the soil onto lower leaves. In addition, removing leaves in the lower 8 to 12 inches (20 to 30 cm) of the plant (as the plant grows) also helps protect lower leaves from infections splashing from the soil (Watt, 2004).

2.3.7 Sprinkler method of irrigation

Overhead sprinkling or irrigation on tomatoes has been associated with high incidence of early blight (Davies *et al.,* 2008). Fungal spores are easily splashed from one leaf to another during irrigation and the pathogen requires water on the plant surface to cause infections. It is advisable to water in the morning in order that plants dry quickly. Plants that remain wet all night from evening watering are prime targets for disease infection (CSU, 2002).

2.3.8 Fertilizer application

A mid-summer loss of plant vigour from inadequate moisture or fertilizer will leave the plant more susceptible to the early blight fungi (Tisserat, 1993). Research has indicated that early blight frequently explodes due to low nitrogen levels in mid to late summer (CSU, 2002). It is therefore recommended that tomatoes should be lightly fertilized as the first fruits reach two inches in diameter. Additional monthly applications may also he helpful (every two weeks on a sandy soil). However, heavy applications of nitrogen should be avoided as that can over-stimulate vine growth at the expense of fruiting and prompt blossom end rot (CSU, 2002).

2.3.9 Removal of infected leaves

Removal of infected leaves as soon as they are noticed, washing of hands with soap and water immediately after touching diseased leaves to prevent spreading spores to other plants, and avoiding of working with the plants when they are wet are some of sanitation practices recommended (CSU, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Experimental Sites

This study consisted of two (2) experiments. The first experiment was carried out in the Laboratory of the Crop Production and Horticulture Department and the second (potted) was done at the Landscape unit, Federal University of Technology, Yola, Adamawa state. Yola, Adamawa state lies between latitude 8^{o} N and 11^{o} N and longitude 11.5^{o} E and 13.5^{o} E (Adamawa State Government Diary, 2008). Adebayo, (1999) reported that the state is located at an altitude of 185.9 m above sea level and lies within the Northern Guinea Savannah zone of Nigeria.

3.2 Experimental Design and Layout

The experiment conducted in the laboratory was laid using a Completely Randomized Design (CRD). Five plants whose extracts were used include Billy Goatweed (*Ageratum conyzoides),* Siam weed *(Chromolaena odorata),* Asian spiderflower or wild mustard *(Cleome viscosa),* Castorbean plant *(Ricinus communis) and* Eucalyptus (*Eucalyptus camaldulensis*) (Plates 1a-1e). Three (3) concentrations each of these extracts and a control to give a total of sixteen (16) treatments were used. The concentrations were *A. conyzoides, C. odorata, C. viscosa, R. communis and E. camaldulensis* at 25 *%*, 50 *%* and 100 *%* each. The potted experiment was carried out using the same plant extracts as was used in the laboratory experiment, and was laid using a Randomized Complete Block Design (Fig 1). Both experiments were replicated three times. The chemical compositions of the five plants extracts used in the study are contained in Appendix 1.

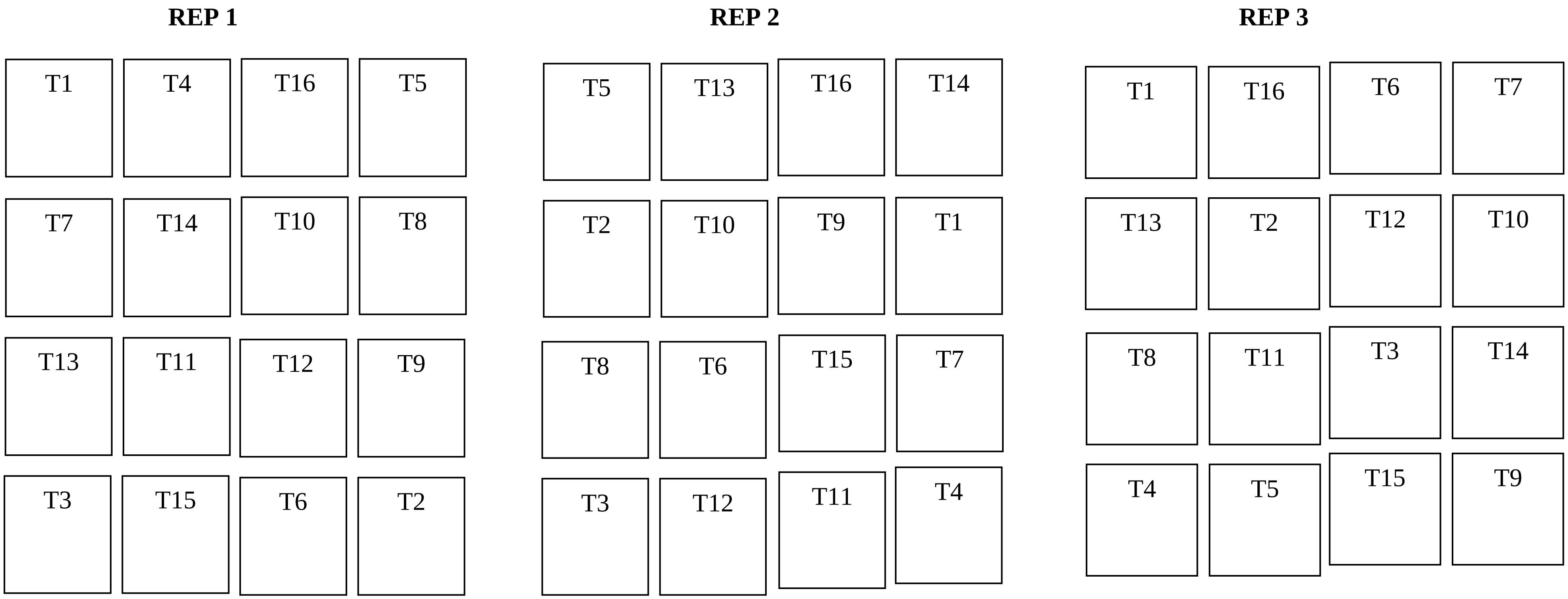
Plate 1a: *Chromolaena odorata*

Plate 1b: *Cleome viscosa*

Plate 1c: *Ricinus communis*

Plate 1d: *Eucalyptus camaldulensis*

Plate 1e: *Ageratum conyzoides*



Key:

T1 = *A. conyzoides 25%*	**T5**= *C. odorata 50%*	**T9**= *C. viscosa 100%*	**T13**= *E. camaldulensis* 25%
T2 = *A. conyzoides 50%*	**T6**= *C. odorata 100%*	**T10** = *R. communis 25%*	**T14**= *E. camaldulensis* 50%
T3 = *A. conyzoides 100%*	**T7** = *C. viscosa 25%*	**T11** = *R. communis 50%*	**T15**= *E. camaldulensis* 100%
T4= *C. odorata 25%*	**T8** = *C. viscosa 50%*	**T12** = *R. communis 100%*	**T16**= No extracts (Control)

Fig. 1: Experimental Layout and Randomization of treatments

3.3 Collection of Plant Materials and Preparation of extracts

The plant materials; *Ageratum conyzoides, Chromolaena odorata, Cleome viscosa, Ricinus communis and Eucalyptus camaldulensis* were collected from within and around the Federal University of Technology, Yola. The plant materials collected were rinsed, washed with 10 % sodium hypochlorite (NaOCl), air dried and later packed in brown envelopes and oven-dried at 70 ^{o}C for 20 minutes according to Akinbode and Ikotun (2008). Thereafter the plant materials were ground using pestle and mortar, sieved using 40 mm sieve and 200 g of the respective plant powder was added into 500 ml of distilled water. The suspension was allowed to stand for 24 hours and the content was filtered using a muslin cloth and kept in glass bottles until needed. The different concentration were then prepared by taking 25 ml, 50 ml and 100 ml of the stock preparation and made up to 100 ml with distilled water to give 25 %, 50 % and 100 % concentrations respectively.

3.4 Source of tomato Seeds

The tomato seed for the experiment was UC82-B procured from the Premier Seeds Limited, Yola.

3.5 Isolation of *Alternaria solani*

Diseased leaves of tomato with the symptom of *Alternaria solani* were collected from farms within the University. A small piece from the advancing margin of a lesion on diseased leaf was cut with a sterile pair of scissors after sterilizing with 10 % sodium hypochlorite (Larone, 1995). The tissues were then washed thoroughly in several changes of sterile distilled water and placed aseptically into 9 cm diameter Petri dishes containing 15 ml of molten Potato Dextrose Agar (PDA). The medium was impregnated with streptomycin, and cultured for 7 days at room temperature (28-30 ^{o}C) in a sterile fume cupboard. Distinct colonies present on the plates were selected, purified by repeated culturing and maintained on PDA slants. The fungus isolated was identified using the macroscopic and microscopic identification guide according to Larone (1995) and the isolate (*A. solani*) was stored in an agar slant for other experiments.

3.6 Identification of *Alternaria solani*

The early blight induced by *Alternaria* spp. was identified through microscopic and macroscopic features. *Alternaria solani* grow rapidly and the colony size reaches a diameter of 3 to 9 cm following incubation at 25°C for 7 days on potato dextrose agar. The colony is flat, downy to woolly and was covered by greyish, short, aerial hyphae in time. The surface is greyish white at the beginning which later darkens and became greenish black or olive brown with a light border. The reverse side is typically brown to black due to pigment production (Larone, 1995). *Alternaria* spp. has septate, brown hyphae. Conidiophores are also septate and brown in color, occasionally producing a zigzag appearance (Larone, 1995). They had simple or branched large conidia (7-10 x 23-34 µm) which had both transverse and longitudinal septations. They were ovoid to obclavate, darkly pigmented, smooth or roughened. The end of the conidium nearest the conidiophore was round while it tapers towards the apex. This gave the typical beak or club-like appearance of the conidia as described by Zitter *et al.* (2005).

3.7 EXPERIMENT I: Laboratory Experiment

3.7.1 Preparation of Growth Medium and Inoculation

About 39 g of Potato Dextrose Agar powder (Sigma GMBH) was dissolved in 1000 ml of distilled water and the content was stirred and autoclaved for 25 minutes at 115 ^{o}C (Awale, 2001). The medium was allowed to cool down and was then aseptically poured into 25 ml flavour bottles. Thereafter, 5 ml of the plants extract prepared in 3.3 above was poured into the Petri dishes. About 15 ml of molten Potato Dextrose Agar (PDA) at 45-50 ^{o}C was poured aseptically onto the plant extract in the Petri dish and swirled round five times for even dispersion of the extract into the agar and allowed to solidify, before the pathogen was inoculated (introduced) into the middle of the 'poisoned agar'. A mycelial plug of 5 mm diameter from 3-days-old fungus was cut using a 5 mm sterile cork borer and transferred to the PDA plate in the center of the Petri dish and was kept in a sterilized fume cupboard kept at room temperature of 28 - 30 ^{o}C.

3.7.2 Pathogenicity Test

The UC82-B tomato seedlings raised were then transplanted into two 15 cm diameter plastic buckets and later thinned to 2 seedlings/bucket. After well established, the seedlings were then sprayed with spore suspension of 5 x 10^4 conidia/ml in distilled water of the pathogen. The plants were then covered with transparent polyethene sheets to build relative humidity and were later observed for early blight symptoms beginning from 4 WAT (Plate 2a and b).

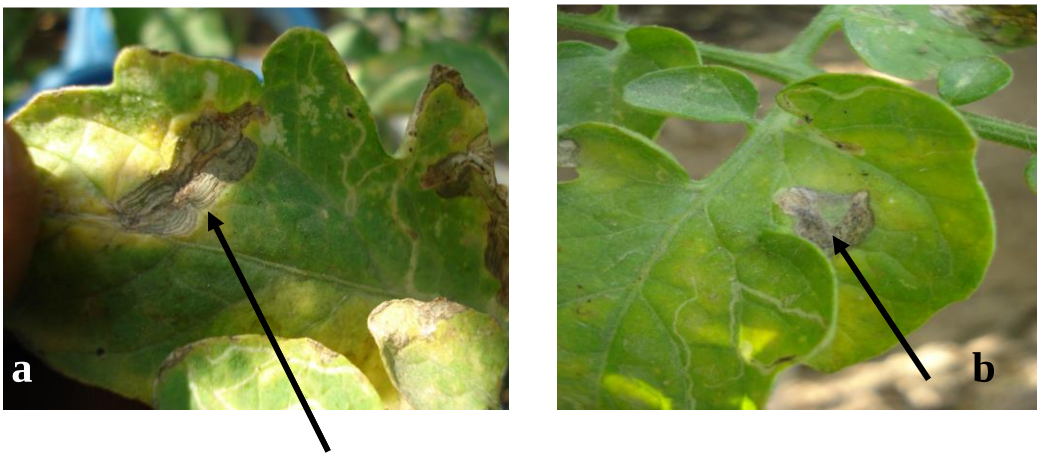


Plate 2a and b: The concentric ring symptoms of early blight on a diseased tomato leaf after pathogenicity test

3.8 Data collected

3.8.1 Fungal Biomass

The fungal biomass was determined by weighing the Petri dish containing the extract, PDA medium and the fungus using Mettler Toledo electric balance and was placed in the sterilized fume cupboard. Thereafter, weighing was done three times at 24 hour interval to determine fungal biomass (Reeslev and Kjoller. 1995).

$$\text{Fungal biomass } (B_r) = \frac{W_1 - W_0)}{(t_1 - t_0)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where W_0 and W_1 are the fungal biomass at time t_0 and t_1, respectively, determined at 72, 96 and 120 hours.

3.8.2 Radial growth

Measurement of the fungal radial growth in centimeters (cm) was done using ruler and the radial growth was determined by using the formula K_r according to (Reeslev and Kjoller, 1995).

$$\text{Radial growth } (K_r) = \frac{(R_1 - R_0)}{(t_1 - t_0)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where R_0 and R_1 are the colony radii at time t_0 and t_1 respectively, determined at 24, 48 and 72 hours interval.

3.8.3 Inhibition percentage

The inhibition percentage was calculated using the formula according to Harlapur *et al.* (2007) from the radial growth measured after 24, 48 and 72 hours of inoculation and the inhibition percentage of fungal growth by the plant extracts determined using the formula:

$$\text{Percent inhibition (I)} = \frac{100\times(C - T)}{C} \quad \ldots\ldots\ldots \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.(3)$$

Where

I = inhibition percentage of pathogen growth,

C = average growth in control and

T= average growth in treatment.

3.9 EXPERIMENT II: Potted Experiment

This experiment was conducted in the landscape unit and consisted of sixteen treatments laid out using a randomized complete block design (RCBD) replicated three (3) times similar to the laboratory experiment as indicated in 3.2 and consisted of 48 plastic pots.

3.9.1 Nursery practices

The tomato seed UC82-B raised in the nursery on a sterilized soil in a plastic seedling tray (30 cm x 15 cm) before it was transplanted into the pots at 3 WAS. The seeds were inoculated with the isolated pathogen by coating the seeds before planting.

3.9.2 Transplanting of seedlings and Inoculation of Soil

The seedlings raised in 3.9.1 were transplanted to the sterilized soil in the pots at 3 WAS by transplanting 2 seedlings into each pot. Thereafter at 2 weeks after transplanting (WAT) the soil in each of the pot was drenched with spore suspension of 5 x 10^4 conidia/ml fungal suspension of *Alternaria solani* isolated and kept on agar slant as stated in 3.5.

3.9.3 Application of Plant Extracts

Four sprayings of plant extracts was done on the tomato plants. The first was done at 3 weeks after transplanting (WAT) and subsequently weekly with the experimental materials prepared in 3.3 above at the rate of 25 ml, 50 ml and 100 ml in 200 ml of water per pot.

3.9.4 Weeds and Insect Control

The pots were kept weed free by hand pulling of weeds and insects were controlled by applying Smash Super® (Cypermethrin 100 EC) at the rate of 30 ml l^{-1} of water weekly which commenced at 3 WAT.

3.9.5 Staking and fertilizer application

The tomato plants were staked at 4 WAT to support the plant for optimum growth and NPK 15:15:15 was applied at the rate of 67 g $plot^{-1}$ in two split doses with the first dose applied at 3 WAT and the second dose at 6 WAT.

3.10 Data Collected

The following data were collected during the course of the experiment;

3.10.1 Plant establishment

Plant establishment was determined by counting the number of plants that have survived after transplanting at 2 WAT.

3.10.2 Percentage severity of early blight

Assessment of early blight on the tomato plants was carried out using a modified scale of Gleason and Edmunds, (2006) on a scale of 1-5 as shown in Table 1. Thereafter percentage disease severity was determined at 4 and 6 WAT by using the formula;

$$\text{Disease severity} = \frac{\text{Sum of individual ratings}}{\text{No. of leaves assessed x 5 (highest score in table)}} \times 100 \quad \text{............(4)}$$

3.10.3 Days to 95 % fruiting

This was carried out by monitoring and recording the time in days from transplanting to the time 95 % of the tomato plants produced fruits.

3.10.4 Number of fruits per pot

Fruits from each pot were counted and expressed as number of fruits per pot.

3.10.5 Percentage number of infected fruits per pot

This was done by counting the number of infected fruits per pot.

3.10.6 Fruit weight per pot (g)

Fruits from each potted plant were counted and weighed and expressed as fruit weight per pot in grams.

3.11 Data Analysis

Data collected were subjected to analysis of variance (ANOVA) for a randomized complete block design using SAS (1999) statistical package. The treatment means that were significantly different was separated using the Duncan's Multiple Range Test (DMRT) at P=0.05.

Table 1: Early Blight Disease Assessment scale

Scale	Description
1	No visible symptoms
2	Small Brown to black spots, 0.25 to 0.5 cm in diameter with dark edges
3	Brown to black spots, more than 0.5 cm in diameter with dark edges on lower leaves
4	Dark, concentric rings appearing in leaf spots, with "target" appearance
5	Necrotic, dead and yellow leaves and stems, drying up and with only a few spots present and concentric rings on fruit.

Source: Gleason and Edmunds, 2006

CHAPTER FOUR

4.0 RESULTS

4.1 EXPERIMENT I: Laboratory Experiment

4.1.1 Fungal biomass of *Alternaria solani* at 72, 96 and 120 hours

The result presented in Fig 2 shows that fungal biomass of *Alternaria solani* at 72 hours shows that *Chromolaena odorata* 25 % concentration and *Cleome viscosa* 100 % concentration recorded the highest fungal biomass with 0.021 g each. *Ageratum conyzoides* 100 % concentration and *Chromolaena odorata* 50 % concentration had 0.020 g each while *Ricinus communis* 50 % recorded 0.018 g. The result further indicated that the lowest fungal biomass of 0.011 g was recorded in *Eucalyptus camaldulensis* 100 % concentration as compared to the control with 0.012 g although not statistically significant. The fungal biomass of *Alternaria solani* at 72 hours after inoculation indicated a highly significant (P=0.01) difference among various treatments in the first and second laboratory experiment respectively (Appendix 2 and 3).

At 96 hours fungal biomass in the first experiment, revealed that *Cleome viscosa* 100 % recorded the highest fungal biomass (0.018 g), with the control recording 0.004 g (Fig 2). At 120 hours result indicated that *Chromolaena odorata* 25 % concentration recorded the highest fungal biomass of 0.012 g, followed by *Ricinus communis* 100 %, *Ageratum conyzoides* 25 %, *Chromolaena odorata* 50 %, *Cleome viscosa* 50 % concentration all recorded means of 0.010 g each. *Eucalyptus camaldulensis* 100 % treatment and the control had the lowest fungal biomass of 0.004 g each in the first experiment (Fig 2).

The result of fungal biomass in the second experiment at 72 hours indicated that the highest fungal biomass (0.020 g) was recorded in *Ageratum conyzoides* 100 % concentration and *Chromolaena odorata* 25 % concentration respectively while, the lowest fungal biomass was recorded in the control with 0.011 g (Fig 3). In the second experiment at 96 hours however, *Cleome viscosa* 50 % recorded the highest fungal biomass of 0.015 g followed by *Cleome viscosa* 25 % and *Ageratum conyzoides* 100 % concentration with 0.014 g each. The result further showed that at 96 hours the control recorded the lowest fungal biomass of 0.004 g (Fig 3). *Chromolaena odorata* 25 % and *Ricinus communis* 100 % treatments record mean number of 0.010 g each at 120 hours in the second laboratory experiment. However, the lowest fungal biomass was recorded in the control with mean of 0.004 g at 120 hours after inoculation (Fig 3).

Table 2 shows the result of the combined analysis of variance which indicated a highly significant (P=0.01) difference among treatments for fungal biomass at 72 hours after inoculation (Appendix 4). The result shows that the highest fungal biomass were recorded in *Ageratum conyzoides* 100 % and *Chromolaena odorata* 25 % concentration with means of 0.020 g each while 0.021 g was recorded in *Cleome viscosa* 100 % (Table 2). Furthermore, the lowest fungal biomass was recorded in *Eucalyptus camaldulensis* 100 % and the control with each having 0.012 g, although not significantly different. The result of the combined analysis of variance at 96 hours indicated that *Cleome viscosa* 100 % concentration recorded the highest fungal biomass of 0.016 g. The lowest means at 96 hours in the combined analysis was recorded in the control with 0.006 g followed by *Eucalyptus camaldulensis* 100 % with 0.007 g (Table 2). In the combine, results revealed that *Chromolaena odorata* 25 % was superior with mean of 0.011 g with the lowest mean of 0.004 g recorded in the control at 120 hours (Table 2).

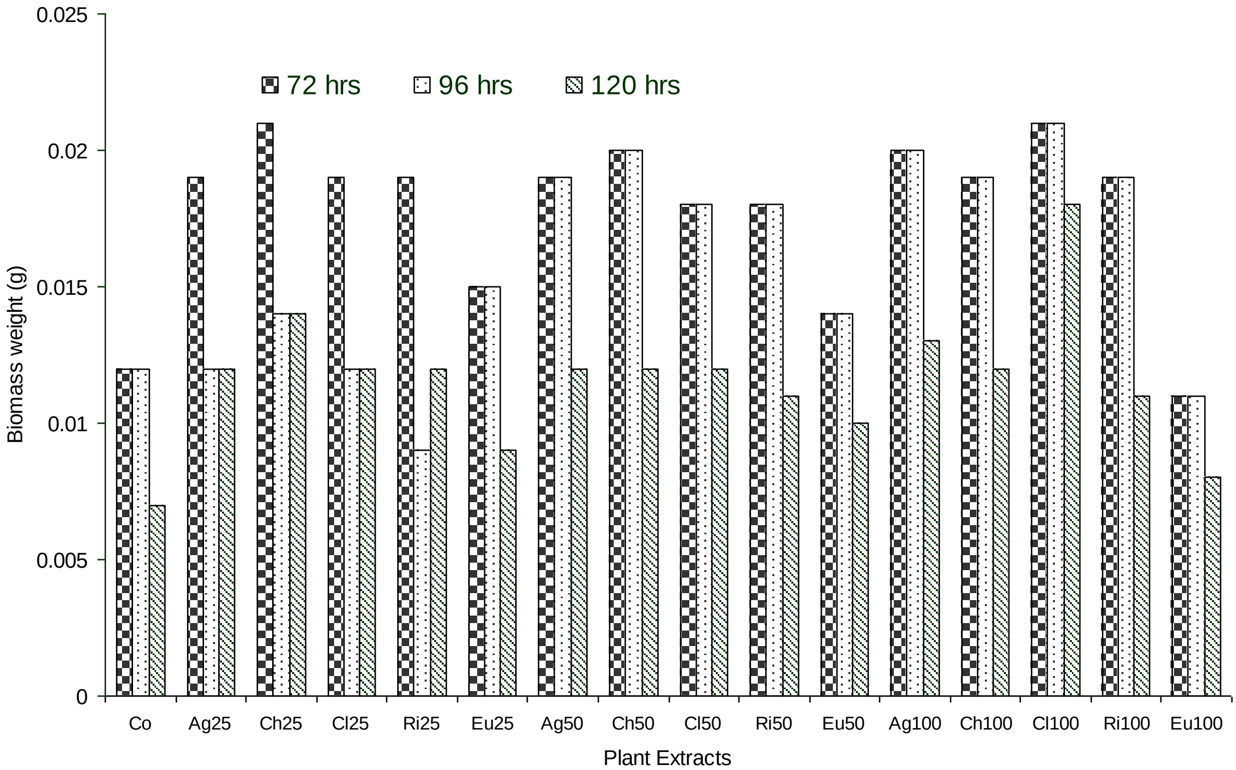


Fig 2: Effect of plant extracts on inhibition of *Alternaria solani* fungal biomass weight first experiment

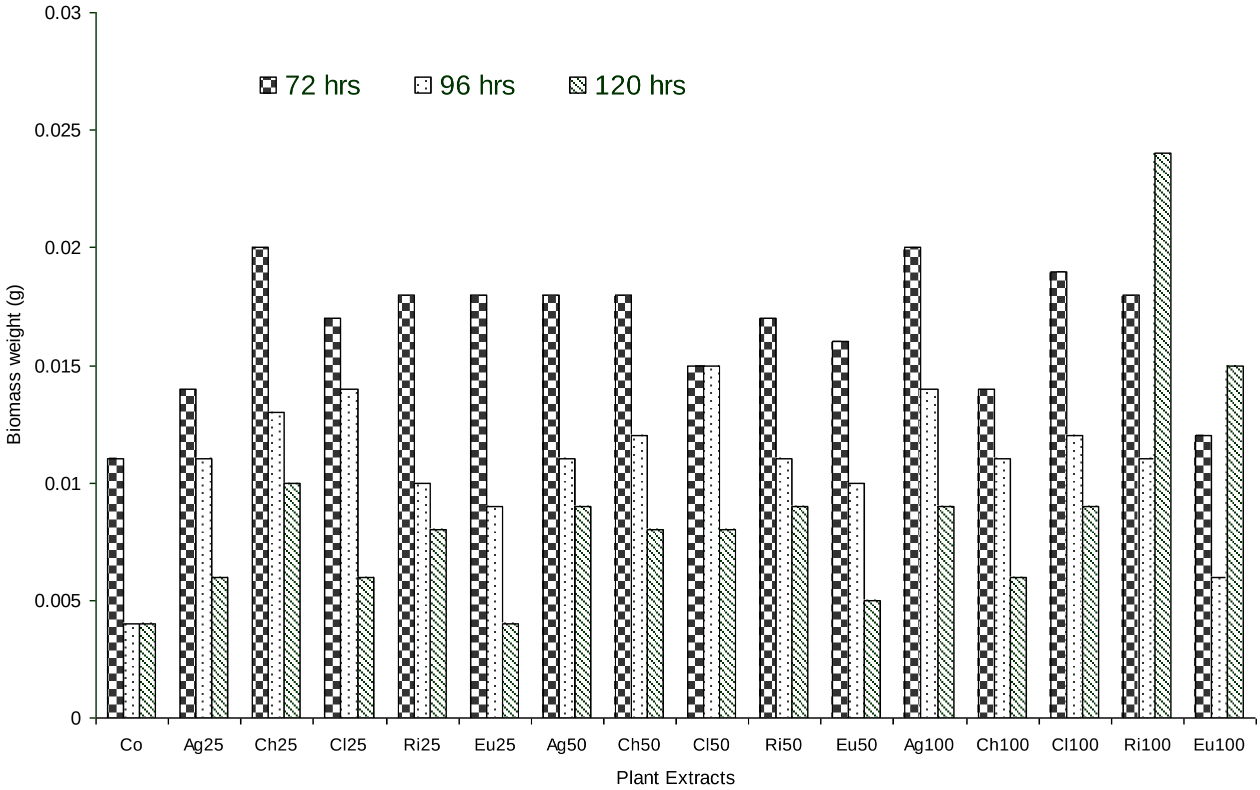


Fig 3: Effect of plant extracts on inhibition of *Alternaria solani* fungal biomass weight second experiment

Table 2: Combined means of the effect of plant extract on fungal biomass (g) of *Alternaria solani* in the laboratory

Treatment	72 hrs	96 hrs	120 hrs
Agcon25	0.017cd	0.011bcd	0.008b
Chord25	0.020a	0.014ab	0.011a
Clovis25	0.018bc	0.012bc	0.008b
Ricom25	0.019abc	0.011bcd	0.009b
Eucam25	0.017cd	0.009cde	0.005c
Agcon50	0.018bc	0.012bc	0.009b
Chord50	0.019abc	0.012bc	0.009b
Clovis50	0.017cd	0.013ab	0.009b
Ricom50	0.018bc	0.011bcd	0.010a
Eucam50	0.015d	0.010bcd	0.006d
Agcon100	0.020a	0.013ab	0.010a
Chord100	0.017cd	0.012bc	0.008b
Clovis100	0.021a	0.016a	0.009b
Ricom100	0.019abc	0.011bcd	0.010a
Eucam100	0.012e	0.007de	0.010a
Control	0.012e	0.006e	0.004e
Mean	**0.017**	**0.011**	**0.008**
Prob. F	**	**ns**	**

Means in the same column followed by the same letters are not significantly different (P=0.05) using Duncan's Multiple Range Test; ** = highly significant (P=0.01) and ns = not significant

Agcon = *Ageratum conyzoides*; Chord = *Chromoleana odorata*; Clovis = *Cleome viscosa;* Ricom = *Ricinus communis*; Eucam = *Eucalyptus camaldulensis*

4.1.2 Radial Growth of *Alternaria solani* at 24, 46 and 72 hours

The result of the analysis of variance (ANOVA) for the effect of plant extracts on radial growth in the first and second laboratory experiments revealed a highly significant difference (P=0.01) at 24, 48 and 72 hours after inoculation (Appendix 5 and 6). Treatment means for the first laboratory experiment at 24, 48 and 72 hours shows that the lowest radial growth were recorded in *Ricinus communis* 100 % concentration with means of 1.57 cm, 2.16 cm and 2.88 cm (Plate 3) respectively with the control recording the highest radial growth of 3.53 cm, 4.09 cm and 4.59 cm (Plate 4) respectively (Table 3). In the second experiment the treatment means also showed that *Ricinus communis* 100 % concentration recorded the lowest radial growth with 1.30 cm, 1.83 cm and 2.55 cm at 24, 48 and 72 hrs respectively. In addition the control recorded the highest radial growth with mean of 3.40 cm, 3.98 cm and 4.43 cm at 24, 48 and 72 hrs respectively (Table 3). In addition, the combined results in Table 4 revealed that at 24, 48 and 72 hours *Ricinus communis* 100 % concentration had the lowest means of 1.43 cm, 2.00 cm and 2.72 cm respectively, followed by *Ricinus communis* 50 % concentration with 1.83 cm, 2.32 cm and 2.93 cm respectively. While the control which recorded the highest radial growth with 3.47 cm, 4.04 cm and 4.51 cm followed at 24, 48 and 72 hours respectively.

4.1.3 Inhibition percentage of radial growth of *Alternaria solani* at 24, 48 and 72 hour

The inhibition percentage for the combined results (Table 5) revealed that *Ricinus communis* extracts inhibited radial growth by 41-59 % after inoculation at 24 hours in comparison to the control. At 48 hours after inoculation, the result indicated *Ricinus communis* extracts inhibited fungal radial growth by 33-51 %, while *Eucalyptus camaldulensis* inhibited radial growth by 15-17 %. At 72 hours, the result of the calculated inhibition percentage showed that *Eucalyptus camaldulensis* inhibited radial growth by 5-14 %, as against *Ricinus communis* extracts which showed inhibitory effect on the radial growth by 26-40 % as indicated in Table 5.

Comparing the plants extract results on radial growth alone (Fig. 4) showed that *Ricinus communis* treatments highly inhibited *Alternaria solani* at 24, 48 and 72 hours followed by *Chromolaena odorata* while the least effective was in *Eucalyptus camaldulensis* treatments.

Table 3: Effects of plant extracts on radial growth (cm) of *Alternaria solani* first and second laboratory experiments

Treatment	24 hrs		48 hrs		72 hrs	
	First experiment	Second experiment	First experiment	Second experiment	First experiment	Second experiment
Agcon25	3.13ab	2.88b	3.57abc	3.34cd	4.20abcd	3.85bc
Chord25	2.38cdef	2.58d	3.10bcd	2.70ef	3.75bcde	3.67bc
Clovis25	2.23fg	2.24e	3.13bcd	2.76ef	3.68cde	3.21d
Ricom25	2.27efg	1.85f	2.66def	2.76ef	3.35efg	3.33d
Eucam25	2.78bcdef	2.83bc	3.65ab	3.20d	4.42ab	3.93b
Agcon50	2.85bcde	2.22e	3.30bcd	2.92e	4.12abcd	3.35d
Chord50	2.20fg	2.30e	2.95cde	2.65f	3.72cde	2.90e
Clovis50	2.52cdef	2.27e	3.38bc	2.65f	3.67cde	3.13de
Ricom50	1.75gh	1.91f	2.43ef	2.20g	2.95fg	2.90e
Eucam50	2.96bc	2.87b	3.43abc	3.47bc	4.33abc	4.27a
Agcon100	2.84bcde	2.61cd	3.21bcd	2.86ef	4.35abc	3.95b
Chord100	2.28defg	2.30e	2.90cde	2.33g	3.57def	3.63c
Clovis100	2.87bcd	2.54d	3.50abc	3.58b	4.08abcd	3.91bc
Ricom100	1.57h	1.30g	2.16f	1.83h	2.88g	2.55f
Eucam100	2.62bcdef	2.28e	3.47abc	3.27cd	4.03abcd	3.75bc
Control	3.53a	3.40a	4.09a	3.98a	4.59a	4.43
Mean	**2.55**	**2.40**	**3.18**	**2.91**	**3.86**	**3.55**
Prob. F	**	**	**	**	**	**

Means in the same column followed by the same letters are not significantly different (P=0.05) using Duncan's Multiple Range Test; ** = highly significant (P=0.01)

Agcon = *Ageratum conyzoides*; Chord = *Chromoleana odorata*; Clovis = *Cleome viscosa;* Ricom = *Ricinus communis*; Eucam = *Eucalyptus camaldulensis*

Table 4: Combined means of the effect of plant extracts on radial growth (cm) of *Alternaria solani* in the laboratory

Treatment	24 hrs	48 hrs	72 hrs
Agcon25	3.01b	3.46b	4.03bcd
Chord25	2.48de	2.90def	3.71def
Clovis25	2.24ef	2.95def	3.45fg
Ricom25	2.06fg	2.71ef	3.34g
Eucam25	2.81bc	3.43bc	4.18abc
Agcon50	2.53cde	3.11cd	3.73def
Chord50	2.25ef	2.80def	3.31g
Clovis50	2.39e	3.02de	3.40fg
Ricom50	1.83g	2.32g	2.93h
Eucam50	2.92b	3.45b	4.30ab
Agcon100	2.72bcd	3.03de	4.15bc
Chord100	2.29ef	2.62fg	3.60efg
Clovis100	2.70bcd	3.54b	4.00bcd
Ricom100	1.43h	2.00h	2.72h
Eucam100	2.45de	3.37bc	3.89cde
Control	3.47a	4.04a	4.51a
Mean	**2.47**	**3.06**	**3.70**
Prob. F	**	**	**

Means in the same column followed by the same letters are not significantly different (P=0.05) using Duncan's Multiple Range Test

** = Highly significant (P=0.01)

Agcon = *Ageratum conyzoides*; Chord = *Chromoleana odorata*; Clovis = *Cleome viscosa;* Ricom = *Ricinus communis*; Eucam = *Eucalyptus camaldulensis*

Table 5: Combined results on inhibition percentage (%) of radial growth in the laboratory experiment

Treatment	Concentration	% Inhibition		
		24 hours	48 hours	72 hours
Control	0	0	0	0
Ageratum conyzoides	25	13	13	11
	50	27	23	17
	100	22	25	8
Chromolaena odorata	25	29	28	18
	50	35	31	27
	100	34	35	20
Cleome viscosa	25	35	27	26
	50	31	25	25
	100	22	12	11
Ricinus communis	25	41	33	26
	50	47	43	35
	100	59	51	40
Eucalyptus camaldulensis	25	19	15	7
	50	16	15	5
	100	30	17	14

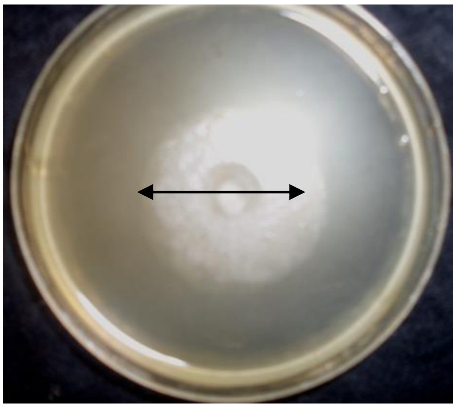


Plate 3: The lowest radial growth in *Ricinus communis* 100 % at 48 hours

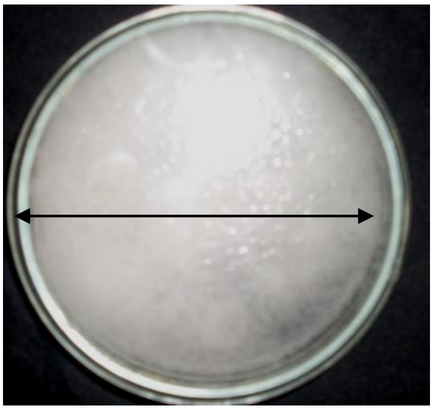


Plate 4: The highest radial growth in control 48 hours

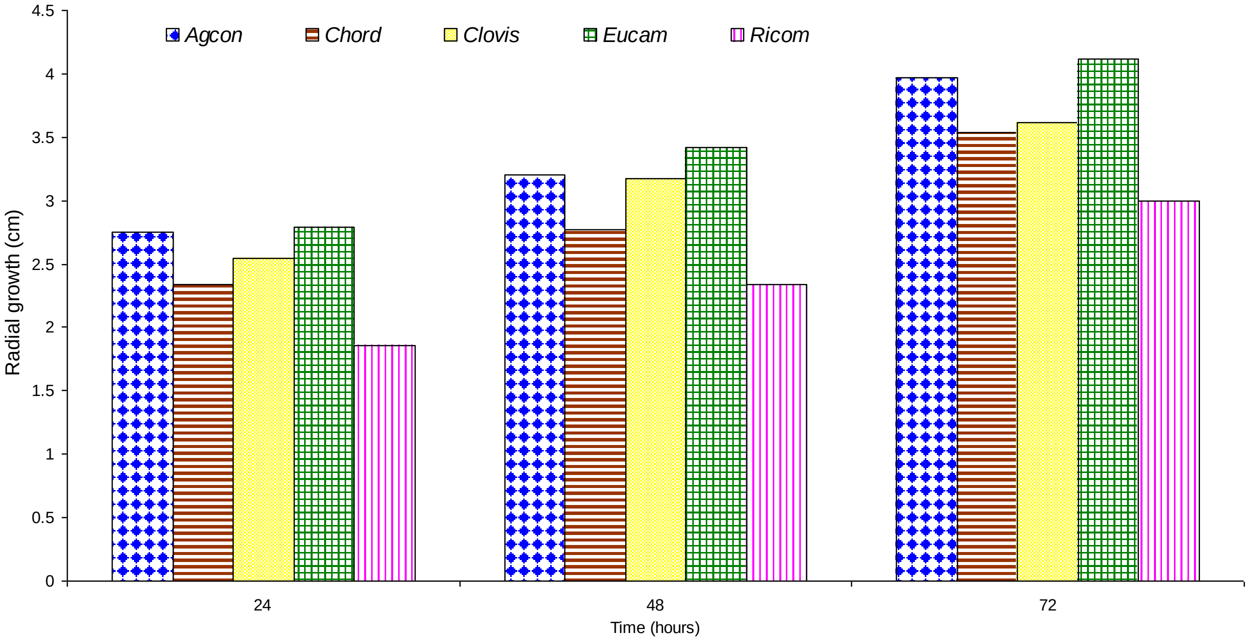


Fig 4: Effect of plant extracts on inhibition of *Alternaria solani* radial growth

4.2 EXPERIMENT II: Potted Experiment

4.2.1 Plant Establishment

The result contained in Table 6 shows that plant establishment was not significantly (P=0.05) different among the various treatments. The highest plant establishment means of 100 were recorded from *Chromolaena odorata* 50 %, *Ricinus communis* 50 %, *Eucalyptus camaldulensis* 50 %, *Ageratum conyzoides* 100 %, *Chromolaena odorata* 100 %, *Ricinus communis* 100 % and *Eucalyptus camaldulensis* 100 while treatments with 25 % concentration of *Ageratum conyzoides* and *Cleome viscosa* recorded means of 83.33 % with the lowest mean establishment (66.67 %) recorded in *Ageratum conyzoides* 50 % and the control treatment.

4.2.2 Percentage disease severity at 4 and 6 WAT

The percentage disease severity result is contained in Table 6 taken at 4 and 6 WAS indicated highly significantly (P=0.01) differences between treatment means. The highest score of 83 % was recorded in the control followed by *Cleome viscosa* 100 % with 77 % severity. While *Ricinus communis* 100 % and *Eucalyptus camaldulensis* 100 % recorded severity of 60 % and 63 % respectively, with the least severity of 34 % recorded in *Ricinus communis* 50 % concentration. At 6 WAS, the lowest disease severity were recorded in *Ricinus communis* 50 % and *Chromolaena odorata* 50 % with 39 % each. More so *Cleome viscosa* 50 % and *Chromolaena odorata* 25 % recorded severity of 48 % and 47 % respectively. The result further revealed that the control followed by *Eucalyptus camaldulensis* 100 % treatment recorded 94 % and 70 % respectively (Table 6).

4.2.3 Days to 95 % Fruiting

Table 6 contains result of days to 95 % fruiting for the various treatments. There were no significant (P=0.05) difference among the various treatments with regards to this parameter. However, the lowest days to 95 % fruiting was recorded in *Chromolaena odorata* 50 % with 70.00. Furthermore, the result further revealed that *Cleome viscosa* 50 %, *Ageratum conyzoides* 50 % and *Ricinus communis* 100 % all recorded 73.00 days to 95 % fruiting.

4.2.4 Number of fruits

The result presented in Table 6 shows that, there were no significant (P=0.05) difference among treatments with regards to number of fruits. However, treatment means indicated that *Eucalyptus camaldulensis* 25 % recorded the highest number of fruit (15) while *Chromolaena odorata* 100 % and *Eucalyptus camaldulensis* 100 % recorded 14 and 13 fruits respectively. Similarly, the lowest number of fruits was recorded in *Cleome viscosa* 100 % with only one fruit; however the control recorded 7 fruits.

4.2.5 Percentage number of infected fruits

Table 6 contains the percentage number of infected tomato fruits for the various treatments. There were no significant difference (P=0.05) between the plant extracts treatments. However, control recorded the highest percentage infected fruits with 71.00 %, while the least infected fruits were recorded in treatments with *Ageratum conyzoides* 25 %, *Chromolaena odorata* 25 %, *Cleome viscosa* 25 %, *Ricinus communis* 25 % and *Ricinus communis* 100 % respectively with zero percentage fruit infected.

4.2.6 Fruit weight (g)

The result presented in Table 6 is the fruit weight which revealed no significant (P=0.05) difference among treatments. *Chromolaena odorata* 100 % recorded the highest weight of 14.94 g, followed by *Eucalyptus camaldulensis* 25 % and *Eucalyptus camaldulensis* 100 % with 12.82 g and 10.49 g respectively. The lowest fruit weight of 0.67 g was recorded in *Cleome viscosa* 100 % concentration followed by the control having 1.49 g.

Table 6: Effect of plant extracts on some parameters taken on manifestation of early blight disease on potted tomato plants

Treatment	Plant Estab.	Percentage Disease severity (WAT)		Days to 95 % fruiting	No. of fruits	Percentage no. of infected fruits	Fruit weight (g)
		4	6				
Agcon25	83.33ab	70.00c	57.00f	71.00a	4.00a	0.00c	4.42ab
Chord25	100.00a	68.00d	47.00g	72.00a	7.00a	0.00c	4.42ab
Clovis25	83.33ab	55.00g	54.00f	71.00a	3.00a	0.00c	2.95ab
Ricom25	100.00a	53.00g	42.00h	72.00a	7.00a	0.00c	5.03ab
Eucam25	100.00a	62.00e	55.00f	70.00a	15.00a	0.00c	12.82a
Agcon50	66.67b	35.00j	39.00i	73.00a	3.00a	0.00c	3.93ab
Chord50	100.00a	40.00i	59.00e	70.00a	12.00a	0.00c	7.33ab
Clovis50	100.00a	47.00h	48.00g	73.00a	8.00a	51.63b	4.41ab
Ricom50	100.00a	34.00j	39.00hi	71.00a	8.00a	0.00c	5.73ab
Eucam50	100.00a	35.00j	40.00h	72.00a	4.00a	0.00c	6.80ab
Agcon100	100.00a	66.00d	62.00d	71.00a	11.00a	51.63b	9.67b
Chord100	100.00a	35.00j	39.00i	71.00a	14.00a	0.00c	14.92a
Clovis100	83.33ab	77.00b	66.00c	72.00a	1.00a	0.00c	0.67b
Ricom100	100.00a	60.00f	66.00c	73.00a	8.00a	0.00c	6.02ab
Eucam100	100.00a	63.00e	70.00b	72.00a	13.00a	51.63b	10.49b
Control	66.67b	83.00a	94.00a	73.00a	7.00a	71.00a	1.49ab
Mean	92.71	55.19	54.81	72.00	7.81	14.12	6.32
Prob. of F	0.10ns	0.001**	0.001**	0.70ns	0.67ns	0.55ns	0.53ns

Means in the same column followed by the same letters are not significantly different (P=0.05) using Duncan's Multiple Range Test; ** = highly significant (P=0.01) and ns = not significant; WAT = weeks after transplanting; DAT= days after inoculation

Agcon = *Ageratum conyzoides*; Chord = *Chromoleana odorata*; Clovis = *Cleome viscosa;* Ricom = *Ricinus communis*; Eucam = *Eucalyptus camaldulensis*

CHAPTER FIVE

5.0 DISCUSSION

5.1 Fungal biomass of *Alternaria solani* at 72, 96 and 120 hours

In this study, screening *in vitro* the effect of various plants extracts for antifungal activity against *Alternaria solani* Ell. and Mart. responsible for early blight of tomato was carried out using different concentrations. The effect of plant extracts on fungal biomass varied among the different plant materials as well as the concentrations. The result of the study showed that the plant extracts tested had effect on fungal biomass at high concentration as revealed by the high fungal biomass recorded in *Cleome viscosa* at 100 %, *Chromolaena odorata* at 25 and 50 % concentration at 72 and 96 hours after inoculation in the first and second experiments. This however, differs slightly with fungal biomass at 120 hours in the first experiment where *Chromolaena odorata* at 25 % concentration had the highest mean weight of 0.012 g. This result may be due to its effects on biomass production as Prapagdee *et al.* (2008) reported that plants extracts causes abnormal hyphal structures such as thickness and bulbous roundedness of the inhibited fungal hyphae resulting from diffusible secondary compounds. Similar result has been previously reported by Taechowisan *et al.* (2005). While, Getha and Vikineswary (2002) found out that antifungal activity of *S. violaceusniger* G10 showed *in vitro* antagonistic effects against *F. oxysporum* f.sp. *cubense*, such as hyphal swelling and the inhibition of spore germination. Thus the high fungal biomass recorded in the *Cleome viscosa* and *Chromolaena odorata* could also be attributed to the fungal growth inhibition by the plant extracts because of the presence of extracellular metabolites both hydrolytic enzymes and secondary antifungal compound(s).

Results further showed that three of the plants extracts had antifungal potential, with significant difference in activity between the different extracts on fungal biomass *in vitro*. The most antifungal active plants were *Cleome viscosa*, *Ageratum conyzoides* and *Chromolaena odorata*, whereas, the least active plant was *Eucalyptus camaldulensis*. Only two of the tested plant extracts were active against fungal biomass of *Alternaria solani* with the most active from *Cleome viscosa* and *Chromolaena odorata* and the least active from *Eucalyptus camaldulensis*. The relationship between concentration and fungal biomass revealed significant differences in their efficacy to fungal biomass of *A. solani*. This could be seen from the effect of *Chromolaena odorata* at 25 % being at par with *Cleome viscosa* in the first, second and combined analysis of variance mean. This indicated that *Chromolaena odorata* at low concentration could inhibit growth of *A. solani* which consequently affected its fungal biomass. The high fungal biomass recorded in *Cleome viscosa* at 100 % concentration could be as a result of changes in morphology of the fungi when grown under *Cleome viscosa* and *Chromolaena odorata* plant extracts due to hyphal swelling or by the formation of very short hyphae with multiple distorted branch is in line with the findings reported by Bajwa *et al.* (2004). Furthermore, Bajwa *et al.* (2004) found out that biomass production in the early growth phase of five days after incubation (5 DAI) revealed a negative response at lower concentrations of 10-30% than at a higher concentration of 60-70% with respect to biomass production. This findings further revealed that mycelial yield of the test fungal species of *Drechslera hawaiiensis* was enhanced, particularly at 60 and 70% concentrations with a very sharp stimulated growth, even significantly higher than the control.

Furthermore, cleomiscosin D, a coumarino-lignan and diterpene were reported from the seeds of *Cleome viscosa* by Kumar and Ray (1988) which could have inhibited radial growth but with changes in structure and morphology resulting in high fungal biomass production. *Cleome viscosa* extracts also demonstrated a contact insecticidal activity on adult *Cylas formicarius elegantulus* Summer (*Coleoptera*: Curculionidae). It was also reported that the extract also had high nematicidal activity with a percentage Abbott's value of 72.69 on the plant parasitic nematode *Meloidogyne incognita* (Spjut, 2007). The plant extract of *Chromolaena odorata* is second best, in the sense that at least the extract was able to control the growth of the fungi *in* *vitro* as revealed by the fungal biomass. The medicinal efficacy of *Chromolaena odorata* has been reported to lie in their component phytochemicals such as tannins, steroids, terpenoids, flavonoids and cardiac glycosides as well as alkaloids detected in methanolic extract (Akinmoladun *et al.,* 2007).

Similarly, higher concentration of 100 % extracts as revealed by the highest mean fungal biomass recorded in the combined at 72 and 96 hours from *Ageratum conyzoides, Cleome viscosa* and *Ricinus communis* may be attributed to the high concentration of certain plant chemicals that stimulated or altered the morphology and structure of the fungus. Baldrian and Gabriel (2002) reported that the presence of cadmium (Cd) at 50, 100, or 250 µM in solid growth media caused reduction of radial growth rate and increased the length of the lag phase. In liquid culture, the mycelium formed a mat, which in the control and at 10 µM cadmium extended wide, whereas at 100 µM Cd the "colony" was smaller in size, but denser (Baldrian and Gabriel, 2002). The mycelia grown at higher cadmium concentrations both in liquid culture and on agar plates were denser, which can probably be attributed to changes in hyphal branching. This is in agreement with the results of Darlington and Rauser (1988), who found that in *Paxillus involutus* cadmium decreases the rate of elongation of fungal hyphae, but significantly decreases the distance between branch points and increases the number of laterals per branch point. This may be the reason why the fungal biomass in this study differed from other studies possibly because the extracts caused changes in the fungal structure and morphology. The enhancement of biomass production of *D. hawaiiensis* at higher concentration of shoot extracts may be attributed to detoxifying ability of the fungi, to allelochemicals as reported by Sicker (1998). It may also be due to the ability of some allelochemicals to enhance the growth of mycoflora (Mughal *et al*., 1996) or ability of particular species to exploit them as source of nutrition. In the case of *A. alternata*, the comparative effectiveness of aqueous extracts of selected test species revealed that 10-60% concentrations were relatively more allelopathic as compared to other species while 70%

concentration supported the mycelial biomass production during the early phase. These results are supported by the fact that the allelopathic substances have selective effects, depending upon their concentrations, either inhibitory or stimulatory to the growth of fungi (Paruis *et al.*, 1985; Cheema, 1988).

Ming (1999) reported that *Ageratum conyzoides* contains flavonoids, alkaloids, cumarins, essentials oils and tannins. While Borthakur and Baruah (1986) identified prococene I and prococene II in plant collected in India. These compounds have also been shown to affect insect development. Almagboul *et al.* (1985) reported methanolic extracts of the whole plant to inhibit action in the development of *Staphylococcus aureus, Bacillus subtillis, Escherichia coli* and *Pseudomonas aeruginosa;* while the presence of thioglucosinolates in *Cleome viscosa* might have inhibited the biomass. Thus, this result is in line with the findings of Luna-Zarate *et al.* (2004) who reported that increasing cinnamon oil concentration delayed fungal growth and reduced radial growth rate.

Also Varaprasad *et al.* (2009) reported that the antifungal activity of some fourty nine plants extracts tested against *Aspergillus niger* was enhanced by increase in the concentration of the extracts. The difference observed at 120 hours which showed *Chromolaena odorata* at 25 % concentration to having the highest mean fungal biomass could probably be due to the other higher concentration of plant extracts reaching their stationary or death phase in fungal microbial growth curve earlier than the low concentration. This is as indicated by the low fungal biomass of *Cleome viscosa* 100 % concentration at 120 hours probably because growth factors are limiting.

The lowest fungal biomass recorded consistently in the control in the first, second and combined result could be indication of the free growth of the pathogen without inhibition. Tariq (2009) reported that at exponential phase of growth of fungi, growth is rapid and will continue until one or more nutrients become limiting or depleted. This luxuriant growth made the fungus to sporulate and grow uninhibited. This finding is buttressed by the report of Reeslev and Kjoller (1995) who stated that specific growth rate of fungi is constant unless when the nutrient composition varies or after treatment with inhibitors or paramorphogens. Hence the control here grew faster and used up the medium hence the low fungal biomass as compared to medium with growth inhibitors. The low fungal biomass in the control could be attributed to exponential phase of fungal kinetics which takes place in a media until one or more nutrients become limiting, oxygen becomes depleted and/or metabolic by-products accumulate to toxic levels then growth decelerate (Tariq, 2009). This is later followed by a stationary phase, during which no discernible change in cell concentration or biomass takes place. Tariq (2009) opined that at this phase cell death and lysis results in a decrease in cell number and/or biomass. Similarly, Bajwa *et al.* (2004) has reported that at 5 days after incubation, the aqueous extract of *Parthenium hysterophorus* interfered with the growth of *Dreschslera hawaiiensis, Alternaria alternata* and *Fusarium moniliforme* at higher concentration of 60 % - 70 % by giving marked positive effect on fungal biomass than at lower concentration of 10 % - 30 %.

5.2 Radial Growth of *Alternaria solani* at 24, 46 and 72 hours

Result on the effect of plant extracts on radial growth of *A. solani in vitro* revealed variation among the extracts and concentrations unlike the biomass result. Although there were differences in radial growth among the extracts and concentration for the first, second and combined result. This investigation revealed that *Ricinus communis* was able to record the lowest mean radial growth rate and at 100 % concentration when compared to the same extract at 50 %. This is clear indication that *Ricinus communis* at 100 % inhibited growth of the pathogen. This may be attributed to the presence of ricin and ricinine in the extracts as reported by Ukpabe (2002) who stated that leaf extracts of *Ricinus communis* inhibited the growth of *Fusarium oxysporum*. In addition, it has been reported that dried ground leaves of *Ricinus communis* when used at 16 g/kg admixed with cowpea caused 100 % mortality in adult *Callosobruchus maculatus* within seven days and reduced the first generation emergence (FAO, 1992). *Chromolaena odorata* is second to *Ricinus communis* in inhibiting radial growth as indicated by the result earlier presented in Tables 4, 5 and 6. Ngane *et al.* (2006) reported that aqueous ethanol extract of leaves of *Chromolaena odorata* and some of its fractions examined has antifungal properties by dilution methods on solid and liquid media, using yeasts and filamentous fungi. The result revealed that extract and fractions inhibit the *in vitro* growth of *Cryptococcus neoformans*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*. Chemical analysis of the extract and fractions showed the presence of biologically active constituents such as coumarins, flavonoids, phenols, tannins and sterols which they attributed made the extracts fungicidal (Ngane *et al.*, 2006).

Mishra *et al.* (1989) have reported that essential oils from leaves of *Chenopodium ambrosioides, Cinnamomum zeylanicum, Citrus medica, Melaleuca lucadendron, Ocimum canum* and *O. grattissium* to have demonstrated fungitoxicity against *Aspergillus flavus* at 200, 300, 400 and 500 ppm concentration and were even more effective than synthetic fungicides viz; Agrosan, Copperoxychloride, Ceresan, Thiovit and Dithane M45. Asthana *et al*. (1986) have found that the leaf extract of *Ocimum adscendens* to be fungitoxic against *Aspergillus flavus.* This findings is similar to the results of this study which showed that extracts of *Ricinus communis, Chromolaena odorata* and *Cleome viscosa* inhibited the radial growth of *Alternaria solani in vitro.*

Comparing the rate of radial growth in *Ricinus communis* with that of the control; it could be deduced that the pathogen grew freely and penetrated the medium, establishing itself and using up the food as compared to the “poisoned food” in the PDA containing the plants extracts. The inhibitory effects of the extracts might be due to the chemicals present in the plant, ricin a protein toxin as reported by Lowery *et al.* (2007). Similarly *Ricinus communis* had been reported to have inhibited number of eggs and percentage hatch in 100 % concentration of root extracts in *Meloidogyne incognita*. Similarly, Siam weed and Neem gave maximum inhibition of egg hatching (100 %) and larval mortality followed by Lemon grass and Castor bean with 95 and 93 % inhibition of egg hatch respectively (Adegbite and Adesiyan, 2005). This therefore suggests that the plant extracts (*Ricinus communis*, *Chromolaena odorata* and *Cleome viscosa*) showed inhibitory effects on the growth of the fungus. Tariq (2009) in his report observed that growth in fungi is affected by the availability of substrate. He further stated that the growth of most fungi is rapid at the exponential phase until one or more nutrient becomes limiting or depleted and or metabolic products accumulates to low level, the fungi will grow if uninhibited. A similar result was reported by Tongle *et al.* (2002) in their study on extracts from five Chinese medicinal herbs *Galla chinensis, Rheum palmytum* (root), *Sophora flavescens* (root), *Terminalia chebula* (fruit) and *Magnolia officinalis* (bark) had inhibitory effects against *Phytophthora infestans in vitro* and *in vivo* by inhibiting sporangia germination, mycelial growth and infection on potato leaves.

5.3 Inhibition percentage of radial growth of *Alternaria solani* at 24, 48 and 72 hour

Results on inhibition percentage of the plant extracts as observed in this study showed that *Ricinus communis* at higher concentration inhibited radial growth by as much as 41 % to 59 % in the combined results. This finding concurs with that of Baldrian and Gabriel (2002) who reported that *Piptoporus betulinus* growth was found to be concentration dependent as fungal growth was inhibited at higher concentration. This could be inferred that *Ricinus communis* could inhibit the growth of *Alternaria solani*. In addition, the fungus is much more sensitive to *Ricinus communis* in affecting radial growth than to the other plant extracts.

5.4 Potted experiment

Results of the potted experiment regarding the performance of tomato variety UC-82B on manifestation of early blight symptoms revealed that plant establishment was high at higher concentration (100 %) except in *Cleome viscosa*. Good establishment of crops is critical to the performance of transplanted crops if good yield is to be obtained. In the result of the potted experiment, result show that the control recorded the lowest plant establishment as well as recording the highest percentage disease severity. This low percentage establishment in the control could be attributed to the high percentage disease severity which could have resulted in low photosynthate production. Harper (1999) stated that foliar pathogens are some of the most serious diseases of crop plants as a result of their effects on photosynthesis. In addition to the managing effect on growth and yield, the quality of yields may be affected through reduced fruits sizes due to smaller supply of assimilates from the leaves. This result clearly indicates the effect of the various plant extracts in reducing the early blight disease on tomato. The lowest percentage disease severity was recorded in *Chromolaena odorata* 100 % and *Ricinus communis* 50 % as well as in *Cleome viscosa* 50 %. This relationship exhibited by the extracts in comparison to the control shows that the plant extracts have some fungicidal or fungitoxic properties. Sudhakar *et al.* (2006) reported that *Cleome viscosa* exhibited a broad spectrum of antimicrobial activity against *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. In addition Ngane *et al.* (2006) has reported the efficacy of *Chromolaena odorata* as possessing antifungal properties when extract and fractions inhibit the *in vitro* growth of *Cryptococcus neoformans*, *Microsporum gypseum,* and *Trichophyton rubrum* with a minimal inhibitory concentration range from 62.5 to 500 µg/ml. Also Enikuomehin (2005) reported that extracts of *C. odorata* substantially reduced the number of infected leaves and number of lesions on foliage in the control of *Cercospora* leaf spot of sesame (*Sesamum indicum* L.)

More so, the result of days to 95 % fruiting indicated no significant differences (P=0.05) among the various treatments although there were a mixed effects which show *Chromolaena odorata* and *Eucalyptus camaldulensis* 25 % recording in the shortest means while the longest days to 95 % fruiting were recorded in the control and *Cleome viscosa* 50 %. This result indicates that the plant extracts at the various concentrations probably had no effect on days to fruiting. This could possibly be as a result of the possible effect of container grown plant on availability of nutrients. This could also be seen from the fruit weights which show that those with short days to 95 % had higher fruit weight than those that reached 95 % fruiting later with the exception of *Eucalyptus camaldulensis.* This trend was observed by Xu and Kafkafi (2001) when they reported the effect of container volume on flowering, fruiting and nutrient uptake in sweet pepper. They stated that small container induced early flowering and led to early fruit set and maturation.

Tomato which is usually grown for its soft edible and succulent juicy fruits requires good crop management and proper harvesting in order to get marketable fruits. Therefore, infected fruits due to early blight could be a detriment. The result in this study revealed that the highest number of percentage infected fruits was recorded in the control which incidentally also recorded the highest percentage disease severity. This could explain the reason why the control had the highest in terms of percentage infected fruits. A similar result was reported by Abadie *et al.* (2008) in Cameroon in banana when the effect of *Mycosphaerella* leaf spot had impact on fruit quality by malformation of the fruit. This subsequently affected market price and export due as a result of poor fruit quality (Abadie *et al.,* 2008). Similarly, *Cleome viscosa* and *Eucalyptus camaldulensis* had percentage infected fruits. It also concurs with the findings of Sudhakar *et al*. (2006) who reported that *Cleome viscosa* and *Eucalyptus camaldulensis* have mild effects on fungi.

The highest fruit weight was recorded in 100 % concentration of *Chromolaena odorata.* This could be attributed to the high initial establishment and low percentage disease severity as compared to the control which recorded the lowest fruit weight. The high fruit weight could be attributed to the source strength in this treatment as it is disease free which did not impede assimilate utilization or production. Nahunnaro (2006) reported that in cotton, increased susceptibility of SAMCOT-8 due to *Xanthomonas campestris* pv. *malvacearum* was attributed to its consistently low yield. The study further revealed the relationship between angular leaf spot, vein blight, boll blight and yield to have highly significant negative correlation which is an indication of the reduction in yield of the genotypes due to the effect of the disease (Nahunnaro, 2006). Hence the tomato fruits with high disease severity produced less fruits and low fruit weight. More so, *Chromolaena odorata* could be considered as possessing some chemicals that could greatly reduced the manifestation of early blight disease of tomato in the field.

Although the field (*in vivo*) result is different from the laboratory (*in-vitro)* experiment which indicated *Cleome viscosa* and *Ricinus communis* to be effective in comparison to the potted (*in vivo*) experiments which showed *Chromolaena odorata* to have inhibited radial growth in the pathogen. Amadioha (2001) have reported similar result in his study in which *Citrus limon* effectively reduced radial growth of *Rhizoctonia solani in vitro* but failed to check the disease in the field. This variability in the efficacy of the plant extracts in the laboratory and potted experiments could also be attributed to factors such as temperature and relative humidity. Disease incidence has been reported to be highly variable across space and time, and much of the variability appears to be influenced by climatic and environmental variables according to Jarosz and Davelos (1995). Suleiman and Emua (2009) also reported the result of a study to control root rot of cowpea using four plant extracts which revealed that the laboratory *in vitro* was more effective when compared to the field (*in vivo*) study conducted. In addition, Hadizadeh *et al.* (2009) reported that essential oils from nettle (*Urtica dioica* L.) and thyme (*Thymus vulgaris* L.) controlled *Alternaria alternate in vitro* but failed to control the disease under field condition. Therefore, these findings indicate that there are plants with fungicidal properties and which may have the potential of being used in plant disease control.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Early blight of tomato has been controlled using cultural, physical, chemical, and biological methods. However, the very large host range, and absence of durable resistance of tomato cultivars in the field is a major problem. In addition, fungicides being used to control the disease have been reported to put enormous selection pressure on the pathogen leading to development of resistance by the early blight pathogen. Moreover, the toxicity posed to man and the environment prompted many researchers to look for safe and effective alternatives to chemicals in the control of plant diseases. Hence the desire to search for alternatives from plants as well as ascertain their effectiveness in the control of early blight of tomato. The objectives of this study were to determine the effectiveness of plant extracts for the control of early blight of tomato as well as to ascertain the concentration that is most effective. The experiment consisted of five (5) plant materials (*Ageratum conyzoides, Chromolaena odorata, Cleome viscosa, Ricinus communis* and *Eucalyptus camaldulensis*) at three (3) concentrations and control.

The fungicidal activity of the cold water extracts of the five plants was tested against *Alternaria solani* both in the laboratory on potted tomato plants. Laboratory evaluation of the plants extracts for their effectiveness on fungal biomass at 72 and 96 hours after inoculation revealed that *Cleome viscosa* has inhibited the growth and sporulation of the fungus. The other extracts that show promising activity in suppressing *Alternaria solani* growth are *Chromolaena odorata* and *Ricinus communis* which recorded high fungal biomass as compared to the control which recorded the lowest weight followed by *Eucalyptus camaldulensis*. This result indicated the ability of the plant extracts (*Cleome viscosa, Chromolaena odorata* and *Ricinus communis*) to have chemicals that inhibited the growth of the fungus. The result from this study further revealed that *Eucalyptus camaldulensis* has low fungicidal activity (*in vitro*) as compared to the control which probably is due to utilization of the medium uninhibited as well as reaching it death or lysis phase early resulted in the low fungal biomass. The uninhibited growth in the medium without inhibitors allowed the growth and sporulation of the fungus as recorded by the low fungal biomass in the control which made the fungus grow freely.

Furthermore, from the results *Ricinus communis* at 100 % concentration exhibited the most potent fungicidal activity in reducing radial growth taken at 24, 48 and 72 hours after inoculation. The lowest radial growth recorded consecutively at 24, 48 and 72 hours is an indication that *Ricinus communis* inhibited the growth of *Alternaria solani in vitro* by preventing utilization of the growth media (Potato Dextrose Agar). In this study, highest radial growth was recorded in the control which has no plant extracts added in the medium (PDA) which allowed the fungus to growth uninhibited and fast compared to the plant extracts. Although *Eucalyptus camaldulensis* in the combined result at 72 hours recorded high radial growth means the extract has low fungicidal activity.

The result of inhibition percentage of the various extracts on the fungus revealed that *Ricinus communis* has the highest inhibition percentage by the high inhibition of 50 - 61 % whereas *Eucalyptus camaldulensis* has low to moderate inhibition of 4 -33 % of growth of the fungus. The effect of concentration indicated that high concentration of 100 % extracts has more effect in inhibiting fungal growth as recorded by its effect on radial growth and percentage inhibition of growth. This is evident in the *Ricinus communis* and *Chromolaena odorata* extracts which revealed high inhibition at higher concentration. Thus there is a negative relationship between inhibition of radial growth and concentration in the growth of *Alternaria solani in vitro*. Evaluating the plant extracts further in potted plants revealed a different trend in terms of the most effective plant material in preventing the manifestation of the early blight symptom *in vivo* (field). The percentage disease severity revealed that *Chromolaena odorata* treatment recorded the highest fruit weight when compared to the control which recorded the highest percentage disease severity and recording a low fruit weight. This may not be unconnected with the effect of the disease on the control which affected assimilate production and interfered with photosynthesis resulting from diseased leaves and reduction in chlorophyll content of the leaves. Thus it is evident from this study that plant extracts has potential for use in reducing foliar disease of tomato.

6.2 Conclusion

In conclusion, the evaluation of the various plant extracts at different concentrations tested on *Alternaria solani* showed promising prospects for the utilization of natural plant extracts in plant disease control. *In vitro* experiments showed that *Ricinus communis* 100 % could reduce radial growth in *Alternaria solani* and inhibited its growth while *in vivo* potted experiment indicated that *Chromolaena odorata* 100 % has fungicidal effects in inhibiting the manifestation of early blight symptom of tomato caused by *Alternaria solani*. Therefore, plant extracts can be used as a potential source of sustainable ecofriendly botanical fungicides, after successful completion of wide range trials.

6.3 Recommendations

Based on the findings of this study, the following recommendations are made:-

1) In view of high fungal biomass recorded in *Cleome viscosa* and *Chromolaena odorata* plant extracts there is need for further research as very few information are available to support this finding.
2) *Ricinus communis* 100 % and *Chromolaena odorata* 100 % which proved effective should be put to further *in vitro* and field trials to confirm their effectiveness.

3) Similarly the differences in the effectiveness of the plant extracts *in vivo* and *in vitro* as observed in this research, calls for field study to ascertain the factors responsible for this variation.
4) There is the need for further research on these plant extracts using other methods such as hot water or methanol as solvents to evaluate their efficacy on early blight disease control using the same procedure.
5) Lastly, more plant materials should be evaluated to study their effectiveness on controlling early blight symptoms.

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Appendix 1: Phytochemical composition of the five plants extracts

Plants	Chemical composition
Billy Goatweed (*Ageratum conyzoides)*	The composition of *A. conyzoides* includes flavonoids, alkaloids, cumarins, essential oils, and tannins. Vyas and Mulchandani (1984) identified conyzorigum, a cromene. Also identified are precocene I and precocene II, in India as well as 11 cromenes in essential oils, including cromene, 6-angeloyloky-7-methoxy-2,2-dimethylcromen. Trigo *et al.* (1988) found several alkaloids, including 1,2- desifropirrolizidinic and licopsamine.
Siam weed (*Chromolaena odorata)*	Chemical analysis of the extract and fractions showed the presence of coumarins, flavonoids, phenols, tannins and sterols (Ngane *et al*., 2006). Tests for tannins, steroids, terpenoids, flavonoids and cardiac glycosides were positive in methanolic and aqueous extracts (Akinmoladun *et al.*, 2007).
Asian spiderflower (*Cleome viscosa)*	The seed contains 0.1 % viscosic acid and 0.04 % viscosin, cembranoid diterpene, Cleomiscosin D, a coumarino-lignan from seeds (Rukmini and Deosthale, 2007). Seeds of *Cleome viscosa* also contained thioglucosinolates (Chopra *et al.*,1986)
Castorbean (*Ricinus communis)*	Castor seed is composed of 80 % to 90 % ricinoleic acid (12-hydroxyl-cis-9-octadecenoic acid). The seeds, leaves, and stems of the plant contain ricin and ricinine, dihydroxystearic, linoleic, oleic, and stearic acids, β-sitosterol. Sprouting seeds contain catalase, peroxidase and reductase (Oplinger *et al.,* 1997).
Eucalyptus or redgum (*Eucalyptus camaldulensis)*	The analysis of *Eucalyptus* spp. revealed the presence of saponin, saponin glycosides, steroid, cardiac glycoside, flavonoids, tannins, volatile oils, phenols and balsam (Essien and Akpan, 2004). In the leaves betulinic acid, eucalyptic acid, eucalyptolic acid, oleanolic acid and ursolic acid (Siddiqui *et al.,* 1997).

Source: Vyas and Mulchandani, 1984; Chopra *et al.,* 1986; Trigo *et al.,* 1988; Oplinger *et al.,* 1997; Siddiqui *et al.,* 1997; Essien and Akpan, 2004; Ngane *et al*., 2006; Rukmini and Deosthale, 2007; Akinmoladun *et al.,* 2007

Appendix 2: Mean squares of the effect of plant extracts on fungal biomass (g) first laboratory experiment

Source of variation	Df	72 hrs	96 hrs	120 hrs
Treatment	15	0.000027**	0.000019*	0.00024ns
Error	32	0.000007	0.0000080	0.00020
Total	47			

* = Significant (P=0.05), ** = highly significant (P=0.01) and ns = not significant

Appendix 3: Mean squares of the effect of plant extracts on fungal biomass (g) second laboratory experiment

Source of variation	Df	72 hrs	96 hrs	120 hrs
Treatment	15	0.000027**	0.000022*	0.00024ns
Error	32	0.000007	0.000008	0.00020
Total	47			

* = Significant (P=0.05), ** = highly significant (P=0.01) and ns = not significant

Appendix 4: Combined mean squares of the effect of plant extracts on fungal biomass (g)

Source of variation	Df	72 hrs	96 hrs	120 hrs
Treatment	65	0.000023**	0.000022**	0.00016ns
Error	30	0.000004	0.0000053	0.00014
Total	96			

** = highly significant (P=0.01) and ns = not significant

Appendix 5: Mean squares of the effect of plant extracts on radial growth (cm) first laboratory experiment

Source of variation	Df	24 hrs	48 hrs	72 hrs
Treatment	15	0.70**	0.93**	0.57**
Error	32	0.14	0.17	0.18
Total	47			

* = Significant (P=0.05), ** = highly significant (P=0.01) and ns = not significant

Appendix 6: Mean squares of the effect of plant extracts on radial growth (cm) second laboratory experiment

Source of variation	Df	24 hrs	48 hrs	72 hrs
Treatment	15	9.22ns	0.83**	0.74**
Error	32	8.98	0.02	0.02
Total	47			

* = Significant (P=0.05), ** = highly significant (P=0.01) and ns = not significant

Appendix 6: Combined mean squares of the effect of plant extracts on radial growth (cm)

Source of variation	df	24 hrs	48 hrs	72 hrs
Treatment	15	4.86ns	0.93**	0.67**
Error	32	4.56	0.10	0.10
Total	47			

* = Significant (P=0.05), ** = highly significant (P=0.01) and ns = not significant

Appendix 7: Mean squares of parameters taken on the effect of plant extracts on manifestation of early blight (potted) experiment

Source of variation	df	Plant estab.	Percentage disease severity (WAT)		Days to 95 % fruiting	No. of fruits	Percentage no. of infected fruits	Fruit weight
			4	6				
Treatment	15	440.97ns	762.55**	664.00**	2.37ns	52.77ns	0.35ns	46.36ns
Error	32	260.42	1.69	2.33	3.06	66.06	0.29	50.32
Total	47							

* = Significant (P=0.05), ** = highly significant (P=0.01) and ns = not significant