Antifungal Activity of Selected Crude Plant Extracts on Bean Rust *(Uromyces appendiculatus)* and Their Effects on Physiological Activities of French Beans.

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other university.

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DEDICATION

This work is dedicated to my beloved mum and dad who despite the loneliness occasioned by my absence, believed in me. To my lovely siblings Nancy, Lamech, Lydia and Ezra who hopefully understood that I had to be away for good things to come. Above all to God, the creator of all beings, who provided strength, health and favor to enable me see this output.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CAN	Calcium Ammonium Nitrate
CV	Coefficient of Variation
DAP	Di-Ammonium Phosphate
DTPA	Diethylene triamine pentaacetic acid
EU	European Union
EurepGAP	European standards
FPEAK	Fresh Produce Exporters' Association
GK	Government of Kenya
HCDA	Horticultural Crop Development Authority
HPLC	High Pressure Liquid Chromatography
IRGA	Infrared Gas Analyzer
JKUAT	Jomo Kenyatta University of Agriculture and Technology
LSD	Least Significant Difference
PAR	Photosynthetic Active Radiation
RBD	Randomized Block Design
Rubisco	Ribulose-1,5-Bisphosphate Carboxylase Oxygenase
S E D	Standard Error of Differences
SAS	Statistical Analysis System
UK	United Kingdom

ABSTRACT

Rust (*Uromyces appendiculatus*) is a major foliar disease that reduces yields and pod quality in beans. There is need to introduce effective and environmentally friendly pest control products. The objective of this study was to evaluate the performance of commercial fungicide (Kocide DF) and antifungal plant extracts in the control of this disease. A total of 9 plants belonging to different genera were selected from native flora of Eastern, Western and Rift Valley provinces in Kenya.

The antifungal activities against *U. appendiculatus* by the crude extracts of selected plants were studied *in vitro* and *in vivo* experiments. French bean (*Phaseolus vulgaris* L.) variety Amy that is susceptible to bean rust (*U. appendiculatus*) was used in evaluation. *In vitro* evaluations was performed on extracts from *Maesa lanceolata, Tithonia rotundifolia, Aloe secundiflora, Carisa edulis, Urtica dioica, Boscia angustifolia, Zanthoxylum chalybeum, Melea volkensii* and Kocide DF as treatments. A field trial was established at JKUAT-Kenya in a Completely Randomized Block Design replicated 4 times. The plots were 3 × 4 m with 0.5 m paths between plots and 1.5 m between blocks. Variety Amy was planted at a spacing of 30 × 10 cm within and between rows. Single plant extracts and combinations of *Boscia angustifolia, Zanthoxylum chalybeum* and *Melea volkensii* were used to evaluate their effect on *U. appendiculatus* in the field. The treatments were applied once in every week. Major carotenoids from the pods of French beans were isolated and profiled using High Performance Liquid Chromatography (HPLC) peaks to determine the consistency of the compounds in the pods.

Physiological responses such as carbon dioxide assimilation, Photosynthetic active radiation (PAR), Transpiration, Stomatal conductance (gs), leaf temperature and Photosynthetic rate (Pn) of French beans treatments were examined using Infrared gas analyzer (IRGA) in all treatments.

Differences were found between the inhibitory effects *in vitro* and *in vivo*. *B. angustifolia, Z. chalybeum* and *M. volkensii* inhibited efficiently spore germination of *U. appendiculatus*. Extracts of *B. angustifolia, Z. chalybeum* and *M. volkensii* showed significant levels ($P<0.05$) of disease inhibition activities against *U. appendiculatus* on bean leaves and pods. The most effective treatment was *M. volkensii* followed by *B. angustifolia- Z. chalybeum*. There were significant differences among treatments in marketable yields. The high regressions between stomatal conductance and rate of transpiration in the all treatments indicated that stomatal conductance and rate of transpiration were interdependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. A total of eight treatments were used in the study. A combination of *Z. chalybeum and M. volkensii* appeared to have caused reduction in bacterial population. *M. volkensii* and *B. angustifolia - Z. chalybeum* treatments caused significant increase in fungal population. In general, results revealed bioactive potential of the flora from *M. volkensii* and a combination between *B. angustifolia* and *Z. chalybeum* to produce metabolites with potential applications as botanical pesticides.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Introduction

French beans (*Phaseolus vulgaris L.)* are important in the farming systems of East and Central Africa. They are also called Haricot beans, string beans, snap beans or fillet beans and belong to the family of plants called Leguminosae (Fabaceae). The crop has great potential for addressing food security, income generation and poverty alleviation (Ugen *et al.,* 2005). In 2009, Kenya produced 29,923 metric tonnes of vegetables valued at KES 4.2 billion that were marketed to various destinations as fresh produce and processed products. French beans accounted for a significant proportion of total horticultural exports (HCDA, 2009).

Bean rust, caused by the fungus *Uromyces appendiculatus,* is a common and serious disease of French beans worldwide but is most prevalent in tropical and sub-tropical areas (Robert, 1991). It causes 25 - 100% yield loss depending on the stage of infection and the prevailing weather conditions (Schwartz *et al.,* 2004; Robert, 1991). Kenyan French beans are largely exported to the European markets where consumers demand aesthetic quality products that are disease free. This has generally encouraged excessive use of chemical pesticides in French bean production in Kenya (Mwanthi and Kimani, 1990; Okado, 2001; Jaffee, 2003; Farina and Reardon, 2000). Chlorothalonil and copper based fungicides have been effective in the control of bean rust (Gerhardson, 2002) but indiscriminate use of these chemicals has often resulted in adverse environmental effects, development of pest resistance and negative

effects on human health (Slusarenko, 2008). Concerns over the adverse effects of chemical fungicides on the health of consumers have lead to revision of food safety standards in regard to pesticide residue in fresh produce.

The revision of international food safety standards has introduced a new order in the use of pesticides in production of fresh vegetables destined for markets in developed countries. Alternative non-chemical disease management strategies which are based on naturally occurring compounds need to be developed to ensure safe trading (Oniang'o, 2003). A possible alternative is the use of antifungal plant extracts. The plant world comprises a rich storehouse of biochemicals that can be used as biological pesticides which are environmentally safe (Hashim and Devi, 2003).

Extracts from plant such as *Melia azedarach*, Euclyptus *citriodora, Azadirachta indica, Allium sativum, Lippea javanicum, Urtica massaica, Satureia biflora, Warburgia ugandensis, Zingiber officinales* and *Alstonia scholaris* have showed antimicrobial activity against a wide range of plant pathogens (Charmaine *et al.*, 2005; Makeredza *et al*., 2005; Otanga, 2005). Identification of indigenous plants with antifungal activity against *U. appendiculatus* would contribute substantially in the development of an environmentally friendly control method for bean rust in French beans. This line of research has not been given attention in Kenya, and is therefore the subject of this study.

1.2 Statement of the problem

Most of the popular French bean varieties grown in Kenya such as Amy, Julia, Samantha and Paulista are susceptible to rust. Rust is a major foliar disease

particularly, where overhead irrigation is practiced because of the splashing of uredospores by water, which aids dissemination of the pathogen. Application of fungicides to control rust twice a week, as practiced by some farmers, is overuse of fungicides and could lead to high residue levels in the harvested produce. Effectiveness of some chemical fungicides in controlling bean rust is questionable. Agricultural farm workers who are exposed to pesticides for a long time have been found to exhibit health problems such as immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities and different kinds of cancer. Exposure to all the commonly used pesticides such as phenoxyherbicides, organophosphates, carbamates, and pyrethrins has been associated with these adverse health effects.

Famers' failure to observe the pre-harvest intervals, poor calibration of sprayers and use of contaminated water to mix chemicals lead to residue levels in agricultural produce that are above the recommended. Many pesticides are not easily degradable; they persist in soil, leach to groundwater and surface water and contaminate the environment. Pesticide residues have also been found in soil, air, surface and ground water across the nation.

The presence of pesticides directly or indirectly influences the microfauna in the soil and can alter decomposition and circulation of nutrients. The non-selective effect of chemical fungicides results in a profound long-term disturbance of the ecosystem. The majority of chemical fungicides do not specifically target specific pathogens only; during their application they also affect non-target plants. In addition to

controllling diseases, chemical fungicides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants.

The main European markets are increasingly becoming intolerant to residues in the horticultural produce. Farmers lack a control product that is environmentally friendly as well as leaving no harmful residues in the beans. Plant extracts could be tried as safe potential alternative to chemical control of plant pathogens. In the search for new eco-friendly and non-toxic agrochemicals to control fungal phytopathogens, extracts from nine indigenous plants from Eastern and Rift Valley provinces in Kenya were tested as potential biopesticides against bean rust (*U. appendiculatus)* in French beans.

1.3 Justification

The proper use of pesticides takes on significant meaning in Kenya especially with quality regulations and requirements for horticultural imports under review in the European Union (EU). In East Africa, the use of synthetic pesticides has been the major method of pest control. These pesticides have been found to be hazardous to man and environment and are therefore not conducive to support sustainable agriculture.

Pesticides residues bio accumulates in soft tissues of humans when contaminated food product is consumed, leading to acute and chronic health hazards. Pesticides can cause adverse health impacts notably death, disease, and birth defects (teratogenic) among humans and animals. The massive use of pesticides leads also to the environmental pollution in many different forms inflicting global warming and

depletion of ozone layer, pest migration and expansion that affects productivity, profitability and safety of food products. Pesticide overuse can cause pollution of soil, water, and air making unstable ecosystem unsustainable for agriculture. They also cause death of wildlife and bees disturbing the ecosystem chain. Heavy use of pesticides has been reported in many developing countries leading to concerns over medical health effects of pesticide residues on consumers and farm workers. To reduce the recurrence of food safety failures and allay consumer fears over the safety of the food, developed country governments have enacted stringent legislations relating to pesticide residue limits and packing hygiene. In view of the increasing levels of pesticides in the environment, it would seem important to have a better understanding of how these environmental changes will impact the physiology of plants in agriculture.

This project sought to explore use of antifungal plant extracts in addressing the problem of bean rust disease in French beans which will contribute to reduction of pesticide use and Kenyan exporters comply with safety standards set by European importers.

1.4 Objectives

1.4.1 Overall objective

To determine the antifungal activity of selected crude plant extracts for control of bean rust and their effects on physiological activities in French beans.

1.4.2 Specific objectives

- To evaluate selected plant extracts for management of bean rust
- To evaluate the effect of selected plants extracts on growth and physiology of French beans
- To assess possible inclusion or accumulation of extraneous compounds in experimental plants
- Assessment of effects of selected plant extracts on soil fungal and bacterial population

1.5 Hypothesis

- Selected crude plant extracts have antifungal activity against *U. appendiculatus L.*
- Chemical fungicide suppresses growth and lower physiological activities of French bean plants.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Kenya's horticulture Industry

Kenya's horticultural sector has received a great deal of attention over the past decade due to the rapid and sustained growth of its exports to Europe (Jaffee, 2004). In 2004, it exported over 30,000 tons of French beans to European markets. Despite the lack of consensus on the actual contribution of small landholders to total horticulture exports, there is evidence suggesting that this contribution has declined over time, largely due to the cost and difficulty of complying with the new export production requirements (Okello and Swinton, 2007).

These requirements are established in the protocol for Good Agricultural Practices (GAP) of the retailer members (mostly supermarkets) of Euro-Retailer Produce Working Group (EUREP) and are a response to rising litigation from European consumers following several food safety scandals (Mungai, 2004). Most Kenyan exporters have reduced their involvement with small-scale growers after the introduction of EurepGap (Asfaw *et al.*, 2007). Farmers are aware of harmful residues in beans due to chemicals used that contribute to a high rate of rejection but lack alternative disease management strategies (Monda *et al,* 2003).

Horticultural exports have demonstrated huge potential in terms of both growth rates and overall demand, generating jobs that directly support a half million workers,

small scale farmers, and their families (Jaffee, 2003). The European Union remains the principal market for Kenya horticultural export produce; with the United Kingdom, Netherlands and France in the leading positions. Other important markets of the EU are Germany, Switzerland, Belgium and Sweden. The Middle East and South Africa are vital markets outside the EU.

2.2 French beans ecological requirements

French beans are polymorphic, annual herbaceous species. They are grown where temperatures are warm, ranging between 12° C and 34° C. The optimal temperature for French beans is 20° C. French beans are good source of protein, carbohydrates, vitamin B, nicotinic acid, calcium and essential amino acids. French beans grow in a wide variety of soils ranging from light sand to heavy clays but does best in well-drained loam soils rich in organic manure (HCDA, 1996). They need a well distributed rainfall of 600 to 1500mm throughout the growing season. Frost, dry winds, long rains and fog periods are harmful (MOALDM, 1995). Irrigation is vital to maintain continuous production (Legget, 1992). Harvest time of beans depends on the climatic conditions in which they are grown and also the bean variety but generally picking of pods begins seven to eight weeks after planting and may go on for two months. Some of the major French bean production areas in Kenya are located in Kirinyaga and Machakos districts (KARI, 2005).

2.3 Pests and diseases

Pests and diseases are known to cause high economic losses in French bean production. Among them bean rust caused by *Uromyces appendiculatus L.* is a major

disease in both temperate and tropical bean production regions. It causes 25 - 100% losses depending on stage of infection and the prevailing weather conditions (Schwartz *et al.,* 2004). Rust causes maximum effect on yield if it infects beans between the third trifoliate leaf and pre-flowering stages (Mwangi *et al*., 1993). Ideal conditions for rust are moderate temperatures of 18-27° C with relative humidity of 95% for periods of 7-8 h. Severe rust infection results in defoliation, stunted growth and subsequent reduced yields while infected pods may be rejected in the market due to the development of disfiguring lesions (Jacques, 2002). There is also growing concerns among consumers for food safety certification and compliance with environmental and ethical standards (Will, 2003).

The main European markets are increasingly becoming intolerant to residues in the horticultural produce that are exported from Kenya (Mulandi, 1998; Cesnik *et al.,* 2006). Pesticide residues are often attributed to the failure of farmers to restrain applications before harvest not observing pre harvest interval and to the use of prohibited pesticides (Shopper, 2006). Apart from their effect on consumer health and the environment; pesticide residues have significant trade implications (Weinberger and Lumpkin, 2005). Mausch *et al*. 2006 reported that export standards introduced by the food industry, like EurepGAP, present a challenge for Kenyan export-oriented horticulture, which is targeting almost exclusively the European market.

2.3.1 Bean rust disease

The bean rust fungus (*Uromyces appendiculatus*) is of worldwide importance as a yield-reducing disease of *Phaseolus vulgaris* L., potentially causing yield losses up to

50% (Venette and Jones, 1982; Berger *et al*., 1995; De Jesus Junior *et al*., 2001). Heavy epidemics occur especially in the tropics and subtropics, because of the climatic conditions favoring the spread and infectiousness of *U. appendiculatus* (Stavely, 1991). *U. appendiculatus* is an obligate parasite completing its entire life cycle on the host.

The potential source of rust inoculum (spores) that initiate an epidemic is air-borne uredospores blown. Rust development is favored by cool to moderate temperatures with moist conditions that result in prolonged periods of free water on the leaf surface for more than 10 h. Repeating disease cycles may occur at 10 to 14 day intervals under favorable conditions (Steadman *et al.,* 2002). Bean rust increases the potential for significant yield loss in the event that a rust epidemic occurs. Rust affects leaves, stems and pods of bean plant. The common symptom is the brown orange pustules on leaves. Under severe disease, it completely defoliates the plant and can cause 100% crop failure (Steadman *et al.*, 2002). All French bean varieties such as Amy, Paulista, Regina, Samantha and Julia grown by farmers are susceptible to rust.

Amy is a popular variety due to extra fine quality pods and a longer harvesting period but reduces the quality as the pods tend to bend due to rust pustules (Monda *et al*., 2003).

2.3.1.1 Life cycle of Bean rust disease

The common bean rust disease is caused by the basidiomycete fungus *U. appendiculatus*. This fungus cannot be cultured on artificial media in the laboratory (Pastor-Corrales, 2006). The rust pathogen completes its entire life cycle on the

common bean host; thus, this rust is autoecious. This pathogen is also macrocyclic; that is, it produces several different types of spores that include the urediniospores, teliospores, basidiospores, pycnyospores and aeciospores (Liebenberg and Pretorius, 2010). The rusty, cinnamon brown type of spores, named urediniospores, gives this disease its name.

The urediniospores are contained within the reddish brown uredinia (known as pustules) which are observed on infected leaves, and sometimes on pods. The urediniospores are the most commonly observed type of spores of the bean rust fungus (Pastor-Corrales, 2006). Repeated infections by urediniospores occur during the summer (planting) season on toward the end of bean plant growth cycle, telia (dark pustules containing black teliospores) are usually seen on old infections (Liebenberg and Pretorius, 2010). These teliospores are the overwintering, resting spores. When the teliospores germinate, they produce basidia and basidiospores that infect the leaf tissue of their bean host. Following these infection the next stage in the cycle of the rust pathogen is produced. These are the pycnia (the fruiting structure) that produce pycnyospores.

Following cross fertilization of the bean host with the pycnyospores a fruiting structure (aecium) is produced (Liebenberg and Pretorius, 2010). The aecium produces the aeciospores. When the aeciospores infect the leaf tissues of young bean plants during the spring, uredia pustules are produced, completing the cycle of the bean rust pathogen (Souza *et al.*, 2008).

2.3.1.2 Control strategies

Fungicides for bean rust management are most effective when used in the very early stages of the epidemic and preventatively. Effective fungicides include protectants such as chlorothalonil and dithiocarbamates, and systemic chemicals such as triazoles and carboxins (Liebenberg & Pretorius, 2010). Control of the bean rust fungus is achieved by application of several disease management measures like cultural practices, cultivation of rust-resistant varieties, and the use of protectant and systemic fungicides (McMillan *et al*., 2003). In the last century, pesticides were largely adopted to counteract the action of pests and disease and to increase plant health and yield. Efficacy levels of commercial fungicides in terms of reducing rust disease severity reach over 90% (Stump *et al.,* 2000; Gent *et al.*, 2001). However, continuous use of chemical fungicides for plant defense caused great environmental impact, the onset of resistance phenomena within some populations of fungal pathogens as well as acute and general toxicity on humans and non-target organisms. This situation has prompted an increased demand for more environmentally-friendly products in order to reduce the side effects of chemical fungicides in crop protection (Coats *et al*., 2003).

Natural oil-based fungicides such as neem could represent a good alternative to chemical fungicides (Wicks *et al*., 1999). They are effective in controlling some plant pathogens at low doses and induce little or no resistance in target fungi (Martin *et al*., 2005). Furthermore, they have excellent spreading and leaf surface adhesion

characteristics, and due to their rapid biodegradation have a low toxicity for human beings and cause little environmental impact. Kenya's small farmers are facing a serious threat in French bean production due to new pesticide controls (Jaffee, 2003).

2.4 Antifungal activity of crude plant extracts

Some plant extracts could be a potential alternative to control of plant pathogens. On global scale, studies have shown that some plant species have antifungal compounds (Fabry *et al.,* 1996; Okemo *et al.,* 2003).

Neem cake *(Azadirachta indica*) significantly suppressed population of fungal pathogens such as *Fusarium oxysporum, Urtica massaica L.* leaf extracts reduced the severity of potato late blight (*Phytophthora infestans)* and *Maesa lanceolata* has been reported to have antifungal activity (Okemo *et al.,* 2003).

Products based on *Azadirachta indica* (neem) are also known for their antifungal and pest control properties (Singh, 2003). Fungal diseases such as downy mildew of plants are one of the major causes of agricultural losses, followed only by insects. Researchers (Monda *et al* 2003; Okemo *et al.,* 2003) have increased the search to find natural alternatives to control fungal diseases of crops in agriculture, and to reduce their negative impact over soil, air, water and all living forms. One alternative is to use plant extracts, also known as botanical pesticides. It is widely recognized that plants biosynthetize a vast array of secondary metabolites, such as phytoalexins which are antifungal compounds for self defence (Kim *et al*., 2004).

With this strategy, many plants have been submitted to screening programs searching for natural alternatives to control crop pests and diseases (Quiroga *et al*., 2004).

2.5 Hazards of chemical pesticides

Pesticides cause: acute and chronic human health effects, contamination of atmospheric, ground and surface water (Matthews, 2006). In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants.

Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risks to non-target organisms (Galloway and Handy, 2003). Different pesticides have been implicated in chronic neurotoxicity, endocrine disruption, immune impacts, genotoxicity, mutagenicity and carcinogenesis (Abou-Donia, 2003; Choi *et al.*, 2004).

Certain environmental chemicals including pesticides termed as endocrine disruptors are known to elicit their adverse effects by mimicking or antagonising natural hormones in the body and it has been postulated that their long-term, low-dose exposure are increasingly linked to human health effects such as immunosuppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Liroff, 2000). Non target organisms such as plants, earthworms, termites, ant colonies, snakes, birds, toads, lizards and other amphibians have been affected negatively by pesticide use (Mosleh *et al.*, 2003; Larson *et al.*, 2005). The heavy treatment of soil with pesticides can cause populations of beneficial soil microorganisms to decline (Sardar and Kole, 2005).

A recent review of pesticides effects on earthworms showed on negative effects on growth and reproduction by many pesticides (Shahla and D'Souza 2010). A laboratory experiment that reproduced vineyard conditions in France showed that mixture of insecticides and/or fungicides at different environmental concentrations caused a neurotoxic effect in earthworms.

After a long period of exposure or high concentrations, earthworms were physiologically damaged and could not cope with the high toxicity (Schreck *et al.*, 2008). Several articles reported negative effects of pesticides and intensive agriculture on butterflies' populations (Longley and Sotherton, 1997; White and Kerr, 2007; Adamski *et al.*, 2009), and showed positive impact of organic farming (Saarinen, 2002; Feber *et al.*, 2007). Glyphosate affected predatory arthropods (spiders and ground beetle) in agricultural field, caused behavioural changes and influenced long-term surviving even in residual exposure (Evans *et al.*, 2010). Carbaryl has been found toxic for several amphibian species, additional combination with predatory stress caused higher mortality (Relyea, 2003). Also herbicide Roundup, glyphosate, caused high mortality of tadpoles and juvenile frogs in outdoor mesocosms study (Relyea 2005).

2.6 Effects of pesticides on physiological activities of plants

Photosynthesis is connected to water relations due to gas exchange. Direct or indirect heavy metal effect on stomatal functions may be responsible for the decline in transpiration. Changes in the morphology of plants caused by heavy metals may indirectly have effects on the cell walls and cell membranes which may directly

influence the water uptake and transport of mineral salts in plants (Barcelo and Poschenreider, 1990). Decreased photosynthesis can be caused by the decrease in the level of photosynthetic pigments related to breakdown or the inhibition of synthesis (Garnczarska and Ratajczak, 2000). The decrease of chlorophyll content as the effect of toxic amount of Cu^{2+} was reported for barley, spinach and rice (Lidon and Henriques, 1991). Chlorosis of leaves associated with simultaneous destruction of the inner structure of chloroplasts was also observed during a long exposure to Cu^{2+} (throughout the vegetative period) (Eleftheriou and Karataglis, 1989).

Copper is essential as a micronutrient but at high concentrations it is toxic for photosynthetic organisms (Maksymiec, 1997). Copper an ingredient of copper based fungicides inhibits the photosynthetic electron transport at elevated concentrations (Baron *et al.*, 1995). Evidence that photosynthesis is affected by pathogens can be seen in infected plants through the development of chlorotic or necrotic tissues, loss of leaves, reductions in chlorophyll and numbers of chloroplasts, and stomatal closure.

The major resistance pathways between water uptake at the root and transpiration through the stomata influence leaf transpiration, whereas additional resistance pathways exist between the stomata and the bulk atmosphere that can feed back on ecosystem evapotranspiration (Bazzaz and Sombroek, 1996). Short term lower stomatal conductance at elevated CO_2 will increase leaf temperature and, in turn, water vapor pressure deficit, which will tend to increase transpiration partially offsetting the response of stomatal conductance to CO_2. In the longer term,

conservation of soil moisture due to decreased stomatal conductance may result in increased leaf growth and, in turn, more transpiration (Arnell and Liu, 2001).

2.7 Effects of pesticides on microbial Population

Reduced numbers of bacterial decomposers in soil due to pesticides application could have important consequences relative to the mineralization of organic material and recycling of elements essential to plants, such as nitrogen and sulfur (Liebich *et al.*, 2003). Fungicides have been found to be toxic to soil fungi and actinomycetes and caused changes in microbial community structure (Pal *et al.*, 2005). Other bacterial species, such as nitrification bacteria, are very sensitive to pesticides influence. Inhibition of nitrification was proved by sulphonylurea herbicides (Gigliotti and Allievi, 2001). Chlorothalonil and dinitrophenyl fungicides such as Mancozeb, Maneb or Zineb have also been shown toxic to nitrification and denitrification bacterial processes (Kinney *et al.*, 2005; Lang and Cai, 2009).

A few studies show that some organochlorine pesticides suppress symbiotic nitrogen fixation resulting in lower crop yields (Fox *et al.*, 2007; Potera, 2007). Some pesticides (Benomyl, Dimethoate) can also negatively affected symbiotic mycorrhizal fungi, which facilitate plant nutrient uptake (Menendez *et al.*, 1999). Moreover, agricultural practices such us tillage, crop rotation, fertilization, pesticide application, irrigation can also reduce root colonisation by myccorhizal fungi (Jansa *et al.*, 2006). Cypermethrin and monocrotophos had adverse effects on the total number of soil bacteria in the soil (Ajaz *et al.*, 2005). Bacteria and fungi are the most important contributors to biomass decomposition (De-Lorenzo *et al.*, 2001).

The bacterial populations in soil are dominated by species of *Pseudomonas, Arthrobacter, Bacillus, Micrococcus, Clostridium, Achromobacter,* and *Flavobacterium* (Coleman *et al*., 1992). Dilution plate techniques measure only a small portion of the total soil community nevertheless it is a useful tool for studying the relative abundance of culturable populations and the changes in population density which occur according to the medium used or the proximity to plant roots.

2.8 Role of nutrients in plant growth and development

A good supply of nitrogen stimulates root growth and development, as well as the uptake of other nutrients therefore direct applications of fertilizer was done. Plants deficient in nitrogen tend to have a pale yellowish green color (chlorosis), have a stunted appearance, and develop thin, spindly stems (Brady and Weil, 1999). Phosphorous enhances many aspects of plant physiology, including the fundamental processes of photosynthesis, nitrogen fixation, flowering, fruiting (including seed production), and maturation. In bean plants, phosphate supply increases shoot growth sufficiently to dilute the zinc concentration and to induce or enhance zinc deficiency (Singh *et al.*, 1988).

However, it has been observed that the combination of low zinc and high phosphorus levels may enhance the absorption and transport of phosphorus in plants, inducing the accumulation of phosphorus to toxic levels in old leaves (Cakmak and Marschner, 1986).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Efficacy of different plant extracts on bean rust

3.1.1 Study sites

Experiments were carried out in the laboratory, green house and field at Jomo Kenyatta University of Agriculture and Technology (JKUAT) in Thika District. The university is located at latitude 1°05° S and longitude 37°00° E. It lies at an altitude of 1525 meters above sea level and it receives an annual rainfall of 850mm. Temperatures range from 13°C and 26°C.

3.2 Collection and processing of plant materials

The samples of nine desired plants from previous studies (Omwenga, 2009; Kiswii, 2009) that showed antifungal activity were collected from different parts of the country (Samburu, Mombasa, Mwingi, Kakamega forest and Nakuru) in clean sacks (Table 1). The plants were stored at Jomo Kenyatta University of Agriculture and Technology (Taxonomy unit, Department of Botany). Voucher specimens were deposited in the Herbarium. The samples were labeled and deposited in the GK Botany laboratory at a room temperature for three weeks. The plant leaves and roots were dried separately at room temperature for a period of 3 weeks and then ground separately to powder using a grinding mill at 8000rpm (Type 8 lab mill). The powder was stored in plastic bags at room temperature until the time required.

Table 1. Selected plants for the study and parts of the plants used

Family	Scientific Name	Common/local name	Parts used
Fabaceae	*Senna didymobotrya*	Popcorn senna	Whole plant
Maesaceae	*Maesa lanceolata*	Oljaninyuki (Maasai)	Leaves
Asteraceae	*Tithonia rotundifolia*	Mexican sunflower	Leaves
Asphodelaceae	*Aloe secundiflora*	Imugutan (Samburu)	Whole plant
Annonaceae	*Carisa edulis*	Coast rubber vine	Leaves
Urticaceae	*Urtica dioica*	Stinging nettle	Leaves
Capparidaceae	*Boscia angustifolia*	Mulule (Kamba)	Leaves, Stem
Rutaceae	*Zanthoxylum chalybeum*	Mjafari (Swahili)	Leaves, Stem
Rutaceae	*Melea volkensii*	Mukau (Kamba)	Leaves, Stem

3.3 Effect of plant extracts on uredospore germination.

Spores of *U. appendiculatus* were scraped from infected leaves using a normal tooth brush and stored at 4°C. Inoculum was prepared in distilled water and spore concentration adjusted to 10^6 spores/ml using a hymocytometer. One kilogram of each plant sample was soaked in five liters of water and left overnight to allow extraction of the crude active compounds. A drop of 0.04 ml spore suspension with about 80 spores was placed on each sterile cavity slide containing 0.5ml of plant extracts. Negative control slides without plant extract contained distilled water and 0.04 ml spores only.

All cavity slides were placed individually in sterile Petri dishes lined with sterile moist filters and incubated at 25˚C (Fitzel, 1988). Eleven treatments were replicated four times in complete random design. The treatments consisted of nine plant extracts, Kocide DF containing copper hydroxide as an active ingredient (metallic copper 40%) and formulated as a dry flowable as a positive control and a negative water control. The fungicide was applied according to the manufacturers' recommendations at the rate of 125g/20l of water. Percentage uredospore germination was arcsine transformed

for homogeneity. The data were analyzed subjected to analysis of variance (ANOVA) and the LSDs' test at 5% level of probability was used to test the differences among mean values. Observations were made under a light microscope after 6, 12, 24 and 48 h and germination percentages recorded.

3.4 Effect of plant extracts on bean rust under greenhouse conditions

French bean (Amy) seeds were planted in 15cm pots in sterile soil mixed with manure, sand and ballast (2:1:1). Extra pots were planted to allow for poor or late germination. The seeds that took longer to emerge were pre-germinated in order to obtain a more uniform germination. When the trifoliate leaves reached approximately 2/3 of their full development, about 10 days after sowing, inoculation was performed according to the method of Carrijo *et al.,* 1980. The concentration of the uredospores was adjusted to 2.0×10^4 uredospores/ml using a haemacytometer. First trifoliate leaves were inoculated with rust by spraying 5ml of spore suspension on both leaf surfaces.

Each of the eleven treatments was replicated eight times giving a total of eighty eight plants in a completely randomized design. Spore suspension was applied (5ml per plant) with a hand-held garden sprayer to all eighty eight plants. One kilogram of each plant sample was soaked in five liters of water and left overnight to allow extraction of the crude active compounds. The fungicide was applied at a rate of 125g/20l of water according to the manufacturers' recommendations. A bar soap ground to powder and dried was used as a sticker at a rate of 1 g per litre of extracts.

The treatments consisted of nine plant extracts, Kocide DF: copper hydroxide 61.4% (metallic copper equivalent 40%) formulated as a dry flowable as a positive control and a negative water control. The first trifoliate leaves were sprayed with antifungal plant extracts and commercial fungicide (Kocide DF) in separate lots. After inoculation, the plants were covered with polythene bags for 24 h to increase relative humidity. After 48 h, the plants were transferred to a greenhouse (20 ± 5º C), where they were kept until symptom development and evaluation. The leaf size affects the size of the pustule hence only the plants in the nursery with the same degree of leaf expansion were used for data collection.

3.4.1 Disease severity

Bean rust severity was determined by estimating the percentage of the total leaflet area covered by the sporulating of pustules using disease severity scale by Stavely (1985), which is a scale of 1-5, where 1 = (0-5%) leaf damage, 2 = (6-10%), 3 = (11-25%), 4 = (26-50%) and 5 = over (50%) leaf damage.

3.5 Field experiment

3.5.1 Study site soil analysis

Plant nutrition is one of the environmental factors which, along with others such as temperature, humidity, and soil moisture may have a measurable effect upon the course of disease development. Soil analysis of study site was conducted. Sample comprised of approximately 1 kg of soil taken from a depth of 6 inches (15 cm. At least 25 cores were taken at random from each area to be sampled and together

formed a single representative sample. All cores were mixed thoroughly and a sub-sample taken to the laboratory.

Soil analysis for nitrogen was done using Kjeldahl digests by titration, steam distillation and colorimetric methodologies, while phosphorus concentration was determined using sodium acetate, Bray, sodium bicarbonate (Olsen), ammonium bicarbonate-DTPA (Diethylene triamine Pentaacetic Acid), Morgan extracting solutions (Ryan *et al*., 2001). Potassium and sodium were determined by atomic absorption spectrophotometry. Zinc and copper were determined using DTPA and ammonium bicarbonate-DTPA extracting solutions. Organic matter was determined following combustion (Ryan *et al*., 2001).

3.5.2 Field layout and planting

Seeds were obtained from Regina Seed Company and planted at a spacing of 30cm between rows and 10cm between plants within the rows (Monda *et al,* 2003). French bean seeds commercially available coated with thiram were used to control root rots. French bean variety Amy seeds were planted in 4×3m plots each separated by a 1m path between the treatments and the replications. Amy is high yielding compared to other varieties therefore it is grown by most farmers. Di-ammonium phosphate (DAP) was used at planting at a rate of 200kg/ha mixed well before seed placement. Calcium ammonium nitrate (CAN) was applied at a rate of 100Kg/ha at trifoliate leaf stage.

3.5.3 Treatment application

The best three extracts in the *in vitro* and greenhouse experiments namely *B. angustifolia, M. volkensii*, and *Z. chalybeum* were used in the field experiment using natural inoculums (spores dispersed by wind naturally found in the field where previously beans were planted). The percentage uredospore germination and disease severity were used to determine the best extracts in bean rust control. The extracts were used as single treatments and combined with each other namely; *Z. chalybeum- M. volkensii, B. angustifolia - Z. chalybeum* and *B. angustifolia - M. volkensii* making a total of eight treatments including a negative and positive controls.

The treatments consisted of six plant extracts, copper hydroxide 61.4% (Kocide DF: metallic copper equivalent 40% formulated as a dry flowable) as a positive control and a negative water control. Combinations of powders in a 1:1 ratio were soaked in water overnight and strained. Two kilogram of each plant sample was soaked in ten liters of water and left overnight to allow extraction of the crude active compounds. The supernatant of each plant extract was filtered in several layers of muslin cloth and volumes adjusted to 20 L (Stoll, 2000). A bar soap ground to powder and dried was used as a sticker at a rate of 1 g per litre of water extracts.

A spray regime of once a week using a knap sack was employed from the fifteen days after planting until flowering. The extracts used were used as protectants. The fungicide was applied at a rate of 2.5kg ha^{-1} according to the manufacturers' recommendations. Eight treatments were replicated four times in a randomized block design (RBD) making a total of thirty two plots. There were a total of seven hundred

and sixty plants per replicate. Overhead irrigation twice a week and weeding were done as necessary.

3.5.4 Disease severity

Disease severity is the area of plant tissue that is (visibly) diseased. It is a measure of the quality of plant tissue. Disease severity was evaluated on ten randomly selected plants from inner rows in each plot using disease severity scale by Stavely (1985), which is a scale of 1-5, where 1 = (0-5%) leaf damage, 2 = (6-10%), 3 = (11-25%), 4 = (26-50%) and 5 = over (50%) leaf damage. It was done once a week for 6 weeks, for every treatment.

3.5.5 Disease incidence

Disease incidence was evaluated once a week on plants from each plot. The numbers of plant units that are (visibly) diseased, usually relative to the total number were assessed.

3.5.6 Yield and pod quality

Pod quality was evaluated on two hundred harvested pods per plot in four replicates of each treatment at every harvest of twice a week. The sampled pods were rated by the Stavely (1985) visual rating scale of 1-5 where 1= unblemished pods (0%) 2 = 1-10% (slight damage), 3 = 11-25% (moderate damage), 4 = 26-50% (severe damage), 5 = over 50% (very severe damage). Harvesting of fresh pods was done twice a week and weights of yields (to the nearest 0.1 kilogram) taken at every harvest. Harvest period started at nine weeks after sowing and continued for about for weeks.

3.6 Effect of plant extracts on growth and physiology of French bean plants

3.6.1 Biomass measurements

Biomass measurements were done only in the field experiment. Ten representative plants from each of the eight treatments were randomly harvested after three weeks of planting French beans to estimate the initial biomass. Plant growth parameters that include plant shoot height and dry shoot weight were used to assess the effects of various treatments on bean plant performance.

Shoot height was taken from the first node to the leaf apex where ten plants were randomly selected in each plot making a total of forty plants per treatment, marked and shoot heights taken once every month from the start of foliar (six weeks after planting) sprays until flowering. The plants were then harvested and dried separately in an oven at 80°C for 72 h. The dry shoot weights were recorded on ten sampled plants from each treatment.

3.6.2 Leaf area measurement

A destructive method of leaf area estimation was carried out to determine plant growth. Ten plants sampled in each of the four plots in a treatment were uprooted and sampled leaves plucked and area measured. This was done at the 1^{st}, 2^{nd} and 3^{rd} months of plant growth. Leaf area for the ten sampled plants from each plot was used to estimate the area. A total of one hundred and twenty plants per treatment were sampled at three intervals of three months during the season. A total of two hundred and forty plants were sampled for the experiment. These methods involved the use of

leaf area meter (Nobel *et al.*, 1993). The mean area of the ten sampled leaves was multiplied by the total number of leaves present to provide an estimate of the total leaf area for each plant. The aim was to establish whether treatments had impact on leaf expansion.

3.6.3 CO_2 exchange measurements

Three different types of leaf gas exchange measurements were made on plants from the interior rows of the plots. First, once a week, measurements of carbon dioxide assimilation rate were made at 0900hrs, 1200hrs, and 1500 hours on the plants in the field experiment in each three months of growth. Mature, fully illuminated upper canopy leaves were measured at their nominal daytime growth. Daylight patterns of carbon dioxide assimilation rate were measured by the infrared gas analyzer (IRGA). IRGA was used as a null point instrument that allows the flow of carbon dioxide into the system at a rate equivalent to the rate of uptake of the leaf.

The amount of carbon dioxide assimilated by the leaf was read directly from the IRGA. French bean leaf tissues from ten selected plants from each treatment were enclosed in the leaf chamber (Leaf chamber =2.5cm^2) one at time. A total of eighty plants were sampled from the eight treatments which included single plant extracts and combinations; *Z. chalybeum- M. volkensii, B. angustifolia - Z. chalybeum* and *B. angustifolia - M. volkensii*. The air flow rate through the chamber remained fixed. The carbon dioxide assimilation was monitored for 1 min for each leaf by the IRGA connected in an open gas flow system. During measurement of CO_2 assimilation rate

the following parameters were also recorded using IRGA; stomatal conductance, photosynthetic active radiation (PAR), transpiration and leaf temperature.

3.7 Residue analysis in bean pods

About 250g of freshly harvested bean pods from each treatment were dried and grounded to fine powder using a grinding mill at 8000rpm (Type 8 lab mill). The bean pods harvested within two days were dried at 70°C for two days and ground into fine powder. The powder was dissolved in methanol and filtered using Whitman filter papers.

The filtrate was concentrated using rota-evaporator and samples dispersed in vials and run through the column with silica gels. The extracts were filtered through micro filters and injected into HPLC (reverse phase for polar solution elution) using micro syringe of 20µm. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with two Shimadzu LC-10 ATVP reciprocating pumps, a variable Shimadzu SPD-10 AVP UV VIS detector and a Rheodyne Model 7725 injector with a loop size of 20 µl.

Reverse-phase chromatographic analysis was carried out in binary gradient conditions using a C-18 reverse phase column (250 x 4.6 mm, particle size 5 µm, Luna 5µ C-18(2); phenomenex, Torrance, CA, USA) at 25°C. Running conditions included: injection volume, 5µl; mobile phase, methanol: water (80: 20 v/v); flow rate, 0.8ml/min; and detection at 230 nm. The peak profiles obtained were used to determine consistency of major bean pod compounds in comparison with untreated control and treatments.

3.8 Phyto-toxicity of plant extracts

The number of flower drops from bean plants was assessed to evaluate the phytotoxic effect of the plant extracts to the bean plant. Ten randomly selected plants from each treatment were marked and number of flower drops picked and counted on daily basis for three weeks starting from four days after foliar spray.

3.9 Effect of plant extracts on soil microbes

Before field experiment was carried, plots were prepared and the bacterial and fungal population established. Each sampled plots designated for a specific treatment were analyzed before and after the experiment to determine the change in fungal and bacterial population. A total of thirty two sample plots each measuring (4×3) m were needed. Random sampling of plots was done and plots were marked. This was on assumption that the study area was homogenous. Soil was taken from the rhizosphere of ten plants in each treatment and mixed to make a bulk sample of 1kg. Soils from each of the sampled plots of each treatment were dissolved in sterilized distilled water separately.

Three representative isolates in each for bacteria and fungi were chosen. Serial dilutions were made on each of the dissolved soil samples up to 10^{-6} per ml. Fungi and bacteria were isolated on Potato Dextrose Agar (PDA) and Nutrient Agar respectively using standard microbiological methods. Sterile Petri dishes were used and media prepared under aseptic techniques (Cappuccino and Sherman, 2002).

Pour plate methods were used to obtain both bacteria and fungi. In isolation of bacteria, 1ml of each soil sample was put into Petri dishes and molten nutrient agar was poured. To isolate fungi, 1ml of each soil samples was put into Petri dishes and molten Potato Dextrose Agar was poured. The plates containing Nutrient Agar were incubated at 25º C for 48 h to establish colony forming units. PDA media plates were incubated at 28º C for 2-3 days. The culture plates were observed and the number and type (morphology and color) of each colony in each media plate was assessed and recorded.

For bacteria, Gram staining procedure was carried out to establish whether Gram positive and Gram negative groups. On each plate different colonies were identified and gram staining performed beginning with primary staining using crystal violet for 1 min. All the cells stained blue-purple. The slides were washed gently by tap water. This was followed by the application of Gram's iodine solution for 3 min. All cells remained purple. The slides were then washed with tap water. The slides were decolorized with acetone-alcohol for about 20secs. Counterstaining with Safranin stain for 1 to 2 min was done. After completion of the Gram stain procedure observations were made using a light microscope at low and high magnifications.

For fungi lacto phenol staining was done by adopting Cappuccino and Sherman (2002) method and colony colors, spore shape observations made using a light microscope at low and high magnifications. Soil bacterial population was estimated by Waksman`s (1952) method using the nutrient agar medium. Fungal population was estimated by dilution plate method (Johnson and Curl, 1972) using PDA medium. The inoculated Petri-dishes were incubated at 37°C for 24 h and 25°C for 5 days for

bacteria and fungi respectively. To calculate the populations of bacteria and fungi, colonies developed on Petri dishes were counted with the help of digital colony counter and expressed as numbers of colony forming units (cfu/g) dry soil. Representative isolates of fungi were identified under microscope with the help of standard manuals (Domsch *et al*., 1980). Logarithmic transformation was performed on data for fungal and bacterial population before a paired T test was performed to determine changes in population.

3.10 Data processing and analysis

Data entry management and preliminary summaries were done in Microsoft Excel Spreadsheet. Percentage data such as disease incidence, disease severity and percentage uredospore germination were arcsine transformed for homogeneity. Mean separation of treatments was accomplished using Least Significant Difference statistical procedure. Data from repeated experiments for both the *in vitro* bioassays were subjected to analysis of variance (ANOVA) for each treatment and means separated using Duncan test (SAS/IML software; Version 9.1; SAS Institute 1999).

Regression analysis was used to determine the relationship between stomatal conductance, transpiration rate, CO_2 assimilation and photosynthetic rate. Probability value of $P<0.05$ was used for entire tests to show statistical significance of mean values for parameter analyzed.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of selected plant extracts on uredospore germination

Crude extracts of all the nine plant species inhibited *U. appendiculatus* germination and their impact was as shown in Table 2. In order to evaluate the application potential of the crude extracts, their *in vitro* antifungal activities were compared to that of standard commercially available fungicide Kocide DF (Table. 2). The spore germination inhibitory activity of a *M. volkensii* leaf extract was almost similar with that of Kocide DF against *U. appendiculatus* (>30%). Among the test plants, the root extract of *B. angustifolia, Z. chalybeum* and *M. volkensii* significantly ($P<0.05$) inhibited spore germination of *U. appendiculatus* (Table 2).

The untreated control had the highest percentage spore germination (30.57±4.23) compared to other treatments followed by *S. didymobotrya* (24.48±0.52), *M. lanceolata* (12.69±2.54) and *A. secundiflora* (9.23±1.73) respectively. *T. rotundifolia* recorded the fourth highest spore germination percentage. *C. edulis, U. dioica, B. angustifolia* were not significantly different from each other and they had higher percentage spore germination than *Z. chalybeum, M. volkensii* and commercial fungicide (Kocide DF). *Z. chalybeum* (1.19±0.21), *M. volkensii* (1.02±0.16) and Kocide DF (0.94±0.16) inhibited spore germination and therefore they produced the lowest percentage spore germination (Table 2).

The commercial fungicide had significantly the lowest percentage spore germination followed closely by *M. volkensii, Z. chalybeum* and *B. angustifolia* plant extracts (Table 2). Among the test plants, the leaf extract of *M. volkensii* showed the most promising potential in significantly (P<0.05) inhibiting the spore germination of *U. appendiculatus* compared to the other plant extracts. Spore growth inhibition by the extracts of *A. secundiflora, U. dioica, C. edulis, S. didymobotrya, M. lanceolata* and *T. rotundifolia* were insignificant.

Table 2. *In vitro* percentage germination of spores in selected plant extracts and commercial fungicide (Kocide DF)

Treatment	**% spore germination**
Untreated control (negative control)	*30.57±4.23[a]
S. didymobotrya	24.48±0.52[ab]
M. lanceolata	12.69±2.54[b]
T. rotundifolia	9.29±1.82[c]
A. secundiflora	9.23±1.73[bc]
C. edulis	7.24±1.36[cd]
U. dioica	5.74±1.45[cd]
B. angustifolia	4.68±1.36[cd]
Z. chalybeum	1.19±0.21[d]
M. volkensii	1.02±0.16[d]
Kocide DF (positive control)	0.94±0.16[d]
LSD	3.157
CV%	59.4%

*Numbers represent percentage mean spore germination for each treatment.
*Means separated using LSD test by the same letter are not significantly different (P<0.05) from each other.

Treatments varied significantly with time (h) in all treatments studied (P<0.05). At 6h, treatments were significantly different (P=0.0004). After 12h there were spore germination percentage differences (P=0.002) and Kocide DF, *M. volkensii, Z. chalybeum* and *B. angustifolia* had lower spore germination percentages compared with *A. secundiflora, U. dioica, C. edulis, S. didymobotrya, M. lanceolata* and *T. rotundifolia* (Table 3). The treatments maintained the same trend in significantly

inhibiting spore germination at 24h and 48h. *A. secundiflora, U. dioica, C. edulis, S. didymobotrya, M. lanceolata* and *T. rotundifolia* had significantly higher percentage spore germination *in vitro* throughout the evaluation period (Table 3).

Table 3. Percentage spore germination in different plant extracts at different incubation periods.

Treatments	**6 h**	**12 h**	**24 h**	**48 h**
Kocide DF (positive control)	*0±0.00	1.23±0.03	1.39±0.25	1.12±0.24
M. volkensii	0±0.00	1.325±0.12	1.35±0.19	1.41±0.11
Z. chalybeum	0±0.00	1.62±0.30	1.57±0.26	1.58±0.31
B. angustifolia	0±0.00	6.63±3.40	4.73±1.42	7.33±3.52
U. dioica	0±0.00	6.16±2.97	8.085±2.49	8.72±3.33
C. edulis	0±0.00	8.92±1.78	10.34±2.35	9.68±2.16
A. secundiflora	0±0.00	13.27±1.94	11.23±1.90	12.39±0.23
T. rotundifolia	0±0.00	12.09±1.36	8.41±2.74	16.65±2.39
M. lanceolata	0±0.00	16.21±4.06	16.76±2.03	17.82±2.12
S. didymobotrya	0±0.00	23.24±0.23	32.12±0.08	42.56±0.04
Untreated control (water)	8.76±1.06	27.06±2.73	37.22±2.28	49.26±4.03
***P*-value**	0.0004	0.002	0.003	0.005
CV%	14.3	7.8	12.2	20.3

*Mean ± S.E calculated from untransformed data. Statistical analysis was performed on arcsine transformed data. Each column represents mean of 3 experiments.

Also compared Kocide DF, the leaf extract of *Z.chalybeum* was effective against *U. appendiculatus.* The performance of the *M. lanceolata* extract was rather poor compared to the standard fungicide except for slight inhibition of *C. edulis* (Table 3).

4.2 Effect of plant extracts on bean rust under greenhouse conditions

There were significant differences ($P<0.05$) among treatments*time on rust severity throughout the growth period. Among the promising, commercial fungicide (20%), *Z. chalybeum* (30%) and *B. angustifolia* (25%) exhibited the lowest leaf disease severity scores compared to others. However, there were significant differences ($P=0.0002$) among the treatments 14 days after inoculation (Fig. 1). Untreated control (75%), *S.*

didymobotrya (60%), *C. edulis* (85%), *U.dioica* (70%), *A. secundiflora* (60%) recorded the highest percentages of rust disease severity. *T. rotundifolia* (40%) and *M. volkensii* (40%) recorded moderate percentage rust severity at the 14th day of the inoculation (Fig. 1).

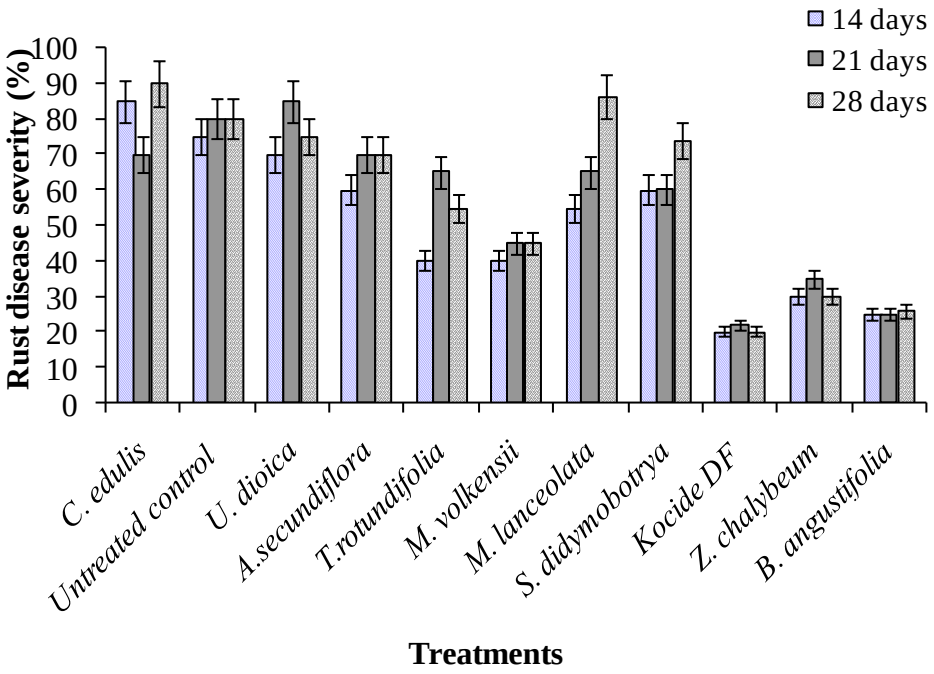


Figure 1. Rust severity at 14, 21 and 28 days under greenhouse conditions

There were significant differences in disease severity at 21 days (P<0.0001). Commercial fungicide (22%) and *B. angustifolia* (25%) had significantly high inhibitory effects on rust severity in the 21st day compared with others. *C. edulis (70%), S. didymobotrya* (60%), *U.dioica (85%), M. lanceolata (65%), A. secundiflora (75%), T. rotundifolia (65%)* and the untreated control (80%) had highest percentage rust disease severity scores. *M. volkensii* (45%) recorded moderate percentage rust severity at the 21st day of the inoculation. *B. angustifolia* treated bean plants had the highest inhibition to rust compared to all plant extracts (Fig. 1).

On the 28th day after inoculation, there were significant differences among the treatments studied (P<0.0001). The untreated control (80%), *M. lanceolata* (86.4%) and *U. dioica* (75%) treated plants recorded the higher disease severity to rust compared to the rest of the treatments meaning they were ineffective in rust control. Commercial fungicide (20%), *Z. chalybeum* (30%), and *B. angustifolia* (26%) recorded lowest percentages of rust disease severity compared to others at 28 days and these were the most effective in rust control just as the commercial fungicide (Kocide DF). *M. volkensii* (45%) recorded moderate disease severity at 28 days of inoculation at green house level (Fig. 1).

The mean disease severity for various treatments during the whole treatment period is shown in Table. 4. There were significant differences (P=0.003) in mean disease severity among various treatments studied. Mean disease severity was lower in plants treated with Kocide DF (20.82%), *Z. chalybeum* (31.48%), *M. volkensii* (43.32%) and *B. angustifolia* (25.36%) in the green house experiment. *C. edulis* (81.65%), *U. dioica* (76.65%), *S. didymobotrya*, *T. rotundifolia* (53.27%) and *M. lanceolata* (68.84%) and the control (78.27%) treated plots had significantly higher mean rust disease progression compared to all other treatments meaning their impact on bean rust disease was insignificant (Table 4).

Table 4. Mean Disease severity of rust on French beans sprayed with plant extracts and Kocide DF.

Treatments	Mean rust disease severity (%)
C. edulis	81.65±0.02[a]
Untreated control	78.27±0.06[b]
U. dioica	76.65±0.41[c]
A. secundiflora	66.60±0.07[e]
T. rotundifolia	53.27±0.06[g]
M. volkensii	43.32±0.01[h]
M. lanceolata	68.84±0.16[d]
S. didymobotrya	64.57±0.10[f]
Kocide DF	20.82±0.15[k]
Z. chalybeum	31.48±0.19[i]
B. angustifolia	25.36±0.03[j]
LSD	0.3086
CV%	0.2525

*Numbers represent percentage mean rust disease severity for each treatment.
*Means separated using LSD test by the same letter are not significantly different (P<0.05) from each other.

4.3 Evaluation of field soil for its nutritional status

The baseline values for the soil characteristics at the start of the field experiment were recorded. There were 13.6 mgkg-1 of copper (Cu), 199.1mgkg-1 of Zinc (Zn), 120.5 mgkg-1 of sodium (Na), 818.513.6 mgkg-1 of potassium (K), 5.4 mgkg-1 of Phosphorus (P) and 15.0 mgkg-1 of Nickel (Ni) ions present in the soil. The soil had a higher organic matter content recording 30300 mgkg-1; however there was no nitrogen (N) found.

4.4 Efficacy of plant extracts under field conditions

4.4.1 Disease incidence

Disease incidence was significantly different among the treatments* time (P<0.05). The treatments varied with time in percentage disease incidence from the 1st week to the 6th week of evaluation. At the 1st week, the treatments were significantly different

(P=0.02312) from each other (Table 5). *Z. chalybeum –M. volkensii* treated plots had the highest disease incidence. During the 2nd week, there were significant differences (P=0.0021) in disease severity whereby Kocide DF (4.25%), *B. angustifolia - Z. chalybeum* (6.24%), *B. angustifolia - M. volkensii* (7.40%), *B. angustifolia* (6.24%) and *M. volkensii* (8.75%) treatments had lower percentage disease incidences compared to *Z. chalybeum* (15.15%) and *Z. chalybeum- M. volkensii* (11.26%) treated plots (Table 5)**.** The untreated plots (18.75%) had the highest percentage disease incidence in the 2nd week (Table 5).

There were significant differences (P=0.0187) in disease incidence among treatments in the 3rd week. Kocide DF (6.24%) and *B. angustifolia - Z. chalybeum* (8.75%) treatments had the lowest percentage disease incidences in the 3rd week. *M. volkensii* (12.5%) and *B. angustifolia* (16.25%) treated plot had relatively lower percentage disease incidence in the 3rd week. Untreated control (36.25%) had the highest disease incidence in the 3rd week followed by *Z. chalybeum* (31.25%) and *Z. chalybeum- M. volkensii* (31.23%) treated bean plants (Table 5).

The diseased progressed overtime and by the 4th week, there were significant differences among the treatments (P<0.0001). Kocide DF (6.25%) had significantly the lowest percentage disease incidence compared to other treatments. *B. angustifolia - Z. chalybeum* (12.5%) and *M. volkensii* (13.5%) treatments had relatively lower percentage disease incidences in the 4th week (Table 5). *B. angustifolia - M. volkensii* (26.4%) treatment revealed higher disease incidence but not higher than *Z. chalybeum* (38.5%), *Z. chalybeum- M. volkensii* (42.4%) and control (48.75%) treated bean plants respectively.

Table 5. Rust Incidence with single and combinations of treatments in the field

Treatments	Rate of application (dosage)	Percentage Disease incidence over time (weeks)						Mean % incidence
		1st week	2nd week	3rd week	4th week	5th week	6th week	
B. angustifolia	2kg/20l	*1.24±0.01^{d}	6.24±0.01^{f}	16.25±0.01^{d}	21.25±0.01^{e}	22.65±0.15^{e}	36.12±0.12^{e}	17.25±0.05^{d}
B. angustifolia – M. volkensii	2kg/20l	0.00±0.25^{e}	7.40±0.10^{e}	22.65±0.15^{c}	26.40±0.10^{d}	28.61±0.14^{d}	45.17±0.18^{d}	21.71±0.09^{c}
B. angustifolia –Z. chalybeum	2kg/20l	1.25±0.01^{d}	6.24±0.01^{f}	8.75±0.01^{f}	12.50±0.05^{g}	17.46±0.04^{f}	20.35±0.10^{f}	11.08±0.02^{f}
Kocide DF (positive control)	2.25kg ha^{-1}	1.25±0.01^{d}	4.25±0.01^{g}	6.24±0.01^{g}	6.25±0.01^{h}	9.50±0.03^{h}	9.75±0.01^{h}	6.21±0.01^{g}
M. volkensii	2kg/20l	1.25±0.00^{d}	8.75±0.01^{d}	12.50±0.20^{e}	13.50±0.00^{f}	16.75±0.01^{g}	17.75±0.01^{g}	11.75±0.26^{e}
Untreated control (Water)	20l	2.55±0.05^{c}	18.75±0.01^{a}	36.24±0.01^{a}	48.75±0.01^{a}	66.25±0.01^{a}	81.25±0.01^{a}	42.29±0.02^{a}
Z. chalybeum	2kg/20l	3.75±0.01^{b}	15.15±0.15^{b}	31.25±0.01^{b}	38.50±0.03^{c}	50.04±0.05^{c}	76.22±0.03^{b}	35.79±0.06^{b}
Z. chalybeum –M. volkensii	2kg/20l	8.75±0.01^{a}	11.26±0.01^{c}	31.23±0.02^{b}	42.40±0.10^{b}	53.20±0.55^{b}	67.45±0.05^{c}	35.83±0.04^{b}
	LSD	0.2942	0.2091	0.2899	0.1762	0.6794	0.2792	0.6356
	CV%	5.0247	0.9291	0.6097	0.2917	0.8913	0.2736	0.3326

*Numbers represent percentage mean disease incidence for each treatment at time intervals.
*Means separated using LSD test by the same letter along the column are not significantly different (P<0.05) from each other.

At the 5th week there were significant differences among the treatments (P=0.0346), Kocide DF (9.5%) had significantly the lowest percentage disease incidence compared to all other treatments (Table 5). *M. volkensii* (16.75%) and *B. angustifolia - Z. chalybeum* (17.46%*)* treated plants had relatively lower incidences compared to other plant extracts and combinations.

B. angustifolia - M. volkensii (17.5%) and *B. angustifolia* (22.65%) treated plots had relatively higher disease incidences while *Z. chalybeum* (50.04%) and *Z. chalybeum- M. volkensii* (53.20%) had the highest disease incidence in the 5th week when compared to other plant extracts. Untreated control (66.25%) had the highest disease incidence in the 5th week (Table 5). At the 6th week, there were significant differences among the treatments (P=0.0003). Kocide DF (9.75%) had the lowest percentage disease incidence followed by *M. volkensii* (17.75%) and *B. angustifolia - Z. chalybeum* (20.25%) respectively. *B. angustifolia - M. volkensii* (45%) and *B. angustifolia* (36%) treatments had higher disease incidences at week 6 compared to other treatments (Table 5).

Z. chalybeum (76.22%) and *Z. chalybeum - M. volkensii* (67.45%) recorded highest percentage disease incidences throughout the evaluation period while Kocide DF revealed low disease incidences during the same period. Untreated control revealed over 81% disease incidence at the 6th week (Table 5). There were significant differences in the mean disease incidence of the treatments (P<0.0001). Kocide DF (6.21%) had the lowest mean percentage disease incidence followed by *B. angustifolia - Z. chalybeum* (11.08%) and *M. volkensii* (11.75%*)* which were not different from each other. *Z. chalybeum*

(35.79%) and *Z. chalybeum- M. volkensii* (35.83%) treated plots had the highest mean percentage disease incidence compared with other plant extracts. The untreated control (42.29%) however, had the highest mean disease incidence throughout the evaluation period (Table 5).

4.4.2 Disease severity

Spore growth inhibition and greenhouse performance by the extracts of *B. angustifolia*, *Z. chalybeum* and *M. volkensii* was significant hence were used in the field experiment. There combinations which include *Z. chalybeum- M. volkensii*, *B. angustifolia - Z. chalybeum* and *B. angustifolia - M. volkensii* were used in the field experiment**.** Plate 1 shows rust disease in the 6th week of the French beans growth.

There were significant differences (P=0.06621) in all treatments in the 1st week. *Z. chalybeum* (17.65%) treatment had the highest disease severity while *Z. chalybeum –M. volkensii* (7.85%) had the lowest disease severity (Table 6). The 2nd week recorded significant differences between the treatments (P=0.0298). *B. angustifolia - Z. chalybeum* (15.35%), *B. angustifolia- M. volkensii* (18.6%), *M. volkensii* (17.55%) and Kocide DF (11.6%) treated plots had lower disease severity at the 2nd week. *Z. chalybeum* (33.65%) and untreated control (35.30%) plots exhibited the highest disease severity scores followed by *Z. chalybeum- M. volkensii* (27.65%) treated plots (Table 6).

Table 6. Percentage disease severity for single treatments and combinations of plant extracts.

Treatments	Rate of application (dosage)	Percentage Disease severity over time (weeks)						Mean % disease severity
		1st week	2nd week	3rd week	4th week	5th week	6th week	
B. angustifolia	2kg/20l	*12.50±0.50^{c}	16.05±0.05^{f}	20.50±0.20^{f}	29.55±0.12^{e}	33.5±0.05^{f}	46.60±0.10^{e}	26.33±0.000^{e}
B. angustifolia – M. Volkensii	2kg/20l	15.00±0.50^{b}	18.60±0.10^{d}	36.20±0.10^{d}	46.40±0.2^{c}	52.15±0.15^{c}	53.15±0.15^{d}	36.92±0.000^{d}
B. angustifolia –Z. Chalybeum	2kg/20l	15.10±0.10^{b}	15.35±0.35^{g}	23.40±0.05^{e}	24.45±0.0^{f}	30.5±0.05^{g}	29.10±0.10^{g}	22.92±0.002^{g}
Kocide DF (positive control)	2.25kg ha^{-1}	8.40±0.10^{e}	11.60±0.10^{h}	15.06±0.01^{g}	20.55±0.11^{g}	24.2±0.05^{h}	24.05±0.05^{h}	17.29±0.002^{h}
M. volkensii	2kg/20l	10.55±0.05^{d}	17.55±0.05^{e}	20.55±0.05^{f}	24.55±0.07^{f}	34.5±0.05^{e}	35.15±0.15^{f}	23.73±0.020^{f}
Untreated control (Water)	20l	15.00±0.15^{b}	35.30±0.30^{a}	68.15±0.15^{a}	70.45±0.17^{a}	76.60±0.10^{a}	81.55±0.05^{a}	57.72±0.025^{a}
Z. chalybeum	2kg/20l	17.65±0.15^{a}	33.65±0.15^{b}	59.10±0.10^{b}	66.50±0.21^{b}	69.55±0.05^{b}	77.25±0.25^{b}	53.91±0.003^{b}
Z. chalybeum –M. volkensii	2kg/20l	7.85±0.15^{e}	27.65±0.15^{c}	40.45±0.05^{c}	44.35±0.15^{c}	51.45±0.05^{d}	67.70±0.20^{c}	39.92±0.100^{c}
	LSD	2.3060	0.6128	0.3459	0.6262	0.2513	0.4789	0.0373
	CV%	3.006	1.2096	0.4235	0.6647	0.234003	0.4007	0.0463

*Numbers represent percentage mean disease severity for each treatment at time intervals.

*Means separated using LSD test by the same letter along the column are not significantly different (P<0.05) from each other.

The 3rd week experienced changes (P=0.0001) in disease severity scores whereby the untreated control (68.15%) and *Z. chalybeum* (59.1%) had the highest disease severity scores. *B. angustifolia - M. volkensii* (36.20%) and *Z. chalybeum- M. volkensii* (40.45%) treated bean plants had the second highest disease severity percentages. Kocide DF (15.06%), *M. volkensii* (20.55%) and *B. angustifolia - Z. chalybeum* (23.4%) treated plants recorded the lowest bean rust disease compared to other treatments (Table 6).

During the 4th week, there were significant differences in disease severity among treatments (P<0.0001). Commercial fungicide (Kocide DF) had the lowest percentage disease severity (20.55%) among all treatments followed by *B. angustifolia - Z. chalybeum* (24.45%) and *M. volkensii* (24.55%) treated plots**.** Untreated control (70.45%) and *Z. chalybeum* (66.5%) treated bean plants had significantly the highest severity scores compared to others (Table 6).

B. angustifolia - M. volkensii (46.5%) and *Z. chalybeum- M. volkensii* (44.5%) treatments had significantly moderate disease severity while Kocide DF, *M. volkensii* and *B. angustifolia - Z. chalybeum* recorded the lowest rust severity scores meaning the were better in inhibiting bean rust. There were significant differences in disease severity at week 5 (P<0.0001). At the 5th week rust disease severity had progressed and *B. angustifolia - Z. chalybeum* (30.5%), *M. volkensii* (34.5%) and *B. angustifolia* (33.5%) plant extracts performed better than *Z. chalybeum- M. volkensii* (51.45%), *B. angustifolia- M. volkensii* (52.15%) and Z. *chalybeum* (69.55%) treatments. Untreated control had the highest percentage disease severity revealing 76.6% in the 5th week

(Table 6). In the 6th week the rust continued to increase in most treatments and there were significant differences (P<0.05). Kocide DF (24%), *B. angustifolia* - *Z. chalybeum* (29%) and *M. volkensii* (35%) exhibited lower rust disease severity compared to all other treatments however untreated control (81.55%), *Z. chalybeum* (77.25%), *B. angustifolia* (46.6%), *B. angustifolia*- *M. volkensii* (53.15%), *Z. chalybeum*- *M. volkensii* (67.7%) treated plots continued to have the highest rust disease severity (Table 6).

Plate 1. The common orange-brown pustules are surrounded by a pale halo, and leave a rusty deposit when wiped with the finger.

In the mean leaf disease severity scores, there were significant differences in all treatments examined (P<0.05). The control (57.72%) and *Z. chalybeum* (53.91%) treated plots had the highest mean bean rust disease severity while *B. angustifolia* - *M. volkensii* (36.92%) and *Z. chalybeum*- *M. volkensii* (39.91%) had the second highest severity scores. Commercial fungicide (Kocide DF) had significantly the lowest mean leaf disease severity (17.29%) followed by *B. angustifolia* - *Z. chalybeum* (22.92%) and *M. volkensii*

(23.73%) treated bean plants. *B. angustifolia* (26.33%) treatment had relatively lower mean rust disease severity **(**Table 6)**.**

4.5 Effect of plant extracts on some selected C_3 parameters of French beans

Field performance in respect to reduction of disease severity and incidence by the extracts of *Z. chalybeum, Z. chalybeum- M. volkensii, B. angustifolia* and *B. angustifolia - M. volkensii* were insignificant hence were not considered in C_3 parameters assessment. *B. angustifolia - Z. chalybeum* and *M. volkensii* treatments were considered because of their ability to inhibit rust.

4.5.1 Stomata conductance (gs) Transpiration, Photosynthetic Active Radiation (PAR) and leaf temperature

The diurnal changes in stomata conductance (gs), rate of transpiration, photosythetic active radiation and leaf temperature in month 2 under the antifungal treatments were as shown in Figures 2 a, b, c and d. The stomatal conductance in Fig 2 (a) followed the same pattern in all the treatments being highest at 9:00am dropped at midday and maintained low levels in the late afternoon. However, there were significant differences in stomatal conductance (P=0.0173) in the treatments at 9:00am and was rated as untreated control (95.8mol/$m^{-2}sec^{-1}$) having the highest stomatal conductance followed by *B. angustifolia - Z. chalybeum* (77.7 mol/$m^{-2}sec^{-1}$), *M. volkensii* (46.3 mol/$m^{-2}sec^{-1}$) and Kocide DF (39.18 mol/$m^{-2}sec^{-1}$) respectively.

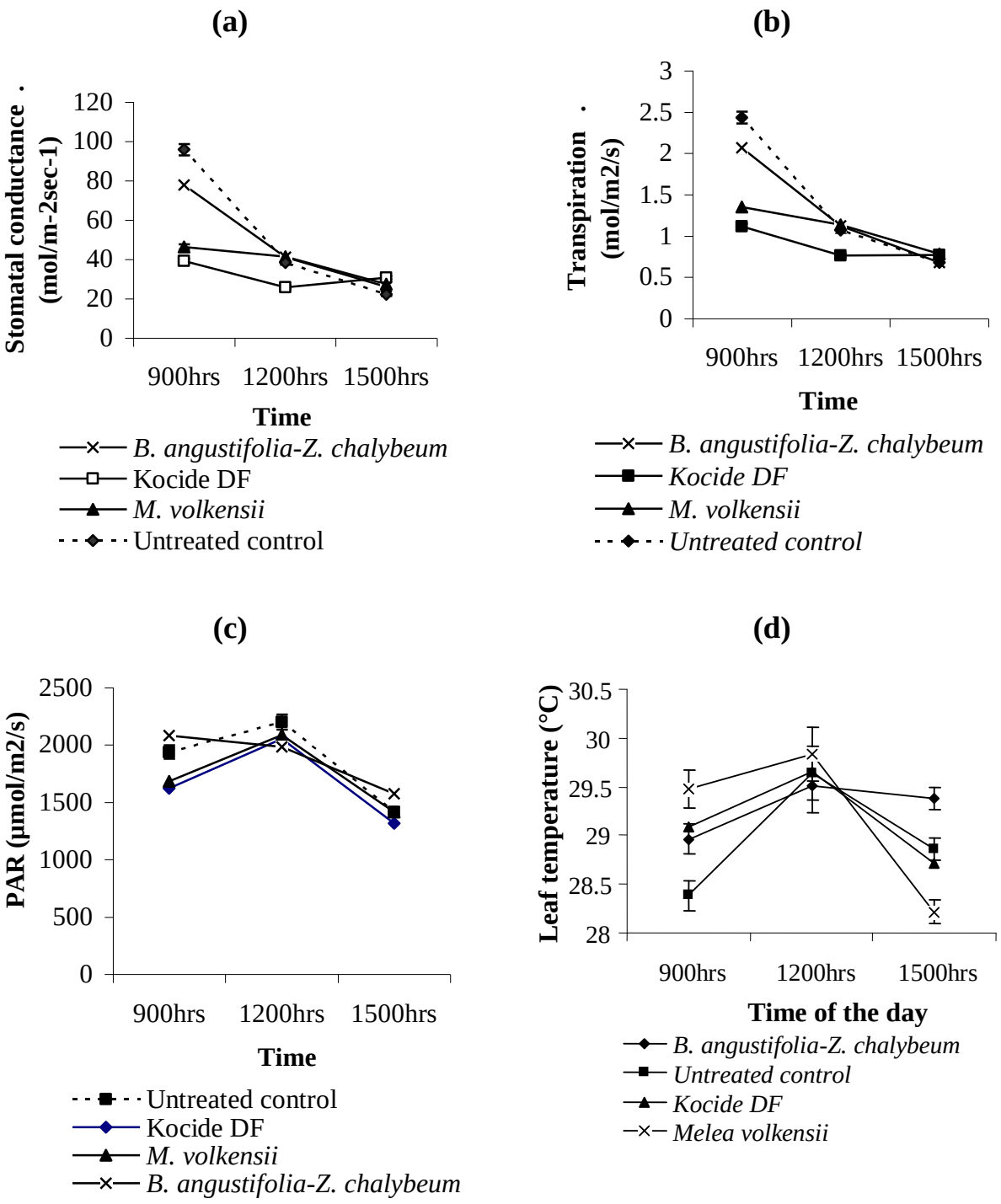


Figure 2. Daily diurnal courses of stomatal conductance (a), rate of transpiration (b), Photosynthetic Active Radiation (c) and leaf temperature (d) in French beans (Amy variety) exposed to four treatments.

The stomatal conductance for commercial fungicide (Kocide DF) was significantly lower (25.6 mol/$m^{-2}sec^{-1}$) than other treatments at 12:00pm. *B. angustifolia - Z. chalybeum* (41 mol/$m^{-2}sec^{-1}$), *M. volkensii (*41.5mol/$m^{-2}sec^{-1}$) and untreated control (38.3mol/$m^{-2}sec^{-1}$) were not significantly different from each other at 12:00pm. There were no significant differences in stomatal conductance at 15:00pm of all treatments (P=0.1235). This showed that apart from controlling fungal attack the treatments had influenced stomatal conductance. This behavior was observed in all the four treatments during the three months growth period of the crop. The rate of transpiration was highest at 9:00am coinciding with highest stomatal conductance and dropped at noon when stomatal conductance also dropped and maintained low levels in the early afternoon and evening when stomatal conductance and PAR were low (Fig. 2,b).

There were significant differences (P=0.003) in transpiration rates of the treatments at 9:00am. Untreated control (2.432mol/m^2/s) had significantly the highest rate of transpiration compared to other treatments at 9:00am followed by *B. angustifolia-Z. chalybeum* (2.065 mol/m^2/s), *M. volkensii* (1.353 mol/m^2/s) and Kocide DF (1.116 mol/m^2/s) respectively. There were significant differences (P=0.0015) in the rates of transpiration among the treatments at 12:00pm. Kocide DF (0.76 mol/m^2/s) had significantly the lowest rate of transpiration while there were no significant differences in *B. angustifolia - Z. chalybeum* (1.12 mol/m^2/s), *M. volkensii* (1.135 mol/m^2/s) and untreated control (1.067 mol/m^2/s) (Fig. 2,b).

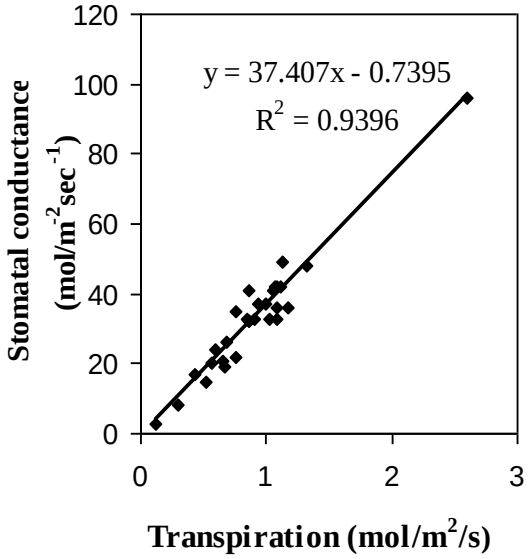
There were significant differences ($P<0.05$) in transpiration rates of the treatments at 15:00am. *B. angustifolia - Z. chalybeum* (0.67 mol/m^2/s), *M. volkensii* (0.78 mol/m^2/s) and Kocide DF (0.77 mol/m^2/s) treated beans had no significant differences in the rate of transpiration at 15:00pm while the untreated control (1.3 mol/m^2/s) beans had significantly the highest rate of transpiration at 15:00pm (Fig. 2,c). There were no differences in changes in PAR in all treatments at 9:00am ($P=0.25$), 12:00pm ($P=0.32$) and 15:00pm ($P=0.43$).

The relationship between PAR and stomatal conductance was that stomatal conductance was highest at 9:00am while PAR was still low and by noon when PAR was intense stomatal conductance decreased indicating that there was partial stomata closure at noon. The leaf temperature was low at 9:00 am and rose gradually reaching a peak at noon when PAR was highest and dropped in the afternoon following decrease in PAR. These changes in leaf temperature were significantly different at 9:00am ($P=0.001$). *M. volkensii* (29.48°C) treated bean plants had significantly the highest leaf temperature compared to all other treatments (Fig. 2,d).

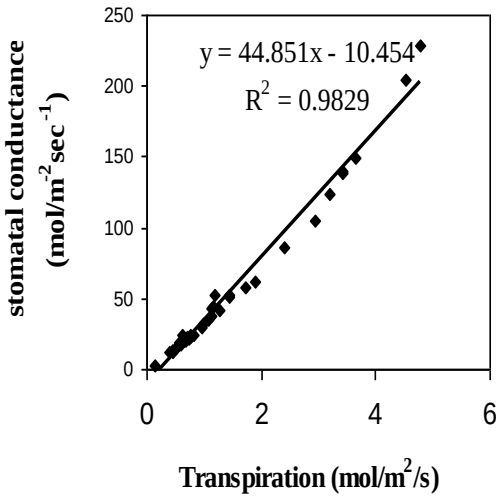
The untreated control (28.38°C) had the lowest leaf temperature while there were no differences between *B. angustifolia - Z. chalybeum* and Kocide DF at 9:00am. There were no significant differences ($P=0.2563$) in leaf temperature among all treatments at 12:00pm, however at 15:00pm there were differences ($P<0.05$) revealed. *M. volkensii* treated plants recorded the lowest leaf temperature while *B. angustifolia - Z. chalybeum* treated bean plants had significantly the highest leaf temperature at 15:00pm (Fig. 2,d).

The regressions between stomatal conductance and rate of transpiration in the four treatments were as shown in Figures 3 a, b, c and d. The high positive regressions (r^2 >0.9) and the regression equations are summarized in table 7.

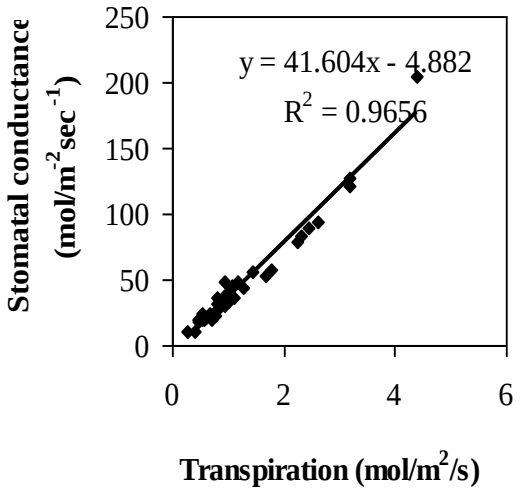
(a). M. volkensii treated bean plants



(b). *B. angustifolia-Z. chalybeum* treated bean plants



(c) Untreated control treated bean plants



(d). Kocide DF treated bean plants

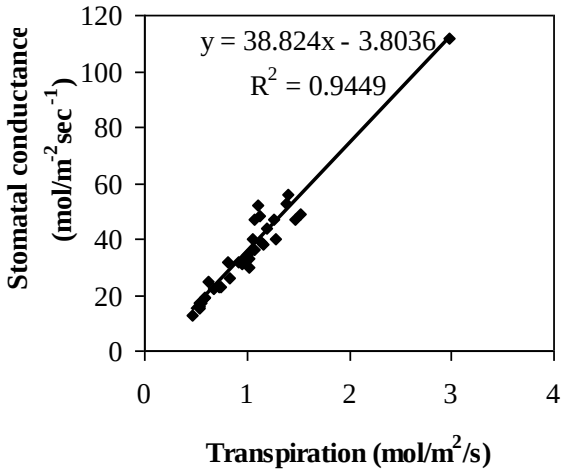


Figure 3 (a), (b), (c), (d). Linear relationship between stomatal conductance (gs) and rate of transpiration (E) in the four treatments in month 2 of growth

Table 7. Relationship between stomatal conductance and rate of transpiration with different treatments

Treatments	Equation	R^2
B. angustifolia-Z. chalybeum	y =44.851x -10.454	0.9829
Untreated control	y = 41.604x -4.882	0.9656
Kocide DF	y = 38.824x -3.8036	0.9449
M. volkensii	y = 37.407x -0.7395	0.9396

4.5.2 Effect of treatment on CO_2 assimilation and photosynthetic rate (Pn)

The more the negative CO_2 assimilation the more CO_2 is absorbed from the environment as shown in Fig. 4. The CO_2 assimilation reached a peak at 9:00am and decreased sharply at noon and eventually maintained low levels in the afternoon. CO_2 assimilation followed the same pattern as that of stomatal conductance. There were significant differences ($P<0.001$) in CO_2 assimilation rates among treatments at 9:00am. *B. angustifolia - Z. chalybeum* (577.933ppm) treated bean plants had significantly lowest CO_2 assimilation rate while there were no differences between *M. volkensii* (679.5ppm), Kocide DF (641.364ppm) and untreated control (651.154ppm) in CO_2 assimilation rate at 9:00am (Fig. 4).

There were no significant differences ($P>0.002$) in CO_2 assimilation rate of all treatments at 12:00pm however they ranged from untreated control (362ppm) being the highest then followed by *B. angustifolia - Z. chalybeum* (328.33ppm), *M. volkensii* (320.33ppm) and Kocide DF (304.18ppm) respectively. Likewise, at 15:00pm there were no differences ($P=0.1425$) in CO_2 assimilation rates of all treatments (Fig. 4).

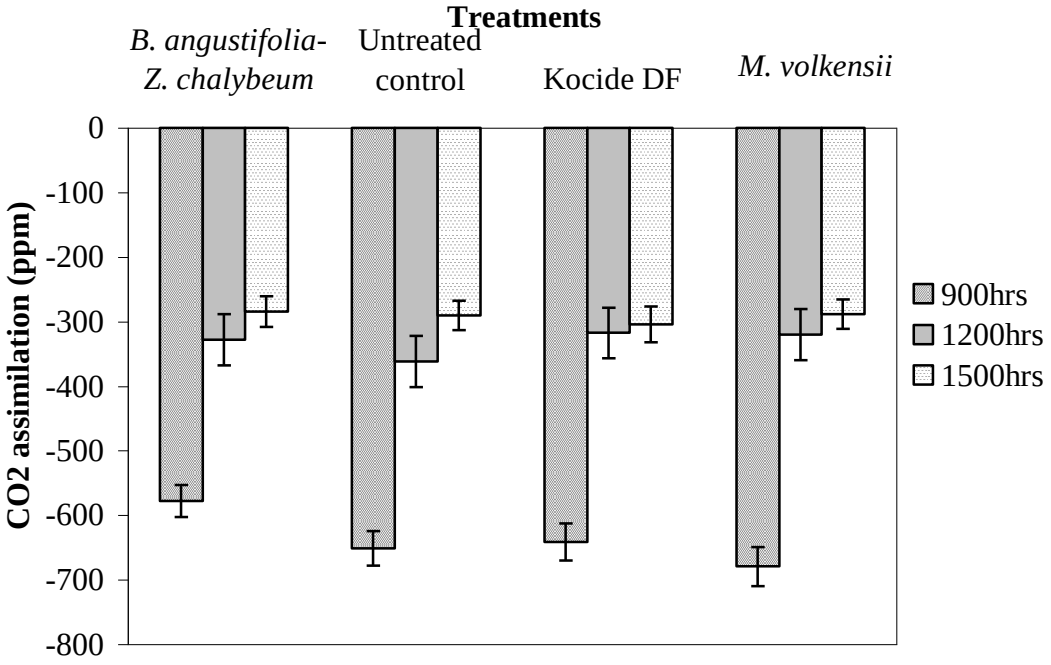


Figure 4. Daily courses of CO2 assimilation in French beans exposed to four treatments under field conditions**.**

The relationship between stomatal conductance and CO_2 assimilation was described by low insignificant positive regressions in each treatment as shown in Table 8**.** The low R^2 indicated the two parameters were very slightly interrelated.

Table 8. Linear relationships between CO_2 assimilation and stomatal conductance

Treatments	**Equation**	**R^2**
B. angustifolia-Z. chalybeum	y = 4.0963x + 74.228	0.5873
Untreated control	y = 0.0369x + 39.852	0.0801
Kocide DF	y = 0.1134x + 7.1438	0.4250
M. volkensii	y = 0.0994x + 27.677	0.6596

The diurnal pattern of rate of photosynthesis among the treatments was similar with other parameters being highest at the morning 9:00am dropped at noon and remained low in the afternoon (15:00pm) following decrease in PAR.

There were significant differences (P=0.0021) in the rate of photosynthesis among the treatments at 9:00am. The rate of photosynthesis was rated highest in *M. volkensii* (99.9µmolCO_2/m^2/s) followed by Kocide DF (80.72µmolCO_2/m^2/s), *B.angustifolia-Z.chalybeum* (72.5µmolCO_2/m^2/s) and untreated control (53.9µmolCO_2/m^2/s) respectively **(**Fig. 5**).**

There were significant differences (P=0.0132) in the rate of photosynthesis among the treatments at 12:00pm. *M. volkensii* (68.38µmolCO_2/m^2/s) had significantly the highest photosynthetic rate at 12:00pm followed by *B.angustifolia-Z.chalybeum* (59.1µmolCO_2/m^2/s). However, there were no differences between Kocide DF (23.51µmolCO_2/m^2/s) and untreated control (24.4µmolCO_2/m^2/s) at 12:00pm **(**Fig. 5**).**

M. volkensii (50.77µmolCO_2/m^2/s) and Kocide DF (50.7µmolCO_2/m^2/s) revealed significantly the highest rates of photosynthesis although there were not different from each other at 15:00pm. Untreated control (39.98µmolCO_2/m^2/s) had the lowest photosynthetic compared to other treatments at 15:00pm. The diurnal oscillations in photosynthesis in Kocide DF were greatest **(**Fig. 5**).**

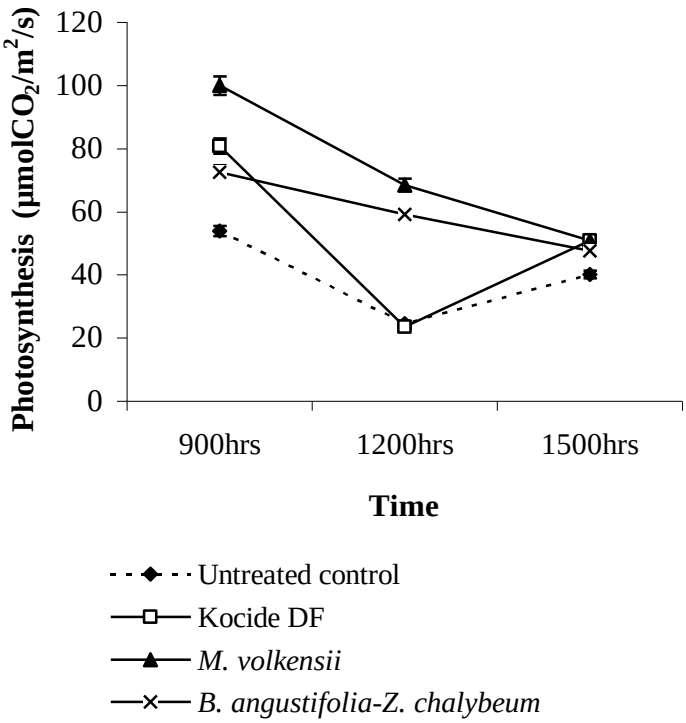


Figure 5. Daily courses of the rate photosynthesis in French beans exposed to two antifungal plant extracts and a commercial fungicide under natural conditions.

4.6 Effect of plant extracts on different growth parameters

4.6.1 Effect of leaf area

In the 1st month of growth, there were significant differences among the treatments (P=0.0199). Mean leaf area in the 1st month were; 10.81±1.4 for *B. angustifolia* and 7.47±0.7 for Kocide DF. *Z. chalybeum*, *B. angustifolia* - *Z. chalybeum*, untreated control had a mean leaf area of 10.46±1.2, 10.03±0.9 and 10.01±0.9 respectively. *B. angustifolia* -*M. volkensii* (8.35±1.0) had the second lowest mean leaf area (8.35±1.0) **(**Table 9**).**

Table 9. Mean leaf area for sampled plants within each treatment in 1st, 2nd and 3rd months of plant growth.

Treatments	Leaf area (cm^2) per plant			Mean Total leaf area per plant
	1st month	**2nd month**	**3rd Month**	
B. angustifolia	*10.81±0.003[a]	41.66±0.003[g]	3000.3±0.200[d]	3052.97±0.049[d]
Z. chalybeum	10.43±0.031[b]	41.65±0.050[g]	2711.6±0.100[h]	2763.70±0.100[h]
B. angustifolia -Z. chalybeum	10.04±0.010[c]	59.52±0.011[a]	2714.9±0.250[g]	2784.19±0.049[g]
Untreated control	10.01±0.001[c]	44.33±0.020[e]	3115.5±0.150[c]	3169.76±0.000[c]
Z. chalybeum -M. volkensii	9.78±0.004[d]	54.43±0.002[b]	3206.4±0.400[b]	3270.38±0.162[b]
M. volkensii	9.00±0.002[e]	44.03±0.007[f]	2783.5±0.150[f]	2836.59±0.146[f]
B. angustifolia -M. volkensii	8.35±0.004[f]	50.55±0.021[c]	3262.5±0.200[a]	3321.28±0.057[a]
Kocide DF	7.45±0.018[g]	46.07±0.006[d]	2841.1±0.200[e]	2894.43±0.003[e]
LSD	0.0433	0.0689	0.7269	0.3240
CV%	0.1978	0.0625	0.0106	0.0043

*Numbers represent mean leaf area for each treatment at time intervals.
*Means separated using LSD test by the same letter along the column are not significantly different ($P<0.05$) from each other.

There were significant differences in the mean leaf area of treatments ($P=0.0003$) in the 2nd month. *B. angustifolia - Z. chalybeum* treated bean plants had the highest mean leaf area (59.52±0.011) while *Z. chalybeum* sprayed plots had the lowest mean leaf area (41.65±0.050). *Z. chalybeum - M. volkensii* (54.43±0.002), *B. angustifolia - M. volkensii* (50.55±0.021) and Kocide DF (46.07±0.006) treatments had average mean leaf area. Plots sprayed with untreated control, *M. volkensii*, *B. angustifolia* and *Z. chalybeum* recorded lower leaf areas (Table 9). The 3rd month of growth experienced no significant differences within the treatments ($P=0.2327$). Mean leaf area of treatments ranged from *B. angustifolia - M. volkensii* sprayed plots with the highest (3262.5±0.200) to *Z. chalybeum* treated plots having the lowest mean leaf area (2711.6±0.100).

B. angustifolia, untreated control, *X chalybeum -M. volkensii* and Kocide DF sprayed plots had a mean leaf area of 3000.3±0.200, 3115.5±0.150, 3206.4±0.400 and 2841.1±0.200 respectively (Table 9). *B. angustifolia -Z. chalybeum* and *M. volkensii* had lower mean leaf area compared to most treatments in the 3rd month indicating 2714.9±0.250 and 2783.5±0.150 respectively. There were no significant differences (P=0.2365) in mean total leaf area of all treatments studied. However, mean total leaf area ranged from *B. angustifolia - M. volkensii* treatments having the highest total leaf area (3321.28 cm^2) followed by *Z. chalybeum - M. volkensii* (3270.38 cm^2), Untreated control (3169.76cm^2), *B. angustifolia (*3052.97 cm^2*)*, Kocide DF (2894.43 cm^2), *B. angustifolia - Z. chalybeum* (2784.19 cm^2) and *Z. chalybeum* (2763.70 cm^2) respectively (Table 9).

4.6.2 Effects of plant extracts on shoot height

In the 1st Month there were no significant differences between the treatments (P=0.2048). However, *B. angustifolia – Z. chalybeum* treated plants had the highest mean shoot height (15.21±0.96) while *Z. chalybeum* treated plants had the lowest mean shoot height (11.46±1.09). Kocide DF had the second highest mean shoot height (14.82±1.17) while *M. volkensii* treated plots had the third highest mean shoot height (14.16±1.05). Untreated control was second last in terms of mean shoot height measurements (12.06±1.02). These results are shown in Fig 6.

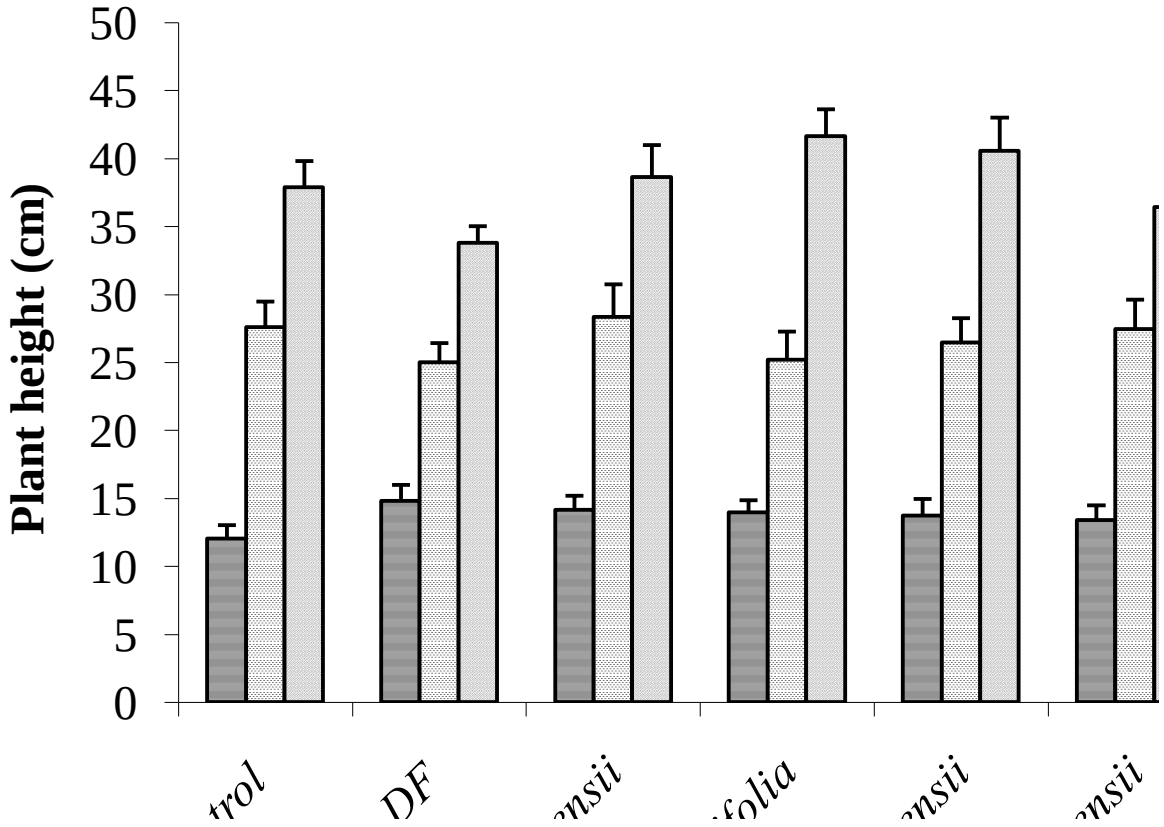


Figure 6. Mean shoot height of French beans sprayed with selected plant extracts and Kocide DF in the field experiment

In the 2nd month, there were no significant differences in different treatments (P=0.6603) in shoot height. However, plant shoot height differed from *B. angustifolia –Z. chalybeum* treated plants (29.29±1.91) with the highest while Kocide DF treated plants had the lowest mean shoot height (25.04±1.62). *Z. chalybeum* treated plots had the second highest mean shoot height (28.67±1.38) while *B. angustifolia* plants had the second lowest mean shoot height (25.2±1.27) compared to other treatments (Fig.17). The 3rd

month experienced significant differences among the treatments (P<0.05). *Z. chalybeum* treated plants had the highest mean shoot heights (42.41±1.90) compared to other treatments in the 3rd month followed by *B. angustifolia* (41.66±1.21) and *B. angustifolia* - *M. volkensii* (40.59±2.34) treated plants (Fig. 6).

B. angustifolia – *Z. chalybeum* (39.78±1.97), *M. volkensii* (38.69±2.43), *Z. chalybeum* - *M. volkensii* (36.44±2.31) and untreated control (37.93±1.61) treatments had moderate shoot heights. Kocide DF plots had the lowest mean shoot heights (33.82±1.46) compared to all other treatments. There were no significant differences (P=0.1256) in mean total shoot height of all treatments including the controls (Fig. 6).

4.6.3 Effect of plant extracts on dry shoot weight

There were no significant differences between treatments in the 1st month (P=0.217). However, *B. angustifolia* had the highest mean dry shoot weight (11.9±1.46) followed by untreated control (9.836±1.30) and the combination *B. angustifolia* - *Z. chalybeum* recorded the third highest mean dry shoot weight (8.94±1.98). *Z. chalybeum* had the lowest mean dry shoot weight (7.856±0.94) among all treatments in the 1st month (Fig. 7). There were significant differences in treatments in the 2nd month (P=0.0279). *B. angustifolia* - *Z. chalybeum* (17.952±2.06) and *M. volkensii (*16.239±2.11) treated plots had the highest mean dry shoot weights compared with other treatments. *B. angustifolia (*14.8±1.14), kocide DF *(*14.754±1.58), *Z. chalybeum* - *M. volkensii* (14.471±1.51) and *B. angustifolia* - *M. volkensii (*13.463±1.57) treatments had better dry shoot weights in the 2nd month of growth than untreated control and *Z. chalybeum* treated plants. Untreated

control (10.336±2.10) and *Z. chalybeum* (10.115±1.54) treated plots had the lowest mean dry shoot weights compared to other treatments (Fig. 7).

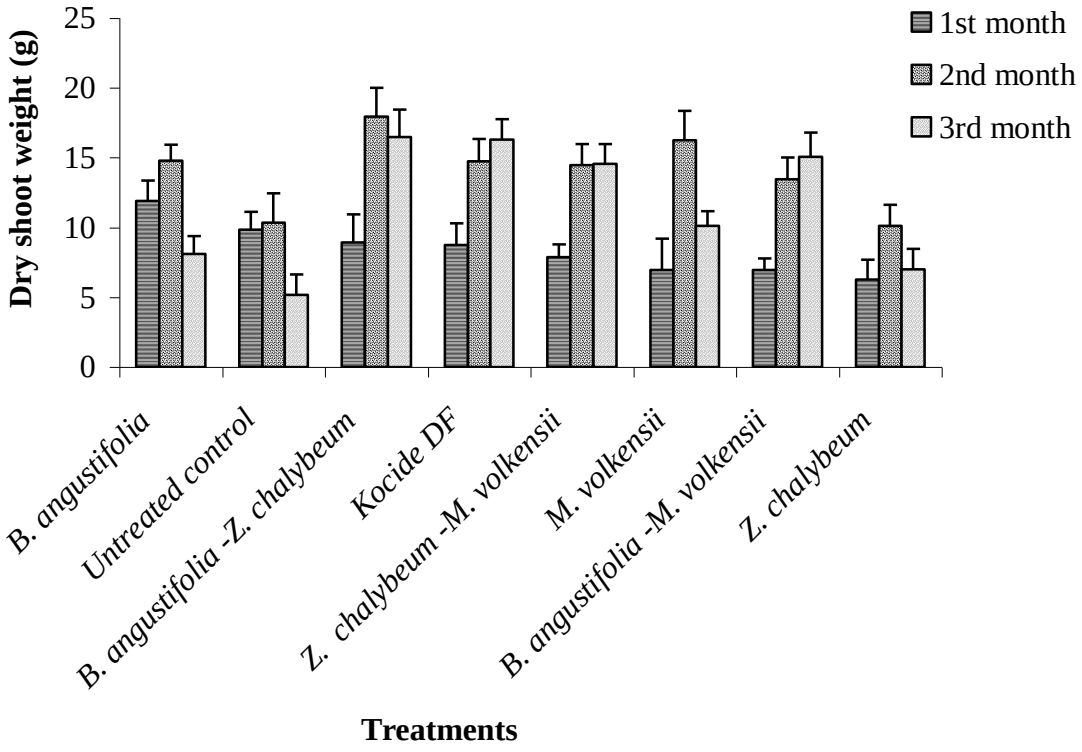


Figure 7. Dry shoot weight of experimental plants for all treatments in a period of three months

In the 3rd month, treatments were significantly different from each other (P<0.0001) in dry shoot weight (Fig. 7). *B. angustifolia* (8.099±1.27), Kocide *DF (*16.31±1.45), *B. angustifolia - M. volkensii (*15.065±1.73) and *Z. chalybeum - M. volkensii (*14.567±1.42) treated plots had the highest mean dry shoot weights of bean plants compared to other treatments (Fig. 7). *M. volkensii* treated plants had higher mean dry shoot weight (10.115±1.04) compared to *B. angustifolia* (8.099±1.27) *and Z. chalybeum* treated plants

(6.996±1.45). Untreated control plants had the lowest mean dry shoot weight (5.166±1.46).

There were differences in mean total dry weight of the treatments (P<0.0001). *B. angustifolia* (34.799g), *B. angustifolia –Z. chalybeum* (43.368g), Kocide DF (39.799g), *Z. chalybeum - M. volkensii* (36.894g), *M. volkensii* (33..326g) and *B. angustifolia - M. volkensii* (35.488g) treated bean plants had the highest mean total dry weights. Untreated control (25.338g) and *Z. chalybeum* (23.395g) plants had significantly the lowest means of the total dry weight compared to other treatmnts (Fig. 8).

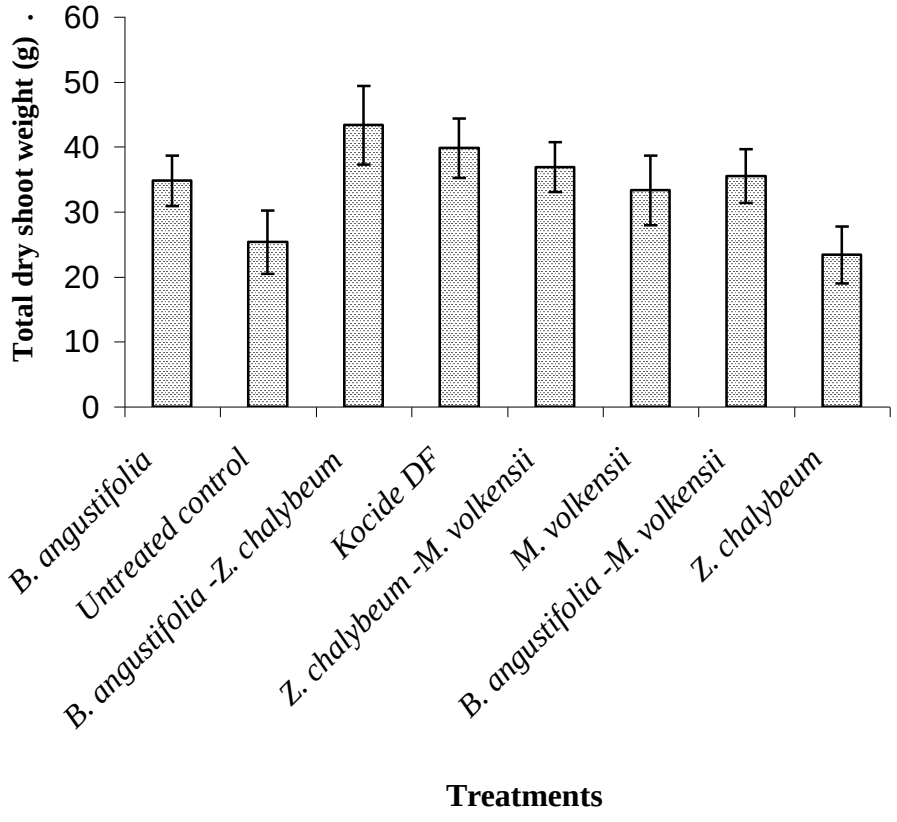


Figure 8. Mean total dry weight for the sampled bean plants from all treatments

4.7 Residue analysis

The main compounds targeted were major carotenoid and chlorophyll constituents of bean pods. The aim was to establish whether bean pods had accumulated any foreign compounds after treatments. HPLC separation of compounds extracted from pods of French bean plants treated with different treatments revealed the elution of major peaks. There were differences in retention times of various treatments at various compounds (Table 10). The 1st compound in all treatments was eluded between 5.009 and 5.697 retention times for untreated control and Kocide DF treated pods respectively. *Z. chalybeum* treated pods had their 2nd compound eluded at 7.29 while untreated control and *M. volkensii* recorded 5.691 and 5.902 respectively. Other treatments had their retention times at 6.051 and 6.175. Most treatments eluded the 3rd compounds between 6.402 and 6.492 retention times (Table 10).

Kocide DF and *B. angustifolia – Z. chalybeum* treatments had different retention times compared to others revealing 7.272 and 7.572 retention times. *Z. chalybeum* treated pods recorded the highest retention time at the elution of the 4th compound (9.987) followed by *B. angustifolia –Z. chalybeum* treatment at 9.684 retention time. Kocide DF and *Z. chalybeum - M. volkensii* treated pods almost similar retention times at 8.423 and 8.871 respectively. *M. volkensii* treated pods eluded the 5th compound at 8.626 retention time compared to the untreated control at 10.741 and Kocide DF (10.796). All treatments eluded the 6th compound between 10.397 for *M. volkensii* and *Z. chalybeum* treated pods at 12.778. The control had elution of the 6th compound at 11.841. There were no

differences between the retention times of the 6th compound except *Z. chalybeum* and *M. volkensii* treated pods. *Z. chalybeum* and *B. angustifolia –Z. chalybeum* treatments did not elude the 7th compound while *Z. chalybeum - M. volkensii* and *B. angustifolia - M. volkensii* treated pods recorded 14.518 and 14.511 respectively.

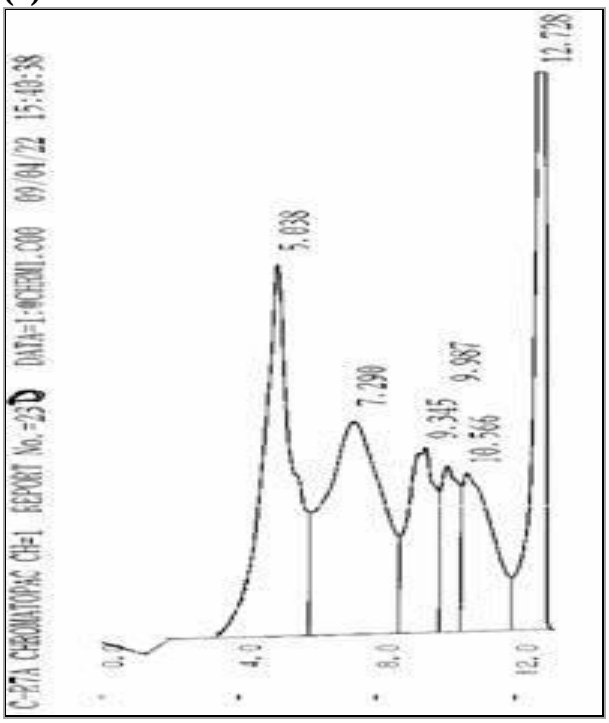
The control, *M. volkensii* and *B. angustifolia* treatments eluded the 8th compound at 13.777, 12.825 and 12.784 respectively (Table 10). HPLC profiles revealed that the bean pods contain similar compounds it is only the amount of the secondary metabolites that may have varied. This was and indication that the plant fungicides had no contaminants.

Table 10. Retention times (in minutes) for eluded compounds from pods of plant extracts and Kocide DF

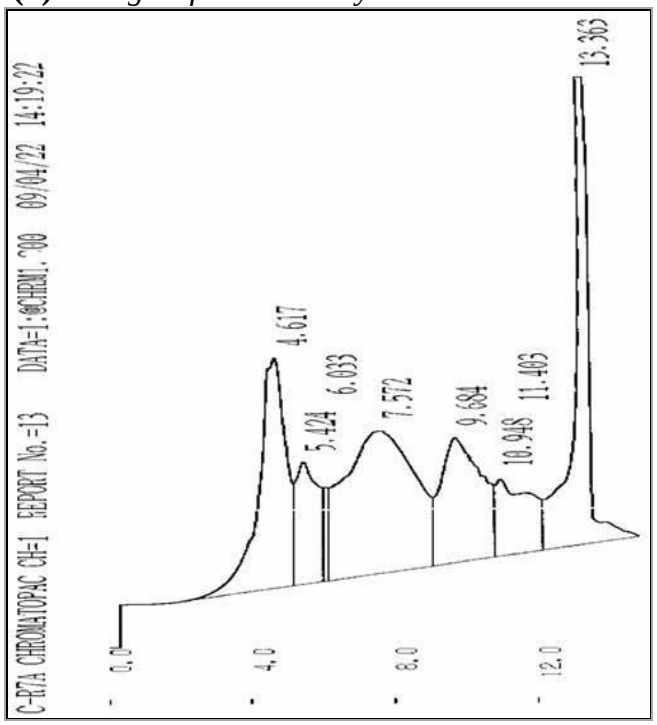
	1st	2nd	3rd	4th	5th	6th	7th	8th
Untreated control	5.009	5.691	6.412	7.767	10.741	11.841	12.653	13.777
Z.chalybeum	5.038	7.29	9.345	9.987	10.566	12.778		
Z. chalybeum – M.volkensii	5.665	6.075	6.441	8.871	11.05	11.505	14.518	
Kocide DF	5.697	6.405	7.272	8.423	10.796	11.482	13.9	
B. angustifolia – Z.chalybeum	5.244	6.033	7.572	9.684	10.948	11.408		
B. angustifolia –M. volkensii	5.665	6.175	6.441	8.811	11.05	11.515	14.511	
M.volkensii	5.341	5.902	6.402	7.919	8.626	10.397	11.808	12.825
B. angustifolia	5.292	6.051	6.492	7.003	9.625	11.216	11.849	12.784

HPLC data was analyzed qualitatively by comparing the presence and absence of peaks in chromatograms obtained with the different treatments (Figs 9 and 10).

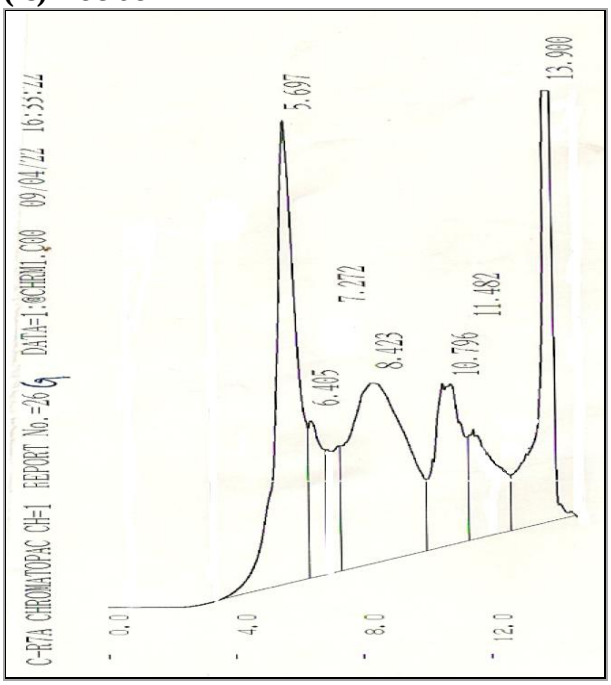
(a) Untreated control



(b) *B. angustifolia- Z. chalybeum*



(c) Kocide DF



(d) *Z. chalybeum –M. volkensii*

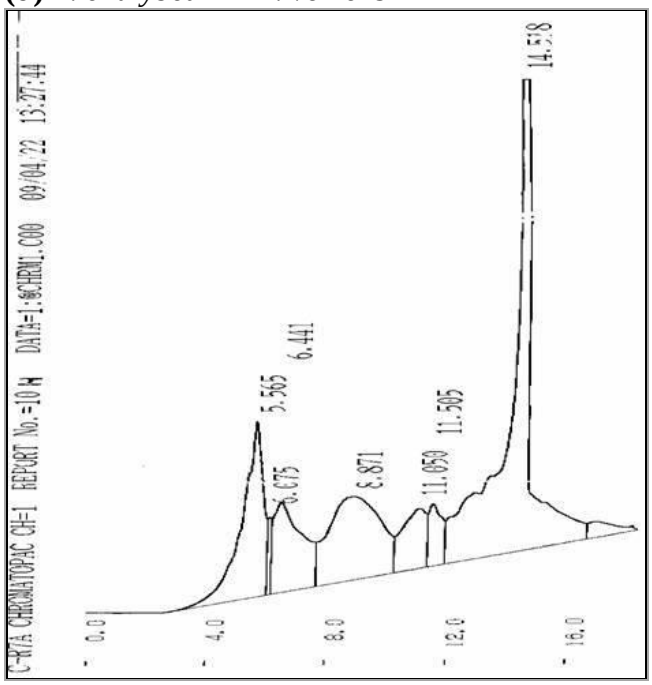
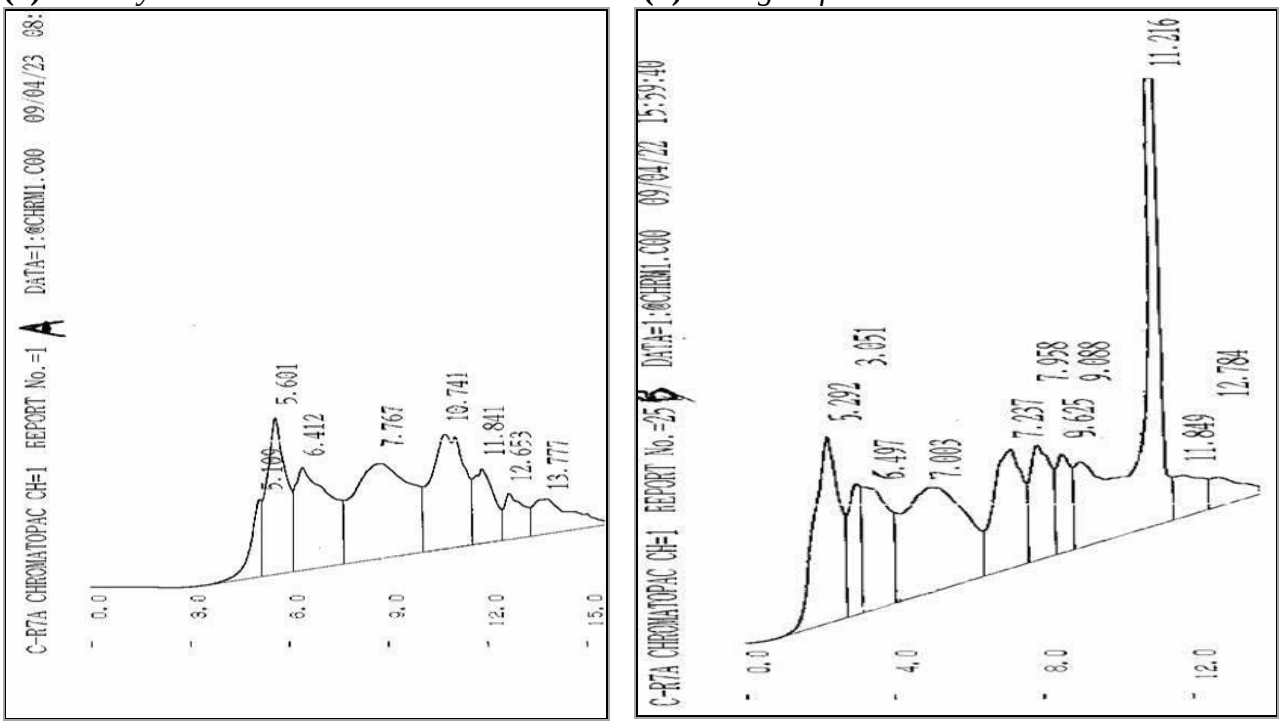


Figure 9 (a), (b), (c) & (d). Represents High pressure liquid chromatography profiles for untreated control, *B. angustifolia - Z. chalybeum*, Kocide DF *and Z. chalybeum – M. volkensii* treatments respectively.

(a) *Z. chalybeum* **(b)** *B. angustifolia*



(c) *M. volkensii* **(d)** *B. angustifolia–M. volkensii*

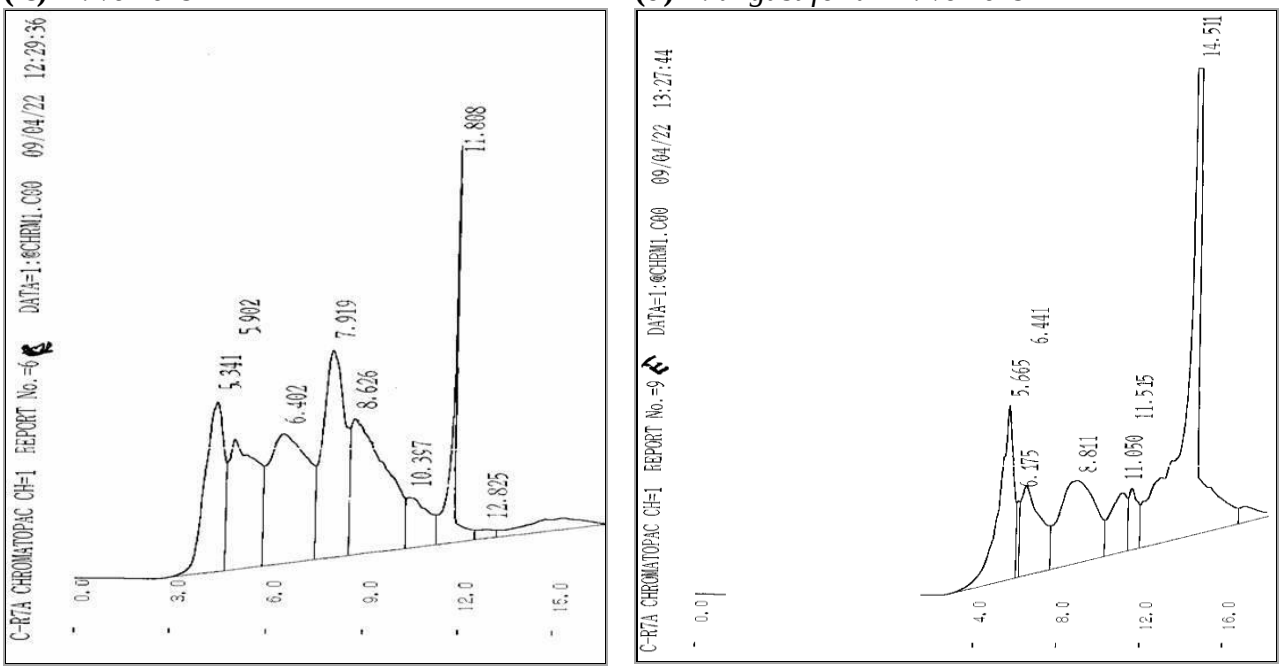


Figure 10 (a), (b), (c) & (d). Represents High pressure liquid chromatography profiles for *Z. chalybeum, B. angustifolia, M. volkensii* and *B. angustifolia – M. volkensii* treatments respectively.

4.8 Phyto-toxicity of plant extracts

4.8.1 Flower drop

There was a significant difference (P<0.05) among the treatments. The control recorded the highest mean flower drop while *M.volkensii* and *Z. chalybeum-M. volkensii* recorded the second and third highest means of flower drop among the treatments respectively. Treatments with *B. angustifolia -M. volkensii* and *B. angustifolia - Z. chalybeum* recorded the lowest mean flower drop. Plots treated with *Zanthoxylum,* Kocide DF and *B. angustifolia* recorded average mean flower drop (Table 11).

Table 11. Mean flower drop of French bean plants sprayed with plant extracts and Kocide DF in the field experiment.

Treatments	**Mean flower drop**
Untreated control	*1.28[a]
Zanthoxylum	0.96[bc]
Z. chalybeum-M. volkensii	1.05[b]
B. angustifolia -M. volkensii	0.72[d]
B. angustifolia	1.01[bc]
Kocide DF	1.00[bc]
B. angustifolia - Z. chalybeum	0.81[cd]
M. volkensii	1.15[ab]
LSD	0.2159
CV(0.05)%	25.20%

*Numbers represent mean flower drop of 10 plants from each treatment
*Means separated using LSDs' test by the same letter are not significantly different (P<0.05) from each other.

It was established that the differences in flower drop between treatments and time were insignificant meaning both plant extracts and commercial fungicide had no impact on flower drop.

4.8.2 Effect of selected plant extracts on pod yield

There were significant differences between treatments in the first harvest i.e sixty three days after sowing (P=0.04792). Some treatments indicated various levels of damage on their pods, *Z. chalybeum* treated pods had severe damage (40%) compared to *B. angustifolia -Z. chalybeum* (25%) and *M. volkensii* (25%) treated pods that had moderate damaged pods. *B. angustifolia -M. volkensii* (40%) and untreated control (40%) pods had also severely damaged pods. *B. angustifolia* (35%), *Z. chalybeum -M. volkensii* (35%), Kocide DF (30%) and sprayed pods were severely damaged (Fig. 11).

There were significant differences seventy days after sowing in the extent of damage in pods sampled among the treatments (P=0.04458). *Z. chalybeum* (45%), *B. angustifolia -M. volkensii* (40%) and untreated control (40%) treated pods had severe damages. *B. angustifolia* (30%), *Z. chalybeum -M. volkensii* (35%), Kocide DF (35%), *M. volkensii* (30%) and *B. angustifolia -Z. chalybeum* (30%) treated pods recorded low percentages of damage compared to others (Fig. 11). After seventy seven days of sowing, pods sampled were significantly different from each other (P= 0.0256). *Z. chalybeum* (60%) and untreated control (60%) treated pods were very severely damaged. Kocide DF (25%), *M. volkensii* (30%) and *B. angustifolia -Z. chalybeum* (35%) revealed lower percentages of damage.

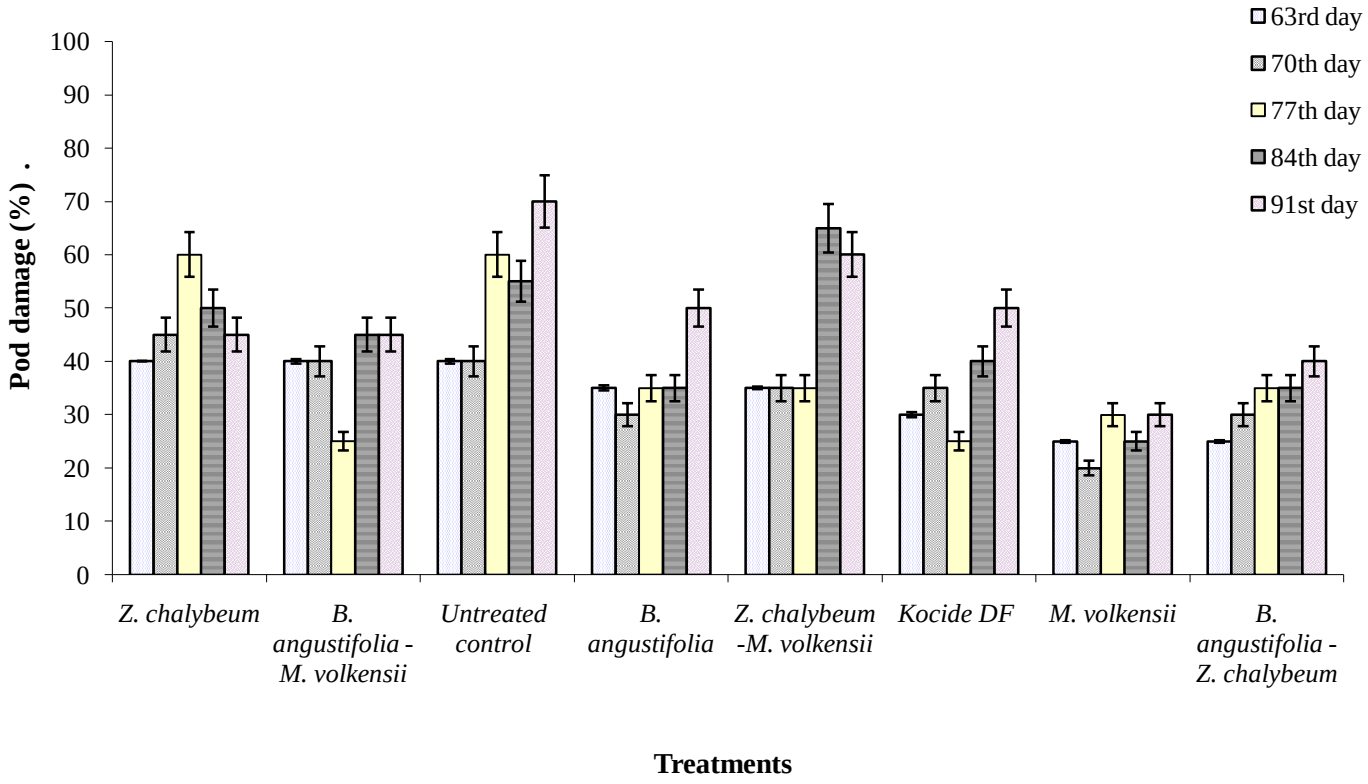


Figure 11. Mean pod damage score of French beans sprayed with selected plant extracts and Kocide DF for the season.

There were significant differences in the pod damage after eighty four days of sowing (P=0.0478). *Z. chalybeum -M. volkensii* (65%) treated pods had very severe damage while *M. volkensii* pods had moderate damage (25%). Ninty one days after sowing recorded no significant differences in pod damage of various treatments (P=0.01683). Pods collected from untreated control (70%) and *Z. chalybeum -M. volkensii* (60%) treated pods had very severe damage compared to the rest of other treatments (Fig. 11).

There were significant differences in mean pod quality among the treatments (P<0.0001). *Z. chalybeum* (48%), untreated control (53%) and *Z. chalybeum -M. volkensii* (46%) treatments had significantly the highest percentage mean pod damage followed by *B. angustifolia -M. volkensii* (39%), *B. angustifolia* (37%), Kocide DF (36%) and *B. angustifolia -Z. chalybeum* (33%) treatments respectively. *M. volkensii* (26%) treatment had significantly the lowest mean pod damage compared to all treatments (Fig 12).

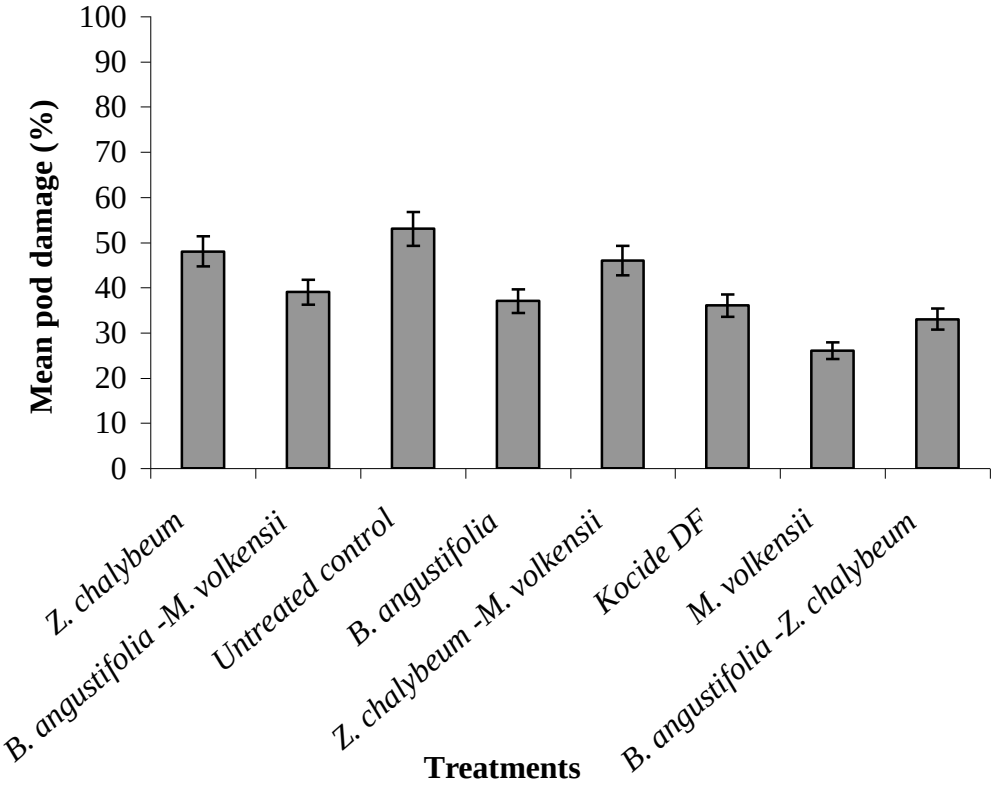


Figure 12. Total mean pod damage for each treatment.

4.9 Effect of different treatments on French beans yield

There were significant mean differences (P= 0.0424) among the treatments in yields. Kocide DF, *B. angustifolia - Z. chalybeum* and *M. volkensii* treatments recorded higher yields while untreated control, *Z. chalybeum-M. Volkensii* and Z. *chalybeum* had lowest yields (Table 12)**.**

Table 12. Mean French bean yield for each treatment

Treatments	Total mean yield (kg)
B. angustifolia	*6.055±1.47^{ab}
B. angustifolia – M. volkensii	4.889±1.27^{abc}
B. angustifolia –Z. chalybeum	6.904±1.71^{a}
Kocide DF (positive control)	6.773±1.78^{a}
M. volkensii	6.782±1.63^{a}
Untreated control (Water)	1.861±0.16^{c}
Z. chalybeum	4.310±1.14^{abc}
Z. chalybeum –M. volkensii	2.723±0.51^{bc}
LSD	3.7186
CV%	18.1641

*Numbers represent total mean yield for each treatment at time intervals.
*Means separated using LSD test by the same letter along the column are not significantly different (P<0.05) from each other.

4.10 Effect of plant extracts on microbial population

There were significant differences in fungal colony forming units in the sampled plots before and after treatments (Fig. 13). There were no significant differences in fungal population in the rhizosphere of bean plants treated with the single treatments of *B. angustifolia* (P=0.5057), *M. volkensii* (P=0.7369) and *Z. chalybeum* (P=0.295).

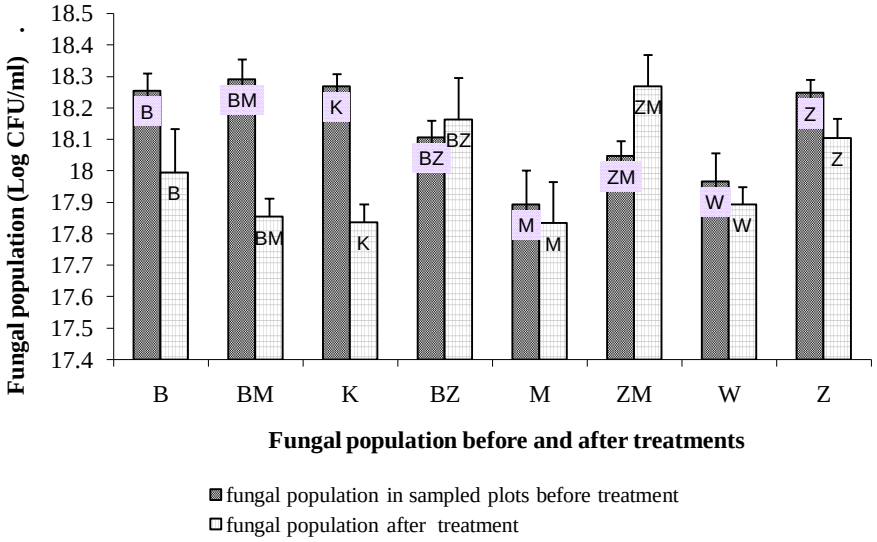


Figure 13. Fungal population before and after the field experiment. Logarithmic transformation was performed on data for fungal population. Letters in each bar represents treatments: B- *B. angustifolia*, W- Untreated control, BZ- *B. angustifolia -Z. chalybeum*, K- Kocide DF, ZM- *Z. chalybeum -M. volkensii*, M- *M. volkensii*, BM- *B. angustifolia -M. volkensii* and Z- *Z. chalybeum*

Plots with *B. angustifolia -M. volkensii* (P=0.004) and *Z. chalybeum -M. volkensii* (P=0.0123) treatments had significant changes in fungal populations before and after treatment. Plots sprayed with commercial fungicide (Kocide DF) had significant changes (P=0.018) in fungal population. *B. angustifolia -Z. chalybeum* (P=0.8145) had no differences in fungal populations (Fig. 13).

There were significant differences in bacterial colony forming units in the sampled plots before and after treatments as shown in Fig. 14. There were no significant differences in bacterial population in the bean plants treated with the following single treatments; *B. angustifolia* (P=0.00298) and *M. volkensii* (P=0.8305). Plots with *Z. chalybeum* treatment had significant changes (P=0.0398) in bacterial population before and after treatment. Plots sprayed with commercial fungicide (Kocide DF) had no significant changes (P=0.2372) in bacterial population. *B. angustifolia -Z. chalybeum* sprayed plots had no significant differences (P=0.5556) in bacterial population. *Z. chalybeum - M. volkensii* revealed significant differences (P=0.0029) in bacterial population (Fig. 14).

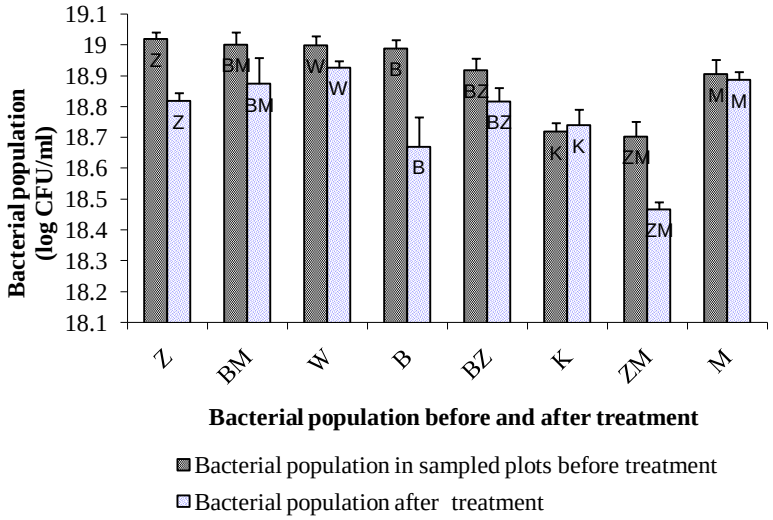


Figure 14. Bacterial population before and after the field experiment. Logarithmic transformation was performed on data for bacterial population. B- *B. angustifolia,* W- Untreated control, BZ- *B. angustifolia -Z. chalybeum*, K- Kocide DF, ZM- *Z. chalybeum -M. volkensii*, M - *M. volkensii*, BM- *B. angustifolia -M. volkensii* and Z- *Z. chalybeum*

The test result of Gram stain showed that unknown organism is Gram negative (Plate 2). Observation with naked eye while staining shows that the alcohol decolorizes the crystal-violet iodine complex. Observation under oil microscope confirmed that unknown organism is Gram negative rods. The cells were single.

To confirm that Gram negative has a rod shape, negative staining was done. The contrast background proved to be an important tool to determine the shape of the unknown

present. Gram negative rods bacteria appear to be in single bacillus shape. From this experiment, the numbers of possible unknowns are narrowed down into six genera: *Bacilli spp, Citrobacter spp, Enterobacter spp, Escheria spp, Proteus spp and Pseudomonas spp.* Plates 2 and 3 shows gram stain reactions of microorganisms.

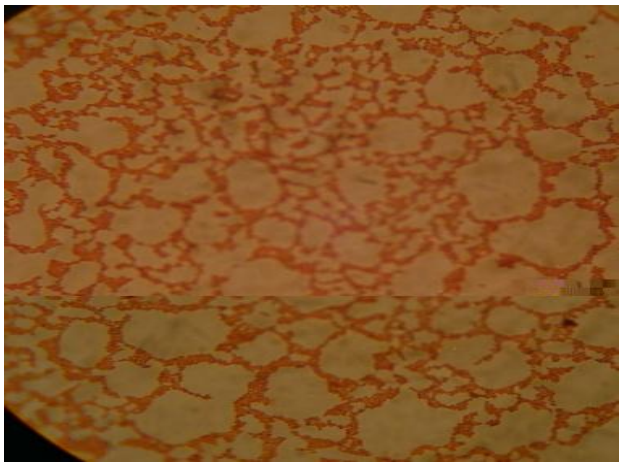


Plate 2. Gram-ve stain reaction on the isolated bacteria species.

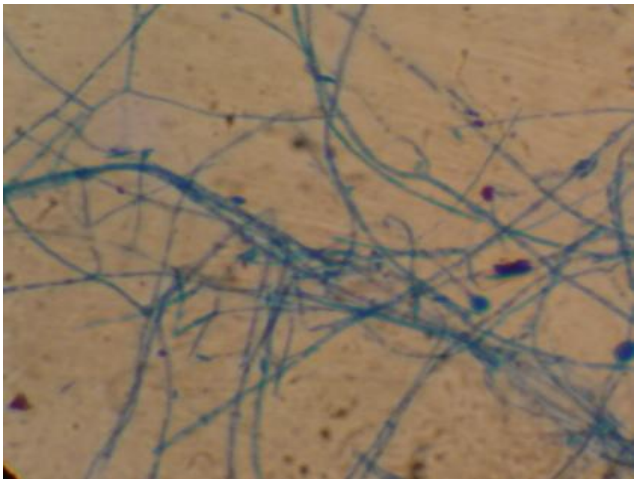


Plate 3. Gram stain reaction on the isolated fungal species.

Fusarium spp and *Mucor spp* were common fungal species isolated from all treated and control plots.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of crude extracts on spore germination

Plant extracts showed significant differences on spore germination time. There were significant differences (P=0.02130) within the 6^{th}, 12^{th}, 24^{th} and 48^{th} hrs of the treatments on the uredopsores germination except the commercial control. Changes in percentage uredospore germination from the time of inoculation could be attributed to the qualitative and quantitative changes of the bioactive compounds in the plant extracts with time. This indicated decrease with time in sensitivity of the uredospores to antifungal agents calls for repeated application of the crude extracts. This indicated that treatments had low levels of potency throughout the period. The commercial fungicide had significantly the lowest percentage spore germination followed closely by *M. volkensii, Z. chalybeum* and *B. angustifolia* plant extracts. This revealed the possibility of these plant extracts reducing the inoculum potential thus reducing the disease progression.

These results are in conformity with earlier *in vitro* research by Hassan *et al* in 2006 who studied antibacterial activity and phytochemical analysis of crude root extracts of *B. angustifolia*. Considering the fact that the extracts were applied in crude form at a rather low rate of 1 g, it is speculated that the possibility exists to improve its efficacy against the more resistant pathogens by concentrating the extract via liquid–solid fractionation using organic solvents in series of increasing polarity.

Compared to *M. volkensii* leaf extracts *U. dioica, A. secundiflora, T. rotundifolia and C. edulis* extracts were less effective in inhibiting the spore germination of *U. appendiculatus.* These plants indicated that they could be having different combinations of secondary products that may be supporting spore germination. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combination of secondary products in a particular plant is taxonomically distinct (Wink, 1999).

U. dioica, A. secundiflora, T. rotundifolia and *C. edulis* extracts had insignificant antifungal activity. This probably was due to the fact that similar plants growing in different geographical locations may be phytochemically very different or the taxonomy in certain cases is somewhat obscure (both scientifically and by local nomenclature). This makes it usually necessary to specify not only the botanical sources used but also give some indication of the chemical composition. This is because apart from the interspecific variation in chemistry, the extent of the variations of active constituents within a species (particularly from different geographical locations) is still not clear (Olila *et al*., 2001).

The suppress effect expressed by *M. volkensii, Z. chalybeum* and *B. angustifolia* plant extracts could be attributed to the presence of bioactive compounds present in these plants. The results of this study indicate the possibility of using plant extract fungicides which are environment friendly and also comparable to commercial ones.

5.2 Effect of plant extracts on incidence and severity of rust disease

Differences in disease incidence between treatments were greatest between 21-42 days and it is probable that these differences resulted from more widespread dispersal of uredospores, at early stage in the 'open' plots. Untreated control and *Z. chalybeum* had higher disease severity throughout the experiment. For all treatments there were significant differences between the different plant ages.

In this study, age of plants played a significant role in disease infection. It was observed that the younger plants at 7 and 14 days less infection for all the treatments than plants at 21-42 days. This agrees with Agrios (2005), who reported that plant age is important in disease infection. He also reported that plants in their reaction (susceptibility or resistance) to disease depends largely on age and for instance in infections caused by *Pythium* (damping off and root rots), downy mildews, bacterial blights and viral infections, the host plants are susceptible only during the growth period when young and become resistant during the adult period. Also depending on the particular plant-pathogen combination, the age of the host plant at the time of arrival of the pathogen may affect considerably the development of infection and of an epidemic (Agrios, 2005).

Interestingly, under field conditions the *B. angustifolia –Z. chalybeum* was most effective against *U. appendiculatus* and showed a competitive advantage over the *M. volkensii* in controlling bean rust. Compared to the untreated control, an extract from *B. angustifolia - Z. chalybeum* decreased bean rust incidence and increased yields significantly over the season, while also comparing favorably to the commercial fungicide Kocide DF.

According to Dimitra *et al.*, 2003, variability of test plants in terms of their *in vitro* and/or *in vivo* antimicrobial properties is not only due to different bio-active substances within plants but can also be related to quantitative differences of the same active substances used in the experiments. Further, the sensitivity of pathogens towards different natural compounds from plants as well as synthetic chemicals can vary due to natural or acquired resistance (Lyr and Werner, 1982; Godet and Limpert, 1998).

Plants in the family of Meliaceae including *M. volkensii* are usually characterized by the presence of a group of secondary metabolites known as limonoids and steroids which could be attributed to be antifungal. *B. angustifolia - Z. chalybeum* combination showed significant fungal inhibition. The synergy between *B. angustifolia* and *Z. chalybeum* may have been enhanced by one compound requiring another compound to enhance antifungal activity.

5.3 Phytotoxic effects of plant extracts

It has been reported that even natural compounds from plants can be highly toxic and can adversely affect the host plant when applied externally (Seddon and Schmitt, 1999). Although in this study, no phytotoxic effects were observed on French bean seed germination or on seedling establishment following treatments.

5.4 Assessing soil characteristics

5.4.1 Field soil characteristics

The quantity type of fertilizer was depended on the results of soil analysis. The major macronutrients are nitrogen (N), phosphorous (P), and potassium (K). Calcium (Ca), magnesium (Mg), and sulfur (S) are also macronutrients. All six nutrients are important constituents in soil that promote plant growth were present in the soil in right quantities. In addition to macronutrients, there are various trace elements that are necessary for plant growth (Brady and Weil, 1999). Trace elements needed in smaller quantities cadmium, copper (13.6 mgkg-1), sodium (120.5mgkg-1) and zinc (199.1 mgkg-1) were also in the soil examined before the field experiment was carried out in correct amounts. This was done to rule out that any plant symptoms were due to fungal/bacterial/viral but not due to nutrient deficiencies.

Potassium is the third most likely, after nitrogen and phosphorous, to limit plant productivity. For this reason, it is commonly applied to soils as fertilizer and is a component of most mixed fertilizers. For this reason N: P: K fertilizer was applied at the planting and a few weeks after emergence of seedlings. In this study the plots where the French beans were grown had nitrogen and phosphorous deficiency. Phosphorus was in low levels ($5.4 mgkg^{-1}$) which was appropriate for French beans hence it was necessary to supply fertilizer of right amounts to supplement the deficiency. This is because it has been established that high levels of available phosphorus in soil or high application rates of phosphate may induce zinc deficiency in plants grown on soils characterized by low

concentrations of available zinc. The interaction of phosphorus and zinc, called phosphorus-induced zinc deficiency, has been observed in many crops, such as bean, wheat, tomato, cotton, flax, soybean, grape and citrus (Cakmak and Marschner., 1986; Singh *et al*., 1988; Webb and Loneragan., 1990).

5.4.2 Effect of treatments on microbial population in the soil

Soil microorganisms are vital for the continuing cycling of nutrients and for driving above-ground ecosystems (Cairney, 2000; Klironomos *et al.*, 2000; Ovreas, 2000). It is unknown how changes in microbial diversity can influence below-ground and above-ground ecosystems. Another problem with this approach is that soil is heterogeneous, containing many microhabitats that are suitable for microbial growth. It was for this reasons that rhizosphere amount of micro-organisms had to be established before treatment.

The effects of foliar application of antifungal crude plant extracts (*Z. chalybeum, Z. chalybeum -M. volkensii* and *B. angustifolia -M. volkensii*) and commercial fungicide (Kocide DF) on bacterial and fungal population of rhizosphere are depicted. Commercial fungicide treated plants resulted into a drop in the fungal population. Some treatments can be directly toxic to certain soil microorganisms and can disrupt important microbial processes in soil, such as nutrient cycling. It could be inferred that the adverse effect of fungicides was severe and prolonged in the treatments in case of fungal population.

The rhizosphere bacterial population of *Z. Chalybeum* and *Z. chalybeum -M. volkensii* treated plants had differences in bacterial population before and after treatments. Fast and rapid recovery of bacteria may be attributed to their tolerance to the action of fungicides (Chaube, 1985) and capacity of quick growth rate (Wainwright, 1977). Kocide DF, *M. volkensii*, *B. angustifolia-Z.chalybeum* treatments had significant differences in rhizosphere fungal population. Continued increase in the microbial population of many treatments and untreated control could be attributed to the more favorable temperature and moisture conditions during the later part of the study. It appears that the treatments that reduced the fungal and bacterial populations of the rhizosphere might have inflicted changes in fungal and bacterial community structure and thus adversely affected the various fungi and bacteria mediated processes of soil and rhizosphere due to pollution.

Copper does not degrade in soil or leach into groundwater, but becomes chemically bound up, especially with organic matter. An application of 1 lb of active ingredient per acre would raise the copper levels of about 0.5ppm. Copper fungicides are protectants, so they must be applied to the foliage before infection. Studies reported lower fungal species diversity in soils sprayed with commercial fungicide Kocide DF showing it pollutes. Copper the main ingredient in this commercial fungicide could have been toxic to this species.

The relatively long residence time of Cu in top soils, largely related to the high affinity of Cu for soil organic matter and hydrous oxides, means that long term accumulation of Cu is likely to occur. The accumulation of Cu in top soils also corresponds to the zone in the

soil profile of greatest biological activity. Detrimental effects of elevated Cu concentrations upon mycorrhizal associations (Georgieva *et al.,* 2002), microbial populations and function (Dumestre *et al.,* 1999) and arrange of mesofauna (Paoletti *et al.,* 1998) have been documented. In this study, Pour plate method revealed a densely diverse microbial population in the soil samples where Kocide DF had not been used.

The colony forming units (cfu/g) from this study are within the range of other findings already reported by others Turco *et al*., 1995 and Hazen *et al*., 1991 reported that under optimal growing conditions, total microbial abundance in soils can exceed about 10^6 to 10^8 colony forming units per gram (dry weight) of soil (cfu/g) for bacteria; 10^6 cfu/g for actinomycetes, and 10^5 cfu/g for fungi. Moreover it has been reported that, due to relatively low recovery efficiencies from soils, population densities of total recoverable heterotrophs within soils usually range between about 10^4 and 10^7 cfu/g (Turco *et al*., 1995). In this study the number of soil microbes (bacteria and fungi) varied slightly within sampled sites but was within normal ranges. This can be attributed by the slight nutrient and organic matter variability of the soil. According to Zhou *et al.,* 2002 spatial and resource factors influence microbial numbers and diversity in soil. Competition also has been reported to drive the structure of the aqueous maintained microbial communities (Rashit and Bazin, 1987).

Moreover, both theoretical and empirical studies suggest that in plant, microbial and animal communities competitive interaction is the key determinant of species abundance and diversity (Huston, 1994).

5.5 Effect on crop yield

Kocide DF (6.773kg), *B. angustifolia* - *Z. chalybeum* (6.904kg) and *M. volkensii* (6.782kg) had the highest mean yields thereby making the treatments the best among all treatments. Untreated control (1.861kg) and *Z. chalybeum* - *M. volkensii* (2.723kg) recorded the lowest mean yields indicating they were not effective fungicides. *B. angustifolia – M. volkensii* (4.889kg) and *Z. chalybeum* (4.310kg) recorded relatively lower mean yields.

5.6 Effect of plant extract treatment on French bean pod composition

The extracts were eluded by binary gradient (methanol: water) using HPLC reverse phase column at a flow rate of 0.8ml/min and a wavelength of 230nm. The binary gradient chromatographic procedure adopted allowed the separation of all the major carotenoid and chlorophyll constituents of bean pods without the need for saponification and removal of chlorophylls and their derivatives as explained by earlier reports (Frederick *et al.,* 1991). High pressure liquid chromatography revealed absence of any new compounds except what could likely be organic chemical components of the pods from all the treatments. Similar compounds were evidently eluded at specific retention times showing no variation in the organic contents of the treated and analyzed pods.

This is also suggestive that the treated and untreated pods showed similar peaks. The isomeric occurrence of the compounds in some treatments pods is suggestive to the fact that the two isomers belong to the same compounds but have different molecular weights.

The general chromatographic profiles of raw green beans were very similar in all treatments. These similarities are consistent with the extensive surveys carried out by Frederick (1991), who showed that the leaves of higher plants usually contain the same carotenoids.

It is expected that Kocide DF treated pods could accumulate copper ions from the active ingredients of the fungicide. However, HPLC being specific to detection of traces of organic compounds used in this experiment could not be used to reveal presence of inorganic compounds in the pods. The major differences in retention times among the pods from the various treatments might have been attributed to concentrations at which the various components were present. Some secondary metabolites in various pods from different treatments could have been affected by environmental factors thus the peak length differing in some of the treatments.

M. volkensii, *B. angustifolia* treated and untreated pods had more compounds than other treatments. These compounds may have existed in multiple forms as free, esterified, glycosylated or polymerized. In addition, these compounds are not uniformly distributed in plants at tissue, cellular and sub-cellular levels and may coexist as complexes with proteins, carbohydrates, lipids or other plants components (Robbins, 2003).

Z. chalybeum treated pods had smaller peaks compared to all other treatments this could have been attributed to pod length which can affect the overall composition of compounds present in pods. Goodwin (1980) reported the effect of environment on the synthesis of carotenoids in plants. These require other analytical techniques such as gas

chromatography (GC) and gas-liquid chromatography, which are sensitive to inorganic compounds. However, residues such as copper due to Kocide DF and other fungicides have been reported extensively by exporters.

Only four treatments elicited 7 compounds, although several factors like the environment could have influenced the number of compounds produced. Some researchers have documented that the quantity of phenolic compounds in foods is influenced by genotype (cultivar or variety), agronomic practices (irrigation, fertilization, and pest management), maturity at harvest, method of storage and climatic conditions (Ninfali and Bacchiocca, 2003; Hakkinen and Torronen, 2000).

5.7 Effect of treatments on some selected C_3 parameters

Generally, French bean leaves showed higher values for stomatal conductance resulting in higher transpiration. The high positive regressions (r^2 >0.9) were obtained in the four treatments. This indicated that stomatal conductance and rate of transpiration were interdependent and it is interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. This result corresponds with Mott and Parkhurst, (1991) who showed that stomata conductance respond to the rate of transpiration.

The response of stomata to transpiration was used by Monteith (1995a), who re-analysed 52 sets of published measurements at canopy scale of humidity responses on 16 species of monocots in terms of the relation between stomatal conductance and transpiration.

However, there were significant differences in stomatal conductance of the treatments ($P<0.05$). *M. volkensii,* and untreated control treatments of French beans showed no significant differences with each other but recorded higher stomatal conductance. This showed that plant extracts had no impact on physiology of French beans.

Commercial control (Kocide DF) had the lowest stomatal conductance of all treatments however; Kocide DF plots had the lowest water loss compared to others, this indicates better water conservation. Stomata showed a slight opening tendency until 1200 noon, when decreases in stomatal conductance occured were likely cut down high transpiration values. Since similar stomatal conductance values were observed during morning, changes in transpiration values suggest that stomatal aperture was more than sufficient to support maximal transpiration values since early hours of morning.

The high regressions between stomatal conductance and rate of transpiration in the four treatments indicated that stomatal conductance and rate of transpiration were interdependent and it was interpreted to mean that stomatal conductance enhanced rate of transpiration at different times of the day. The differences in R^2 values in four treatments were insignificant meaning that concerning these two parameters the French beans responded to the treatments the same way. This pattern was maintained throughout the growing period.

Therefore the sources of variation in stomatal conductance and the rate of transpiration were treatment, time and PAR. The daily diurnal courses conformed to Zeiger *et al*., (1981) study which showed that at dawn, stomatal conductance usually increases very

rapidly because the entrained rhythm is in correct phase, and also there is a great sensitivity to low photon fluxes of blue light at this time. Stomatal conductance then increases gradually towards a maximum value in late morning or early afternoon before declining noticeably later in the day (Zeiger *et al*., 1981).

This partial closure in the afternoon is thought to be driven by the entrained rhythm, and it is not unusual for the stomata to be nearly closed before dusk. However, gs was significantly different during the morning hours (9:00am) at all treatments (P=0.017). *Melea volkensii* treated plants had the highest gs followed by the *B. angustifolia* - *Z. chalybeum* treatment while the Kocide DF had lowest gs throughout the day.

Despite the fact that stomatal conductance is strongly correlated with light intensity. It is not clear whether it operates directly or principally through changes in internal CO_2 concentration caused by photosynthesis. Sharkey and Raschke (1981) reported that stomatal response in beans is caused chiefly by direct response to light and influenced to only a small extent by the internal CO_2 concentration. It was demonstrated that exposure of the epidermis to dry air causes closure of stomata at midday (Sherrif, 1977b). The responsiveness of stomata to light and CO_2 depends on leaf age, temperature and past treatment. As leaves become older, the stomata often become less responsive and may open partly, even at midday (Sherrif, 1977b).

It is difficult to generalize about stomatal behavior because so many contradictory reports occur in the literature. Stomatal activity is affected by numerous internal and external factors which often interact in complex ways that sometimes are overlooked by

investigators. There were no significant differences in photosynthetic active radiation **(**PAR) of the four treatments. At 12:00pm the treatments recorded the highest PAR compared to 9:00am and 15:00pm. The pattern of PAR increased steadily noon then started to decrease. Any slight decrease in PAR would have been linked to slight cloud cover since IRGA is very sensitive.

The treatments followed a trend that revealed an increase in PAR at midday when the sun is at its maximum while at 9:00am and 15:00pm the PAR values were lower probably due to the amount of sunlight at this time. It was established that treatments did not impact in PAR absorption by bean leaves. The amount of photosynthesis varied with time. It was highest at 900hrs coinciding with time when CO_2 uptake was highest but PAR was still low. This implies that CO_2 absorbed was being used in photosynthesis. At noon the amount of photosynthesis dropped coinciding with a big drop in stomatal conductance and a drop in CO_2 uptake. This is an indication of spartial stomata closure at noon in intense sunlight (PAR) which caused decrease in CO2 uptake and hence decreases in rate of photosynthesis. Daily course of CO_2 assimilation (A) was similar for all evaluated treatments. In early morning, the sharp increase in photosynthetic photon flux density (PPFD) seems to be the main cause of CO_2 assimilation increase. Considering the highest CO_2 assimilation values, no significant difference was found between treatments under natural condition.

Maximal CO_2 assimilation rates were reached around 9.00 am until 12:00 pm when reductions were recorded. Low stomatal conductance is known to cause decrease in CO_2

assimilation values by reducing the CO_2 available, which may be indicated by decreased intercellular CO_2 concentration (Ci) values (Jones, 1998). Hence, it could be inferred that the high leaf temperature (29ºC) caused increase in photorespiration and consequent reduction in the photosynthetic activity. There were significant differences among the treatments in CO_2 uptake in each time of the day ($P<0.05$). Commercial control (Kocide DF) treated plants had the lowest carbon dioxide released compared to all other treatments because of its low stomata conductance.

Photosynthetic rates (Pn) among the four treatments followed a trend whereby they were at the peak at 9:00am reducing gradually towards the afternoon and at 15:00pm.The main sources of variation in the Pn might have been due to treatment and the time of the day since there were significant differences ($P<0.05$); between treatments and the times of the day.

The explanation for the above stated interactions being significant could be that these factors were affecting the photosynthesis rates dependently. The plant extracts were even better enhancers of photosynthesis than copper containing Kocide DF. There were no significant differences in leaf temperatures of different (four) treatments studied at 12:00pm ($P>0.05$). However, at 9:00am and 15:00pm there were significant differences in the amount of leaf temperatures of the four treatments.

At 9:00am *Melea volkensii* treated bean plants had significantly the highest leaf temperature while the control bean plants had the lowest leaf temperature at 9:00am. Although there were no significant differences between *B. angustifolia – X .chalybeum*

and Kocide DF treated plants, they had higher leaf temperatures than the control at 9:00am. The highest values of leaf temperature, were observed in the afternoon (12:00 pm), with values of leaf temperature higher than 29ºC this might have enhanced photorespiration. Besides stomatal conductance effects (Jones, 1998), high temperatures also cause impairments in photochemical and biochemical reactions of photosynthesis. Therefore, the results suggest that the photosynthetic capacity of 'commercial fungicide (Kocide DF) treated beans' were constrained at natural condition by low stomata conductance and high temperature.

Thus, in addition to the effect of temperature on photosynthesis arising from the intrinsic temperature dependence of the process in the range over which the functional integrity of the photosynthetic apparatus remains intact, extreme temperatures can drastically inhibit photosynthesis by disrupting the integrity of the system. Low stomata conductance in the commercial control (Kocide DF) treated plants might have affected the photosynthetic activity. The inactivation of Rubisco (ribulose-bisphosphate carboxylase/oxygenase) a key-enzyme of Calvin cycle and its two accompanying enzymes i.e., Rubisco activase (RCA) and carbonic anhydrase (CA) under the stress conditions caused by copper and lead (not examined) may be regarded another possible factor (Vojtechova & Leblova, 1991). This indicated that plant extracts were physiology friendly to beans as compared to the copper containing Kocide DF.

The control treated plots had the highest mean bean rust disease severity compared to other treatments in the green house and field experiments. Commercial fungicide (Kocide

DF) had significantly the lowest leaf disease severity followed by *B. angustifolia* - *Z. chalybeum* and *M. volkensii* treated bean plants had no significant differences among the treatments. Highest transpiration rates in untreated plants might have been caused by high disease severity. Rust caused increased transpiration from infected tissues after sporulation in untreated control.

Rust diseases commonly increase rates of transpiration from host tissues, once rupture of the epidermis has occurred and sporulation commenced (Duniway & Durbin, 1971b). Studies of transpiration from partly infected leaves have practical relevance since in the field it is rare for leaves to be uniformly infected by rust fungi. Transpiration from rusted areas reflects the internal development of the fungus in the infected host tissue. Transpiration before sporulation, which potentially is by a mainly stomatal pathway, is inhibited, probably by stomatal closure; rust is known to inhibit stomatal opening in the light in other diseases, e.g. bean (*Phaseolus vulgaris*) infected by either *U. phaseoli* (Duniway & Durbin, 1971b). In the present study it was noted that at each sample time more variability in transpiration rate occurred in rusted tissue than in healthy tissue.

This variability probably occurred because the number of lesions per unit area of leaf was not controlled. Durbin (1978) stated that when sporulation occurred, transpiration from bean leaves infected with rust increased by as much as 50%.

Where net photosynthesis was concerned, infection induced opposing changes in the four treatments; net photosynthesis in healthy leaves increased because gross photosynthesis was stimulated and photorespiration was inhibited. Net photosynthesis per plant and

ultimately plant growth of the untreated control reduced because infection inhibits the growth of leaf area. Photosynthesis is closely related to crop growth and yield, and higher photosynthetic rate of leaves is one of the important factors for high crop yield. The results showed that after flowering, the leaves gradually aged, the net photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (gs) of leaves gradually declined. Commercial control (Kocide DF) contains copper metal that might have caused low productivity. When applied according to the manufacturers' instructions Kocide DF revealed low amount of photosynthesis in the second month. This could be attributed to its contents that can hamper the process of photosynthesis.

It being a micronutrient, copper improves plant growth at natural concentrations. However, at higher concentrations it also proves very toxic for plants. The phytotoxic effects related to higher concentrations of copper include inhibition of photosynthetic efficiency and as a result reduced crop productivity (Moustakas *et al.*, 1994). The process of photosynthesis (*A*) was adversely affected by Cu toxicity. Plants exposed to copper formulated fungicide (Kocide DF) showed a decline in photosynthetic rate, which might have resulted from distorted chloroplast structure, restrained photosynthesis of chlorophyll and carotenoids, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomatal closure (Moustakas *et al.*, 1994).

A strong relationship exists between Kocide DF application and a decrease in photosynthesis and it is believed to result from stomatal closure. Increased rates of respiration and loss of chlorophyll from the leaf tissue apparently were the major factors

responsible for the reduction of photosynthetic rates on diseased untreated control leaves. The photoinhibition mechanism could have a character of photoprotection or represent damaging in PSII reaction centers (Osmond, 1994). The former is associated to an avoidance of over-excitation of the PSII reaction center by decreased energy absorption or by increased thermal dissipation of excitation energy via xanthophyll cycle, and the later is related to a cycle of PSII reaction center inactivation and repair (Osmond, 1994).

The maximum CO_2 assimilation values observed in this study are in agreement with the measurements performed by Comstock & Ehleringer, (1993) and Souza *et al.,* (2003) in common bean study. Low temperatures reduce assimilation rate because of reduced activity of the Rubisco and of the capacity for electron transport. High temperatures also reduce electron transport capacity and increase the rates of CO_2 evolution from photorespiration and other sources, again causing assimilation rate to decline.

In most plants, changes in photosynthetic rate in response to temperature are reversible over a considerable range (commonly 10°C to 35°C), but exposure to temperatures below or above this range may cause irreversible injury to the photosynthetic system. Transpiration exhibited similar trend to photosynthesis suggesting that an appreciable part of the inhibition of the two processes is related to increased stomatal resistance as a result of stomatal closure.

Decreased photosynthetic rates were the main effects observed during infection by the rust pathogen. Physiological processes other than photosynthesis, but closely related to it, were also affected by rust infection. In the present study, decreased stomatal

conductance, increased respiration, and losses of chlorophyll from leaf tissue were observed in response to increases in rust severity.

5.8 Effect of plant extracts on growth parameters

There were no significant differences in leaf area, shoot height and dry shoot weight among the treatments in the 1st month this could be because at seedlings stage the plants had not been naturally inoculated with rust. *B. angustifolia –Z. chalybeum* and *B. angustifolia –M. volkensii* had higher mean leaf area, this indicated better physiological processes. Leaf area is an essential component to estimate plant growth through its incidence on crop physiology mechanisms (Ramesch and Singh, 1989; Bhatt and Chanda, 2003).

Commercial fungicide (Kocide DF) treatment did not affect the leaf area but *Z. chalybeum* treatment had the lowest mean leaf area throughout the growth period. This may suggest that the commercial fungicide can be tolerated by the plants. *Z. chalybeum* treatment may have had some secondary metabolites that could have been harmful to the bean plants. Phytotoxin in the form of phenols have been found to have an adverse effect on germination and growth parameters (Hafees *et al*., 1988; Ahmed & Siddiqui, 1995; Siddiqui *et al.*, 1997).

Therefore, leaf area measurements for physiological studies is one of the most essential processes, such as one of the physiological determinants of plant growth is the efficiency of the leaves with which the intercepted light energy is used in the production of new dry

matter (Evans, 1972; Uzun, 1996). *B. angustifolia –Z. chalybeum*, *M. volkensii* and Kocide DF treatments had highest mean dry shoot weights throughout the growth period meaning they were friendly to the physiological processes. Untreated control and *Z. chalybeum* had lower mean dry shoot weights; this could be because of the higher disease severity and incidence. Siddiqui *et al.*, 1997 reported that benlate (fungicide) caused an increase in fresh and dry weights of *Sesbania sesban* at 0.25g/l concentration. Commercial fungicide, untreated control and *Z. chalybeum* treatments had lower mean shoot heights compared to other treatments. This indicated that apart from rust disease causing low shoot height in untreated control, Kocide DF and *Z. chalybeum* treatments could have initiated production of compounds that deterred increase in shoot height.

The results confirmed Heisy (1990) research that exposure of plants to fungicide creates chemical stress facilitating the production of compounds that are potential inhibitor of germination and seedling growth. *M. volkensii*, *B. angustifolia –Z. chalybeum* and Kocide DF treatments had no effect on the bean pods as compared to untreated control meaning they had less damage.

5.9 CONCLUSIONS AND RECOMMENDATIONS

5.9.1 Conclusions

- *M. volkensii* and a combination of *B. angustifolia and Z. chalybeum (B. angustifolia - Z. chalybeum)* significantly inhibited growth of *U. appendiculatus* the causal agent of bean rust in French beans.
- *M. volkensii* and a combination of *B. angustifolia* and *Z. chalybeum* extracts significantly enhanced photosynthesis of French beans compared to the control.
- Plant extracts did not have any effect on growth parameters of French beans.
- No extraneous compounds were detected in French beans after application of plant extracts showing that no chemical residues were present.
- The plant extracts had different effects on soil fungal and bacterial populations in the rhizosphere of test plants. A combination of *Z. chalybeum and M. volkensii* appeared to have caused reduction in bacterial population. *M. volkensii* and *B. angustifolia - Z. chalybeum* treatments caused significant increase in fungal population.

5.9.2 Recommendations

- It is recommended that molecular structures of the active compounds in the effective plant extracts from *M. volkensii, B. angustifolia* and *Z. chalybeum.* Be determined, isolated and developed for commercial production so that they are made available to Kenyan French bean growers.
- Further testing is recommended to confirm that the plant extracts do not leave any residues and hence they are safe to use.
- It is recommended that the specific fungal and bacterial micro-organisms affected by the plant extracts be determined. This will help establish whether the plant extracts have adverse effects on beneficial soil microorganisms.

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APPENDICES

Appendix 1. Physiological responses

Appendix 1.1. Transpiration and Stomatal conductance in various treatments

			Transpiration			Stomatal conductance		
Treatment	**Time**		**900HRS**	**1200HRS**	**1500HRS**	**900HRS**	**1200HRS**	**1500HRS**
B.	Month 1	mean	0.72	1.18	1.411667	55.5	37.66667	382.8333
angustifolia-		SE	0.06	0.02	0.017591	5.948389	1.085255	19.17884
Z. chalybeum	Month 2	mean	2.06	1.12	0.678333	77.76923	41	25.94444
		SE	0.35	0.07	0.048184	17.2913	3.34664	2.310069
	Month 3	mean	1.52	1.23	1.237273	36.57143	19.11765	16
		SE	0.01	0.06	0.071834	4.539035	3.721838	3.125157
Untreated	Month 1	mean	0.85	1.02	1.49	73.16667	34.08333	549.1667
control		SE	0.01	0.033	0.014407	1.845688	1.43262	33.18745
	Month 2	mean	2.43	1.06	0.679412	95.86667	38.33333	22.11765
		SE	0.20	0.194	0.048092	8.204403	7.735919	2.196057
	Month 3	mean	1.60	0.92	0.769	27.14286	38.94444	42.3
		SE	0.04	0.066	0.056597	1.895234	1.957844	2.902298
Kocide DF	Month 1	mean	0.87	1.24	1.483333	74.5	39.83333	544.1667
		SE	0.02	0.02	0.01145	4.402651	2.495552	30.44932
	Month 2	mean	1.11	0.76	0.7675	39.18182	25.66667	30.66667
		SE	0.31	0.192	0.055652	14.68566	7.241854	2.717322
	Month 3	mean	1.60	1.27	0.926667	28.8	17.91667	22.58333
		SE	0.02	0.05	0.036873	7.818124	5.562943	3.046255
M. volkensii	Month 1	mean	0.95	1.102	1.56	82.5	41	784.1667
		SE	0.02	0.01	0.014407	3.7183	2.266355	56.00187
	Month 2	mean	1.35	1.135	0.782222	46.3	41.5	27.5
		SE	0.16	0.06	0.051345	6.314224	3.649201	2.589887
	Month 3	mean	1.51	1.03	1.132	34.125	32.83333	18.3
		SE	0.00	0.029	0.114783	5.232005	3.015483	1.042939

Appendix 1.2. Photosynthesis and PAR in various treatments

Treatment	Time	Hours	Photosynthesis			PAR		
			900HRS	1200HRS	1500HRS	900HRS	1200HRS	1500HRS
B. angustifolia-Z. chalybeum	Month 1	mean	62.41	83.43	97.18	429	2394.3	220.33
		SE	11.40	8.191	2.71	14.35	41.50	5.834
	Month 2	mean	72.50	59.1	47.58	2079.9	1982	1574.6
		SE	2.73	4.39	4.14	237.40	347.09	143.75
	Month 3	mean	39.9	28.6	22.23	134	1935.82	307.36
		SE	0	7.07	6.65	111.2	169.85	17.22
Untreated control	Month 1	mean	76.75	99.8	99.9	685.58	1705.83	196.41
		SE	5.82	0.08	0	21.82	195.00	12.19
	Month 2	mean	53.9	24.4	39.98	1937.84	2198.83	672.3
		SE	0	10.46	3.70	29.2181	322.02	147.37
	Month 3	mean	46.61	17.45	20.86	1077.28	1983.55	319.8
		SE	3.28	4.92	6.57	20.16	155.50	22.26
Kocide DF	Month 1	mean	57.18	97.05	99.9	670.33	2151.66	217.5
		SE	8.84	1.33	0	63.99	295.29	14.04
	Month 2	mean	80.72	23.51	50.7	1118.9	2051.33	1315.6
		SE	9.75	11.23	4.21	157.98	190.99	189.58
	Month 3	mean	57.05	14.50	42.87	728.5	2220.83	321.33
		SE	2.85	5.85	2.98	67.59	183.93	22.29
M. volkensii	Month 1	mean	72.67	96.93	99.15	707.33	1331.66	231.5
		SE	4.78	2.96	0.75	42.78	305.05	11.30
	Month 2	mean	99.9	68.38	50.77	1682.8	2084.16	1415.3
		SE	8.35	1.42	3.78	287.22	296.26	157.57
	Month 3	mean	45.3	33.11	15.64	256.75	1269.38	592.9
		SE	1	5.92	7.74	19.41	253.10	149.98

Appendix 2. ANOVA tables

Table 1: ANOVA: Effect of different plant extracts on spore germination

Source of variation	DF	Mean Square	F Value	Pr > F
Hours	3	970.26167	32.91	<.05
Treatments	10	1190.11977	26.83	<.05

Table 2: ANOVA: Effect of various treatments on rust treated with selected plant extracts and Kocide DF in the Greenhouse experiment

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	11	**13.7551**	**21.22**	<.05

Table 3: ANOVA: Effect of various treatments on shoot height (cm) of French beans sprayed with selected plant extracts and Kocide DF in the Greenhouse experiment

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	7	38.159	1.32	.2397
Time	2	12840.80	445.53	<.05

Table 4: ANOVA: Effect of various treatments on dry shoot weight (g) of French bean plants sprayed with selected plant extracts and Kocide DF in the field experiment

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	7	152.640688	5.33	<.05

Table 5: ANOVA: Effect of various treatments on flower drop of French bean plants sprayed with selected plant extracts and Kocide DF in the field experiment

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	7	6.585	5.18	<.05

Table 6: ANOVA: Effect of various treatments on rust treated with selected plant extracts and Kocide DF in the field

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	7	72.1738	83.40	<.05

Table 7: ANOVA: Effect of various treatments on pod quality of French beans sprayed with selected plant extracts and Kocide DF for the season

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	7	3.85357143	5.44	<.05

Table 8: ANOVA: Effect of various treatments on disease incidence of French bean plants sprayed with selected plant extracts and Kocide DF in the field experiment

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	6	1348.73	5.18	<.05

Table 9: ANOVA: Effect of various treatments on yield of French bean plants sprayed with selected plant extracts and Kocide DF in the field experiment

Source of variation	DF	Mean Square	F Value	Pr > F
Treatments	7	**43186049**	**5.06**	<.05

Table 10: ANOVA: Transpiration and stomatal conductance

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	10349712.958	1	10349712.958	220.211	.05