EFFECT OF TREATMENT WITH ESSENTIAL OILS ON ANTHRACNOSE DISEASE DEVELOPMENT, QUALITY AND SHELF LIFE OF BANANA (*Musae spp.*) FRUITS

MSc THESIS

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EFFECT OF TREATMENT WITH ESSENTIAL OILS ON ANTHRACNOSE DISEASE DEVELOPMENT, QUALITY AND SHELF LIFE OF BANANA (*Musae spp.*) FRUITS

By

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As thesis research advisor, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by **Fekiya Mohammed**, entitled with "Effect of treatment with essential oils on anthracnose disease development, quality and shelf life of banana (*musae spp.*) fruits**"**. We recommended that it be submitted as fulfilling of the thesis requirement for the degree of Master of Science.

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DEDICATION

I dedicate this thesis manuscript to my families who played indispensable role and nursing me with affection and love and for their dedicated partnership in the success of my life.

Fekiya Mohammed

STATEMENT OF THE AUTHOR

I, the undersigned, declare that this thesis is my work and is not submitted to any institution elsewhere for the award of any academic degree, diploma or certificate and all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University, College of Agriculture and Veterinary Medicine and is deposited at the University Library to be made available to borrowers under the rules of the library.

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BIOGRAPHICAL SKETCH

The author Fekiya Mohammed was born from her father Mr. Mohammed Idris and mother Mrs. Workenesh Nasir at Arsi Dera, Oromia Region, Ethiopia on February 23, 1989. She attended and completed grade 1 to 8 at Dera elementary school (1996-2003), grade 9 to 10 at Dera secondary school (2004-2005) and grade 11 to 12 at Hawas (Adama) preparatory school (2006-2007). She joined Samara University, Agriculture Faculty in September 2008 and graduated with BSc degree in Horticulture in July 2010. In September 2011, she joined the school of graduate studies of Jimma University to pursue her graduate study leading to MSc degree in Postharvest Management.

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LIST OF ABBREVIATIONS AND ACRONYMS

AS	Anthracnose Symptom
B	Basil
C	Cinnamon
CRD	Completely Randomized Design
DI	Disease Incidence
EO	Essential Oil
GRAS	Generally Regarded As Safe
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
LSD	Least Significant Difference
MGI	Mycelial Growth Inhibition
MIC	Minimum Inhibitory Concentration
PDI	Percent Disease Index
PDA	Potato Dextrose Agar
R	Rosemary
TA	Titratable Acidity
TSS	Total Soluble Solid
WL	Weight Loss

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EFFECT OF TREATMENT WITH ESSENTIAL OILS ON ANTHRACNOSE DISEASE DEVELOPMENT, QUALITY AND SHELF LIFE OF BANANA (*Musa spp.*) FRUITS

ABSTRACT

Banana fruit experiences a different problem from that of most other fruits, because ripe bananas are very perishable. Postharvest diseases such as anthracnose reduced the annual yield of banana fruit. Losses from postharvest banana diseases are severe in Ethiopia. The application of fungicides to fruits after harvest to reduce decay has been increasingly curtailed by the development of pathogen resistance to many key fungicides, the lack of replacement fungicides, negative public perception regarding the safety of pesticides and consequent restrictions on fungicide use. The objective of this study was to determine the efficacy of different essential oils on prolonging the storage life of banana fruit and to reduce postharvest losses by controlling anthracnose (C. musae). Essential oils were extracted by hydro distillation method using Clevenger type apparatus, and then each essential oil was tested in three different concentrations. The first part of the experiment (in vitro) focused on determining the effect of essential oil on mycelia growth of C. musae and it was carried out by using food poison method. The treatments consisted of essential oils of basil (0.10%, 0.15% and 0.20%), cinnamon (0.025%, 0.05% and 0.075%) and rosemary (0.20%, 0.25% and 0.30%) on potato dextrose agar and incubated at 25^{o}c. In the second part (in vivo) fruits were infected artificially by C. musae spore, and then banana hands were sprayed with emulsions of the essential oils of 0.10%, 0.15% and 0.20% Ocimum basilicum, 0.20%, 0.25% and 0.30% Rosmarinus officinalis and 0.025%, 0.05% and 0.075% Cinnamomum zeylanicum and then stored at room temperature. The experimental design for this particular phase was two-factor factorial with the two factors being banana variety (Giant Cavendish and Williams) and the essential oils with different concentrations (basil at 0.10%, 0.15% and 0.20%, cinnamon0.025%, 0.05% and 0.075% and rosemary at 0.20%, 0.25% and 0.30%) and with three replications. Response measurements collected were mycelial growth inhibition (%), physiological weight loss (%), pulp to peel ratio (g), firmness, total soluble solid (°Brix), titratable acidity(%),total soluble to titratable acidity ratio, dry matter (%),pH , disease incidence (%) and percent disease index (%). A significant ($P < 0.01$) inhibition of mycelial growth of C. musae was observed in all treatments of essential oil as compared to the control after 7 days of incubation at 25^{o}c. All essential oils used for this experiment had significant ($p<0.05\%$) effects on both disease incidence and percent disease index but had no significant effect on most of physico- chemical characteristics evaluated. From all essential oils used, basil at 0.20% concentration showed the minimum percent disease index (26.67%) compared with the other treatments 19 days after storage. It can thus be concluded that among the essential oils used, basil oil proved to be effective during in-vivo test; applied at 0.20% (v/v) reduced the development of C. musae growth, maintained the freshness of the banana fruits and increased shelf life of Giant Cavendish and Williams banana fruits for up to 19 days without affecting the physico-chemical properties. Thus, further research efforts are needed to integrate the current findings to be used in integrated disease management methods in the near future.

1. INTRODUCTION

Banana is one of the most widely grown tropical fruits, cultivated over 130 countries, along the tropics and subtropics of Capricorn. Edible bananas are derived from *Australimusa* and *Eumusa* series, which have different origins from same genus (Mohapatra *et al.*, 2010). It is mainly composed of soluble sugars, starch and other polysaccharides. It was reported that its world's production exceeded 91 million tones (FAO, 2012). Banana is the second largest produced fruit after citrus, contributing about 16% of the world's total fruit production. India is largest producer of banana, contributing to 27% of world's banana production (FAO, 2009). In Ethiopia it is a widely produced crop both in home gardens and commercial farms. About 261,059 tones of banana had been produced in Ethiopia from 39428 hectars of land, in the year 2008 (FAOSTAT, 2011). Its wide consumption is due to its sensory characteristics, leading to its demand mainly by developed countries which account for nearly 70% of world's consumption (FAO, 2012) and due to its caloric contribution of vitamins and minerals, mainly potassium (Temple *et al*., 2005).

Banana has many uses. It is highly nutritious and easily digestible than many other fruits (Mohapatra *et al.*, 2010). Bananas are an excellent, low-fat, cholesterol-free source of dietary fiber, potassium, vitamin C, vitamin B6 and manganese. The amount of potassium and fiber in bananas may help combat atherosclerosis, or the hardening of arteries that can lead to heart attack and stroke (Ellyn, 2011). The ripe fruit is pureed, candied, and preserved in various forms when not eaten fresh. Its extract is used in the manufacture of catsup, vinegar, and wine. The unripe fruit is powdered and chipped. Banana serves as an ideal and low cost food source for developing countries where most of the populations rely mostly on bananas for food, the World's Healthiest Foods.

However, banana fruit experiences a different marketing problem from that of most other fruits, because ripe bananas are perishable and even when ripened in the best possible conditions; they still require prompt distribution to retailers and consumers (Abd El-Naby, 2010). During storage, it can develop many postharvest diseases that affect the quality of the

fruit. Postharvest diseases such as anthracnose and crown rot reduced the annual yield of banana fruit. The genus *Colletotrichum* and its teleomorph *Glomerella* are considered to be major plant pathogens worldwide. They cause significant economic damage to crops in tropical, subtropical, and temperate regions (Bailey and Jeger, 1992). Anthracnose of banana is caused by the *Colletotrichum* species and is one of the most serious diseases of ripe banana. In developing world loses up to thirty-seven percent of harvested foods due to problems in storage and transportation. Everyday 1.6 million bananas are thrown in the garbage (Global Food Security, 2011).

Though quality of a produce after harvest cannot be improved, it is possible to reduce the rate of quality loss. The rate of deterioration (physiological decay) of fruit is directly related to the respiration rate (Kader *et al*., 1989). Surface treatments delay physiological decay in fruit tissues, stabilize the fruit surface and prevent degradation that affect the quality of the product (Akhtar, 2010). The application of fungicides to fruits after harvest to reduce decay has been increasingly curtailed by the development of pathogen resistance to many key fungicides, the lack of replacement fungicides, negative public perception regarding the safety of pesticides and consequent restrictions on fungicide use. Particularly during postharvest storage and handling, it is important to encourage the rapid development of alternative approaches to plant disease control. Among the various alternatives, natural plant products, including essential oils that are biodegradable and eco-friendly, are catching the attention of scientists worldwide. Such products from higher plants are bioefficacious, economical, and environmentally safe and can be ideal candidates for use as agrochemicals. Applying postharvest treatment to fruits has advantages such as it maintains quality (appearance, texture, flavor and nutritive value), protects food safety and reduces harvest and postharvest losses.

Storage of banana at room temperature favors decay, fruit weight loss, softening and off-flavor development, while storage at temperatures below 8–10 °C promotes physiological breakdown. Physical damage to the peel induced during handling and storage predisposes banana to be attacked by decay-causing pathogens (Deka *et al.,* 2006). Storability of fruit can however be improved by post harvest treatments. Treatments in the form of essential oil

treatment (Maqbool, 2010) have been developed to control postharvest decay, insect infestation and alleviate storage disorders in a wide range of fresh produce.

Essential oils are volatile, natural, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. In nature, essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reducing their appetite for such plants (Asghari, 2009). The essential oils are reported to have some fungicidal properties against certain postharvest diseases of tropical fruits and vegetables and are also safer for the environment than synthetics (Maqbot *et al*., 2010). Acetaldehyde and ethanol are two products of anaerobic respiration in fruits, they accumulate during ripening, contributing to fruit aroma and ethylene production in plant (Abd El-Naby, 2010). Therefore, using externally these volatile compounds had pronounced effect on the fruit ripening delay. And also consumer demand is increasing for fruit which has been treated non-chemically for postharvest pathogens as well as for shelf life extension (Deka *et al.,* 2006).

General objective

- To determine the efficacy of essential oils on prolonging the storage life of banana fruits without affecting its quality.

Specific objectives

- To assess the effectiveness of various types of essential oils applied at different concentrations to reduce postharvest losses and extend shelf life of banana fruits.
- To evaluate in vitro and in vivo nature of cinnamon, basil and rosemary oils against anthracnose disease causing pathogen of harvested banana fruits
- Assess impacts of essential oils on postharvest quality and shelf life of banana

2. LITERATURE REVIEW

2.1. Historical Background of Banana

Bananas are the fruit of *Musa accuminata*. *Acuminata* means long-pointed or tapering, not referring to the fruit, but to the flowers giving birth to the fruit (Peggy, 2007). The genus name *Musa* is thought to be derived from the Arabic name for the plant (*mouz*) which, in turn, may have been applied in honour of Antonius Musa (63 – 14 BC), physician to Octavius Augustus, first emperor of Rome (Hyam and Pankhurst, 1995). The name 'banana' is derived from the Arabic *banan* = finger (Boning, 2006) and was thought to be used in Guinea (West Africa) concomitant with the introduction of the fruit by the Portuguese. The name then spread to the New World. The genus *Musa* is a member of the family Musaceae, which includes at least one other genus (*Ensete*) and, depending upon the affiliations of the taxonomist, may also include the monotypic genus *Musella* (Constantine and Rossel, 2001). All genera are monocotyledons and, as such, are technically defined as 'herbs' even though some species can grow up to 15 m tall.

Bananas were originally found in South East Asia, mainly in India. They were brought west by Arab conquerors in 327 B.C. and moved from Asia Minor to Africa and finally carried to the New World by the first explorers and missionaries to the Caribbean. The mass production of bananas started in 1834 and really started exploding in the late 1880's (Anonymous, 2007). The modern day edible bananas are a mix of wild and cultivated, species and hybrids associated with *M. acuminata* and *M. balbisiana*. *M. acuminata* is the most widespread of the species in section *Musa* (Daniells *et al*., 2001) and the centre of diversity is thought to be either Malaysia or Indonesia (Horry *et al.*, 1997).

In east Africa and West Africa regions represent two main secondary centers of *Musa* diversity as a result of a long history of cultivation in these regions (De Langhe, 1995). There are approximately 60 cultivars of African Highland bananas unique to East Africa but it is not known whether these derived from traded plants (may be 2,000 years ago) or from indigenous

edible diploids (De Langhe 1995, Daniells *et al*., 2001). These Highland bananas have the AAA genotype (Karamura 1998). It is thought that plantains reached West Africa 3,000 years ago and that they may have initially been propagated for their starchy corms and/or fibres rather than for their fruit. Vegetative propagation eventually led to the evolution of fleshy, seedless fruits that were edible (De Langhe, 1995).

2.2 Importance of Banana Fruit

Bananas constitute the fourth most important global food commodity (after rice, wheat and maize) grown in more than 100 countries over a harvested area of approximately 10 million hectares, with an annual production of 88 million tons (Frison and Sharrock, 1999). The all year round fruiting habit of bananas puts the crop in a superior position in bridging the 'hunger gap' between crop harvests. It therefore contributes significantly to food and income security of people engaged in its production and trade, particularly in developing countries. In Africa they provide more than 25% of the carbohydrate requirements for over 70 million people (IITA, 1998). Eastern and Southern Africa produces over 20 million tones of bananas which accounts for 25.58% of total world output (Karamura *et al.,* 1999). The Great Lakes region covering parts of Uganda, Rwanda, Burundi, Tanzania, Kenya and DRC is the largest producer and consumer of bananas in Africa (Smale, 2006) where per capita consumption has been estimated at more than 250kg; the highest in the world (FAO, 1985).

A healthy diet consists of eating a variety of foods from 5 food groups but in the correct proportions. These include; foods containing starch, fruit and vegetables, milk and dairy food, foods containing protein, and that containing fats and sugars. Bananas fall in the fruit and vegetable group as well as the food group which mostly contain starch. Sweet dessert bananas are generally eaten raw (fruit), while cooking bananas and plantains are boiled, steamed, fried or roasted (food). A person should eat at least 5 portions of fruit and vegetables every day where one whole banana fruit is equivalent to one portion just as two tomatoes and or half cucumber**.** Bananas provide a good source of nutrients for both human and animal consumption and the nutritional values per 100 grams of edible portion are indicated in Appendix Table 1 where the same amount of grams yield up to 120 kcal of energy (ED

informatics, 2006). Compared with many snack foods, the banana provides energy primarily in the form of carbohydrate with minimal contribution to energy from fat. Any food containing carbohydrates should be the main part of our daily meals. More additional nutrients are provided in Appendix Table 2. The nutritional values indicated in the tables below vary between different cultivars, degree of ripeness and the growing conditions. In unripe bananas the carbohydrates are mostly starches. In the process of ripening the starches are converted to sugars; a fully ripe banana has only 1-2% starch (Forsyth, 1980).

Bananas have a lot of health benefits, but we are aware of only very few. Bananas reduce the risk of heart diseases and blood pressure since it is rich in potassium, which is very vital for the muscle contraction and proper functioning of the heart and nervous system. Bananas reduce depression tryptophan acids are present in bananas and these help in reducing depression and thereby improve your mood. Studies show that bananas are also helpful in reducing stress levels. Bananas are not only rich in potassium but also rich in iron; therefore, eating a banana increases iron and thereby high hemoglobin. When hemoglobin content is increased, naturally anemic conditions decreases. A survey was conducted very recently among 200 students at a school in Middlesex. They were given bananas along with their normal diet for their breakfast, break and lunch. Research proved that potassium in bananas helped to increase their mental alertness and boost brain power. Reduce itching of mosquito bites with bananas, what do you normally do when there is an itching or swelling due to mosquito bites? We go in for creams that reduce itching and swelling. Rather using these creams, use banana peels. Rub the itching or swelling area with the inside of banana peels and see for yourself how soon you get relief from mosquito bite itching or swelling (Anuradha, 2010). Bananas can be used to fight intestinal disorders like ulcers. Bananas are one of the few fruits that ulcer patients can safely consume. Bananas neutralize the acidity of gastric juices, thereby reducing ulcer irritation by coating the lining of the stomach. Not only can bananas relieve painful ulcer systems, and other intestinal disorders, they can also promote healing (Kumar, 2012).

2.3. Banana Production

Banana plants reproduce asexually by shooting suckers from a subterranean stem. The shoots have a vigorous growth and can produce a ready-for-harvest bunch in less than one year. Suckers continue to emerge from a single mat year after year, making bananas a perennial crop. In 2009, world production of bananas reached an estimated 97.3 million metric tonnes (mmt), grown on 4.9 million hectares. The 2009 crop represented an increase in production of 49 percent from the 65.1 mmt recorded in 2000 (FAO, 2003).

The top five banana-producing countries of India, the Philippines, China, Ecuador, and Brazil accounted for 61 percent India continues to be by far the largest world producer of fresh bananas; by 2009, India had produced more than 26 mmt of bananas (almost 28% of the global production). Next in line is the Philippines, with a market share of 9.3 percent, followed closely by China (9.2%), Ecuador (7.8%), and Brazil (7%). Of the top five banana-producing countries, only Ecuador and the Philippines are major exporting countries. The bulk of the production is sold to the domestic market in these countries (Edward and Fredy, 2012). The importance of bananas as a food crop in tropical areas cannot be underestimated. In Uganda, for example, annual consumption per capita was some 243 kg in 1996, and between 100 and 200 kg in Rwanda, Gabon and Cameroon. In these 4 countries, bananas account for between 12 percent and 27 percent of daily calorie intake of their populations (FAO, 2003).

Total fruit production in Ethiopia was estimated at about 320,000 tonnes (FAO, 2002). In 2003, Ethiopia exported only about 5,366 tonnes of various fruits (including banana), and earned only about Birr 213.3 million (equivalent to about USD 1.5 million) in foreign currency. Of this only about 1,300 tonnes worth Birr 2.8 million (USD 325,000) was from banana exported mainly to Djibouti (CSA, 2004). Global share of Ethiopia in banana export was only about 0.01% (FAO, 2003).

2.4. Maturity and Maturity Indices

Maturity is one of the major factors that determine the compositional quality of fruits and vegetables (Lee and Kader, 2000). The principles dictating at which stage of maturity a fruit or vegetable should be harvested are crucial to its subsequent storage and marketable life and quality. Postharvest physiologists distinguish three stages in the life span of fruits and vegetables: maturation, ripening, and senescence. Maturation is indicative of the fruit being ready for harvest. At this point, the edible part of the fruit or vegetable is fully developed in size, although it may not be ready for immediate consumption. Ripening follows or overlaps maturation, rendering the produce edible, as indicated by taste. Senescence is the last stage, characterized by natural degradation of the fruit or vegetable, as in loss of texture, flavour, etc. (senescence ends at the death of the tissue of the fruit) (FAO, 2003). Some typical maturity indexes are: harvest age, mean fruit weight, mean finger length, mean finger diameter, Pulp to peel ratio and Skin color (Ramm *et al*., 1999).

Requirements for establishing maturity indices are*:* simple, easy to carry out, objective vs. subjective indicators, related to quality, related to storage life, represents a progressive change with maturity, permits prediction of maturity from year to year and Inexpensive (Cantwell, 2009). Know the consequences of harvesting at different stages of maturity/ripeness on final eating quality. Make sure workers involved in harvest, selection are well trained to ID correct maturity/ripeness. Harvesting at the correct maturity is a key to satisfying quality expectation (Cantwell, 2009).

Traditionally, banana maturity has been judged by the amount of peel chlorophyll and its visual disappearance with ripening. Peel color charts have been developed to help standardize banana maturity ratings for industry and research purpose. High ripening temperatures and low relative humidity's often can make bananas retain chlorophyll, creating a situation in which peel color does not reflect internal changes. An internal indicator of ripeness can provide additional information on the advancement of maturity (Blankenship, 1993).

There are no universally recognized objective criteria for determining harvest time for bananas. Some commonly used indices are angularity of fingers, bunch age and grade (Robinson, 1996). However, the use of a single indicator of maturity may be applicable to one cultivar but not to other cultivars. This makes it necessary to use a combination of several indicators to determine time of harvest. Many features have been used to estimate maturity, among them caliper grade in combination with bunch age (Ramma *et al*., 1999). The indices must measure fruit characteristics and postharvest quality attributes that change consistently as the fruit develops, and correlate well to fruit development so that harvesting the fruit at particular indices enable final eating quality to be predicted (Dadzie, 1997). Banana fruits have rapid growth in the first phase of fruit development representing cell division, while rapid cell expansion occurs after this phase, followed by fruit maturation (Robinson, 1996). Fruits should be harvested at an appropriate time during maturation phase.

2.5. Harvesting

By definition, postharvest handling begins at harvest (Lee and Kader, 2000). Determination of the harvest date is based on yield, visual appearance, anticipated prices, estimated culling losses to achieve shipping quality and field conditions. Harvesting is accomplished by hand, by mechanically assisted picking devices, or by mechanical harvesters (Prussia and Woodroof, 1986). Factors during harvesting operations that can influence postharvest quality include the degree of severity of mechanical damage induced by machine or human, the accuracy of selecting acceptable and unacceptable fruit, the time of day of harvest and the pulp temperature at harvest (Prussia and Woodroof, 1986).

Bananas are harvested raw and ripened artificially. The dwarf bananas are ready for harvest within 11- 14 months after planting, while tall cultivars take about 14-16 months to harvest. The mature fruit becomes pulpy and all the angles are filled in completely. When tapped the fruit gives metallic sound. The method of harvesting depends on the height of the plant. Low growing varieties are harvested by cutting through the bunch stalk about 30-35 cm above the top hand. With taller varieties, the stem of the plant will be partly cut through to bring the

bunch down within the harvester's reach (Anonymous, 2010). The effects of harvesting time according to Chillet (2010) stated that in both lowland and highland zones, harvesting bananas at an earlier physiological age (700 DD instead of 900 DD) reduced the susceptibility of bananas to *C. musae* and significantly increased their green life.

2.6. Ripening

During the growth and development period of bananas, there are many chemical and physical changes that occur. These have an impact on the fruit quality after harvesting. Normally, ripening is the final stage in fruit maturation. During ripening, the fruit changes colour, flavour, texture and aroma to optimal eating sensorial and textural properties. The agent that triggers these changes during maturation of bananas is a chemical called ethylene. Ethylene is a gas naturally produced by plants, for example to trigger leaves to turn yellow and fall off during certain seasons like winter (Joshua, 2010). Among other things, ethylene stimulates the formation of amylase, an enzyme that breaks down starch into sugar, influencing the taste of bananas. The greener, less ripe bananas contain higher levels of starch and, consequently, have a "starchier" taste. On the other hand, yellow bananas taste sweeter due to higher sugar concentrations. Furthermore, ethylene signals the production of pectinase, an enzyme which breaks down the pectin between the cells of the banana, causing the banana to soften as it ripens (Anonymous, 2012).

Fruit ripening is the result of a complex of changes, many of them probably occurring independently of each other. The following are some major changes that occur in most banana, cooking banana and plantain during ripening: Peel and pulp colour changes, Conversion of starch into sugar, Changes in pulp to peel ratio (and ease of peeling), Changes in pulp firmness or pulp softening, Changes in total soluble solids content, Changes in pulp pH and total titratable acidity, Changes in peel and pulp moisture and dry matter content, Changes in respiration rate and ethylene production (Dadzie, 1997).

During ripening, most fruits undergo many physical and chemical changes after harvest that determines the quality of the fruit purchased by the consumer. Fruit ripening quality is an

important postharvest selection criterion, hence new banana, cooking banana and plantain hybrids being screened for their ripening quality. The ripening quality of new Musa hybrids should be consistent with the parents from whom they were developed (Dadzie, 1997).

2.7. Causes of Postharvest Losses of Banana

The most common causes of postharvest losses in developing countries include rough handling and inadequate cooling and temperature maintenance. The lack of sorting to eliminate defects before storage and the use of inadequate packaging materials further add to the problem. In general, minimizing rough handling, sorting to remove damaged and diseased produce and effective temperature management will help considerably toward maintaining a quality product and reducing storage losses. If the temperature during the postharvest period is kept as close to the optimum as feasible for a given commodity, storage life will be enhanced (FAO, 1995).

2.7.1. Physiological reaction

Losses in fruit quality are mostly due to its relatively high metabolism activity during storage (Asghari *et al.,* 2009). During normal ripening, the peel loses water to both the atmosphere and the pulp leading to higher weight loss, desiccation of the fruit and uneven ripening. Bananas ripened naturally lost between 9.0-9.5% of fresh weight, similar to the 8.3% loss reported from Embul banana naturally ripened (Sarananda *et al.,* 2000). During postharvest handling and storage, fresh fruits and vegetables lose moisture through their skins via the transpiration process. Commodity deterioration, such as shriveling or impaired flavor, may result if moisture loss is high. In order to minimize losses due to transpiration, and thereby increase both market quality and shelf life, commodities must be stored in a low temperature, high humidity environment.

In addition to proper storage conditions, various skin coatings and moisture-proof films can be used during commodity packaging to significantly reduce transpiration and extend storage

life (Ben-Yehoshua, 1969). Metabolic activity in fresh fruits and vegetables continues for a short period after harvest. The energy required to sustain this activity comes from the respiration process (Mannapperuma, 1991). Respiration involves the oxidation of sugars to produce carbon dioxide, water and heat. The storage life of a commodity is influenced by its respiratory activity. By storing a commodity at low temperature, respiration is reduced and senescence is delayed, thus extending storage life. Proper control of the oxygen and carbon dioxide concentrations surrounding a commodity is also effective in reducing the rate of respiration. Generally, harvested fruits and vegetables are exposed to high respiration and transpiration rates and subsequently high weight and quality losses (Mohamed, 1997).

2.7.1.1. Transpiration

Transpiration is one of the two major physiological processes that are carried out after fruits and vegetables are harvested. Together with respiration, excessive rates of these two processes lead to physical deterioration of fruit of fruits and vegetable after harvesting. Transpiration, or evaporation of water from the plant tissues, is one of the major causes of deterioration in fresh horticultural crops after harvest. Water loss through transpiration not only results in direct quantitative losses (loss of saleable weight), but also causes losses in appearance (wilting, shriveling), textural quality (softening, flaccidity, limpness, loss of crispness and juiciness), and nutritional quality. Transpiration can be controlled either through the direct application of postharvest treatments to the produce (surface coatings and other moisture barriers) or through manipulation of the environment (maintenance of high relative humidity) (FAO, 2003).

2.7.1.2. Respiration

Despite having been detached from the plant, fruits and vegetables remain as living organs after harvest. Like all living tissues, harvested produce continues to respire throughout its postharvest life. During the process of respiration, carbohydrates are broken down to their constituent parts to produce energy to run cellular processes, thus keeping the cells and

organism alive. Throughout this process, oxygen is consumed and water, carbon dioxide, and energy are released. Because this process occurs from harvest to table, the carbohydrates stored in the harvested plant portion are continually "burned" as energy to keep the vegetable alive; as respiration continues, compounds that affect plant flavor, sweetness, weight, turgor (water content), and nutritional value are lost. Thus, reducing the rate of respiration is an important consideration in extending the postharvest life of a fruit or vegetable and optimizing postharvest quality.

Harvested fruits and vegetables of different plants have different rates of respiration; some respire at a faster rate (and thus are more perishable vegetables), while some respire at a relatively slow rate (less perishable vegetables). In addition, storage conditions affect respiration, with higher temperatures leading to a faster rate of respiration; for every 10°C rise in temperature, the respiration rate will double or even triple. Because of the significant effect of temperature on respiration, the amount of time a harvested product is exposed to heat should be minimized; the fruit or vegetable should be quickly brought to its optimal storage temperature (Silva, 2008). In the case of the banana, fruit with a climacteric pattern, it was found by Kidd and West that storage in 2.5 and 5.0 % oxygen did not materially decrease the rate of ripening. On the other hand, a reduction in CO, liberation by fruit stored in oxygen concentrations lower than air and no effect in concentrations higher than air (Young, 1962).

2.7.2. Ethylene

Ethylene is a colorless gas that is naturally produced by plants and functions as a plant growth regulator. In this way, ethylene behaves in the same way as hormones in mammals. It triggers specific events during a plant's natural course of growth and development, such as ripening. The presence of ethylene is not always beneficial, especially in terms of postharvest shelf life. The effect of ethylene is accumulative so continuous exposure to a low concentration of ethylene throughout marketing can cause significant harm (Wills *et al*., 2000). According to Mohson *et al.* (2006), effect of ethylene treatment on quality of banana fruit, ethylene treated bananas ripened earlier than un-treated bananas. The negative impact of ethylene is that it

increases pathogen susceptibility, physiological disorders and senescence, with a net reduction in postharvest life (Martinez-Rmero *et al.*, 2007).

Fruits and vegetables may be classified depending on their response to ethylene. Climacteric species produce ethylene as they ripen, and the harvested produce is capable of ripening during the postharvest period. These commodities, such as bananas, apples, and peaches, tend to get sweeter and softer after harvest. Non-climacteric plants, such as leafy vegetables, do not continue to ripen after harvest; they will soften and rot, but this is due to moisture loss, decay, and tissue deterioration (Silva, 2008).

In addition to being naturally produced by plants, ethylene is produced by a variety of other sources. These include internal combustion engines, cigarette smoke, and natural gas leaks. Even low concentrations of ethylene throughout the postharvest life of a commodity can affect quality, so care must be taken to minimize exposure from both natural sources (i.e. climacteric fruit or veggies being stored with non-climacteric ones) or to artificial sources (engine exhaust, heaters, etc). All ethylene-producing sources should be considered when optimizing postharvest storage conditions as inadvertent exposure to ethylene can contribute to loss of quality in some fruits and vegetables.

2.7.3. Mechanical injury

Mechanical losses to the fruits are caused by careless handling during harvesting, packing, transportation, storage etc. Mechanical injuries may be defined as plastic deformations, superficial ruptures and destruction of vegetal tissues led by external factors. Such injuries lead to physical modifications (physical damages) and/or physiological, chemical and biochemical alterations that alter color, aroma, flavor and texture of vegetables (Mohsenin, 1986). Injuries can be classified as compression, impact or cut. Impact is generally caused by the collision of the fruit against solid surfaces or against other fruits during harvest, handling and transport. Compression injuries are caused by a variable pressure on the fruit surface exerted by an adjacent fruit or by the container holding the fruits. Cut injuries are generally

due to the collision of the fruit against a sharp surface that ruptures the epidermis, or due to the pressure exerted by uneven surfaces, such as the container edges, against the fruit (Chitarra and Chitarra, 2005).

Mechanical injuries are among the main factors affecting postharvest losses in bananas. Different injuries may cause different effects on agricultural products, mainly changes in color and appearance, fast ripening (due to increased respiratory rate and ethylene production), increase in loss of water and in deterioration by microorganisms, thus, directly affecting fruit quality and retail prices (Dadzie and Orchad, 1997). Mishandling, vibration, impacts, compression and/or superficial bruises are the basic causes of banana fruit injuries leading to fruit deterioration and favoring the development of diseases (Cortez *et al.,* 2002).

2.7.4. Chilling injury

Chilling injury is primarily a disorder of crops of tropical and subtropical origin, although certain physiological disorders will appear in temperate crops only when they are stored at low temperatures. Chilling injury is not the same as freezing injury, which is a result of damage from ice crystals formed in tissues stored below their freezing point. The minimum safe temperature for chilling sensitive commodities will be well above their freezing point. The critical temperature for chilling injury varies with the commodity, but it generally occurs when produce is stored at temperatures below 10°–13°C. Therefore, crops which are susceptible to chilling injury often have a short storage life as low temperatures cannot be used to slow deterioration and pathogen growth. Chilling injury may occur in the field, in transit or distribution, in retail or home refrigerators. The effects of short periods of chilling may be cumulative in some commodities (Jiang *et al.,* 2004).

The primary cause of chilling injury is thought to be damage to plant cell membranes. The membrane damage sets off a cascade of secondary reactions, which may include ethylene production, increased respiration, reduced photosynthesis, interference with energy production, accumulation of toxic compounds such as ethanol and acetaldehyde and altered cellular structure. The symptoms include retarded development of yellow skin color and

failure of the fruit to soften. The reason of chilling injury of banana fruit stored at low temperature is due to reduced ability of tissue to respond to ethylene (Jiang *et al.*, 2004). The main mechanisms suggested for the prevention of chilling and heat injury are the synthesis of small heat shock proteins, changes in the saturation of fatty acids of the membranes and suppression of the ethylene production and respiration rates (De Villiers, 2008).

2.7.4. Postharvest rots of banana

The two primary postharvest rots of banana (*Musa* spp.) fruits are crown rot and anthracnose. The diseases usually appear on ripening fruits either at points of sale (farmers' markets, grocery stores) or later, after purchase. Occurrence of these two diseases is closely linked to poor cultural and disease management practices in the banana field, to unclean packinghouses, and to improper postharvest handling. The diseases can be serious problems for growers who fail to manage them with a combination of integrated practices. Infected fruits are safe for humans to consume; however, the infections reduce fruit quality, shelf life, and marketability (Nelson, 2008).

The fungus *Colletotrichum musae* can cause both crown rot and anthracnose; in addition, crown rot diseases may also be caused by fungal pathogens in the genera *Fusarium*, *Acremonium*, *Verticillium*, and *Curvularia*. These pathogens are found wherever bananas are grown but are more prevalent in high-rainfall areas, and especially where growers do not follow good field and packinghouse sanitation practices.

To manage this rotting problem some growers apply copper fungicide spray to banana fruits after deflowering fingers and before bagging (Preharvest fungicides). On other hand, some banana growers sometimes use dips or sprays of fungicides such as thiabendazole to deter crown rot and anthracnose disease development (postharvest fungicide).

2.7.4.1. Causal agent and symptom of banana anthracnose

Anthracnose disease caused by *Colletotrichum musae* is considered of the most important worldwide diseases of banana fruits, and it is particularly associated with losses due to scratches and wounds caused by handling and transport processes. Since the disease can infect ripe banana fruits, more losses can also occur at market. Banana fruits can be infected by the fungus in the field at any time within growing season (Simmonds and Mitchell, 1940). Banana anthracnose often begins as an invisible disease on green fruits in the field. As the fruits ripen, the accumulation of phytoalexins restricts the successful penetration of the fungus (Turner, 1995). The shipping of banana fruits as bunches and with subsequent ripening at high temperature, may magnify the anthracnose problem (Meredith, 1960). Fungus can invade green finger necks when damaged by flexing.

Symptoms of anthracnose include black and sunken lesions with spore masses or acervuli in the lesion. Infection on the banana usually starts during the development of the fruit but remains quiescent until the fruit ripens; symptoms often manifest during storage and marketing (Prusky and Plumbley, 1992). Anthracnose becomes severe when the banana fruits are wounded by scratches during handling and transportation, making the fruit unmarketable.

In the case of post harvest anthracnose, developing fruit are infected in the field, but infection remains quiescent until the onset of repining, which occur after harvest. Once the climacteric period of the fruit starts, lesions begin to develop. There is usually no fruit-to-fruit infection; hence post harvest anthracnose is considered a monocyclic disease (Appendix Figure 1) (Aruz, 2000).

Anthracnose is a postharvest disease that develops during the storage and ripening of the banana. *C. musae* is the most common species associated with anthracnose of banana and has been widely accepted as the causal agent of the disease (Meredith 1960). Besides causing anthracnose on banana fruits, *C. musae* can also infect the bracts, flowers, petioles and leaves of banana plants (Jones and Slabaugh, 1998). Anthony *et al*. (2004) reported that *C. musae*

was isolated from anthracnose lesions of three banana cultivars and was also associated with crown rot disease of banana in Sri Lanka.

2.7.4.2. Economic importance of banana anthracnose

Losses due to postharvest disease may occur at any time during postharvest handling, from harvest to consumption. When estimating postharvest disease losses, it is important to consider reductions in fruit quantity and quality, as some diseases may not render produce unsaleable yet still reduce product value. For example, blemished fruit may not be sold as fresh fruit but may still be suitable for processing, in which case, it brings a lower price. It is also important to take into account costs such as harvesting, packaging and transport when determining the value of produce lost as a result of postharvest wastage (http://www.appsnet.org).

Postharvest diseases are often classified according to how infection is initiated. The so-called 'quiescent' or 'latent' infections are those where the pathogen initiates infection of the host at some point in time (usually before harvest), but then enters a period of inactivity or dormancy until the physiological status of the host tissue changes in such a way that infection can proceed. The dramatic physiological changes which occur during fruit ripening are often the trigger for reactivation of quiescent infections. Examples of postharvest diseases arising from quiescent infections include anthracnose of banana fruit caused by *Colletotrichum* spp. It deteriorates the quality and nutritive value of the fruits and renders them unfit for marketing and consumption, thereby causing severe loss to farmers and traders (Prema *et al.*, 2011).

2.8. Treatments to Reduce Postharvest Losses of Banana

Reduction of postharvest losses can increase food availability to the growing world population, decrease the area needed for production, and conserve natural resources. Strategies for loss prevention include (Kader and Rolle, 2004): (1) use of genotypes that have longer postharvest life; (2) use of integrated crop management systems and Good Agricultural

Practices that result in good keeping quality of the commodity; and (3) use of proper postharvest handling practices in order to maintain the quality and safety of fresh produce.

In some instances, it is desirable to maintain the fruits in the unripe state to regulate their marketing life, such treatments currently used to prevent decay or preserve texture and color, can compromise aroma quality. Chemical treatment is necessary for precooled fruits because it can reduce the incidence of physiological disease and improve the storage performance. Many kinds of chemical solutions are used for immersion-cleaning, including calcium chloride solution, ethoxyquin solution, thiabendazole solution and others. The use of a combination of such techniques in the postharvest handling of fresh products is increasing.

Fresh produce is at peak quality when picked; its quality can only be maintained (or deteriorated) as it is handled and stored. Maintaining crop quality after harvest is an important consideration for any fresh market produce grower or handler (Silva, 2008). There are different ways to maintain the quality of fresh produce such as postharvest treatments. According to Abd El- Naby (2010), investigation on effect of postharvest treatment with ether or hot water, mandarin volatile oil, calcium chloride or potassium permanganate on enhancing or delaying of the ripening and quality aspect of mature Maghrabi banana fruit shows that; untreated and treated banana fruit had a normal ripening process and similar good freshness at the ripening time. Color development of peel, easy peeling condition, loss of firmness and increasing pulp to peel ratio and total soluble solids/titrable acidity ratio.

2.8.1. Essential oil

Spices, herbs, and their derivatives such as essential oil and oleoresin are used in foods for their flavors and aroma. There has been considerable emphasis on studies involving essential oils and extracts of spices and their constituents for inhibiting the growth of microbes (Ozcan *et al.,* 2006). Essential oils and their constituents have a long history of applications as antimicrobial agents. But starting few years ago, in different parts of the world, attention has

been paid to exploiting plant products as novel chemotherapeutant and preservatives in plant protection and food storage (Özcan and Erkmen, 2001).

Essential oils are typically volatile substances produced by many plant species, however, when they was absorbed into packaging paper, used to protect fruits from water loss and pathogens. The essential oils extracted from tender stems, leaves and flowering tops are used in cosmetics, perfumeries and toiletries and for flavouring liquors, soft drinks, beverages and pharmaceutical preparations (Shylaja and Peter, 2004). The essential oils of thyme, cinnamon, anise and spearmint have more effect on fungal development and subsequent mycotoxin production in wheat grains. The extent of inhibition of fungal growth and mycotoxin production was dependent on the concentration of essential oils used (Soliman and Badeaa, 2002).

In some studies, it is reported that the essential oils may affect the metabolic pathways of microorganisms. Nychas (1995) found that phenolic compounds in low concentration disrupt proteins and in high concentrations damaged the enzymes outbreak in production of energy. Generally according to many researchers, essential oil treatment had less adverse effect on quality parameters of pear fruits; essential oil treatment increased the taste of treated fruits. Similarly Maskouki and Mortazavi (2004) reported that *Carum copticum* oil increased the taste of pear fruits in comparison with controls. Essential oils and their constituents have often been used as biological control agents because of their therapeutic activity and their toxicity to fungi, bacteria and insects. The oils of herbs such thyme (Thymus), marjoram (Origanum), and basil (Ocimum) contain compounds, such as cinnamaldehyde, acetaldehyde and eugenol, which are generally regarded as safe (GRAS). Some have been tested on fruits such as mandarin, kiwi, and rambutan to control postharvest diseases caused by fungi (Thanassoulopou- los and Yanna, 1997).

2.8.1.1. Basil

The genus *Ocimum*, (Lamiaceae formerly Labiatae), collectively called basil (*Ocimum basilicum* L.) has long been recognized as a diverse and rich source of essential oils. *Ocimum*

contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia, Africa, and Central and South America (Simon *et al.*, 1990). Cultivated as a culinary herb, condiment or spice; source of essential oil for use in foods, flavors, and fragrances; garden ornamental. The green aromatic leaves are used fresh and dried as flavorings or spices in sauces, stews, salad dressings, vegetables, poultry, vinegar, confectionery products, and the liqueur chartreuse. Basil extract has been reported to have antioxidant activity. Cultivars with purple foliage, such as 'Dark Opal' and var. *auranascens*, are grown as ornamentals, but can also be used as flavorings. The essential oils and oleoresin are used extensively, reducing the need for dried leaves in the food industry; used in perfumes, soaps, and shampoos (James, 1995).

Traditionally, basil has been used as a medicinal plant in treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions. It is also thought to be an antispasmodic, stomachache, carminative, stimulant and insect repellent. The oils of basil, especially the camphor-containing oil, have antibacterial properties. Volatile compounds produced by sweet basil have been shown to influence the composition, distribution, and spore germination of some fungal populations. The volatile terpenes camphor and 1, 8-cineole present in basil and other members of the Lamiaceae have been suggested as agents in allelopathic reactions. Sweet basil is generally recognized as safe as a spice/natural flavoring and as a plant/oil extract.

The essential oils of basil extracted via steam distillation from the leaves and flavoring tops are used to flavor foods, dental and oral products, in fragrances, and in traditional rituals and medicines. Extracted essential oils have also been shown to contain biologically-active constituents that are insecticidal, which have antimicrobial properties (Ntezurubanza *et al.*, 1984). These properties can frequently be attributed to predominant essential oil constituents, such as methyl chavicol, eugenol linalool, camphor, and methyl cinnamate. According to many researchers report the major compounds found in essential oils of basil was linalool. the result of the experiment done by Abdolahi *et al.* (2010) shows that essential oil treatment and different concentrations of essential oils had a good antifungal effect on treated grape clusters,

but counter-effects (reciprocal effects) of different type and concentrations of essential oils were not significant. With increasing of essential oil concentrations, the antifungal activities of essential oils were increased.

2.8.1.2. Rosemary

Rosemary plant with scientific name of *Rosmarinus officinalis* L. is of Lamiaceaa (Labiatae) family. In traditional medicine, rosemary is used to treat different diseases including depression, insomniac, gout and arthritic pains (Zargari, 1995). With due attention to their anti-oxidant and anti-bacterial effects and that give flavor to meat, fish and chicken they are used to keep the quality of fasts and meats. Pharmacopeial grade dried rosemary leaf contains at least 1.2% essential oil. Rosemary is a fragrant and bushy evergreen shrub with linear aromatic leaves, native to the Mediterranean basin and Portugal, now cultivated in England, France, Spain, Portugal, Morocco, South Africa, India, China, Australia, the United States, and along the Crimean peninsula in Transcaucasia. Rosemary is often grown as a garden ornamental. A common spice, rosemary is used to flavor meat, soups and stews (Wichtl, 1994). Rosemary has a long tradition of use as a tonic, stimulant, and to relieve headaches, head colds, nervous tension and indigestion. The herb is used to relax spasms, relieve pain, stimulate the liver, and improve digestion.

Rosemary contains volatile oils, flavonoids and phenolic acids. Some notable constituents include camphor, cineole, alpha pinene and borneol. The powerful antioxidant proerties of rosemary make the herb and its preparations valuable for food preservation. Phytochemicals in rosemary demonstrate hundreds of biological activities, including antioxidant, anti-inflammatory, antifungal, antibacterial, antispasmodic, antiviral, anticancer, antitumor against various tumors, anesthetic, analgesic, cancer-preventive and liver protective properties (Bown, 1995). The essential oil of rosemary plant has been studied in Iran and in the world. The chemical compounds, genetic differences, antimicrobial and antifungal impacts of rosemary plant have been studied (Angioni *et al.,* 2004). The use of plants is as old as the mankind. Natural products are cheap and claimed to be safe. They are also suitable raw material for production of new synthetic agents. The most important constituents of rosemary

are caffeic acid and its derivatives such as rosmarinic acid. These compounds have antioxidant effect. The phenolic compound, rosmarinic acid, obtains one of its phenolic rings from phenylalanine via caffeic acid and the other from tyrosine via dihydroxyphenyl-lactic acid (Moghtader *et al.,* 2011).

2.8.1.3. Cinnamon

Cinnamon (*Cinnamomum verum*, synonym *C. zeylanicum*) is a small evergreen tree, 10-15 meters tall, belonging to the family Lauraceae. The flowers, which are arranged in panicles, have a greenish colour and have a distinct odour. The fruit is a purple one-centimeter berry containing a single seed. Its flavour is due to an aromatic essential oil which makes up 0.5 to 1% of its composition (Vaibhavi *et al.,* 2010). The Cinnamon is having essential oils, resinous compounds, Cinnamic acid, Cinnamaldehyde and Cinnamate. Essential oil such as trans-cinnamaldehyde, caryophyllene oxide, L-borneol, L-bornyl acetate, eugenol, b-caryophyllene, E-nerolidol, and cinnamyl acetate was reported by Tung et al. Some other constituents are Terpinolene, α-Terpineol, α-Cubebene, and α-Thujene (Tung *et al.,* 2008).

In medicine it acts like other volatile oils and once had a reputation as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system. Cinnamon is high in antioxidant activity. The essential oil of Cinnamon also has antimicrobial properties, which aid in the preservation of certain foods. "Cinnamon" has been reported to have remarkable pharmacological effects in the treatment of type II diabetes. Cinnamon has traditionally been used to treat toothache and fight bad breath and its regular use is believed to stave off common cold and aid digestion (Vaibhavi *et al.,* 2010).

Cinnamon is an ancient spice mentioned several times in the Old Testament. It is a bushy evergreen tree of the Laurel family. Native to Sri Lanka, India, and Burma, Cinnamon is also cultivated in South America and the West Indies for the spice consisting of its dried inner bark. Cinnamon is used as a spice, for incense, and in aromatherapy. The oil is distilled from bark fragments for use in food, medicine, liqueur, and perfumery. The cinnamon oil is

commonly used in cooking as a flavoring agent and is also a safe preservative (Ranasinghe *et al*., 2003). Cinnamon extracts have fungistatic and fungicidal activity against the anthracnose and crown rot pathogens and spraying them on banana prior to storage, controlled crown rot and extended shelf life (Ranasinghe *et al.,* 2003). According to Win *et al*. (2007) experiments, all treated (5 g/l cinnamon essential oil) banana fruit were stored at 13 ◦C for 7 weeks. For the other experiment, fruit were stored for 1 month at 13 ◦C and removed to room temperature for 7 days as a measure of shelf-life.

2.8.2. Mode of action of essential oils

Although some studies have reported on the antimicrobial activity of essential oils, the mechanism(s) of action of such oils is poorly understood. However, some researchers reported that there is a relationship between the chemical structure of the most abundant compounds in the essential oils and the antimicrobial activity. According to Faid *et al.* (1996), antimicrobial activity of major oil compounds is in the order: phenols (highest activity) > alcohols > aldehydes > ketones > ethers > hydrocarbons. In general, the mode of action of essential oils is concentration dependent (Prindle and Wright, 1977). Low concentrations inhibit enzymes associated with energy production while higher amounts may precipitate proteins. However, it is uncertain whether membrane damage is quantitatively related to the amount of active antimicrobial compound to which the cell is exposed, or the effect is such that, once small injuries are caused, the breakdown of the cell follows (Judis, 1963). Essential oils damage the structural and functional properties of membranes and this is reflected in the dissipation of the two components of the proton motive force: the pH gradient (ΔpH) and the electrical potential (Δψ) (Ultee *et al*., 2002).

It is known that the cell wall of pathogens is the main target of phenolic compounds and these compounds may disrupt the permeability barrier of cell membrane and inhibit respiration. Hydrophobic nature of essential oils and their components enables these compounds to penetrate lipid of fungal cell membrane and mitochondria as a result disturbing their structure (Cox *et al*., 2000) and these compounds accumulate in the cell membrane of pathogen causing energy deletion (Conner, 1993). In addition, in some studies, it is reported that the essential

oils may affect the metabolic pathways of microorganisms. Nychas (1995) found that phenolic compounds in low concentration disrupt proteins and in high concentrations damaged the enzymes outbreak in production of energy.

Farrag *et al*. (1989) described that the antimicrobial activity of essential oils could be related to the presence of an aromatic nucleus and OH group that can affect hydrogen bonds of enzymes in microorganisms. According to these observations, it is expected that high antifungal activity of cinnamon, basil and rosemary oil could be related to their phenolic or alcoholic components (eugenol, linalool and α-pinene) respectively, and/or could be related to synergism of their minor and major components. Also the antifungal activity of essential oils is attributed to the type of essential oil and chemical composition of essential oil, the method of assay as an antifungal activity of essential oils under in vitro were higher than in vivo conditions and susceptibility of fungi to essential oils as *P. expansum* were highly sensitive to essential oils in comparison to *B. cinerea*.

Sharma and Tripathi (2007) showed that *Citrus sinensis* essential oil caused bifurcation of apical hyphae and profuse budding in vegetative hyphae leading to complete loss of cytoplasm from the hyphae. In spite of that, they reported that the mode of activity of the *C. sinensis* essential oil is a result of attack of oil on the cell wall and reaction of cytoplasm in the hyphae and ultimately death of the mycelium. Such modifications induced by essential oil may be related to the interference of essential oil components with enzymatic reactions of wall synthesis, which affects fungal morphogenesis and growth. The antifungal activity of essential oils may be due to synergism between their components. This synergism would be beneficial in postharvest protection of fruits and vegetables because the pathogen would not easily produce resistant races against the components (Edris and Farrag, 2003).

3. MATERIALS AND METHODS

The present study was undertaken at Jimma University, College of Agriculture and Veterinary Medicine, in laboratory of Postharvest Management and Plant Pathology. The details of materials used and the methodology followed during the course of the present research were described in the subsequent pages.

3.1. Description of the Study Area

The study was conducted in Jimma University College of Agriculture and Veterinary Medicine, (JUCAVM) located at 356 km Southwest of Addis Ababa at about 7^0 33' N latitude and 36^0 57' E longitude and altitude of 1710 meter above sea level (m.a.s.l). The mean maximum and minimum temperature are 26.8°C and 11.4°C, respectively and the mean maximum and minimum relative humidity is 91.4% and 39.92%, respectively (BPEDORS, 2000). At the time of investigation the average temperature and relative humidity of the laboratory was 24 $\pm$ 0.5 and 59 $\pm$ 0.5, respectively.

3.2. Experimental Materials

Plant material: Fresh banana fruits of two varieties namely Giant Cavendish and Williams at month of March were obtained from the Jimma Agricultural Research Center, South West Ethiopia and fruits were selected for uniformity in size, appearance, ripeness and the absence of physical defects. Three spice plants were selected for extraction of essential oils. Botanical samples used for the experiment (Cinnamon and rosemary leaf) were collected from Jimma University College of Agriculture and Veterinary Medicine, horticulture garden and areal part of basil were collected from farmers around Jimma.

Table 1: Plants used for essential oil extraction

Name	Family	Plant part used
Ocimum basilicum L.	Lamiaceaa	Leaf and flower
Rosmarinus officinalis L.	Lamiaceaa	Leaf
Cinnamomum zeylanicum	*Lauraceae*	Leaf

3.3. Experimental Design and Layout

Experiment one (Determination of *in vitro* mycelia growth of *C. musae*)

The first part of the experiment was designed using completely randomized design with three replications. The experiment had one factor which was essential oils with 10 different concentration levels (0%(no E.O treatment), basil oil 0.1%, 0.15% and 0.2%, Cinnamon oil 0.025%, 0.05% and 0.075% and rosemary oil 0.2%, 0.25% and 0.3%).

Model

$$Yij = \mu + T_i + E_{ij}$$

Where

Y i represents measurement for all the i^{th} observation (mycelia growth), for which X_1

μ = represents overall mean effect,

T= represents is the effect treatment factor (i = 1)

E_{ij} = the error associated with replication 3 of the factor combination i

Experiment two (Determination of in vivo antifungal activities of essential oils against anthracnose and their effect on quality of banana fruit)

The experiment was designed using completely randomized design (CRD) with factorial combination and three replications. The experiment had two factors which are: factor one was banana varieties (Two 2 levels), factor two was essential oils with different concentration (10 levels). Banana varieties are Giant Cavendish and Williams and the 10 levels of the essential oil are 0%, basil oil 0.1%, 0.15% and 0.2%, Cinnamon oil 0.025%, 0.05% and 0.075% and rosemary oil 0.2%, 0.25% and 0.3%. Therefore there were 2x10 treatment combinations and total number of treatment combinations was 20 and the experiment had three replications hence the total experimental units were 60 (20x3). All banana cartons were arranged according to a complete randomized design (CRD) with three replicates.

Model

$\mu_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$

Where

μ_{ij} represents measurement for all the ij^{th} observation (overall quality of banana),

μ represents overall mean effect,

A_i represents the main effect of i^{th} level of banana variety, $i = 1,...,a$,

B_j represents the main effect of j^{th} of type essential oil, $j= 1,...,b$,

$(AB)_{ij}$ represents the interaction of factor A level i with factor B level j.,

e_{ijk} is the error associated with replication 3 of the factor combination ij

Table 2: Details of treatment combination

Varity of banana	Oil type with different conc.	Treatment combination
V_1	B C_1	V_1B C_1
	B C_2	V_1 B C_2
	B C_3	V_1 B C_3
	R C_1	V_1 R C_1
	R C_2	V_1 R C_2
	R C_3	V_1 R C_3
	C C_1	V_1 C C_1
	C C_2	V_1 C C_2
	C C_3	V_1 C C_3
	0 C_4	$V_1$0C_4
V_2	B C_1	V_2 BC_1
	B C_2	V_2 B C_2
	B C_3	V_2 B C_3
	R C_1	V_2 R C_1
	R C_2	V_2 RC_2
	R C_3	V_2 R C_3
	C C_1	V_2 C C_1
	C C_2	V_2 C C_2
	C C_3	V_2 C C_3
	0 C_4	$V_2$0C_4

Whereas; V_1 (Variety1) = Giant Cavendish, V_2 (variety2) = Williams
B = basil, R = rosemary and C = Cinnamon, $C_{1, 2, 3,4}$ = Concentration
B C_1 = 0.1%, B C_2 = 0.15%, B C_3= 0.2%
C C_1=0.025%, C C_2= 0.05% and C C_3 =0.075%
R C_1=0.2%, R C_2=0.25% and R C_3= 0.3%
V_1C_4 = control treatment (0%)

3.4. Experimental Procedures

3.4.1. Extraction of essential oils

In this study, the essential oils were extracted in Wondo Genet Agricultural Research Center and Chemistry Department laboratory of Jimma University. After drying the plant materials (basil, rosemary and cinnamon*)* in shade, 2kg of each dried samples were extracted by hydro distillation method using a Clevenger-type apparatus. The oils were separated, dried over anhydrous sodium sulfate and kept in airtight sealed dark glass at 4^{o}C until used (Abdolahi *et al.*, 2010b).

3. 4. 2. Culture media preparation and fungal culture

3.4.2.1. Culture media preparation

The media used for this experment was potato dextrose agar which is most commonly used for *Colletotrichum musae* identification. Potato dextrose agar (PDA) was prepared by dissolving commercially formulated powder PDA. The Potato dextrose powder were then mixed with sterilized water in a flask at the rate of 39 g/l and heated until melting. The mixtures were boiled while stirring with a magnetic stirrer for 15 minutes to completely dissolve the powdered agar. The solution was then autoclaved at 121°C at atmospheric pressure for 15 minutes to sterilize the media. The liquid media were maintained under aseptic condition and allowed to cool to about 45^{o}C. Then Streptomycin sulphate powder were added to the nutrient media at the rate of l gm/1 to avoid bacterial contamination and the media were poured into sterilized Petri dishes and the PDA were then allowed to cool completely and solidified before being used for maintaining fungal cultures.

3.4.2.2. Isolation and purification of test pathogen

Banana fingers that displayed symptoms of anthracnose were collected from retail market of Jimma town. The test fungi *C. musae* were isolated from decaying banana fruits and identified in the Jimma University College of Agriculture and Veterinary Medicine, Plant Pathology laboratory. Diseased banana fruit tissues (4-mm^2) were cut from anthracnose lesions surface under aseptic conditions. The tissues were surface sterilized by soaking the sections in freshly prepared NaOCl (3% w/v) for 3 minutes. After three serial washings in sterile distilled water, banana tissues were placed (4 pieces per plate) on Potato Dextrose Agar (PDA) and incubated 25^{o}C in the incubator (MJX-150B model). Potential pathogens were transferred to new PDA plates in order to obtain pure cultures (Beasley and Shivas, 2005; Narayanasamy, 2011). The fungal pathogens were identified by studying their morphological characteristics using slide cultures and by comparison with literature (Jinyoung, 2002; Abd-Elsalam, 2010). The identity of test pathogens was confirmed by Mycologist. The isolated fungi were maintained on potato

dextrose agar (PDA) medium at 4°C for further studies. A 7-day culture of each fungus was used for bioactivity tests. Conidia were scrapped out from agar surface and suspended in sterile distilled water and its concentration was adjusted using a hemocytometer 10^5 conidia/mL for in vivo studies.

3.4.3. Pathogenicity test for anthracnose disease

The pathogenicity and the virulence of pure cultures of fungal pathogens isolated from anthracnose lesions was established by inoculating of fully matured green unripe Cavendish and Williams cultivars obtained from Jimma Agricultural Research Center. Banana fruits were washed thoroughly by tap water. The fruits were air dried and surface sterilized with 3 per cent sodium hypo chlorate. The fruits were injured (pinprick) with sterilized needle and the spore suspension (1×10^5 spores/ml) of the pathogen was prepared using a seven days old PDA culture by rubbing with a glass rod with sterile distilled water and sprayed over the sterilized fruits. The fruits inoculated with sterile distilled water after pin prick served as control. The inoculated fruit surface was kept in cartons lined with polyethylene and Manila paper and inside the carton at two side moist sponge is kept to increase the relative humidity and stored at room temperature. The infection and evaluation of disease was recorded after seven days. The fungus was reisolated from the artificially inoculated fruits showing typical anthracnose symptoms and the culture obtained was confirmed for its morphology and colony characters, the method was adapted from Natalia *et al.* (2002) with some of modification.

3.5. In vitro Antifungal Bioassays with Essential Oils

3.5.1. In vitro activity of essential oils against mycelia growth

The antifungal activities of essential oils against *C. musea* were tested by poison food medium method. The extracted essential oils of basil, cinnamon and rosemary were tested using the following concentrations: basil oil (100 µl,150µl and 200µl), cinnamon (25µl, 50µl, 75µl) and rosemary (200µl, 250µl and 300µl) were added aseptically to 100ml of sterile cooled PDA

medium (45°C) to prepare 0.10%, 0.15% and 0.20% (v/v) for basil; 0.025%, 0.05% and 0.075% (v/v) for cinnamon and for rosemary 0.20%, 0.25% and 0.30% (v/v) concentrations with a drop of Tween 80 (emulsifying agent of food grade). The mixture was stirred using a magnetic stirrer to obtain an emulsion and mixed well by shaking. The resulting media were immediately dispensed (20 ml) into sterilized Petri plates (90 mm). A mycelial disk of 5 mm diameter of the test pathogens were taken from the 7 day old cultures, with the help of a sterilized cork borer, and placed at the center of the Petri plates contained test materials.

In the controls, water and Tween 80 were used instead of essential oils. Inoculated Petri plates were sealed with sterile laboratory paraffin to avoid contamination, then incubated at 25°C in darkness and observations were recorded at the 7th day of incubation, the time by which the growth of the controls would have reached the edge of the plate. Four Petri plates were used per treatment. Each treatment was replicated three times and fungi toxicity of essential oils was measured in terms of percentage mycelial growth inhibition (MGI %).

3.5.2. Determination of minimum inhibitory concentration (MIC)

The minimum concentration of essential oils (expressed as % (v/v)) required to give complete control or the minimum inhibitory concentration (MIC) for fungal growth was calculated. The MIC of each of the essential oils was classified as fungicidal or fungistatic in its effect. A fungicidal effect was observed where there was no growth, whereas a fungistatic effect was where temporary inhibition of fungal growth occurred. The media disk of the fungi tested, which failed to grow, were transferred to the fresh PDA without oils and incubated for 7 days. Activity of the MIC of the various oils was considered fungicidal if the pathogens did not grow or fungistatic if the pathogen growth occurred (Abdolahi *et al.*, 2010b).

3.6. In vivo Activity of Essential Oils

Fungal suspensions of *C. musae* were prepared from 7 to 13 day old cultures. PDA plates with growing fungi were flooded with sterilized water and surface gently rubbed with a sterile

grass rod. The concentration of conidia mL^{-1} was determined using a haemacytometer and adjusted to $1x10^5$ mL^{-1}.

Treatment of banana fruits

Bunches of banana were dehanded; hands were selected (by avoiding the first and last) from one bunch to avoid differences in physiological development. Hands were washed, treated by Sodium hypochlorite (3%), drained and randomized to the treatment combinations shown in Table 3. Then fruits were sprayed by prepared suspension and located in room temperature for 10 minutes in order to dry it. Then each of selected banana fingers was sprayed with essential oil. At the end of the treatments, all fingers were allowed to dry and transferred to cartons lined with pinpricked polyethylene plastic and Manila paper and inside the carton at two sides moistened sponges were kept to increase the relative humidity and stored at room temperature in the Post harvest management laboratory bench.

Each experimental unit consisted of 12 banana fingers from that one banana fruit was randomly removed for evaluation time, and the entire experiment was replicated three times. Then data was taken with three days interval from treated and control samples until the overall acceptability became unsatisfactory for each lot of samples.

3.7. Data Collected

3.7.1. Mycelia growth inhibition

Daily radial growth measurements were taken until the fungus reached the edge of the control plate by using a caliper and percentage inhibition of mycelia growth was calculated using the formula (Abdolahi *et al.,* 2010b).

$$MGI\,(\%) = \frac{(dc - dt)}{dc}\, x\, 100 \quad \text{..... (Equation 1)}$$

Where dc and dt represent mycelial growth diameter in control and treated Petri plates, respectively.

3.7.2. Weight loss (WL) (%)

The mass of banana fruits were weighed and recorded using a balance (model of TWIII and made in USA). Weight loss was calculated as a percentage of the initial weight measurement. Percentage weight loss was calculated as follows:

$$Weight\ loss\ (\%) = \frac{Initial\ \ weight(g) - Final\ \ fruitweigh\,t(g)}{Initial\ \ fruit\ weight(g)}\ x\,100 \quad \text{..... (Equation 2)}$$

3.7.3. Pulp to peel ratio

To determine the pulp to peel ratio, first the fruits were peeled and the weights of the pulp and peel were taken separately on a sensitive balance. Then the weight of the pulp was dived to that of the peel to arrive at the ratio.

3.7.4. Firmness

It was determined by an objective test by means of a penetrometer. The model of penotrometer which was used for this experiment was 40570. Fruits were held firmly with one hand; rest it on a rigid surface, such as a table top or the plate at the base of the stand. The choice of plunger size and scale range used was depend on the type and the variety of the produce being tested and its stage of maturity and ripeness. The penetrometer was tarred to zero and the plunger head was placed against the fruit.

3.7.5. Total soluble solid (TSS)

The total soluble solids of the banana fruits was determined using a hand refractrometer (made in United Kingdom and its model was Bellinghama+ stonley 45-02.) and the results were expressed in °Brix. Drops of distilled water, juice were placed on the prism surface then

prism lid was closed. The position at which the demarcation line between the light and dark regions crosses the vertical scale gives the percentage soluble solids reading.

3.7.6. Titratable acidity

It is total titratable acidity of the filtrate from pulp samples was determined by titration of the sample with sodium hydroxide to the phenolphthalein end point and calculation of acid present as malic acid (Hewage,1996). Percentage of titratable acidity was calculated using the following formula:

$$Titratable\ acidity\ (TA) = \frac{V1\ xN\ x\ E}{V2}\ x\ 100 \quad \text{..... (Equation 3)}$$

Where,

N = Normality of NaOH

V1 = Volume of NaOH used

E = Equivalent weight of acid,

V2 = Volume of sample taken for estimation

A laboratory burette of 25 or 50ml capacity, A 10ml pipette, beaker (250ml), a filter (muslin cloth or fine filter), an extractor or homogenizer, A bottle of distilled water, Sodium Hydroxide (NaOH) (The Standard Laboratory solution of 0.1M which was used in the actual titration was considered to be dilute, and can readily be purchased in this form) and Phenolphthalein was used. 3 drops of phenolphthalein was added to the juice/water solution in each beaker from a dropping pipette which was specifically kept for that purpose. After ensuring the tap on the burette was shut and using a funnel pour the 0.1M solution of NaOH into the burette until it reaches the zero mark. Slowly the sample was titrated with the NaOH into the juice/water solution (with a 25ml burette). Using phenolphthalein as an indicator, the point of neutrality was reached when the indicator changes from colourless to pink (A.O.A.C., 1995).

3.7.7. TSS to TA ratio

The ratio between total soluble solids and titratable acidity was determined by dividing the TSS to that of TA.

3.7.8. p^H

p^H is the equilibrium measure of hydrogen ion concentration in a juice. It was measured with a standard calibrated p^H meter its model was CP-50-5. The general procedure to measure a p^H was: Make sure the p^H meter had warmed up before use - allow about 30 minutes. The electrode was removed from the distilled water in the storage beaker and dried. Then it was placed into the beaker containing a buffer solution of p^H 7 and the meter was calibrated to the same figure. The electrode was removed and rinsed in distilled water and then it was placed in the solution to be tested. Finally the p^H meter reading was recorded.

3.7.9. Dry matter

A small slice of the fruit less than 4 mm in thickness was taken and placed in the oven(model M200CF and made in England) at 70^{o}C for two days. Weight of the slice will be measured before and after drying. Dry matter percentage (DM %) was calculated using equation (4) (A.O.A.C. 1995).

$$Dry\ Mtter\ (\%) = \frac{Final\ dry\ weight\ (g)}{Initial\ wet\ weight\ (g)}\ x\ 100 \quad \text{……..…} \text{ (Equation 4)}$$

3.7.10. Disease evaluation parameters

The infection was identified on basis of symptoms i.e., blackening of banana fruit. Therefore, disease incidence (DI) was calculated as number of infested fruits showing any single symptoms out of total numbers of banana fruits stored. Five banana fingers were used for disease incidence and percent disease index evaluation.

$$Disease\ Incidence\ (DI) = \frac{Number\ of\ \text{inf}ected\ \ fruits}{Total\ number\ of\ \ fruits}\ x\,100 \quad \text{..... (Equation 5)}$$

Disease severity evaluation was ranked by observing the anthracnose symptom record of disease levels according to the infected surface area on the fruit (Duamkhanmanee, 2008), in which no infected surface area scored 1, whereas the infected surface areas of >0%–5%, >5%–25%, >25%–50%, >50%–75% and >75% scored 2, 3, 4, 5 and 6, respectively. Based on the numerical ratings given above a 'Percent disease index' for fruit rot was calculated using the formula (Mayee and Datar, 1986) given below:

$$(\%)\ Disease\ Index\ (PDI) = \frac{Sum\ of\ \ numerical\ ratings}{Number\ of\ \ fruit\ exa\min ed\ \ x\max imum\ grade}\ x\,100 \quad \text{..... (Equation 6)}$$

3.7.11. Storage life (day)

The shelf life of the banana fruits was calculated by counting the days required for them to attain the last stage of ripening, but up to the stage when they remained still acceptable for marketing (Mondal, 2000).

3.8. Data Analysis

Analysis of variance (ANOVA) was performed using SAS statistical program (Version 9.2). The Least Significant Differences (LSD) test will be performed following the ANOVA for treatments showing statistically significant difference at $P < 0.05$ level. Disease incidence data was transformed (arcsine transformation) before analysis. Mean separation for interaction which shows significant difference was performed using MSTAT-C software.

4. RESULTS AND DISCUSSION

The analysis of variance shows that there was a significant difference on mycelia growth inhibition ($p< 0.01$). The effect of essential oils on disease incidence and percent disease index showed a significant difference. The effect of essential oils on physico- chemical properties was non-significant except in the case of total soluble solids, titratable acidity and their ratio. Hence the details of the result will be discussed below.

4.1. Pathological Properties

4.1.1. Cultural characterization of *Colletotrichum musae* isolates

Cultural characteristics of purified isolates depicted that: Colony diameter of *C. musae* reached up to 90 mm on PDA after 7 days at 25^{o}C (Figure :1B&B'). The color of the mycelia appeared like blackish white from the front side and yellowish on the back side when checked seven days after incubation. The morphological characteristics observed under microscope showed that conidia shape was cylindrical (Figure 1C) and had brown color. This result is in line with the finding of Latiffah, (2009), Jinyoung *et al.* (2002) and Photita *et al.* (2005).

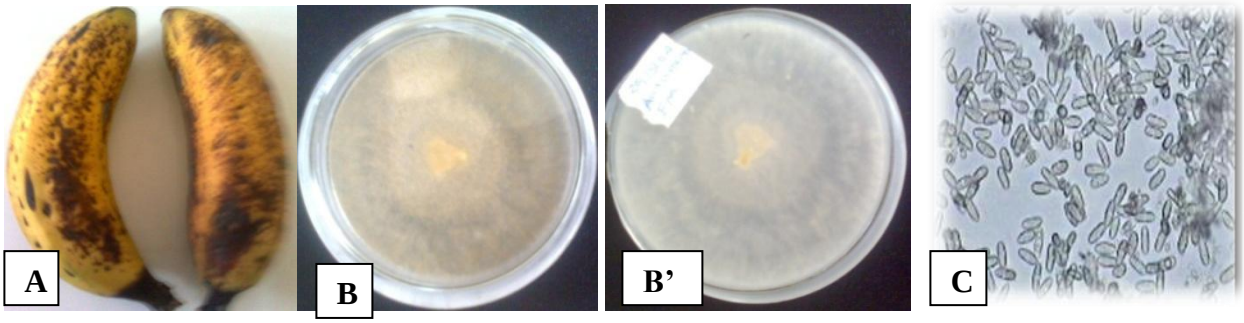

Figure 1: A. Banana fruits with anthracnose symptoms, B and B' front and back side of *C. musea* on PDA plate and C shows Morphological characteristics of *C. musae*

4.1.2. Pathogencity test

During assessment of the pathogenicity of *C. musae*, the fruits of green mature Giant Cavendish and Williams banana varieties revealed dark-brown necrotic and sunken lesions on wound area 7 days after incubation at room temperature (Figure 2). The result of pathogenicity test confirmed that *C. musae* is the virulent causative agent of anthracnose in banana (Shivas and Beasley, 2005). In order to confirm the observed symptom, purification and identification was done from inoculated banana fruit and it was verified to be the same result.

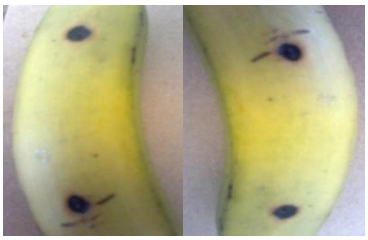

Figure 2: Symptom of anthracnose on banana fruits which confirms the pathogenicity of *C. musae*

4.1.3. In vitro mycelium growth inhibition of tested essential oils

Mycelial growth of *C. musae* was significantly ($P < 0.01$) affected by all essential oils treatment (basil, cinnamon and rosemary oils) as compared to the control during the incubation period of 7 days at 25°C. Treatments with cinnamon and basil essential oil showed high antifungal effects on the mycelial growth of tested fungi at lower concentrations while rosemary essential oil was found to effective at high concentration as compared with the other two treatments. The myceilal growth inhibition was directly related to the concentrations and type of essential oil. The findings of the present study are in agreement with Palhano *et al.* (2004) found that the inhibitory effects of citral and lemongrass crude oil on spore germination of *C. gloeosporioides* were greater with increasing oil concentrations. Similarly, a study done on banana fruit by Thangavelu *et al.* (2004) showed that extracts of *Solanum torvum, Jatropha glandulifera* and *Emblica officinalis* were highly inhibitory to mycelial growth of *C. musae* and the inhibitory effect was directly related to the quantity of extract

added to the medium. The essential oils from cinnamon, basil and rosemary at concentration of 0.025%, 0.1% and 0.2% respectively also significantly ($P < 0.05$) suppressed the mycelial growth of *C. musae* as compared to the control. The percentage of mycelial growth inhibition (MGI) depended on the type and concentration of essential oils used for treatments. In another study Abdolahi *et al*. (2010) also confirmed that the inhibitory effects of sweet basil, fennel, summer savory and thyme on mycelial growth of *Botrytis cinerea* were higher with an increase in the concentration of essential oil and different inhibition with different essential oil type.

Ocimum basilicum oil completely (100%) inhibited the growth of the test pathogen, *C. musae*, at a concentration of 0.15-0.2% (v/v) (Figure 4). Basil oil was reported to be fungicidal to *Aspergillus flavus* and *A. parasiticus* at a concentration of 6.0 ml/L (0.6% v/v) (Dube *et al*., 1989). According to Marandi *et al*. (2011), in vitro assay investigation on pear fruit showed that *Ocimum basilicum* essential oil treatments showed high antifungal effect on mycelial growth of *Penicillium expansum,* fungi.

In this finding, cinnamon leaf oil applied at concentrations ranging from 0.05–0.075% (v/v) was noted to have relatively higher antifungal efficacy against *C. musae* which was at low concentration (Figure 3). The cinnamon oil at 0.025% also suppressed the mycelial growth of *C. musae* as compared to the control but not as effective as 0.05 and 0.075% (Figure 3). In the control plates the mycelia growth of *C. musae* covered almost the whole surface of the plate at the end of the incubation period. In conformity with the present work that cinnamon essential oil was efficient in inhibiting the growth of fungi, Thanaboripat *et al.* (2007) reported that essential oil of cinnamon was effective in inhibiting growth of *A. flavous*. The authors stated that the effects of 16 essential oils from aromatic plants were tested for their inhibitory effect on *A.flavus* on PDA. The results showed that the essential oil of white wood (*Melaleuca cajeputi*) gave the highest inhibition followed by the essential oils of cinnamon (*Cinnamomum cassia*) and lavender (*Lavandula officinalis*) respectively (Thanaboripat *et al.*, 2007).

Experment done by Laura *et al.* (2008) to identify the efficacy of nine essential oils on *C. gloeosporiodes* isolated from papaya indicated that *Cinnamomum zeylanicum* and *Syzygium aromaticum* oils have shown better antifungal effect which had dose dependent mycelia growth inhibition. Similarly Zaika (1988) reported that cinnamon essential oil had strong antimicrobial effect. Cinnamon leaf oil was fungicidal against *C. musae*, *Lasiodiplodia theobromae* and *Fusarium proliferatum*; in vitro test of this essential oil was effective at low concentration against the pathogenic organisms isolated from banana and the main constituent of cinnamon leaf oil was eugenol (Ranasinghe, 2002). Another study indicated that 1, 8-cineole and eugenol exhibited strong antifungal activity against *F. moniliformae* which has been newly classified as *F. proliferatum* (Garg and Siddigui, 1992). Cinnamaldehyde, linalool, eugenol and 1, 8 cineol have been reported as active components in inhibiting the growth of Monilia, Botrytis and Mucor (Goubran and Holmes, 1993).

Experiment done on the antifungal impacts of the essential oil of rosemary plant against *Aspergillus flavus* showed the high controlling and antifungal power of rosemary essential oil under investigation (Moghtader *et al*., 2011). The extent of inhibition of fungal growth varied depending on the levels of essential oil used in experiment (Ozcan and Chalchat, 2008), this is in accordance with present study. The antimicrobial impacts of the essential oil of rosemary plant under investigation can be related with the high percentage of α-pinene, camphor, verbenone and 1, 8-cineole (Moghtader and Afzali, 2009). The antifungal effects of rosemary essential oil can be attributed to the Monoterpenes combination and in particular α-Pinene whose antifungal effects of this combination has been proved (Okamura *et al*., 1994).

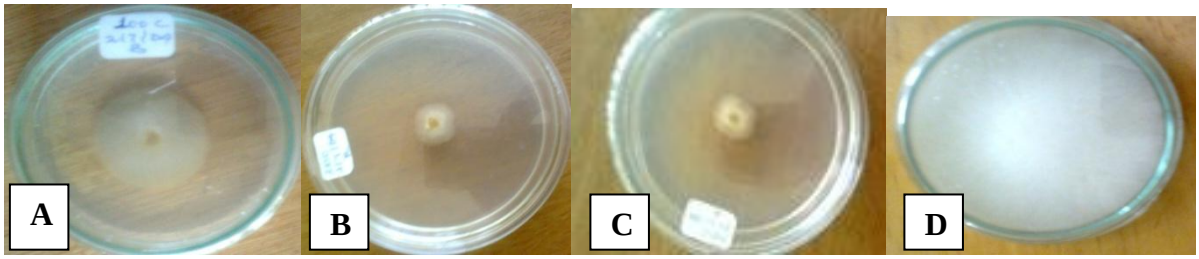

Figure 3: Radial growth of *C. musae* after 7 days incubation with different Oil: (A) 0.1% basil oil (B) 0.2% rosemary (C) 0.25% cinnamon (D) control

Generally, essential oils of cinnamon, basil and rosemary at concentration of 0.05 and 0.075%, 0.15 and 0.20% and 0.25 and 0.30% respectively had significant inhibition on mycelia growth of *C. musae*. But there was a significant difference among the low concentrations used in this experiment and with the control, basil oil (0.10%), cinnamon (0.025%) and rosemary (0.20%) (Figure 4). In the poisoned food bioassay, complete growth (90.0 mm) of each test pathogen was observed by the end of the incubation period in the control set of plates where test oils were not included into the medium having only the media mixed with Tween 80 (emulsifying agent) and distilled water in other plate. This means that there was zero percent inhibition of mycelia growth *C. musae* (Figure 3) in the control plates.

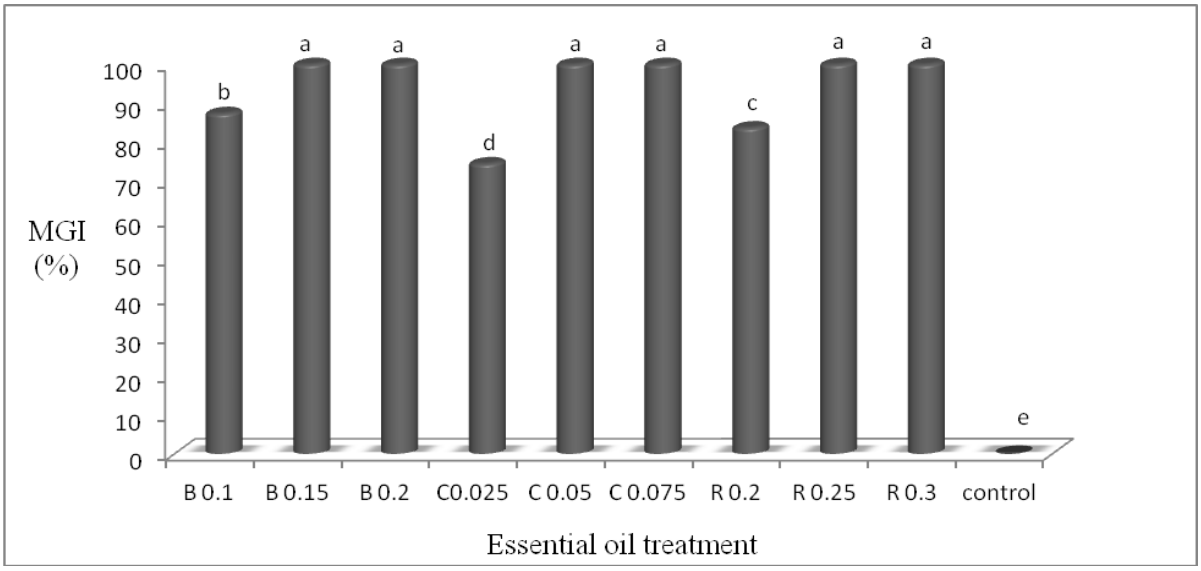

Figure 4: Effect of basil, cinnamon and rosemary oil with different concentrations on mycelial growth inhibition (MGI) of *C. musae* during a 7 days incubation period

4.1.4. Determination of minimum inhibitory concentration (MIC)

The mycelial growth of *C. musae* was inhibited highly significantly over control by all essential oils at 1% level of significance (Table 5). The highest percentage inhibition (100%) was observed at basil 0.15%, 0.20%, cinnamon 0.05%, 0.075%, rosemary 0.25% and 0.30% and that was statistically different from the other treatments at 1% level of significance. The second highest percentage inhibition (87.4%) was observed at basil 0.10% which was statistically different with the above ones (100% inhibited essential oils). The third highest

percentage inhibition (83.71 %) was observed at rosemary 0.20% which was statistically different with the others. The lowest percentage inhibition (72.33%) was observed at cinnamon 0.025% concentration which was statistically different from the other treatments. The mycelial growth inhibitions in different essential oils significantly increased with the increase of concentrations.

Most effective inhibition percentages were basil 0.15%, 0.20%, cinnamon 0.05%, 0.075%, rosemary 0.25% and 0.30%. The result (Table 5) has revealed that at 0.15% basil, 0.05% cinnamon and 0.25% rosemary essential oils inhibited and totally prevented the growth of *C.musae,* therefore these are the minimum inhibitory concentration for the growth of *C. musae*. According to Ranasinghe *et al.* (2002), the minimum inhibitory concentration (MIC) of cinnamon leaf essential oils against anthracnose pathogen was 0.05% (v/v). According to Maqbool *et al.* (2010), maximum inhibition in mycelial growth was observed with application of 0.4% cinnamon oil. Moreover, *O. basilicum* oil had been shown to be fungistatic to mycelial growth of *F. moniliforme, Botrydiplodia theobromae* and a *Colletotrichum* sp. at a low concentration of 0.15% (v/v) (Dube *et al.,* 1989). The oil of *O. basilicum* was the most efficacious, demonstrating fumigant activity against *Colletotrichum musae, Fusarium moniliforme and Lasiodiplodia theobromae* at relatively low concentration (MIC=0.03$\pm$0.01-0.06$\pm$0.01% and MLC = 0.05$\pm$0.02% - 0.2$\pm$0.01%) (Krishanthi *et al.,* 2003).

According to Soylu *et al*. (2006), during their study on antimicrobial activities of the essential oils of various plants against tomato late blight disease agent *Phytophthora infestans*, complete growth inhibition by essential oils of rosemary was at concentrations of 1.2 g/ml, which was relatively high concentration as compared to the other essential oils used for this experiment. Also in the present study the concentration of rosemary essential oil used was higher as compared to both cinnamon and basil oil.

Table 3: Inhibition of radial growth (mm) of *C. musae* by different essential oils

Essential oils in % (v/v)	Radial growth(mm)	Radial growth inhibition (%)
Basil 0.10%	11.33[d]	87.40
Basil 0.15%	0.0[e]	100
Basil 0.20%	0.0[e]	100
Cinnamon 0.025%	24.90[b]	72.34
Cinnamon 0.050%	0.0[e]	100
Cinnamon 0.075%	0.0[e]	100
Rosemary 0.20%	14.67[c]	83.70
Rosemary 0.25%	0.0[e]	100
Rosemary 0.30%	0.0[e]	100
Control (0%)	90[a]	0.00
LSD (5 %)	2.04	0.92
CV (%)	8.51	0.64

Means followed by similar letter (s) with in a column are not significantly different

4.1.5. In vivo activity of essential oils

4.1.5.1. Disease incidence

The disease incidence on banana fruits increased with the passage of time and almost all treated banana fruits showed the disease symptom after nineteen days of storage and all of the control fruits showed the symptom after seven days of storage. Different type of essential oils (basil, cinnamon and rosemary) with different concentrations not only delayed the occurrence of anthracnose disease but also maintained the freshness of the fruits during first two weeks of storage and later on showed few symptoms. All essential oils used for this experiment showed significant ($p<0.05$) effects on disease incidence of banana fruit on the 15^{th}, 17^{th} and 19^{th} days of storage (Table 6). Based on regular observation, the maximum average disease incidence (100%) was observed on the control treatments on all days of assessment with the entire samples of fruits showing anthracnose symptom. On the other hand, the minimum disease incidence in the 15^{th} day of storage (0%) was observed for basil 0.15% and 0.20%, cinnamon 0.075% and rosemary 0.30% essential oil treated banana fruits. Even after 17 days

of storage, basil at 0.20% concentration showed the smallest anthracnose incidence compared with the other treatments.

According to Maqbool *et al.* (2010), investigation on effect of cinnamon essential oil on incidence of anthracnose disease on banana fruit indicated that cinnamon oil had a significant effect on disease incidence (DI) of banana fruit wherein the DI on fruits increased with the passage of time and reached up to 100% after 28 days of storage in control fruits. Treatment with *Ocimum basilicum* oil controlled crown rot and anthracnose, enabling bananas to be stored for up to 21 days at $13.5 \pm 1^{o}C$ without any detrimental effect on their organoleptic properties (Anthony *et al.*, 2003). Similarly Ranasinghe *et al.* (2005) reported that treatment with emulsions of cinnamon oils combined with MA packaging can extend the storage life of Embul bananas up to 21 days in a cold room and 14 days at $28 \pm 2^{o}C$ without any disease incidence. Results of *in vivo* assay showed that essential oils of *Thymus kotschyanus, Ocimum basilicum* and *Rosmarinus officinalis* treatment had a good inhibitory effect on number of infected pear fruits and with increase of essential oil concentration, the antifungal activity of oils increased (Marandi *et al.*, 2011).

In this experiment all banana fruits were infected at the end of the storage period, which is after 19 days of storage time. This result was in accordance to the work of Abdolahi *et al.* (2010b), who reported ajowan, fennel and caraway essential oil treatments had no effect on the control of fungal infection in inoculated tomatoes after 20^{th} day of storage. The ineffectiveness of essential oils in full controlling anthracnose pathogen at ambient temperature could be due to their high volatility. Low temperatures also lower respiration rate, reduce moisture loss and inhibit the growth of decay organisms (Aharoni *et.al.*, 1997).

Table 4: Interaction effects of variety and essential oil on disease incidence at the subsequent days of storage

Variety	Essential Oil (%)	Days After Storage		
		15th	17th	19th
Giant Cavendish	B 0.10	(30.79)26.20^{de}	(43.07)46.66 ^{de}	(88.72)100^{a}
	B 0.15	(1.28)0^{f}	(35)33.33^{efg}	(71.86)90.31^{bc}
	B 0.20	(1.28)0^{f}	(1.28)0^{h}	(42.7)46^{e}
	C0.025	(35)33.33^{cd}	(55.37)67.7^{bc}	(88.72)100^{a}
	C 0.05	(26.56)20^{e}	(46.92)53.33^{cd}	(88.72)100^{a}
	C 0.075	(1.28)0^{f}	(35)33.33^{efg}	(54.99) 67.01^{d}
	R 0.20	(30.79)26.20^{de}	(43.07)46.66^{de}	(88.72)100^{a}
	R 0.25	(26.56)20^{e}	(43.07)46.66^{de}	(88.72)100^{a}
	R 0.30	(1.28)0^{f}	(30.78)26.67^{fg}	(54.99)67.01^{d}
	Control	(88.72)100^{a}	(88.72)100^{a}	(88.72)100^{a}
Williams	B 0.1	(30.79)26.20^{de}	(59.21)73.8^{b}	(88.72) 100^{a}
	B 0.15	(1.28)0^{f}	(46.92)53.33^{cd}	(80.29)97.02^{ab}
	B 0.2	(1.28)0^{f}	(26.56)20^{g}	(54.99)67.01^{d}
	C0.025	(39.23)40^{c}	(59.21)73.8^{b}	(88.72)100^{a}
	C 0.05	(26.56)20^{e}	(46.92)53.33^{cd}	(88.72) 100^{a}
	C 0.075	(1.28)0^{f}	(39.23)40^{def}	(59.21)73.8^{d}
	R 0.2	(35)33.33^{cd}	(59.21)73.8^{b}	(80.29) 93.33^{ab}
	R 0.25	(30.79)26.20^{de}	(43.07) 46.66^{de}	(63.44) 80^{cd}
	R 0.3	(1.28)0^{f}	(35)33.33^{efg}	(59.21) 73.8^{d}
	Control	(88.72)100^{a}	(88.72) 100 ^{a}	(88.72)100^{a}
	LSD (5%)	6.616	11.89	12.24
	CV (%)	19.46	15.55	9.82

The data in the bracket are transformed data

Each data point represents the mean of three replicates ± SE. Different letters in the same column denote a significant difference (P > 0.01)

4.1.5.2. Percent disease index (PDI)

Essential oil treated fruits were better maintained and have low severity of decay scores, whereas non-treated fruits showed increased fruit deterioration. The anthracnose symptoms appeared on the control fruits after seven days of storage and after 19 days most of the bananas were spoiled due to severe disease infection. The effect of basil, cinnamon and rosemary essential oil treatment had a significant ($p<0.01$) effect in all evaluated days.

However, there is no significant difference between the two varieties of banana used namely, Giant Cavendish and Williams. There was a highly significant difference between the control and most of the treatments for almost all sampling days (Table 7). The PDI on bananas reached up to 100% (6 score), after 19 days of storage in the control fruits.

The highest fungicidal effect was observed in those bananas treated with 0.2% basil oil, the average percent disease index was 22.5% for Giant Cavendish and 27.00% for Williams banana variety indicating fruit surface infection close to 1.3 and 1.6, respectively. Treatment of bananas with cinnamon (0.025% and 0.05%), rosemary (0.2% and 0.25%) and basil (0.1%) oil resulted in a small reduction in disease severity of anthracnose compared with other essential oil treatments but had a significant reduction as compared to the controls in both varieties (Table 7). This indicates that the antifungal efficiency of these essential oils has a direct relationship with the concentration of the essential oils.

Essential oils have been known as secondary metabolites in plants responding to biotic stress and to undergo profound changes in plants interacting with fungal (and other) pathogens (Bakkali, 2008). Banana fingers treated with *O. basilicum* oil almost had no symptom of anthracnose (AS$\leq$2) even after at 15 days after storage unlike cinnamon and rosemary treated fingers, both of which had shown some anthracnose symptom (AS $\geq$2), the control treatments (AS $\geq$4 after15 days of storage). The result was in agreement with Anthony *et al.* (2003) who reported that *Ocimum basilicum* was the most efficacious essential oil. It controlled fungal pathogens of Embul banana to the same extent as Benomyl and has potential as an alternative to synthetic fungicides in the horticultural industry. Moreover according to Dube *et al.* (1989) *Ocimum basilicum* oil can be more effective against *F. moniliforme, B. theobromae* and *C. musae* than fungicides such as Agrazim and Bavistin. These two fungicides are also carbendazims, and contain the same class of active ingredients as benomyl. In another investigation *O. basilicum* oil had been shown to be inhibitory to the common spoilage fungus *Aspergillus niger*, at a concentration as low as 1 µl/ml (0.1% v/v) of a broth (Baratta *et al.*, 1998).

However, cinnamon leaf and rosemary oils were ineffective in controlling anthracnose disease as basil oil did. This might be because anthracnose is likely to begin as a latent infection from conidia of *Colletotrichum* that germinated and produced appressoria before the fruits were harvested. Rather treatment of bananas with cinnamon (leaf and bark) oil resulted in a significant reduction in disease severity of crown rot compared with the controls (Ranasinghe *et al.,* 2005). The leaf extracts of some medicinal plants, such as *Calotropis procera*, *Vitex negundo* and *Azadirachta indica*, could delay the appearance of the first disease symptom of infected banana (Singh *et al.,* 1993). Recently Mansour *et al.* (2012) reported that effect of essential oils on the severity of gray mold and keeping appearance quality of inoculated grape berries was significantly different as compared with the control treatments. On the same year Samane and Aminifard (2012) reported that essential oils treated peach fruit better maintained than untreated fruit (control) and had low severity of decay scores, whereas non-treated fruits showed increased fruit deterioration.

Table 5: Effect of essential oil on percent disease index at the 15, 17 and 19th days of storage

Essential oils (%)	Days After Storage		
	15	17	19
B 0.10	21.67^{bc}	29.44^{bc}	37.78^{cd}
B 0.15	18.33^{cd}	23.33^{ef}	36.66^{cd}
B 0.20	16.67^{d}	21.11^{f}	26.67^{f}
C0.025	23.82^{b}	31.67^{b}	46.11^{b}
C 0.05	19.93^{bcd}	26.68^{cd}	34.44^{d}
C 0.075	16.67^{d}	23.8^{def}	27.78^{ef}
R 0.20	23.33^{b}	30.55^{b}	42.77^{bc}
R 0.25	20.55^{bcd}	25.56^{de}	36.11^{d}
R 0.30	16.67^{d}	25.002^{de}	33.8^{de}
Control	63.89^{a}	77.22^{a}	100^{a}
LSD (5%)	3.9	3.29	6.5
CV	14.04	9.61	13.24

Each data point represents the mean of three replicates ± SE. Different letters in the same column denote a significant difference (P > 0.01)

4.2. Physico-chemical Properties

4.2.1. Weight loss (WL)

Basil, cinnamon and rosemary essential oils had no substantial effect on weight loss of banana fruits throughout storage. Weight loss of fruits in all treatments increased throughout the storage. However, there were no significant ($P<0.05$) treatment effects on weight loss during subsequent sampling dates throughout the storage period. Among samples, the highest value for weight loss (11.00%) was obtained from cinnamon essential oil (0.025 % v/v) treated fruits as well as for the control of Cavendish variety. Whereas, the lowest weight loss (9.03%) was recorded from rosemary oil (0.30% v/v) treated Williams banana variety and the grand mean for weight loss was 9.55%. According to the result, essential oil treatment had no vivid and significant effect on weight loss of banana fruits.

This result was in accordance with different research findings that reported cinnamon essential oil had no effects on weight loss throughout storage period of banana fruit (Maqbool *et al.*, 2010; Win *et al*., 2007) also Similarly, Anthony *et al.* (2003) reported that weight losses were not significantly different ($P<0.05$) among all treated fruits with *Ocimum basilicum*, *Cymbopogon nardus* and *Cymbopogon fexuosus* oils when banana fruits were incubated at ambient temperatures. Moreover Marandi *et al.* (2010) indicated that *Thymus kotschyanus* and *Carum copticum* essential oil treatments on table grape had no significant effect on weight loss (%). Strawberries treated with *Eucalyptus globulus* and *C. zeylanicum* oils did not differ in respect of weight loss, organic acid content and sweetness compared with untreated fruits (Tzortzakis, 2007). Additionally, these results were in accordance with Ranasinghe *et al.* (2003) who stressed that *C. zeylanicum* oil had no effect on weight loss of banana fruit. On the other hand, results of present investigation do not agree with Abdolahi *et al.* (2010a) who reported that *T. vulgaris*, *S. hortensis*, *F. vulgare* and *O. basilicum* essential oils reduced weight loss (%) of table grape. The positive effect of eugenol, thymol, and menthol in reduction of weight loss in essential oil treated sweet cherries was reported by Serrano *et al.* (2005).

4. 2.2. Firmness

Firmness of the banana fruits showed a significant ($p<0.05$) difference between the treatments on day three of storage but there was no significant difference between the varieties. However no significant difference was observed with regard to the fruit firmness of treated fruits compared to the controls at all other determination times. These results are in accordance with Ranasinghe *et al.* (2003) who stressed that *C. zeylanicum* oil had no significant effect on the fruit firmness of banana fruits. Additionally, according to Ranasinghe *et al.* (2005) cinnamon leaf essential oil treatments had no effect on flesh firmness of bananas after 14 days of storage at 28 ^{o}C or 21 days at 14 ± 1 ^{o}C. Similarly, cinnamon essential oils were reported to have no effect on fruit firmness values of bananas after 28 days storage at 13±1°C (Maqbool *et al.*, 2010). According to Anthony *et al.* (2003) investigation on effect of basil, lemon grass and Ceylon citronella oil on postharvest diseases and storage life of Embul banana, fruit firmness fell over time in all treatments regardless of whether fruit ripening was natural or induced. But only, fruit treated with citronella oil was significantly firmer ($P<0.05$) than fruit from all other treatments.

4. 2.3. Pulp to peel ratio

All treatment couldn't show significant difference in respect of pulp to peel ratio of the banana fruits throughout the storage period. Additionally, there is no significant difference in pulp to peel ratio between the two varieties, namely Giant Cavendish and Williams banana varieties. As the ripening proceeds, pulp to peel ratio was increased when the fruits become fully ripened. This could be due to the osmotic transfer of moisture from the peel to the pulp as sugar content of pulp increased. It has been suggested that pulp to peel ratio can be considered as a coefficient of ripeness (Loesecke, 1950).

4. 2.4. Total soluble solid (TSS)

The total soluble solids contents of treated fruits were significantly ($p<0.05$) affected by essential oils in all treated fruits throughout storage period. This is possibly due to increased

respiration rates and fungi infection (Figure 5). The total soluble solids values for all treatments were within the range 3.3-3.7 (^{o}Brix) before ripening with no significant difference between any treatment and the control ($P<0.05$). Total soluble solids increased as ripening progressed. Basil essential oil treated fruit had the lowest value 8.93 and 8.99 in both varieties Cavendish and Williams respectively followed by cinnamon essential oil treated (10.6 and 10.88), rosemary essential oil treated (11.5, 11.8) and untreated fruits (14.63 and 16.9) at the end of storage in both varieties, Cavendish and Williams, respectively. However, there was no significant effect of variety and the interaction of variety and essential oil on the TSS of the fruits during the storage period.

The present result was in agreement with that of Abdolahi *et al.* (2010a) who reported that TSS level was lower in oil treated grapes rather than controls. The effect of ammi, anise, ziziphora and cinnamon essential oils on TSS content of peach fruit indicated that significant differences were observed in TSS content among treated fruits and control (Samane and Aminifard, 2012). On other hand Marandi *et al.* (2010) reported that *Thymus kotschyanus* and *Carum copticum* oils treatment had not significant effect on TSS of table grapes.

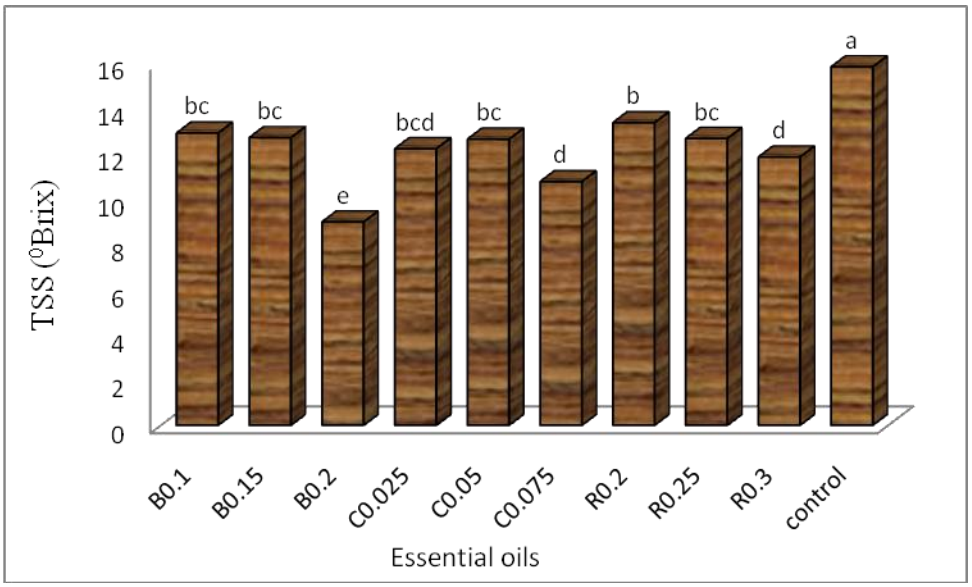

Figure 5: Effect basil, cinnamon and rosemary essential oils with different concentration on TSS of banana fruits

4. 2.5. Titratable acidity

Titratable acidity (TA) increased with ripening. The TA contents of treated fruits were significantly ($p<0.05$) affected in all treated fruits throughout storage (Figure 6). There was a significant difference between control and all treatments except rosemary 0.20% treated fruits. The minimum (0.23) TA value was recorded from banana fruits which were treated with 0.20% basil and 0.30% rosemary oils the maximum was registered in the control treatment which was 0.33.

The result of present study is in accordance with that of Asghari *et al.* (2009) who reported that treatment of strawberry fruits with cumin essential oil had significant influence on the titratable acidity of fruits on the 3rd, 12^{th} and 15^{th} days storage ($p < 0.05$). Similarly, increased TA level has been reported in oil-treated clusters compared to the control (Abdolahi *et al.*, 2010a). Moreover, the effect of ammi, anise, ziziphora and cinnamon essential oils on TA content of peach fruit were observed to be significant in TA content among treated fruits and control (Samane and Aminifard, 2012). On the other hand, the findings of the present investigation is in disagreement with that of Marandi *et al.* (2010) who reported that *Thymus kotschyanus* and *Carum copticum* oil treatment had no significant effect on titratable acidity of table grapes. Similarly Maqbool *et al.* (2010) stated that cinnamon essential oil had no effects on titratable acidity values of bananas after 28 days of storage at 13±1°C.

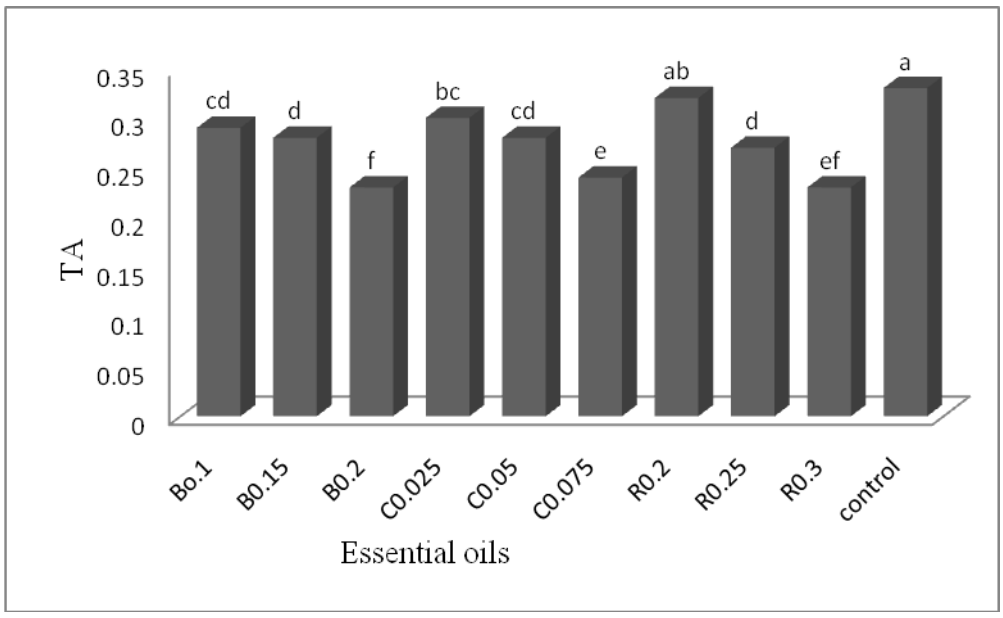

Figure 6: Effect basil, cinnamon and rosemary essential oils with different concentration on TA of banana fruits

4. 2.6. TSS: TA ratio

The effect of essential oil on total soluble solid to titratable acidity ratio of banana fruits showed a significant ($p < 0.05$) difference between treatments. The control treatment was only significantly different with treatments: 0.2% basil, 0.025% cinnamon and 0.3 rosemary essential oil treated banana fruits. But there was no significant difference between the control and other treatments. Investigation done by Samane and Aminifard (2012) on effect of ammi, anise, ziziphora and cinnamon essential oils on post harvest decay and some quality factors of peach fruit indicated that there was no significant difference in respect of total soluble acid to titratable acidity ratio content of fruits among treatments.

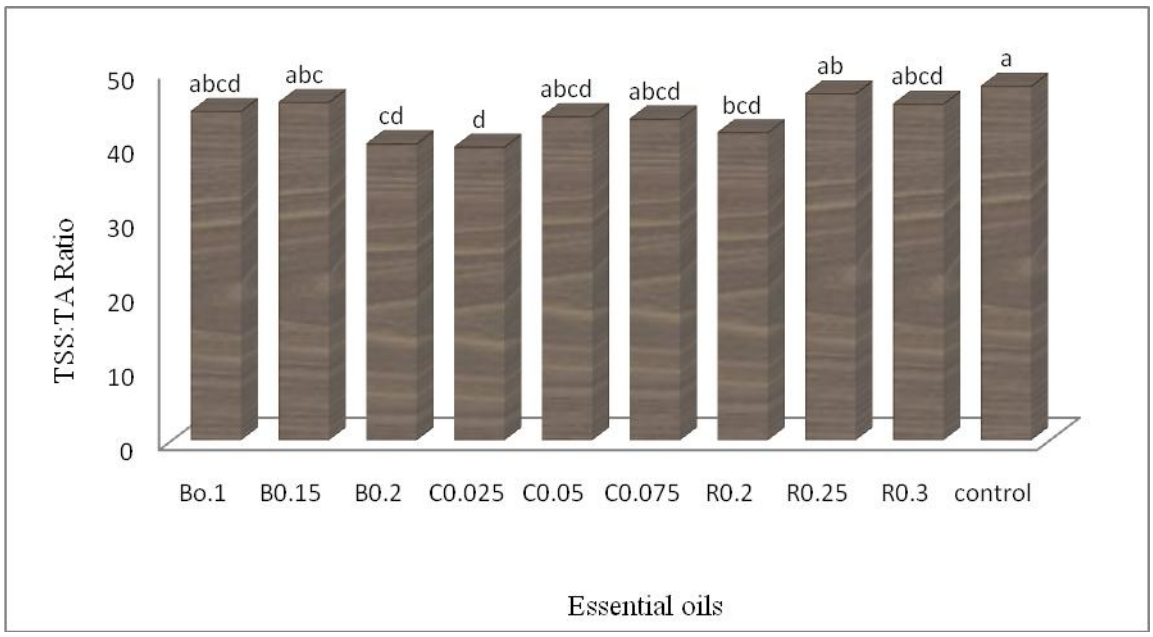

Figure 7: Effect basil, cinnamon and rosemary essential oils with different concentration on the ratio of TSS to TA

4. 2.7. pH

The effects of both essential oil and variety on pH of the banana fruits were not significant throughout the whole storage time. In similar results; basil essential oil spray emulsion (0.16% v/v) treatment on banana to control crown rot disease did not have any significant effect on pH after induced ripening (Anthony *et al.,* 2003). Cinnamon (bark and leaf) essential oil treatments had no effect on pH of bananas after 14 days at 28 ^{o}C or 21 days at 14 ± 1 ^{o}C (Ranasinghe *et al.,* 2005). Also, cinnamon and eucalyptus vapor had no significant effect on pH on tomato (Tzortzakis, 2007).

4. 2.8. Dry matter

No significant effect of essential oils was observed on dry matter of treated fruits compared to the controls for all sampling dates. There was no significant effect attributable to banana varieties and interaction effect of essential oil with variety. According to Abd El Naby (2010)

study on the effect of postharvest treatments on quality aspects of Maghrarbi banana fruits, mandarin essential oil had no significant effect as compared with the untreated banana fruits.

4. 2.9. Storage life

In the present work most of banana fruits were spoiled at the 19 days of storage. At day 7-15 all of the control banana fruits became deteriorated and at day 17 cinnamon, basil (0.1% and 0.15%) and rosemary treated banana fruits became unmarketable. However, only banana fruits treated with basil essential oil (0.2%) stay until 19 days of storage, which could be marketable. This result fits to the work of Abdolahi *et al.* (2010b) who reported essential oils treatments had no effect on the number of infected fruits in the storage end, which on the 20th day of storage, all fruits were infected, on their work on the effect of ajowan, fennel and caraway oils on the control of fungal infections in inoculated tomatoes was evaluated.

5. SUMMARY AND CONCLUSION

Banana fruit experiences different problems compared to most other fruits, because ripe bananas are very perishable. The present investigation consisted of two parts that are in vitro and in vivo bioassay using Cavendish and Williams banana varieties and basil, rosemary and cinnamon essential oils. Results of the present study showed that basil, cinnamon and rosemary essential oils possess strong antifungal activity against the test fungus, *C. musae*, which makes serious postharvest banana loss worldwide. The degree of mycelial growth inhibition was found to be dependent on the essential oil type and concentration. The essential oil from cinnamon has efficaciously inhibited *C. musae* at a very low concentration (0.025%). The difference in efficacy of different essential oils could be attributed to the presence of the active principles that are extracted during extraction time. It is evident from the results that all of the essential oils showed inhibition on the mycelial growth of the isolated fungus.

Among the essential oils examined, basil oil proved to be effective in in-vivo test, when applied at 0.20% (v/v). It showed the minimum percent disease index (26.67%) compared with the other treatments at day 19 of storage and reduced the development of *C. musae* growth, maintains the freshness of the banana fruit, increases its shelf life and had no significant negative effect on physico- chemical properties. All tested concentrations of the essential oils provided significant inhibition. The effectiveness declined when lower essential oil concentrations were used. Utilization of natural substances, can limit development of plant pathogens, increases shelf life of fruit and yet comes into higher and higher prominence, especially restricting traditional chemical application.

The findings of the present study revealed that treatment of banana fruits with essential oils of basil, rosemary and cinnamon at 0.20%, 0.30% and 0.075% (v/v), respectively inhibited the incidence of anthracnose effectively and extended the shelf life of the fruits. It can thus be concluded that among the essential oils used, basil oil proved to be effective in in-vivo test applied at 0.20% (v/v) reduced the development of *C. musae* growth, maintains the freshness of the banana fruit, increases its shelf life of Giant Cavendish and Williams banana fruit for

up to 19 days of storage at 22±1°C temperature and 72% relative humidity without affecting the physico-chemical properties. There were significant ($P < 0.05$) differences between treatments in bananas inoculated with spore suspension of *C. musae* after being treated with different concentrations of the three essential oil types during the 19 days of storage at 22±1°C temperature and 72% relative humidity. The major component of cinnamon, rosemary and basil oil are eugenol, 1, 8-Cineole and linalool which are generally regarded as safe compounds, since their toxicity to the human being was very low. Therefore wholesaler market chains could easily adapt the technology to treat fresh, mature Giant Cavendish and Williams banana in order to increase their shelf life. Giant Cavendish and Williams bananas treated in this way could also be shipped long distance without affecting its quality if treated carefully with appropriate type of essential oil and concentration.

Generally, short shelf life of banana fruit because of high perishability and anthracnose disease is enormous public problem, but it could be controlled by the use of natural preservatives such as essential oils obtained from aromatic plants. The fact that many EOs possess antimicrobial activity has been proved by plenty of investigations in recent years. The type and optimal concentration of EO depend on the commodity used and against which species of bacteria or fungi it is to be used. However if EOs are expected to be widely applied as antimicrobial agents, the physico- chemical property and organoleptical impact should also be considered for the use of naturally derived preservatives can alter the quality of the fruits. Problems that may occur if high concentrations of EOs are used are such as discoloration and change in flavor.

Therefore, research in this area should focus on different types of EOs, concentrations and applications to obtain effective antimicrobial activity at sufficiently low concentrations so as not to adversely influence the quality and acceptability of the fruit. Similar experiment need to be conducted with the same essential oils but on other fruits. Additionally, experiments with the same or other essentials should also be conducted in different fungus and bacteria causing postharvest diseases and loss of fruits.

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7. APPENDICES

Appendix Table 1: The nutritional values of bananas per 100g of edible fresh portion

Nutrients	Amount (%)	Daily recommended values
Water	74	240ml
Carbohydrates	23	300g
Protein	1	50g
Fats	0.5	65g
Fiber	2.5	25g

Source: ED informatics, 2006

Appendix Table 2: Vitamin and minerals content of the banana (nutrients per 100g ripe, edible banana)

No.	Vitamins	Amount (mg)	Minerals	Amount
1	Carotene	21	Sodium	1mg
2	Vitamin E	0.27	Potassium	400mg
3	Thiamin (B1)	0.04	Calcium	6mg
4	Riboflavin (B2)	0.06	Magnesium	34mg
5	Niacin	0.7	Phosphorus	28mg
6	Pyridoxine (B6)	0.29	Iron	0.3mg
7	Folic Acid	14	Copper	0.1g
8	Pantothenate	0.36	Zinc	0.2mg
9	Biotin	2.6	Chloride	79mg
10	Vitamin C	11	Manganese	0.4mg
11			Iodine	8mg

Source: Dickinson, 2000

Appendix Table 3: Means squares for the variance of the effects of essential oils on disease severity, PDI and quality parameters of treated banana fruit

Significance	DF	Disease Incidence	PDI	Weight Loss (%)	TSS	PH	TSS/TA	TA	Dry matter	Pulp/ peel	Firmness
Var.	1	*	*	ns	ns	ns	ns	ns	ns	Ns	ns
EO	9	**	**	ns	*	ns	*	*	ns	Ns	ns
EO * var.	9	**	**	ns	ns	ns	ns	ns	ns	Ns	ns

EO: Essential oil, Var: variety. *, ** and ns: Significant at (P< 0.05, P< 0.01) and not significant, respectively

TSS: Total Soluble solids, TA: Titratable acidity and PDI: percent disease index

Appendix Table 4: Overall ANOVA for Total Soluble solids

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.19	0.12	0.7287
Var	1	1.67	1.06	0.3086
Essen	9	18.59	11.83	<.0001
Var*Essen	9	1.78	1.13	0.3656

CV (%) = 10.1355

Appendix Table 5: Overall ANOVA for PH

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.0207	0.42	0.5216
Var	1	0.066	1.33	0.5216
Essen	9	0.037	1.33	0.6562
Var*Essen	9	0.0397	0.80	0.6167

CV (%) = 4.438

Appendix Table 6: Overall ANOVA for Titratable acidity

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.00030250	0.77	0.3862
Var	1	0.00081	2.05	0.1604
Essen	9	0.0076	19.22	0.0001
Var*Essen	9	0.00071	1.79	0.1007

CV (%) = 7.07

Appendix Table 7: Overall ANOVA for pulp to peel ratio

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.0093	0.30	0.5860
Var	1	0.00000882	0.00	0.9866
Essen	9	0.293	1.06	0.4159
Var*Essen	9	0.3717	1.34	0.2492

CV (%) = 9.7

Appendix Table 8: Overall ANOVA weight loss (%)

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.0255	0.01	0.9102
Var	1	1.184	0.60	0.4438
Essen	9	0.937	0.47	0.8834
Var*Essen	9	0.93034	0.47	0.8856

CV (%) =15

Appendix Table 9: Overall ANOVA dry matter (%)

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.2356	0.25	0.6209
Var	1	0.5415	0.57	0.4544
Essen	9	0.5975	0.63	0.7642
Var*Essen	9	1.8826	1.99	0.0677

CV (%) = 3.04

Appendix Table 10: Overall ANOVA firmness

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.00004	0.05	0.8207
Var	1	0.00028	0.37	0.5483
Essen	9	0.000445	0.58	0.8053
Var*Essen	9	0.00153	1.99	0.0669

CV (%) 1.2081

Appendix Table 11: Overall ANOVA TSS/TA

Source	DF	Mean Square	F Value	Pr > F
Rep	2	1.9981	0.09	0.7719
Var	1	0.8809	0.04	0.8473
Essen	9	62.5712	2.67	0.0162
Var*Essen	9	26.5359	1.13	0.3646

CV (%) = 10.949

Appendix Table 12: Overall ANOVA for disease incidence

Source	DF	Mean Square	F Value	Pr > F
Rep	2	34.04025	1.96	0.1690
Var	1	133.534	7.7	0.0084
Essen	9	2239.861	129.23	<.0001
Var*Essen	9	38.346	2.21	0.0421

CV (%) = 8.51

Appendix Table 13: Overall ANOVA percent disease index

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.0137	0.00	0.9680
Var	1	128.22	15.26	0.0004
Essen	9	16377.4	216.61	<.0001
Var*Essen	9	69.62	0.92	0.5177

CV (%) = 7.71

Appendix Table 14: Overall ANOVA mycelial growth inhibition

Source	DF	Mean Square	F Value	Pr > F
Rep	2	0.305	1.05	0.369
Essen	9	2912.82	10000.8	<.0001

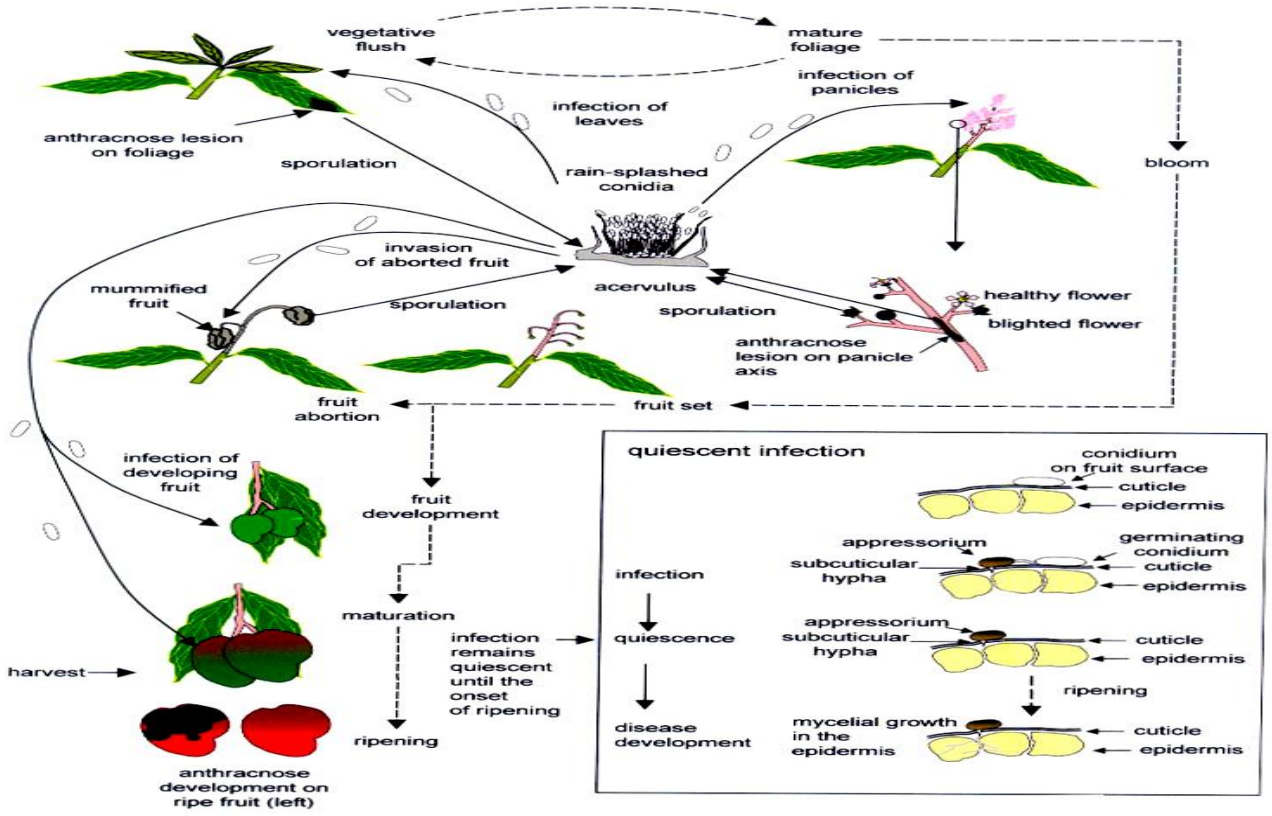
CV (%) = 0.638

TSS: Total Soluble solids, TA: Titratable acidity and PDI: percent disease index

Appendix Table 15: Mean square value for effect of essential oil on TSS, TA and TSS: TA

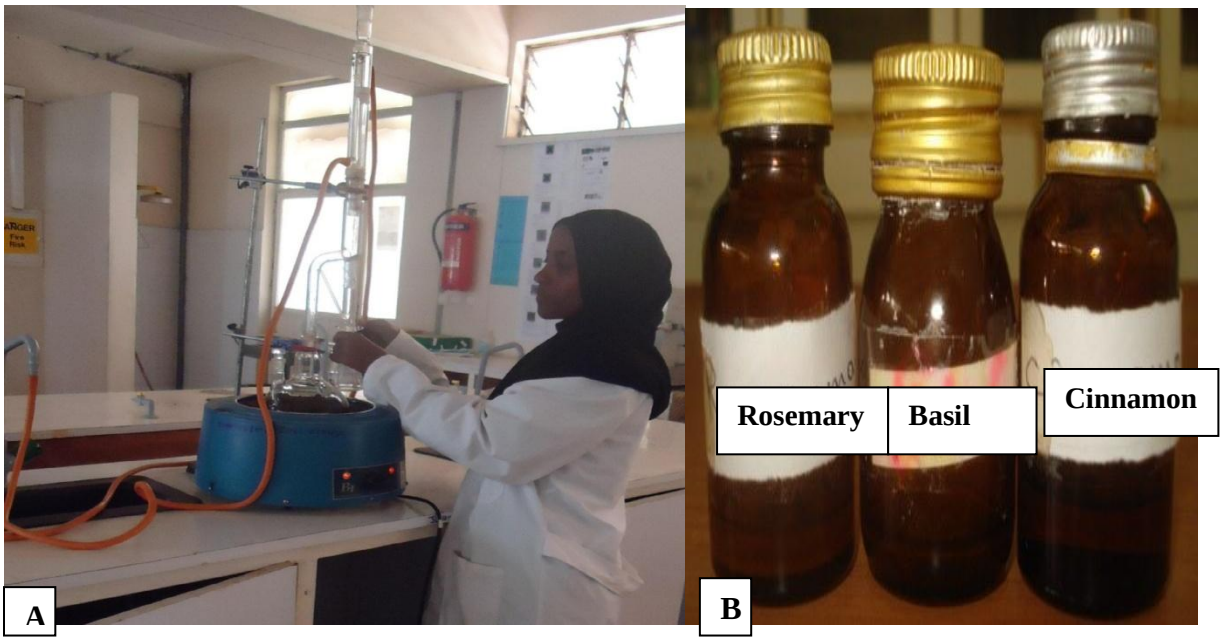
Essential oil	TSS	TA	TSS:TA
B0.10	12.88^{bc}	0.29^{cd}	44.4^{abcd}
B0.15	12.68^{bc}	0.28^{d}	45.66^{abc}
B0.20	8.96^{e}	0.23^{f}	40.09^{cd}
C0.025	12.19^{bcd}	$0.3b^{c}$	39.64^{d}
C0.05	12.61^{bc}	0.28^{cd}	43.76^{abcd}
C0.075	10.74^{d}	0.24^{e}	43.42^{abcd}
R0.20	13.34^{b}	0.32^{ab}	41.59^{bcd}
R0.25	12.64^{bc}	0.27^{d}	46.83^{ab}
R0.30	11.82^{d}	0.23^{ef}	45.43^{abcd}
Control	15.8^{a}	0.33^{a}	47.84^{a}

Each data point represents the mean of three replicates ± SE. Different letters in the same column denote a significant difference (P > 0.05)

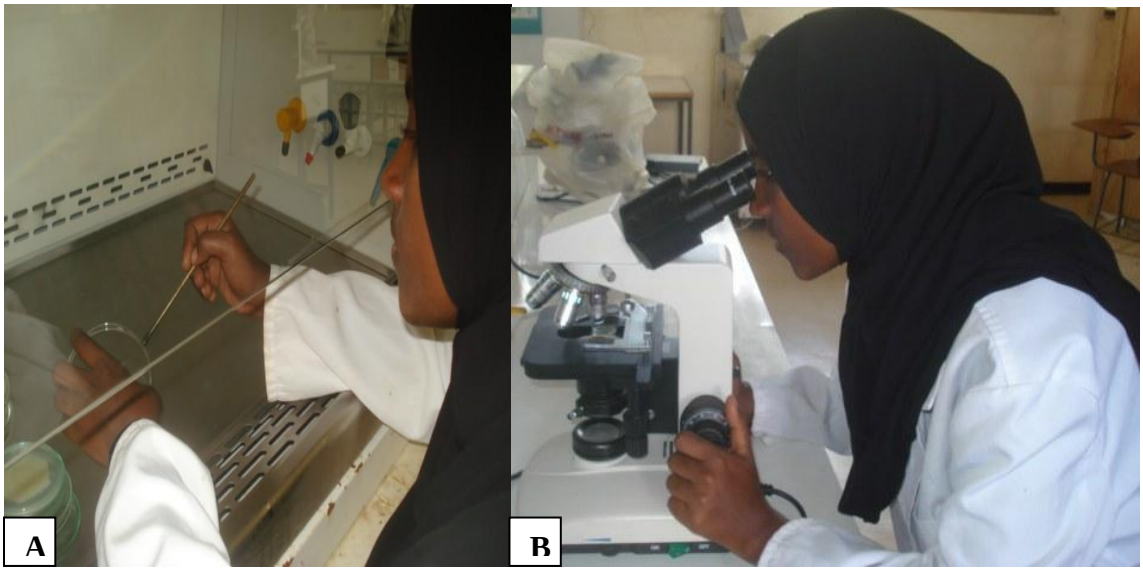


Appendix Figure 1: Anthracnose disease cycle (Arauz, 2000)

Appendix Figure 2: Drying and preparation of plant materials for essential oil extraction



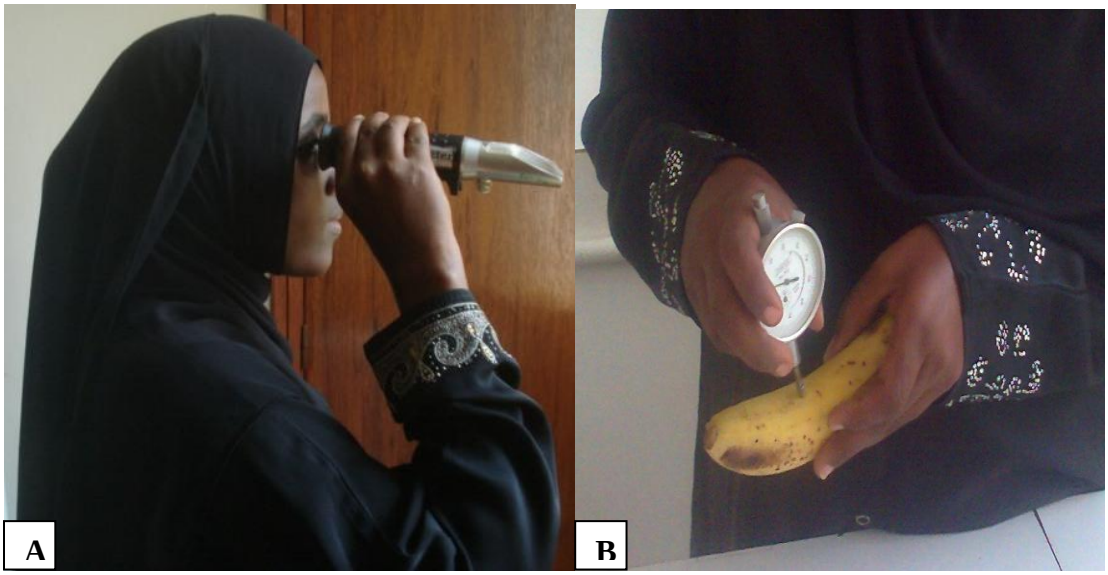
Appendix Figure 3: A. Extraction of essential oils for the study, B. The extracted essential oils



Appendix Figure 4: A. Isolation of C. *musae*, fungi in the laminar flow (safety cabinet) B. Identifying *C. musae* using microscope

Appendix Figure 5: A. Measuring mycelia growth using a caliper and B. Preparation of banana fruits for treatment

Appendix Figure 6: A. Banana fruits wrapped in plastic after pretreatment with Essential oils B. The way banana in the cartons are arranged in laboratory bench



Appendix Figure 7: A. Measuring total soluble solid using hand refractor meter, B. Measuring firmness of banana fruit using hand penetrometer