CHARACTERIZATION OF MICROORGANISMS IN SOIL TREATED WITH *Trichoderma sp* AND PLANTED WITH SOUR SOP *(Annona muricata)* UNDER VARYING WATERING REGIMES

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NIGERIA

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF AGRICULTURE (B. AGRIC) IN CROP SCIENCE

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CERTIFICATION

This is to certify that the work in this report entitled "Characterization of microorganisms in soil treated with *Trichoderma spp* and planted with soursop under varying watering regimes" was carried out by Miss Queenslyn AIBUEDEFE with Matriculation number, AGR1700167 in the Department of Crop Science, Faculty of Agriculture, University of Benin, Edo state, Nigeria.

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(Project Supervisor)

Date: _____

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Prof. K. E. Law-Ogbomo
Head of Department

Date: _____

DEDICATION

This project work is dedicated to God Almighty whose divine wisdom has illuminated my path all through the course of my programme in University of Benin. To my parents, whose unwavering support and sacrifices have made this journey possible. To my brothers and friends, whose constant encouragement has been a source of my strength.

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ABSTRACT

Annona muricata also known as soursop, is a plant in the *Annonaceae* family. Soursop grows in a variety of soil types of soil, which provides a habitat that supports the growth of soil organisms. One of the most important factors influencing microbial activity and diversity in soil is the availability of water. The objective of this study was to identify microorganisms in soil subjected to treatment with *Trichoderma sp* and planted with soursop under varying watering regimes.

The experiment was conducted in the department of Crop Science Laboratory, Faculty of Agriculture; University of Benin, Benin city. The study investigated the influence of watering regime, soil media and soil incubation duration on the growth of Soursop. The experiment was laid out in a Completely Randomized Design (CRD) with two replications and were separated using Student-Newman-Keul test.

The results of this experiment showed the presence of four fungal isolates (*Mucor mucedo, Trichoderma sp*, *Aspergillus niger,* and *Rhizopus arrhizus*) and four bacterial isolates (*Escherichia coli, Bacillus subtilis, Bacillus pumilus*, and *Klebsiella oxytoca*) in soil cultivated with soursop under different watering regimes. The identification of *Trichoderma sp* reaffirms the efficacy of the treatment while the diverse fungi and bacterial species suggest a complex soil ecosystem. It was observed that the varying watering regimes (Watering every day, watering every 3 days, watering every 5 days and Watering every 7 days) showed different degrees of microbial population and diversity, with the highest occurring (12.4 x 10^6 cfu/g and 16.9 x 10^6 cfu/g) in Watering every day while soil-seed treatment; Untreated seed-Untreated soil had the highest microbial population (12.9 x 10^6 cfu/g and 16.4 x 10^6 cfu/g). From the results obtained, the soil and seed treatment, watering regime and soil incubation duration had effect on the microbial population. On the basis of these findings, it is recommended that soil watered daily should be used when growing soursop for optimum growth.

CHAPTER ONE

INTRODUCTION

Annona muricata, also known as soursop, is a plant in the *Annonaceae* family. It is a fruit tree with a long history of traditional use and is an evergreen plant that is mostly found in tropical and subtropical regions of the world. *Annona muricata* fruits are widely used in the production of syrups, candies, beverages, ice creams, and shakes (Sejal Patel, 2016). This plant thrives in hot climates throughout the United States, Europe, India, and Africa. The soursop fruit is green, spiny, spherical, sweet, sour, and delicious, with a diameter of 15 to 20 cm (Gyesi *et al*., 2019). The flesh is white and creamy, with a distinct aroma and taste. When fresh, each fruit may contain 55-170 black seeds, which turn light brown when dry (Awan *et al*., 1980). The flesh ranges in texture from tender and moist to firm and dry (Berumen-Varela *et al*., 2019). The tree blooms and fruits most of the year, however there are distinct seasons depending on altitude. The species is an evergreen, terrestrial, erect tree that grows to a height of 5-8 m and has an open, roundish canopy with large, glossy, dark green leaves (De Souza, 2009). Its oval leaves have axillary buds that are 6 cm broad and 12 cm long when completely mature (Moghadamtousi *et al*., 2015).

Odum (1971) described soil as the upper weathered layer of the earth's crust containing a complex mixture of particle elements produced from abiotic parent minerals, living biota, and particulate organic detritus and humic compounds. Climate (temperature and moisture), parental material, time, terrain, and creatures all

contribute to soil formation (Jenny, 1994), which involves intricate interactions of physical, chemical, and biological processes. The composition of a soil's mineral fraction greatly influences its productive capacity (Brady and Weil, 2002). *Trichoderma* spp. is a cosmopolitan fungus that can be found in soil, dung, and decaying plant tissues (Alexander, 1961). The success of *Trichoderma* spp. in the soil ecosystem and role as a natural decomposer is attributed to its capacity to accelerate growth, nutrient uptake, and rhizosphere modification. It can also survive harsh environments and has a strong destructive ability against plant pathogenic bacteria (Bentez *et al*., 2004). Several recent studies have proven the potential of Trichoderma spp. to detoxify pesticides and herbicides (Vázquez *et al*., 2015).

Water is an important factor in the growth, development and productivity of plants. Water is a factor in seed germination and can have an impact on both the percentage of germination and the rate of germination. Plant species respond differently to water availability. Also, different plant parts adapt differently to varying water stress conditions. Aderounmu *et al.* (2017) mentioned that water has significant effects on growth of plants though watering requirements of different species differ. Water has a strong influence on soil processes such as microbial growth, and changes in precipitation patterns caused by climate change which gives rise to the question of how changes in soil-water availability will affect microbial growth efficiencies. Moisture stress, according to Doescher *et al*. (1985), is one of the parameters that can influence the adaptability and regeneration success of a species population. Levy and

Krikum (1983) opined that insufficient water in plants below a critical level is usually demonstrated by changes in all structures leading to the death of the plants. Similarly, too much water in excess of plant need may retard physiological processes in plants (Isha *et al*., 2013).

1.1 Justification of the study

The introduction of plant pathogenic bacteria in a crop plantation can result in the establishment of pandemic plant diseases. Secondary compounds released by *Trichoderma* spp. have been shown to limit pathogenic microbe growth while encouraging plant growth (Kubicek *et al*., 2001). Because the challenges of dealing with concerns in the agriculture industry have expanded enormously, sustainable techniques based on biological control are required. As a result, using *Trichoderma* spp. as a biological agent appears to be an excellent option.

Trichoderma spp. can be used as an option to enrich the substrates, aiming to improve the availability of nutrients and contributing to the formation of vigorous seedlings, given the perspective of the potential for expanding soursop production.

Gianfreda and Bollag (1996) demonstrated that a drop in soil water content might result in the production of hypertonic osmotic pressure, which reduces microbial activity. As a result, soil water content influences microbial activity in the soil.

1.2 Aim and Objectives of the study

This research aims to characterize microorganisms in soil treated with *Trichoderma spp.* and planted with soursop under varying watering regimes.

The specific objectives of the study were to:

1. isolate and characterize the different microorganisms found in the soil.
2. determine the frequency of the soil microbes.
3. evaluate effect of watering regime on isolation of microorganisms

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany of Soursop (*Annona muricata* L.)

The Annonaceae family consists of approximately 130 genera and 2300 species, while the genus Annona commonly known as custard-apple genus comprises over 70 species among which *Annona muricata* is the most widely grown (Leboeuf *et al*., 1980; Coria-Téllez *et al*., 2016). *Annona muricata* is a small tropical fruit tree known as guanabana or soursop. It is a native plant of Central America which is distributed widely throughout Southeast Asia, South America, and the rainforests of Africa (Moghadamtousi *et al.,* 2015). All portions like the leaves, is used as medicine, but the most widely used in the preparations of traditional medical decoctions are stem barks, roots, seeds, and leaves (Badrie and Schauss, 2009).

Annona muricata is an erect, evergreen, terrestrial tree with large, glossy, dark green leaves that can grow to a height of 5-8 meters. The tree produces huge, heart-shaped, green, and edible fruits that range in diameter from 15 to 20 cm (De Souza *et al*., 2009). It is often cultivated from seeds that can be kept in storage for a number of months before being planted. Seed germination typically takes 3 weeks but in suboptimal conditions, it may take up to 2-3months. Soursop trees bear fruit 3-5 years after planting. The flesh is white and creamy with a characteristic aroma and flavor. The leaves are obovate, oblate, and acuminate, with a dark green, thick, and

glossy upper surface. When fully developed, their oval leaves have axillary buds that are 6 cm broad and 12 cm long (Moghadamtousi *et al*., 2015).

Due to storage circumstances and fruit variety, soursop fruit is vulnerable to phytosanitary issues, one of which is damage from phytopathogenic fungus during the postharvest period, which might result in black spots and limit soursop production and commercialization. One of the primary diseases that attack the soursop fruit is the anthracnose produced by the fungus *Colletotrichum gloeosporioides*, which damages seedlings and adult plants by attacking stems, branches, leaves, flowers, and fruits ((Berumen-Varela *et al*., 2019).

2.1.2 Uses of Soursop

Soursop is a nutritious fruit, rich in ascorbic acid, potash, phosphorous, and calcium. With an edible portion of 62-85%, the crop is very viable for fresh consumption and processing. Different parts of soursop (fruit, leaf, bark, root, and seed) are being used to treat conditions such as diabetes, coughs, skin diseases, and cancers (Syed Najmuddin *et al*., 2016). The fruit flesh is used to increase breast milk production after childbirth and treat rheumatism, arthritic pain, fever, neuralgia, dysentery, heart and liver diseases, and skin rashes, and it has antidiarrheal, antimalarial, antiparasitic, and anthelmintic properties (Moghadamtousi *et al*., 2015; Hajdu *et al.,* 2012). An extract of the stem bark of *A. muricata* has been shown to exhibit antistress activity. Studies have found that an ethanol extract of the stem bark of soursop induces a

significant reduction in stress levels in stress-induced rats, suggesting a role for the extract as an adaptogenic agent (Padma *et al*., 2001).

Soursop pulp is rich in protein, carbohydrate, water, nonreducing sugar, and vitamins B1, B2, and C (Badrie and Schauss, 2010). The medicinal activities and health benefits of soursop have been attributed to their phytochemicals including acetogenins, cyclopeptides, phenolic, alkaloids and essential oils (Moghadamtousi *et al*., 2015).

2.2 Soil microorganisms

Soil microorganisms are a diverse group of organisms that inhabit soil and play crucial roles in maintaining soil fertility and ecosystem health. They can be classified into three major groups: bacteria, fungi, and archaea. These microorganisms participate in a range of ecological processes, including nutrient cycling, decomposition, and soil structure formation (Hartmann *et al*., 2015).

Bacteria are the most numerous and diverse group of soil microorganisms. The population of bacteria in the soil depends on the physical, chemical and biological conditions of the soil. They are involved in key soil processes such as nitrogen fixation, nutrient mineralization, and decomposition of organic matter. For example, nitrogen-fixing bacteria such as Rhizobium, Azotobacter, and Azospirillum are important for supplying nitrogen to plants (Kennedy *et al*., 2004). Bacterial

decomposition of organic matter plays an important role in the release of nutrients such as carbon, nitrogen, and phosphorus into the soil (Chaparro *et al*., 2014).

Fungi are another important group of soil microorganisms. They play key roles in decomposition, nutrient cycling, and the formation of soil structure. Due to their heterotrophic nature, they depend on other living things for both their carbon and energy sources. Most fungi are saprotrophs and decomposers. They decompose all forms of organic materials, including wood and other types of parent material. Fungi can form mutualistic associations with plant roots, known as mycorrhizae, which can improve plant nutrient uptake and resistance to stress (Rillig, 2004). Fungi can also produce glomalin, a glycoprotein that can help to stabilize soil aggregates and improve soil structure (Wright and Upadhyaya, 1996).

Archaea are less well-studied than bacteria and fungi, but recent research has revealed that they play important roles in soil ecosystems. They are involved in nutrient cycling, such as nitrogen fixation and methane cycling (Lehtovirta-Morley *et al*., 2016). Archaea also contribute to soil structure formation by producing extracellular polymeric substances that can bind soil particles together (Ebrahimi and Or, 2016).

2.2.1 Effect of watering frequency on soil microbes

The frequency of watering can have an impact on soil microbes. Soil microbes are a diverse group of microorganisms that play important roles in soil fertility, nutrient cycling, and plant health. They require moisture to survive and carry out their

functions in the soil. Water availability is one of the most critical factors that impact microbial activity and diversity in soil. Research has shown that soil microbial communities are most active and diverse when the soil moisture content is close to its water holding capacity (WHC) (Allison and Treseder, 2008). Ideally, soil should be watered at a frequency that allows it to remain moist but not waterlogged. This will promote the growth and activity of soil microbes, leading to a healthy and fertile soil. In a study by Li *et al*. (2018), the researchers examined the effects of different irrigation frequencies (daily, every two days, every three days, and every four days) on soil microbial biomass, activity, and community composition in a pear orchard. The results showed that microbial biomass and activity were highest in the daily irrigation treatment, while the microbial community composition was more diverse in the less frequent irrigation treatments. Excess water can also negatively impact soil microorganisms, especially if waterlogging persists for extended periods. Waterlogging reduces oxygen availability in soil, which can lead to anaerobic conditions that favor the growth of anaerobic microorganisms (Fenner and Freeman, 2011). Drought can also impact soil microorganisms by reducing water availability and limiting nutrient transport.

2.3 Soil and its physicochemical properties Soil physicochemical properties are basic indicators for estimating the level of soil nutrient contents and characteristics. The physicochemical makeup of the soil affects plant growth, which in turn affects plant morphology. They are important to agricultural chemists for plants growth and

soil management (Kanimozhi and Panneerselvam, 2011). In addition to improving the physicochemical properties of the soil, organic matter also stimulates the microbial growth and increases the enzymatic activity. Physicochemical characteristics of different soils vary in space and time due to variation in topography, climate, physical weathering processes, vegetation cover, microbial activities, and several other biotic and abiotic variables (Paudel and Sah, 2003).

The physicochemical characteristics necessary for plant development include moisture content, specific gravity, and nitrogen as a fertilizer. In addition to being needed for flowering, potassium is also necessary for the synthesis of proteins, photosynthesis, fruit quality, and the prevention of disease. Phosphorus is also utilized by plants to promote the development of their roots. The cell wall of plants, which allows for appropriate movement and retention of other elements, is made up in large part of calcium (Gupta and Varshaney, 1994; Garba *et al*., 2013). It was observed that available nutrient balance in soil was influenced by soil pH; moreover, phytotoxicity of aluminate was reported in alkaline soil (with pH greater than 9). The only approach to provide precise fertilizer recommendations and to assess the amount of accessible nutrients in the soil is through soil testing. Soil electrical conductivity can serve as a measurement of soluble nutrients and it is useful in monitoring the mineralization of organic matter in soil (Ingole, 2015).

In terms of physical characteristics, high quality soil usually has good tilth, sufficient depth, good drainage, and is resistant to soil fertility decline, adverse changes in pH

or salinity, flooding, erosion, and deterioration of the soil's structural condition. The chemical characteristics of high quality soils include sufficient but not excessive levels of nutrients and little or no chemicals or toxins that may harm plants (Gugino *et al*., 2009). Soil physical properties are crucial in determining soil's appropriateness for agricultural applications. Excessive compactness is detrimental to maintaining a good root environment, reducing penetration of water, and increasing runoff and erosion (Mudarisov *et al*., 2020).

2.3.1 Effect of microorganisms on soil physicochemical properties

Soil microorganisms can decompose humus and organic matter in soil and transform them into forms that can be absorbed and utilized by plants, thus changing the content of soil nutrients. At the same time, soil nutrients provide the growing environment and energy for soil microorganisms, thus changing the number and community structure of soil microorganisms (Jacoby *et al*., 2017). Soil quality can be assessed by combining biological, chemical, and physical soil parameters. Microbial activity shows the microbiological operations of soil microorganisms, which vary in proportion depending on the soil system (Chen and Zhang, 2003). According to Zak *et al*. (1993), bacteria and fungi are the most common forms of microorganisms found in soil and play an important role in nutrient transformations and litter decomposition rates. Soil texture is one of the most important elements determining the structure of microbial communities, as are pH, cation exchange capacity, and organic matter concentration. These characteristics can directly alter microbial

community structure by providing suitable habitat for specific microbes, resulting in a maximal degradation process. The interactions between soil organic matter, total nitrogen levels, and soil texture may have an impact on the microbial communities in soil and how well they perform in the process of degrading plant waste (Hamarashid *et al*., 2010).

2.4 *Trichoderma spp.*

The *Trichoderma* genus belongs to the domain Eukaryota, kingdom Fungi, division Ascomycota, subdivision Pezizomycotina, class Sordariomycetes, order Hypocreales, and family Hypocreaceae. Thegenus *Hypocrea*l *Trichoderma* already includes more than 300 molecularly and morphologically characterized species, many of which have not yet been formally described (Bissett *et al*., 2015; Marik *et al*., 2019; Tamandegani *et al*., 2020). *Trichoderma* is a genus of filamentous fungi that are among the most frequently isolated soil microorganisms. They are commonly found in the soil as well as in other environments such as plant roots, decaying wood, and compost (Harman *et al.,* 2004; Druzhinina *et al.,* 2011). *Trichoderma* is a genetically diversified genus that *Trichoderma* spp, a well-known bio-agent is ubiquitous in nature (Khan *et al*., 2017). It is considered beneficial in the field of agriculture because of their high level of antagonism against diverse phytopathogenic microorganisms (Amin *et al*., 2015; Hermosa *et al*., 2012; Pavlovskaya *et al*., 2020). *Trichoderma* spp. increases the level of Co2 and O2 utilization efficiency by controlling expression of genes in plants (Silva *et al*., 2019). In soil, *Trichoderma*

spp. helps to mix the insoluble minerals and makes them available for normal growth and development of plants (Altomare *et al*., 1999).

2.4.1 *Trichoderma* spp. as a plant growth promoter

Trichoderma spp. is known to have the capability of attacking other fungi. They are well known as potential biological control agents among researchers (El Komy *et al*., 2015; Sundaramoorthy and Balabaskar, 2013). *Trichoderma* is able to colonize roots, improving plant nutrition, growth, and development as well as enhancing plant resistance to abiotic stresses. The success of species belonging to the genus *Trichoderma* as biocontrol agents in the soil ecosystems results from their ability to rapid growth, the possibility of utilizing a variety of substrates, and resistance to many toxic chemicals, including fungicides, herbicides, and other organic pollutants (Zin and Badaluddin *et al*., 2020).

Trichoderma species have been used to suppress plant diseases and the growth of pathogens in contact with plant tissues in the soil environment in both greenhouse and field conditions (Harman *et al.,* 2004). *Trichoderma* does not only inhibit pathogen population but also have capacity to develop resistance and enhance many physiological activities in plants. It minimizes the use of chemical NPK fertilizers, increases the uptake of micronutrients and also helps in solubilisation of phosphates (Kamala and Indira, 2011). Previous research showed that inoculating *Trichoderma* sometimes failed to improve crop yield, possibly because the species inoculated without other microbial species could not adapt to the soil

conditions and survive in an established microbial environment (Yan *et al.,* 2011). In the beginning, *Trichoderma* strains appeared white and cottonish, then developing into yellowish-green to deep green compact tufts especially at the center of a growing spot or in concentric ring-like zones on the agar surface (Waghunde *et al*., 2016). The most common and effective methods of applying *Trichoderma* are seed and seedling treatment, soil treatment and foliar application. Soil treatment is the most effective method of *Trichoderma* application for the management of soil-borne diseases (Kumar *et al*., 2014).

2.4.2 Influence of environmental factors on biocontrol potential of *Trichoderma* strains

Among the *Trichoderma* species, the optimum temperature for the growth is different (Samuels, 1996), but mostly they are mesophilic. Some examined *Trichoderma* strains were able to grow in a wide range of pH from 2.0 to 6.0 with an optimum at 4.0. Water conditions have been shown to strongly affect *Trichoderma* activities, most particularly spore germination, germ tube growth, mycelial growth the interaction with other fungi and on enzyme production (Grajek and Gervais, 1987).

2.5 Soil enzymatic activity

Soil enzymatic activity plays a critical role in the decomposition of organic matter, nutrient cycling, and the overall functioning of soil ecosystems. Enzymes produced by soil microorganisms are responsible for breaking down complex organic

compounds into simpler forms that can be used by other soil organisms. The activity of soil enzymes affects the abundance, diversity, and function of soil microorganisms. Bending *et al*. (2002) found that changes in the activity of soil enzymes involved in the breakdown of plant polymers had a significant impact on the ability of soil microorganisms to suppress plant pathogens.

2.5.1 Soil dehydrogenase activity

Dehydrogenases are the most important and major representative of microbial activity among soil enzymes. This enzyme aids in the evaluation of the metabolic condition of soil bacteria and is present intracellularly in all viable cells as a component of their respiratory system (Watts *et al.,* 2010). Dehydrogenase activity (DHA) is one of the best, most crucial, most sensitive bio indicators for assessing soil fertility (Wolinska and Stepniewska, 2012). They are also closely related to microbial oxidoreduction processes (Moeskops *et al.,* 2010). Dehydrogenases play a significant role in the biological oxidation of soil organic matter (OM) by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al*., 2010). Brzezińska *et al*. (2001) ascertained that these enzymes may use not only oxygen molecules as electron acceptors but also other compounds which occur in cells of anaerobic microorganisms. Thus, the activity of dehydrogenases reflects the rate of transformations occurring in the soil.

2.6 Soil microbial diversity

Microbial diversity describes complexity and variability at different levels of biological organization. It encompasses genetic variability within species and the number (richness) and relative abundance (evenness) of species and functional groups (guilds) in communities. Knowledge of microbial diversity and function in soils is limited because of the taxonomic and methodological limitations associated with studying these organisms (Kirk *et al*., 2004). Microbial diversity in soil ecosystems exceeds by far that of eukaryotic organisms. One gram of soil may harbor up to 10 billion microorganisms of possibly 10 thousands of different species (Roselló-Mora and Amann, 2001). Soil microbial diversity, abundance, and community structure are related to the functions of microbial community and soil health. Microbial communities participate in soil functions including nutrient cycling, formation of soil aggregates, and suppression of soilborne diseases (Ruiz Gómez *et al*., 2019).

2.6.1 Effect of *Trichoderma* spp on soil microbial diversity

Trichoderma species directly inhibit the growth of pathogenic bacteria by producing various secondary metabolites (Mironenka *et al*., 2021). On the other hand, they indirectly improve plant disease resistance and promote plant growth by changing soil microbial community structure and increasing the number of beneficial soil microorganisms (Zafra and Cortés-Espinosa, 2015). Bae and Knudsen (2004)

reported that the addition of *Trichoderma* spp. into soils resulted in a higher bacterial population.

2.7 Effect of water on soil microbial frequency

A large number of studies have been conducted to examine the effects of drying and wetting on soil microorganisms and their functions, both in laboratory and field experiment (Gao *et al.,* 2016; Harrison-Kirk *et al*., 2013). Generally, drying can induce osmotic stress on microorganisms, which consequently leads to a decrease in microbial biomass and activity. In addition, drying of soils decreases nutrient and substrate availability through diffusive limitation and reduction in root exudation, which further constrains the growth and activity of soil microorganisms. However, wetting of dry soils typically results in an increase in microbial biomass and activity for short periods. he increased biomass and activity of microorganisms after rewetting dried soils is always coupled with a flush of carbon (C) and nitrogen (N) mineralization (Xiang *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS

This study was carried out at Crop Science Departmental Laboratory, University of Benin, Benin city.

3.1 Collection and preparation of soil samples

The soil samples were obtained from an experimental plot of the Department of Crop Science, University of Benin, Benin city.

3.1.1 Sample preparation

The soil samples were air-dried for one week, ground with a clean porcelain mortar with pestle and passed through a 2.0 mm sieve. The soil samples were kept in the polythene packets for further analysis.

3.2 Microbial analysis

3.2.1 Isolation of bacteria and fungi associated with soil samples treated with *Trichoderma harzianum* and planted with soursop under varying watering regimes

The isolation of bacteria and fungi associated with soil samples treated with *Trichoderma harzianum* and planted with soursop under varying watering regimes was done at an interval of three weeks using soil serial dilution and pour plate method.

Equipment used: Weighing balance, laminar flow hood, incubator, autoclave.

Materials: Conical flask, universal bottle, petri-dish, bunsen burner, sterile water.

3.2.2 Sterilization of Glass Wares and Culture Media preparation

Glass wares used were oven sterilized.

The medium used: Potato Dextrose Agar (PDA), Nutrient Agar (NA).

3.2.3 Preparation of Nutrient Media

Thirty-nine grams (39g) of Potato Dextrose Agar (PDA) was weighed into a conical flask and dissolved in 1000ml of sterile distilled water to give 1000ml of PDA. The dissolved PDA was sterilized in an autoclave at a temperature of 121°C (15psi) for 15 minutes.

Twenty-eight grams (28g) of Nutrient Agar (NA) was weighed into a conical flask and dissolved in 1000ml of sterile distilled water to give 1000ml of NA. The dissolved NA was sterilized in an autoclave at a temperature of 121°C (15psi) for 15 minutes.

3.2.4 Preparation of Stock Solution

A stock solution was prepared by weighing one gram (1g) of the collected samples dissolved in 10ml of sterile distilled water in the first McCartney bottle.

3.2.5 Serial dilution

One gram (1g) of soil was weighed and placed in a McCartney bottle containing 10ml of sterile distilled water and mixed properly to allow for dispersal of microorganisms. One milliliter (1ml) aliquot was taken from bottle 1 (stock solution) to the second McCartney bottle containing 9ml of sterile distilled water. Further dilutions were carried out till the fifth dilution level (bottle 5) was achieved. One

milliliter (1ml) of the suspension from the McCartney bottle 5 was dispensed into sterile petri dishes.

3.2.6 Method of inoculation

The pour plate method of inoculation was used in the isolation of the microorganisms associated with the samples. 1ml each of the serial dilution prepared samples were pipetted with the aid of a syringe and transferred into the corresponding labeled Petri dishes. Twenty (20) ml of molten prepared Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were dispensed into the Petri dishes. The Petri dishes were incubated at room temperature for an interval of 24hrs for bacterial growth and 72hrs for fungal growth.

3.3 Determination of microbial load

The microbial load of the samples was determined visibly by counting the colony-forming unit after 24hrs. The microbial load/ml will then be determined by the formula stated below

Count/ml = No of colonies on plate × dilution factor

3.3.1 Sub-culturing of microorganisms

Freshly prepared NA and PDA were poured into already sterilized Petri dishes, and it is allowed to solidify. From the old plates containing the microorganisms, the streaking method was used to isolate the individual bacteria cultures into a fresh plate containing NA for further studies. The fungal cultures were sub-cultured by

carefully taking a fresh growing portion with a sterilized needle into a sterile petri dish containing PDA.

3.3.2 Macroscopic identification of Fungal isolates

Fungi associated with the soil samples were then subcultured into a freshly prepared PDA on Petri dishes. After growth, it was then examined and identified based on their cultural and microscopic characteristics.

3.3.3 Characterization and identification of Bacterial isolates

For Bacterial isolates, cultural characteristics were observed on Nutrient agar plates. These characteristics include: Size, shape, surface, texture, opacity and pigmentation were determined by visual observation.

Gram reaction

After sterilizing a wire loop in a bunsen burner and letting it cool, a loopful of growth was collected from the agar plate and put to a clean, grease-free slide. A drop of regular saline was then added, and it was emulsified and heated to fixation by passing it three times over a flame. The smear was flooded with Crystal violet for 30-60 seconds and then covered with iodine for 30-60 seconds and washed off. It was decolourized with acetone and rinsed immediately. Safranin was applied on the slide for a minute and the washed with clean water. Afterwards, it was kept in a rack to air-dry after wiping the back with cotton wool. The stained smear was examined microscopically under oil immersion at 100 magnification objective lens. The gram-

positive bacteria appeared dark purple while the gram-negative bacteria appeared pink.

Motility test

Motility test was done by stabbing a slant with the isolated bacteria. If bacteria are not motile, it grows only along the stab line.

Catalase test

A sterile test tube was filled with 3ml of hydrogen peroxide solution. Afterwards, a sterile glass rod was used to collect several colonies and inoculated into the hydrogen peroxide solution. A positive test result is then determined by looking for immediate vigorous bubbling.

Oxidase test

A piece of filter paper was placed in a clean Petri dish and 2-3 drops of freshly prepared oxidase reagent was added. A small portion of culture was placed on the filter paper and smeared on it with the help of a sterile glass rod. A blue-purple colour change was observed within a few seconds and this indicates a positive test.

Urease test

The test organisms were heavily inoculated onto Christensens urea broth in a bijou bottle using a sterile wire loop and incubated at 35-37°C for 18-24 hours and examined. Thereafter, a pink colour in the medium indicated a positive test.

Citrate test

Simmons citrate agar medium was prepared in a slant McCartney bottle. A sterile wire loop was used to inoculate the test organism into the slant medium and incubated at 35°C for 48hours after which it was examined for colour formation. A colour change from green to blue in the medium gave a positive citrate test.

Indole test

A colony of the test organism was inoculated into 2ml of tryptophan-containing peptone water using a sterile wire loop. The tube was stoppered and inoculated for 24 hours at 37°C. Kovac's reagent was then added to the medium, and the mixture was monitored for 10 minutes. A red coloration appeared on the surface layer which indicates a positive result.

Carbohydrate fermentation test

A sterile inoculating loop was use to insert a loopful of test organisms into a pheny-red carbohydrate broth and incubated the tubes for 35°C-37°C for 18-24 hours. A Durham’s tube was injected to measure gas production. A colour change from reddish-orange to yellow denotes positive formation while presence of bubbles in Durham’s tube denotes a positive gas production.

Methyl-red test

The test organisms were inoculated with glucose phosphate peptone water and incubated for 48 hours at 37°C. Afterwards, a few drops of methyl red solution were

added to the culture, and the culture was read immediately. The appearance of red colour indicates a positive test.

Nitrogen content

The Nitrogen content of the soil samples were determined by the Kjeldahl method. This method involves digestion, distillation, and titration.

Digestion: 2g of the soil sample was weighed in a round bottom flask and 25ml concentrated sulphuric acid was added. Afterwards, 0.5g of copper sulphate and 5g of sodium sulphate was added to the samples. The samples were heated slowly using a heating mantle in a fume cupboard to prevent undue frothing, then allowed to digest for 45minutes until the sample became clear pale green. The sample was allowed to cool and 100ml of distilled water was added.

Distillation: This was done against 10ml of the boiled digest using 10ml of sodium hydroxide and 50ml of 2% boric acid containing screened methyl red indicator.

Titration: The alkaline ammonium borate formed was titrated directly against 0.1N HCL. The titre value which is the volume of the acid used was recorded. The percentage of Nitrogen found in the soil samples was calculated with this formula

$$\%N = \frac{14 \times VA \times 0.1 \times w \times 100}{1000 \times 100}$$

VA= volume of acid used

W= weight of the sample

3.4 Soil Physiochemical Analysis

3.4.1 Methods of Soil Analysis

The following methods were used to perform laboratory analysis of the sampled soil:

3.4.2 pH

Soil pH is a measure of the acidity or alkalinity of the soil. The “ideal” soil pH is close to neutral, and neutral soils are considered to fall within a range from a slightly acidic pH of 6.5 to slightly alkaline pH of 7.5. Soil pH can be measure using a glass electrode pH meter or a testing kit. A mixture of 20gm of sample soil and 100ml of distilled water was stirred continuously for an hour prior to dipping the glass electrode in the soil. In order to express pH, 1:5 soil suspensions are used.

3.4.3 Nitrogen (kjeldahl method)

Nitrogen is found in all soils, and is required by all living creatures. In plants, nitrogen is the nutrient required in the largest amounts. Kjeldahl method is a laboratory technique for determining the nitrogen content in organic and inorganic substances such as soil samples, which involves digesting the soil sample and measuring the amount of nitrogen released. 10ml of distilled water was added to a round-bottomed flask containing about 1g of soil sample that had gone through a 2mm screen. The mixture was the left for an hour. 20gm of catalyst digestion mixture and 10ml of conc H_2SO_4 was added to the mixture. The mixture was refluxed for one and half hours and cooled. The mixture was then poured in a volumetric flask and made up to 100ml mark using distilled water. 20ml of sample

was taken from the volume into a distillation flask and 20ml sodium hydroxide was added to it. Conical flask was filled with 20 ml of the volumetric flask sample; 10 ml of 4% Boric acid and 2 drops of mixed indicator was added to it. The conical flask was placed below a condenser so that the condenser's tip would dip into the solution. The assembly was then connected, and distillation was started. About 150ml of condensate was collected and the flask was removed. The mixed indicator in condensate turned blue due to the dissolution of ammonia. The content was the titrated with 0.1N hydrochloric acid until the colour change to light brown pink i.e the original colour of the indicator. Calculation:

% Nitogen = 1.4V ×NW

Where N=normality of standard acid

V = Acid used in titration (in ml)

W = weight of soil (in grams)

3.4.4 Percentage Carbon and Organic Matter

The solid component of soil known as soil organic matter is created by plant detritus and deceased animals. The modified Walkey-Black method was used to calculate the amount of organic matter in the soil sample.

Reagent: 1N K_2Cr_2O7, 85% H_3SO_4, conc. H_2SO_4, Diphenylamine indicator

In this method, an oven dried soil sample was taken and passed through 0.5mm sieve. About 1g of the soil sample was weighed and transferred to 500ml conical flask. 10ml of 1N potassium dichromate and 20ml of concentrated sulphuric acid were

added and mixed by swirling gently. The mixture was allowed to react and was kept for 30minutes. Then the content was diluted with 200ml of distilled water and then 1ml of diphenylamine indicator was added. The sample was then titrated with 0.4N Ferrous Ammonium Sulphate (FAS) till the colour changed to brilliant green at the endpoint. The process was repeated for blank with the same amount of chemicals but without the soil sample.

Calculation:

% Carbon = (1-T÷S) (3.951÷g)

% Organic Matter = % Carbon × 1.724

Where g = weight of soil in grams

S = ml of FAS with blank titration

T = ml of FAS with sample titration

3.4.5 Soil Texture

Materials: Standard soil hydrometer, Dispersing machine, Soil plunger, 100ml measuring cylinder, thermometer, soil shaking bottle.

Reagents: $NaPO_3$, sodium carbonate, Amyl alcohol, Hydrogen peroxide

Procedure

51g of dried soil was weighed in a soil shaking bottle ad 100ml of Calgon was added stirred with a stirring rod for a few minutes. It was dispensed using a dispersing machine and washed into a 100ml measuring cylinder with the aid of a wash bottle.

It was plunged and allowed to stand for 40 seconds, after which the first hydrometer reading was taken using a hydrometer and temperature reading was taken using a thermometer. Afterwards, the suspension was allowed to stand for 2 hours, then the second hydrometer and temperature reading was taken. Calculations were done using the first and second hydrometer and temperature readings.

3.5 Experimental Design

The experiment was set up using a Completely Randomized Design (CRD), and statistical analysis was done on the data gathered. GenStat Version 12 was used for the analysis of variance (ANOVA), which was separated using the Student-Newman-Keuls test.

CHAPTER FOUR

RESULTS

4.1 Physical and Chemical properties of soil sample

Analysis of the soil used is presented in Table 1. The results show that the pH value of the soil was 5.67 which indicates moderately acidic soil, the Exchangeable Cation Exchange capacity (ECEC) was low with a value of 1.58cmol/kg which implies that the soil has limited ability to retain and exchange cations. The organic matter content of the soil was high (17.79g/kg), Total Nitrogen in the soil was 0.62g/kg, Available phosphorus was 18.33mg/kg, the values of calcium, magnesium, sodium, potassium, hydrogen and aluminium in the soil was recorded to be 0.87cmol/kg, 0.21cmol/kg, 0.13cmol/kg, 0.24cmol/kg, 0.10cmol/kg and 0.03cmol/kg respectively. The textural class of the soil used was loamy sand.

Table 4.1 Physical and chemical analysis of the experimental soil prior to treatment with *Trichoderma* spp.

Parameters	Values
pH	5.67
Organic carbon (g/kg)	10.31
Organic matter (g/kg)	17.79
Total Nitrogen (g/kg)	0.62
AvP (mg/kg)	18.33
Ca (cmol/kg)	0.87
Mg (cmol/kg)	0.21
Na (cmol/kg)	0.13
K (cmol/kg)	0.24
H^+ (cmol/kg)	0.10
Al^{3+} (cmol/kg)	0.03
CEC (cmol/kg)	1.55
ECEC (cmol/kg)	1.58
Sand (g/kg)	876
Silt (g/kg)	65
Clay (g/kg)	62
Textural class	Loamy sand

4.2 Microbial Diversity in soil planted with soursop under varying watering regimes on the basis of soil incubation period.

Table 4.2 shows the microbial diversity in soil planted with soursop under varying watering regime on the basis of three different incubation periods (Week 3, Week 6 and Week 9). The presence (+) or absence (-) of specific fungi and bacteria is shown for each period. A total of four fungi isolates comprising of: *Trichoderma sp, Aspergillus niger, Rhizopus arrhizus, Mucor mucedo* and four bacteria isolates, *Escherichia coli, Bacillus pumilis, Bacillus subtilis and Klebsiella oxytoca* was isolated from soil treated with *Trichoderma harzianum* and planted with soursop under varying watering regime. The result of the fungi isolates shows that *Trichoderma sp* are present in all three incubation periods in all the different watering regimes. *Aspergillus niger* are present in week 3 in all watering regimes except watering once in 3 days and also present in week 6 in watering every day and watering once in 3 days but absent at week 9, *Rhizopus arrhizus* are absent at week 3 and week 6 but present at week 9 in the different watering regimes, *Mucor mucedo* are absent at week 3 but present at week 6 in the soil watered every day and week 9 in all different watering regimes.

The result of the bacteria isolates in Table 4.2 shows that *Escherichia coli* was found in week 3 in soils watered once in 3 days and once in 5 days and week 6 in soil watered every day but absent in week 9. *Bacillus subtilis* are present at week 3 in soils watered every day d once in 5 days but absent at week 6 and week 9, *Klebsiella*

oxytoca are absent in week 3 and week 6 but present in week 9 in the various watering regimes.

Table 4.2: Microbial Diversity in soil planted with soursop under varying watering regimes over a 9 week period

Sampling Time	Week 3				Week 6				Week 9			
Watering regimes	**W1**	**W3**	**W5**	**W7**	**W1**	**W3**	**W5**	**W7**	**W1**	**W3**	**W5**	**W7**
Fungal Isolates												
Trichoderma sp	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	+	-	+	+	+	+	-	-	-	-	-	-
Rhizopus arrhizus	-	-	-	-	-	-	-	-	+	+	+	+
Mucor mucedo	-	-	-	-	+	-	-	-	+	+	+	+
Bacterial Isolates												
Escherichia coli	-	+	+	-	-	+	-	-	-	-	-	-
Bacillus pumilus	+	+	+	+	+	+	+	+	+	+	+	+
Bacillus subtilis	+	-	+	-	-	-	-	-	-	-	-	-
Klebsiella oxytoca	-	-	-	-	-	-	-	-	+	+	+	+

Key: + = Present, - = Absent

4.3: Cultural and microscopic characteristics of fungi isolated from soil treated with *Trichoderma harzianum* and planted with soursop under varying watering regimes

The result in Table 4.3 showed that Isolate 1 identified as *Trichoderma sp* displayed a green mycelium on agar plate and pale yellow colour of plate culture reverse. Its hyphae are septate and they produce conidiospores whose size and shape are similar to *Penicillium* and *Aspergillus.* They belong to the class of fungi called Ascomycetes.

Table 4.3 indicates that Isolate 2 identified as *Aspergillus niger* displayed dark colour growth of mycelium and dark colour of plate culture. The nature of its hyphae, its spore type and the class of fungi is same as *Trichoderma sp*.

Isolate 3 identified *as Rhizopus arrhizus* showed white colour of mycelium on agar plate which later turned grey with black dots at aging, it displayed Light grey colour of plate culture reverse. Its hyphae are non-septate. They produce single and unbranched sporangiophore and they belong to the class of fungi called Zygomycetes.

Isolate 4 identified as Mucor mucedo displayed grey to off-white colour of mycelium on agar plate and black colour of plate culture reverse. The nature of its hyphae, its spore type and the class of fungi is same *as Rhizopus arrhizus*.

Table 4.3: Cultural and microscopic characteristics of fungi isolated from soil treated with *Trichoderma* spp. and planted with soursop under varying watering regimes

Cultural characteristics	Isolates 1	Isolates 2	Isolates 3	Isolates 4
Colour of mycelium on agar plate	Green mycelium	Dark coloured growth	Initially white; turning grey with black dots at aging	Grey to off-white or white
Colour of plate culture reverse	Pale yellow	Dark	Light grey	Black
Nature of hyphae	Septate	Septate	Non-septate	Non-septate
Type of Spore	Conidiospore	Conidiospore	Sporangiophores	Sporangiophores
Spore structure/Attachment	Conidia size and shape are similar to *Penicillium* and Aspergillus. Typical green spore clumps are identified as *Trichoderma.*	A. niger consists of a smooth and colorless conidiophores and spores.	Single and unbranched sporangiophore	Sporangiophore
Rhizoids	Absent	Absent	Present	Absent
Appearance of special structure	Conidiophores hyaline and loosely branched at right angles. Phialides flask-shaped and inflated at the base, with very short collarettes.	Conidial heads radiate, becoming columnar when mature; conidiophores are long and smooth-walled; biseriate; two rows of phialides cover the entire vesicle.	Rhizoids occur at the junction of stolon and sporangiophore	Sporangia are produced on the tips of sporangiophores. The sporangia contain spores which are the reproductive units of Mucor
Class of fungi	Ascomycetes	Ascomycetes	Zygomycetes	Zygomycetes
Fungi isolated	***Trichoderma sp***	***Aspergillus niger***	***Rhizopus arrhizus***	***Mucor mucedo***

4.4: Morphological and biological characteristics of isolated bacteria from soil treated with *Trichoderma harzianum* and planted with soursop under varying watering regimes

The result in table 4.4 showed that Isolate 1 identified as *Bacillus subtilis,* displayed a flat elevation, undulate margin, cream colour, irregular shape and large size. It was cultured on BCA agar producing straw colour and appeared purple after gram staining which indicate a positive gram reaction. *Bacillus subtilis* was positive for various biochemical tests such as catalase, citrate, motility, glucose, sucrose, lactose and mannitol fermentation while it displayed a negative reaction for indole, oxidase urease, gas formation and H_2S formation. Its TSI (Slant/Butt**)** reaction was A/A with a positive Esculin hydrolysis.

Isolate 2 identified as *Bacillus pumilus*, displayed a flat elevation, irregular margin, off-white colour, concave shape and large size. It also grew on BCA agar showing similar characteristics as *Bacillus subtilis* with the production of straw colour which appeared purple after gram staining indicating positive gram reaction. *Bacillus pumilis* was positive for catalase, citrate test, glucose fermentation and it was negative for indole, oxidase, motility test, urease, and sucrose and lactose fermentation. Gas formation and H_2S formation was also negative. Its TSI (Slant/Butt**)** reaction was A/A with a positive Esculin hydrolysis.

The result also showed that isolate 3 identified as *Escherichia coli,* displayed same morphological characteristics with *Bacillus subtilis* but *Escherichia coli* grew on EMB agar. It gave a green coloration which appeared pink after gram staining

indicating a negative gram reaction. *Escherichia coli* displayed negative reaction for citrate, oxidase test, urease test, sucrose fermentation and H_2S formation. Its Esculin Hydrolysis was also negative. Catalase test, indole test, motility, glucose fermentation and Gas formation gave positive reaction. Its TSI (Slant/Butt**)** reaction was A/AG.

Isolate 4 identified as *Klebsiella oxytoca,* displayed flat elevation, entire margin, cream colour, circular shape and small size. It grew in EMB agar too and gave a pink coloration before and after gram staining. This indicated a negative gram reaction. *Klebsiella oxytoca* showed positive reaction for catalase, citrate, urease test, glucose and sucrose fermentation. Gas formation also gave positive reaction. Indole test, oxidase, motility test and H_2S formation showed negative reaction. Its TSI (Slant/Butt**)** reaction was A/AG with a positive Esculin hydrolysis.

Table 4.4: Morphological and biological characteristics of isolated bacteria from soil treated with *Trichoderma* spp. and planted with soursop under varying watering regimes

Cultural characteristics	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Elevation	Flat	Flat	Flat	Flat
Margin	Undulate	Irregular	Undulate	Entire
Colour	Cream	Off-white	Cream	Cream
Shape	Irregular	Concave	Irregular	Circular
Size	Large	Large	Large	Small
Gr. Diff. agar	BCA	BCA	EMB	EMB
Colour	Straw	Straw	Green	Pink
Gram stain	+	+	-	-
Cell type	Rod	Rod	Rod	Rod
Arrangement	Disperse	Disperse	Disperse	Disperse
Colour	Purple	Purple	Pink	Pink
Spore staining	+	+	-	-
String Test	-	-	+	+
Catalase	+	+	+	+
Indole	-	-	+	-
Citrate	+	+	-	+
Oxidase	-	-	-	-
Motility	+	-	+	-
Urease	-	-	-	+
Glucose	+	+	+	+
Sucrose	+	-	-	+
Lactose	+	-	+	+
Mannitol	+	+	-	-
Gas formation	-	-	+	+
H_2S formation	-	-	-	-
TSI (Slant/Butt) reaction	A/A	K/A	A/AG	A/AG
Esculin Hydrolysis	+	+	-	+
Identity	***Bacillus subtilis***	***Bacillus pumilus***	***Escherichia coli***	***Klebsiella oxytoca***

4.5 Mean Square Values of the Effect of Soil Media, Watering Regime and Soil Incubation Duration on Bacteria Colony Forming Unit (CFU) and Fungal Colony Forming Unit (CFU)

The result in table 4.5 showed that the Bacteria CFU and Fungal CFU on the soil media were recorded to be 3337 and 5448 respectively which was reported as non-significant values. The Colony Forming Unit of Bacteria and Fungi on watering regimes were 5928 and 18460 respectively with Bacteria being not significant and fungi highly significant. The Colony Forming Unit on Soil Incubation Duration of Bacteria was highly significant with a value of 17610 and that of Fungi were significant with a value of 10856. The Bacteria CFU and Fungal CFU on the interaction between soil media and watering regimes were highly significant with their respective values of 12031 and 18556. The interaction between Soil media and Soil incubation duration had its Bacteria CFU and Fungal CFU values to be 3701 and 3191 respectively which were reported as non-significant. The Bacteria CFU and Fungal CFU on the interaction between the three sources (soil media, watering regimes and soil incubation duration) were highly significant having respective values of 18558 and 14386.

Table 4.5 Mean Square Values of the Effect of Soil Media, Watering Regime and Soil Incubation Duration on Bacteria Colony Forming Unit and Fungal Forming Unit

Source	Df	Bacteria MSV	Fungi MSV
SM	3	3337^{ns}	5448^{ns}
WR	3	5928^{ns}	18460^{***}
SID	2	17610^{***}	10856^{**}
SM×WR	9	12031^{***}	18556***
SM×SID	6	3701^{ns}	3191^{ns}
SM×WR×SID	18	18558^{***}	14386***
RESIDUAL	48	6023^{ns}	7228^{ns}

SM: Soil Media
WR: Watering Regime
SID: Soil Incubation Duration
MSV: Mean Square Values
***: Highly significant
**: Significant
^{ns}: Not significant

4.6 Mean values of Colony Forming Units as Influenced by Soil Media, Watering Regime and Soil Incubation Duration

Table 4.6 showed the mean values of the microbial counts for various soil media, watering regimes and incubation duration (Week 3, 6 and 9) of soil treated with *Trichoderma spp* and planted with soursop. The mean values of the microbial counts on soil media with the combination of Treated seed and Treated soil, Treated seed and Untreated soil, Treated soil and Untreated seed, Untreated seed and Untreated soil had a value of 108.8, 104.7, 104.0, 129.0 respectively with 16.7 as the standard error of differences (sed) of means for Bacteria and 142.1, 139.5, 128.2, 164.2 respectively with 13.14 as the standard error of differences (sed) of means for fungi.

The result also showed that watering everyday had a value of 124.0 for bacteria and 169 for fungi, watering once in three days had values of 108.4 for bacteria and 136.8 for fungi, watering once in five days had a value of 90.7 for bacteria and 107.3 for fungi then watering once in seven days had values of 123.5 for bacteria and 160.9 for fungi.

The result showed that the colonies that were found on the soil incubated for 3 weeks had a mean value of 89.5 for bacteria and 162.5 for fungi while that of week 6 had a value of 109.2 for bacteria and 142.3 for fungi and week 9 had values of 136.2 for bacteria and 125.7 for fungi. The standard error of differences (sed) of means for Bacteria and fungi was 14.69 and 11.38 respectively.

Table 4.6 Microbial population of soil as Influenced by Soil Media, Watering Regime and Soil Incubation Duration

Source	Bacteria CFU($\times 10^5$) (cfu/g)	Fungi CFU($\times 10^5$) (cfu/g)
Soil Media		
Tseed-Tsoil	108.8[a]	142.1[ab]
Tseed-Usoil	104.7[a]	139.5[ab]
Tsoil-Useed	104.0[a]	128.2[a]
USeed-USoil	129.0[a]	164.2[b]
Sed	16.97	13.14
Watering Regime		
W1	124.0[a]	169.0[c]
W3	108.4[a]	136.8[b]
W5	90.7[a]	107.3[a]
W7	123.5[a]	160.9[bc]
Sed	16.97	13.14
Soil Incubation Duration		
3weeks	89.5[a]	162.5[b]
6weeks	109.2[ab]	142.3[ab]
9weeks	136.2[b]	125.7[a]
Sed	14.69	11.38
Cv(%)	52.6%	31.7%

TSeed-Tsoil: Treated seed and Treated soil
Tseed-Usoil: Treated seed and Untreated soil
Tsoil-Useed: Treated soil and Untreated seed
Useed-Usoil: Untreated seed and Untreated soil

W1: Watering everyday
W3: Watering once in three days
W5: Watering once in five days
W7: Watering once in seven days

CHAPTER FIVE

DISCUSSION

In this experiment, the microbial communities inhabiting the soil treated with *Trichoderma sp* and planted with soursop under varying watering regimes were examined, revealing the presence of four distinct fungi (*Mucor mucedo, Trichoderma sp, Aspergillus niger* and *Rhizopus arrhizus*) and four bacteria (*Escherichia coli, Bacillus subtilis, Bacillus pumilus and Klebsiella oxytoca*).

The identification of Trichoderma sp reaffirms the efficacy of the treatment while the diverse fungi and bacterial species suggest a complex soil ecosystem. The impact of changing watering frequencies highlights how soil microbes can respond to environmental factors emphasizing the need for adequate moisture levels for microbial diversity. This is in agreement with (Gilbert *et al*., 2014) who demonstrated that many environmental factors especially soil moisture could affect soil microbes. The absence of some of these microorganisms can be attributed to changes in environmental conditions or variations in the soil's microbial community.

It was observed in this study that the microbial colony forming units was higher in week 9 for bacteria and in week 3 for fungi and that the soil media Untreated seed and untreated soil had the highest colony forming units (129.0×10^5 cfu/g and 164.2×10^5 cfu/g) for both bacteria and fungi respectively while Treated soil and Untreated seed had the lowest colony forming units (104.0×10^5 cfu/g and 128.2×10^5cfu/g) for bacteria and fungi respectively.

This study showed that the effect of watering regime on microbial population had the highest colony forming units (124.0 x 10^5 cfu/g and 169.0 x 10^5cfu/g) at W1 (watering everyday) for both bacteria and fungi respectively while W5 (watering once in 5days) was shown to have the lowest colony forming units (90.7 x 10^5 cfu/g and 107.3 x 10^5 cfu/g) for bacteria and fungi respectively. This is in agreement with the findings of Barnard *et al*., 2013 who observed that changes in water availability can results in shifts in the composition of microbial communities.

5.2 CONCLUSION

The diversity and frequency of soil microorganisms was affected by soil media, watering regime and soil incubation duration.

The effect of the interaction between soil-seed treatment, watering regimes and soil incubation duration was highly significant on the microbial diversity and frequency.

From the results obtained, watering daily had the highest colony forming units while soil media Untreated Seed-Untreated soil and soil incubation duration nine weeks had the highest colony forming units for both bacteria and fungi.

5.3 RECOMMENDATION

On the basis of these findings, it is recommended that untreated Seed-Untreated soil watered daily should be used when growing soursop for optimum growth of the plant.

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